CONTRIBUTORS

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PREFACE

Our editorial policy continues to be to report annually on the more active fields of interest to medicinal chemists and to cover less regularly new or maturing biomedical topics or fields which have passed their prime. Less than half of this year's topics appeared also in last year's Annual Reports.

It may be well to remind the reader that it is not our intent to provide comprehensive literature coverage for the expert in the field; plenty of other publications already serve this purpose. Our aim is to report significant highlights to enable the worker in a related field to recognize developments or concepts which may broaden his approach to his own area or even cause him to delve into a new one. Just as the increasing availability of massive computer-generated bibliographies on a particular topic poses a growing problem for a researcher who wishes to read selectively in a new field, so this same capability makes it more difficult for our chapter authors to be selective in what they choose to discuss. In fact this problem constitutes the editor's most arduous task; the ten-page chapter limit is designed both to achieve desired selectivity of subject matter and to keep the volume within a reasonable price range in this era of soaring costs.

For the first time section editors have been given the opportunity to include one-page editorial overviews of their sections; among the objectives of these overviews are to highlight major new developments, emphasize common features in drug actions or disease etiologies or just to permit speculation as a stimulus for new ideas. While only one section editor chose to accept the invitation this year, it is hoped that more will do so in future volumes.

As always, your constructive suggestions for improvement or for new topics are earnestly solicited.

Kalamazoo, Michigan
June, 1974

Section I - CNS Agents

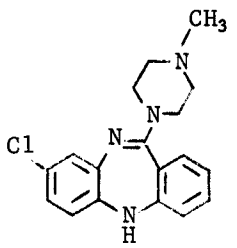
Editor: Maxwell Gordon
Bristol Laboratories, Syracuse, New York

Chapter 1. Antipsychotic and Antianxiety Agents

Charles A. Harbert and Willard M. Welch
Pfizer Inc., Groton, Conn. 06340

Introduction - Although several promising agents have progressed to advanced clinical stages, no fundamentally new antipsychotic or antianxiety agents were introduced in the U.S. in 1973. Clozapine has emerged as the focus of clinical and theoretical interest in the antipsychotic area based on its extremely low incidence of extrapyramidal side reactions. This discovery has given new impetus to research directed toward an understanding of the biochemical and neurophysiological correlates of antipsychotic action and extrapyramidal manifestations. Activity in the antianxiety area again largely focused on agents structurally and mechanistically related to the benzodiazepines. Extensive treatises have covered the chemistry, biochemistry, pharmacology and clinical utility of the benzodiazepines[1] and the neuroleptics.[2]

Antipsychotic Agents - Tricyclics - Structure-activity relationships (SAR) of the tricyclic psychotherapeutics[3] and clinical experience with the long-acting neuroleptics[4,5] have been reviewed. Emphasis continued to shift from structures with 6,6,6 ring systems to the 6,7,6-tricyclics. Among the latter, clozapine (1) has attracted the most interest. A recent review[6] highlights the unique profile of this agent and the author points out some troublesome side effects (hypotension, tachycardia) that may limit its clinical use. Regardless, the emergence of clozapine has reemphasized the feasibility of disassociating antipsychotic activity from the cataleptic properties that have been classically regarded as a prerequisite for clinical therapeutic activity.[7]



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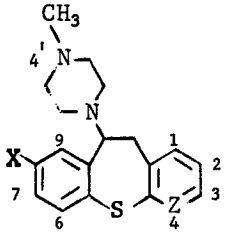
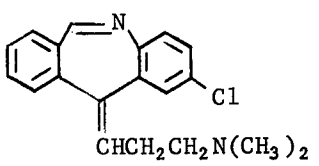


2

a) X = H ; Z = CH
b) X = Cl ; Z = CH
c) X = H ; Z = N
d) X = Cl ; Z = N
e) X = OEt; Z = CH
f) X = OCH_3; Z = CH

Continuing SAR studies in the dibenzo[b,f]thiepin and related 6,7,6 systems have shown that substitution by chlorine in the 9-position of perathiepin (2a) results in diminished activity.[8] All eight possible aryl-monochloro isomers of perathiepin have now been synthesized, and of

these only the 8-Cl isomer (octoclothepin, 2b) is more potent than the parent compound. Octoclothepin enantiomers and the racemate were found to differ little from each other in CNS depressant effects or in toxicity.[9] The 4-aza analogs 2c and 2d showed markedly reduced depressant and cataleptic activity compared with 2a and 2b, respectively.[10,11] Among several 8-alkoxy analogs prepared, only the 8-EtO isomer (2e) exhibited activity (10X chlorpromazine [CPZ] in the rotating rod test) equivalent to the known 8-MeO derivative (2f, octometothepin).[12] Oxidation of the S atom and/or 4'-N atom in a number of dibenzo[b,f]thiepins related to 2 resulted in a uniform loss of CNS depressant activity.[13] EX 11-582A (3) possesses potent activity in the fighting mouse (ED_{50} 0.9 mg/kg) and antiamphetamine toxicity (ED_{50} 0.28 mg/kg) tests.[14] Like clozapine, this compound was inactive against apomorphine-induced emesis.



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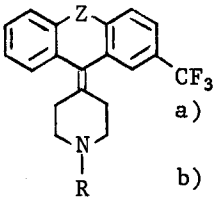
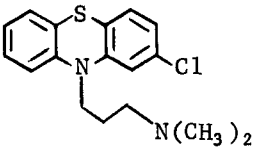


a) R = CH_3; Z = S

b) R = CH_2-◇; Z = S

4



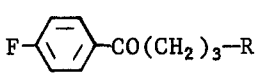
5

A recent paper of theoretical interest reported potent neuroleptic activity for a number of piperidylidene thioxanthenes (4,Z=S) and related structures (4, Z=O or NCH_3); for example, 4a and 4b were respectively 7 and 18X CPZ in the rat ptosis test.[15] Significantly, many of these compounds were more potent than their corresponding tricyclic congeners with nonrigid side chains. Since 4 cannot assume the solid state configuration of CPZ (5), the authors questioned earlier speculations that the active configuration of antipsychotics resembles that of crystalline CPZ. Rather, their findings are consistent with the S-configuration of antipsychotic drugs frequently depicted by Janssen.[7] In a related paper, NMR shift reagents were used to determine the stereochemistry of cis and trans aminoalkylidene xanthenes and thioxanthenes.[16] As expected, neuroleptic activity predominated in the cis series.

An important pharmacokinetic study correlated CPZ plasma levels with therapeutic response and found that plasma levels in the range of 150-300 ng/ml usually corresponded to clinical improvement.[17] Significantly, concomitant administration of an antiparkinson agent (trihexyphenidyl) tended to lower plasma levels of CPZ, an effect ascribed to reduced gastric motility and decreased absorption of CPZ.

Butyrophenones, Phenylpiperazines, and Related Compounds - Bromoperidol (6a) is essentially equal to haloperidol in potency and duration of action.[18] The trans-4-aryl-4-methoxycyclohexylaminobutyrophenone 6b exceeded haloperidol on several behavioral endpoints and exhibited highly selective (vs. 5-HT) NE uptake blockade.[19] Compound 6c, the most potent (15X CPZ in conditioned avoidance response [CAR]) in a series of substituted pyrazino[2',1':6,1]pyrido[3,4-b]indoles, was reported to have a prolonged duration of action and few cardiovascular

effects.[20] Centpyraquin (6d, compound 69-183) combined neuroleptic activity (ca. CPZ in CAR) with significant hypotensive effects.[21] AHR-2277 (lenperone, 6e) exerted a rapid and favorable effect on psychotic symptomatology, and was claimed to produce a very low incidence of neurological side reactions.[22] A double-blind study of AL-1021 (6f) in hospitalized schizophrenics failed to confirm earlier encouraging results obtained in pilot study.[23] Milipertine (7a) produced troublesome EPS side effects in schizophrenics at doses well below those required to produce antipsychotic activity.[24] Compound 7b was one of a series reported to exhibit CNS depressant activity in animals roughly in the CPZ range.[25] Oxazolidinone 7c blocked isolation-induced aggression in mice (ca. 0.5X CPZ);[26] a similar level of activity in this test was seen with the related compounds 8a and 8b.[27]



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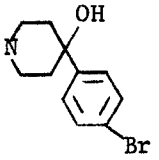
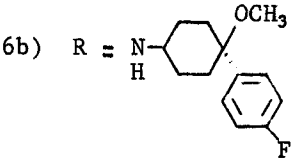
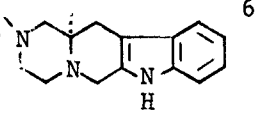
6a) R =



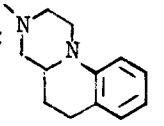
6b) R =



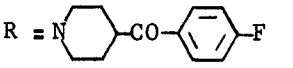
6c) R =



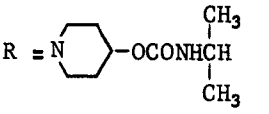
6d) R =

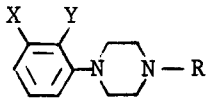


6e) R =



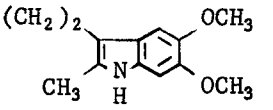
6f) R =



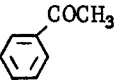


7

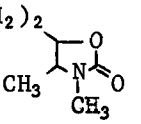
7a) X = H; Y = Cl; R =

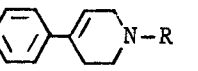


7b) X = OCH_3; Y = H; R = $CH_2CHOHCH_2O$-



7c) X = Y = H; R =





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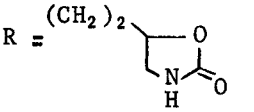
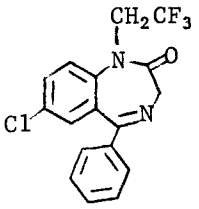
8a) R =



8b) R = $(CH_2)_3OCONHCH_3$

Miscellaneous Compounds - A benzodiazepine, SCH-12,041 (9, halazepam), showed evidence of efficacy against acute psychotic symptoms in an uncontrolled trial.[28] CI-686 (10) was predicted to have major tranquilizer activity of short duration on the basis of quantitative pharmaco-EEG studies.[29] Butaclamol (11, AY-23,028) possessed pharmacological actions resembling fluphenazine, with activity residing solely in the (+)-enantiomer.[30,31] The SAR of several cannabinoids has been reexamined and

found to differ significantly from earlier reports.[32] A comparison study in the same paper showed 12 to be 3-15X CPZ in decreasing motor activity and in blocking fighting behavior in mice.



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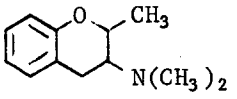

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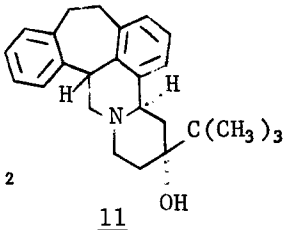

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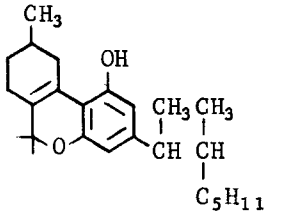
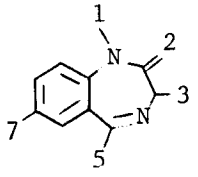


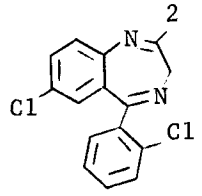
12

Antianxiety Agents-Benzodiazepines and Related Compounds - The drug therapy of anxiety[33,34] and the effect of benzodiazepines in controlling aggressive behavior[35] were reviewed. It was argued that conformational similarities between diphenylhydantoin and diazepam[36] do not constitute an appropriate basis for comparing their biological activities.[37] A series of benzodiazepines with α,α-dihydroperfluoroalkyl groups at N-1 were found to successfully block metabolism at N-1 as predicted. One of these, halazepam (Sch 12,041, 9), appeared to be more specific than diazepam in anticonvulsant (pentylenetetrazole, PTZ) and traction endpoints, with lower side effect potential (therapeutic index 4X diazepam).[38] The related compound, fletazepam (Sch 15,698, 13a),[39] and ID-540 (13b)[40] were claimed to be skeletal muscle relaxants. Clonazepam (Ro 5-4023, 13c) was more active against seizures induced by i.v. lidocaine than either Ro 8-4192 (13d) or diazepam.[41] Compound 13e, from a novel series of 7-azidobenzodiazepines, demonstrated sedative and anticonvulsant properties equivalent to diazepam.[42]



13

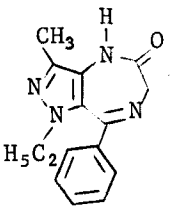
13a) 1-CH_2CF_3, 2-H_2, 5-oFC_6H_4, 7-Cl
13b) 1-CH_3, 2-O, 5-oFC_6H_4, 7-Cl
13c) 1-H, 2-O, 5-oClC_6H_4, 7-NO_2
13d) 1-H, 2-O, 3-$COO(CH_2)_3$N⟨morpholine⟩, 5-oClC_6H_4, 7-NO_2
13e) 1-CH_3, 2-O, 5-C_6H_5, 7-N_3



14

14a) 2-$NHOCH_2CH_2{=}CH_2$
14b) 2-$NHCONHCOOC_2H_5$
14c) 2-⟨imidazolidinone⟩

Pharmacological studies indicated uldazepam (U-31,920, 14a) to be an effective antianxiety drug with weak sedative and muscle relaxant properties.[43] The urea derivative 14b was nearly equivalent to diazepam in several behavioral and convulsant (thiosemicarbazide) end points,[44] whereas the related 2-imidazolidones (14c) were less active in the same tests. A number of 1,3-dialkylpyrazolo[4,3-e]diazepinones exhibited potent CNS activity and one of these, pyrazapon (CI-683, 15) showed anxiolytic activity equivalent to diazepam in man.[45] SAR studies showed that hydrogen is preferred at N-4 and that 1,3-dialkyl substitution is superior to the 2,3-pattern of the isomeric series (16).



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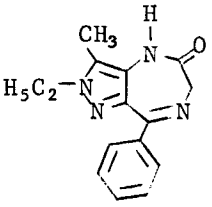

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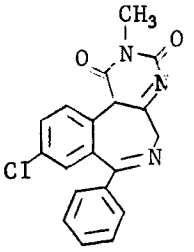

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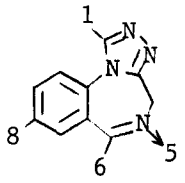


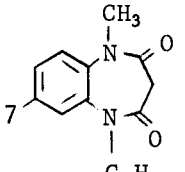
17

17a) 1-H, 6-C_6H_5, 8-Cl
17b) 1-CH_3, 6-oClC_6H_4, 8-Cl
17c) 1-CH_3, 6-C_6H_5, 8-H
17d) 1-CH_3, 6-oClC_6H_4, 8-H
17e) 1-CH_3, 6-C_6H_5, 8-Cl
17f) 3-H, 5→O, 6-C_6H_5

Several series of triazolobenzodiazepines (17) with CNS depressant activity have been reported. D-40TA (17a), the most potent of the 3-unsubstituted triazoles, was highly active as a tranquilizer, sedative-hypnotic and muscle relaxant in animals.[46-48] Triazolam (U-33,030, 17b) exhibited hypnotic activity in man at 0.25-2.0 mg.[49] The anticonvulsant and antiaggressive potencies of U-31,957 (17c) and U-35,005 (17d) exceeded those of the corresponding diazepam analogs, but they were only weakly active in end points measuring overt depression (traction, EtOH).[50] Alprazolam (U-31,889, 17e)[51] demonstrated a marked and rapid anxiolytic effect in man as had been predicted from its EEG profile.[52] Chlordiazepoxide isosteres in the triazolobenzodiazepine series (e.g. 17f) were consistently inactive.[46,53] The triazinodione 18 was equal to diazepam on several behavioral end points and was 0.3X diazepam against PTZ convulsions and EtOH narcosis.[44]

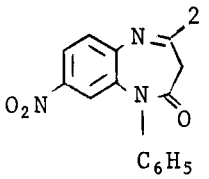
The 1,5-benzodiazepine triflubazepam (ORF-8063, 19a) possessed antianxiety properties in man at 40-160 mg/day,[54,55] with a profile similar to diazepam.[56] Like most 1,4-benzodiazepines, it was primarily metabolized through N-dealkylation, but was otherwise hydroxylated in the N-phenyl ring rather than at C-3.[57] Pharmacokinetic studies with

triflubazepam showed clinical improvement to be correlated with blood levels of 0.5 to 1.0 ng/ml.[58] Clobazam (19b) was claimed to be better tolerated than chlordiazepoxide in pharmacological and toxicological studies.[59] The 1,5-benzodiazepine relatives of chlordiazepoxide, 20a and 20b, protected mice against convulsions produced by PTZ, strychnine (ST) and tremorine, and were less disruptive than chlordiazepoxide on tests of motor coordination.[60]



19

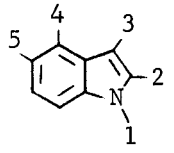
19a) 7-CF_3
19b) 7-Cl



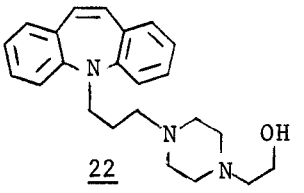
20

20a) 2-NH_2
20b) 2-$N(CH_3)_2$

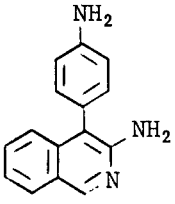
Other Structures with Antianxiety Activity - The oxypertine analogs 21a and 21b induced ataxia, decreased locomotor activity and antagonized electroshock (ES) and ST convulsions in mice (ca. 0.25 diazepam).[61] Indole derivatives U-5092 (21c) and U-13,625 (21d) protected mice against ES-induced convulsions and produced a CPZ-like tranquilizing effect.[62] In a double-blind study against anxiety, 200 mg opipramol (22) was equivalent to 30 mg chlordiazepoxide.[63] The isoquinoline derivative 23 and the benzothiazepine derivative 24 possessed marked CNS activity in mice characterized by general depression and anticonvulsant (ES, PTZ, ST) activity.[64,65] The R enantiomers of a series of 1-aminotetralins (e.g. 25) suppressed conditioned emotional response (CER) in rats at 10 mg/kg. Interestingly, the S isomers were devoid of CER activity, but possessed marked anticataleptic (tetrabenazine) activity.[66] Although 26a and 26b resemble known metabolites of 1,4-benzodiazepine tranquilizers, they exhibited only very weak anti-PTZ and ES activity, and showed no taming effect in isolated mice.[67]



21



22



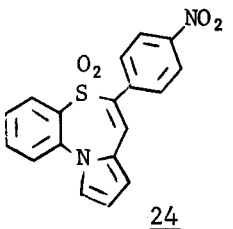
23

21a) 1-H, 2-CH_3, 3-CH_2CH_2-N⟨N⟩N-C_6H_5, 4-CH_3, 5-OCH_3

21b) 1-H, 2-CH_3, 3-CH_2CH_2-N⟨N⟩N-o$OCH_3C_6H_4$, 4-CH_3, 5-OCH_3

21c) 1-CH_3, 3-$CHOHCH_2N(CH_3)_2$, 4,5-H

21d) 1-H, 3-$CH_2NHCH_2CH_2OH$, 4,5-H



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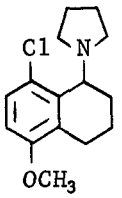
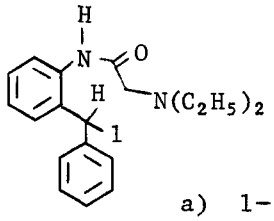


25



26

a) 1-H
b) 1-OH

Biological Observations and Hypotheses - The role of dopamine receptor blocking agents in the treatment of schizophrenia[68] and the mechanistic relationship of the antipsychotic effects of neuroleptics to their extrapyramidal (EPS) effects[69] have been reviewed. Several hypotheses have been advanced to account for clozapine's low incidence of EPS reactions,[6] most of which invoke its atropine-like anticholinergic activity. For example, clozapine causes a greater increase in homovanillic acid levels in the limbic system than in the striatum, while haloperidol shows no selectivity in these portions of the brain. However, when an anticholinergic agent is coadministered with haloperidol an effect resembling that of clozapine is obtained,[70] consistent with recent evidence that cholinergic pathways modulate dopaminergic activity in the striatum, but not in the limbic system.[71] Therefore, clozapine's anticholinergic properties may fortuitously balance its EPS effect in the striatum without attenuating its antipsychotic effect. The recent observation[72] that CPZ enhances acetylcholine output in the striatum of cannulated cats provides the first direct evidence that neuroleptic drugs activate the cholinergic system and supports the postulated existence of an interdependent and antagonistic cholinergic-dopaminergic neuronal network.[73]

Further evidence for the existence of a neuronal feedback mechanism controlling DA turnover in brain came from a report that antipsychotic drugs increase the rate of firing of dopaminergic neurons in the substantia nigra and the ventral tegmental area.[74] Recent papers also suggested that γ-hydroxybutyric acid (GHB)[75,76] and γ-aminobutyric acid (GABA)[77] may regulate this feedback mechanism. If these findings are substantiated GHB and GABA will provide valuable tools to study this feedback mechanism and its relationship to neurological manifestations such as catalepsy in animals, and EPS side effects and tardive dyskinesia in man. The importance of identifying existing or new biological tests that relate more specifically to antipsychotic activity in man was emphasized in two recent papers which claimed that biological endpoints showing tolerance (e.g. stereotyped behavior) do not assess potential antipsychotic effects in man, but rather only measure propensity to cause EPS effects.[78,79]

A new technique to assess changes in NE synthesis in rat brain measures the accumulation of 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG), a major NE metabolite.[80] This method was claimed to be more convenient than conventional methods utilizing exogeneous α-methyl-p-tyrosine or labeled tyrosine, and to avoid errors that might be

introduced by interaction of these agents with the test compound. The sedative activity of neuroleptics appears to correlate well with their ability to increase MOPEG levels in brain.[81]

Several recent reports have implicated enzyme deficiences or abnormalities in the pathophysiology of schizophrenia. Post-mortem brain specimens of schizophrenics showed a significant reduction in dopamine-β-hydroxylase activity vs. normals in all brain regions examined.[82] Monoamine oxidase (MAO) activity was found to be significantly lower in the platelets of schizophrenic and non-schizophrenic monozygotic twin pairs when compared to normal controls, suggesting that decreased MAO activity might be a genetic marker for vulnerability to schizophrenia.[83] An N-methyltransferase capable of forming dimethyltryptamine (DMT), a known hallucinogen, was found in human red blood cells, plasma, and platelets.[84] Significantly the activity of this enzyme in platelets was higher in psychotic subjects than in nonpsychotics, a finding apparently related to an unidentified dialyzable inhibitor present in normal subjects, but not in psychotics. A non-specific N-methyltransferase isolated from rat brain was found to be similarly controlled by a dialyzable inhibitor.[85] DMT and bufotenin have been identified in the urine of autistic children[86] and chronic schizophrenics,[87] but no difference between plasma DMT levels in controls and schizophrenics was evident in a recent study.[88] Although S-adenosylmethionine has been viewed for many years as the methyl donor responsible for the O- and N-methylation of biogenic amines, the involvement of 5-methyltetrahydrofolic acid in these transformations has now been firmly established.[89,90] Accordingly, it has been postulated recently that the cause of schizophrenia might be an overabundance of folate coenzymes, resulting in excessive N-methylation of biogenic amines in brain.[89]

Diazepam appears to exert a direct action at the spinal level by increasing segmental presynaptic inhibition and by decreasing or suppressing presynaptic controls of supraspinal origin.[91] Benzodiazepines appear to decrease NE turnover by reducing impulse activity in the locus coeruleus rather than by a direct interference with release of NE from the nerve terminals.[92] Repetitive dosing may, however, reverse this inhibitory effect in the "reward structures" while maintaining the inhibitory effect upon the "aversive structures."[93] Benzodiazepines may fortify the mechanisms of physiological stabilization of neuronal nerve terminal membranes by facilitating the formation, release or action of prostaglandins.[94] A combination of inhibition of c-AMP phosphodiesterase activity and inhibition of 5-HT turnover in the midbrain raphé region in rats may be responsible for chlordiazepoxide's ability to suppress aggressive behavior.[95]

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Chapter 2. Narcotic Antagonists and Analgesics

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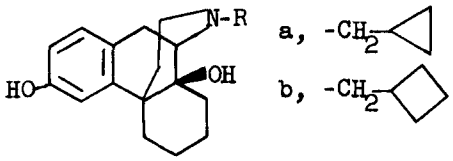
Introduction - During 1973, clinical trial reports on new agents remained few. Further details on agonists and antagonists described in 1972 abstracts and preliminary reports appeared, indicating continuation of these investigations. The variety of new structure types with analgesic or antagonist activity appears to have declined in 1973. Both analgesics and antagonists are treated together in structure-activity discussion as differences between them remain very subtle indeed (e.g. diastereomers and enantiomers with opposite actions).

Several reviews are noteworthy: The well known analgesic publications of the late Dr. Nathan B. Eddy were brought to a close with an overview of 75 years of research toward solutions for morphine-type drug dependence.[1] The proceedings of an International Symposium on Agonists and Antagonists (1971) were published.[2] Reviews on pentazocine[3,4] and strong analgesics[5] appeared.

New Clinical Studies - Current reports do not provide definitive information on any new compounds. Previous investigations are continuing and it remains premature to assess their clinical importance. However, the outlook is optimistic that significant clinical advances are in progress considering the variety of agonist and antagonist agents being studied.

A 10:1 methadone:naloxone combination evaluated in man provoked few side effects,[6] advancing to clinical trials the previously described concept of using a parenterally effective antagonist combined with an orally active narcotic to prevent abuse of oral medication.[7]



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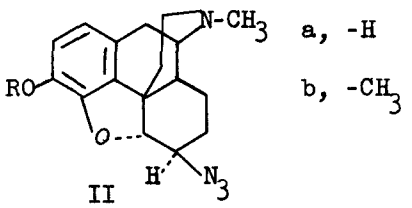


II

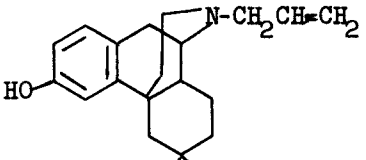
An early clinical trial of oxilorphan [(-)-BC-2605, Ia] as an antagonist for potential narcotic addiction treatment indicated a diuretic effect at low doses (2-4 mg sc).[8] In laboratory animals Ia was a naloxone-like antagonist, was almost equally effective orally as parenterally, and had a prolonged duration.[9] Preliminary clinical results are consistent with this profile. The N-cyclobutylmethyl analog (-)-Ib was a highly potent, dependence-free agonist in animals, and preliminary clinical trials have been initiated with results comparable to the animal model[9] (See also Chapter 5).

Structure-Activity; Agents under Investigation

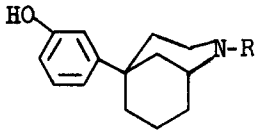
Opiates and Morphinans - Details of the total synthesis of the BC-2605 series (I) were described.[9] A new synthetic approach was utilized representing a major advance in synthetic methods for morphinans and related compounds, including isomorphinans and hasubanan analogs. It provides naloxone analogs which are not derived from the opium alkaloid thebaine, but which have comparable activities. (-)-Ib showed antagonist signs in monkeys, and was a potent analgesic equivalent to morphine in mice (hot-plate and Nilsen tests).[10] Ib racemate was morphine-like in monkeys (suppression of abstinence) and was similar to (-)-Ib in mice.[10] The (+)-isomer was morphine-like in monkeys, less potent but somewhat longer acting, and was weak to inactive in mice.

From a series of morphine derivatives recently synthesized in Hungary, azidomorphine (IIa) and azidocodeine (IIb) proved to be about 300 times and about 13 times more potent than morphine, respectively (rat hot-plate).[11,12] However, physical dependence capacity (PDC) in mice, rats and monkeys was low at equianalgesic doses, unusual for a potent N-methyl opiate structure.

A levallorphan metabolite isolated from rat urine was established by X-ray analysis as the 6β-hydroxy derivative III.[13] It was then synthesized and, like the parent drug, was an antagonist without analgesic activity. An analogous 6β-hydroxy metabolite of naltrexone, formed by reduction, was found in man.[14] These examples represent the first reports of C-6 isomorphine configuration metabolites.



III



IV

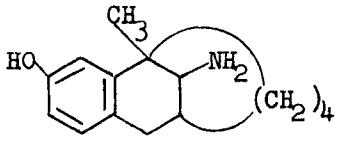
a, $-C_3H_7$
b, $-CH_2CH=CH_2$
c, $-CH_2-$
d, $-CH_3$ (+)

The bicyclic 5-m-hydroxyphenylmorphan series was extended to include the N-propyl, N-cyclopropylmethyl (CPM) and N-allyl derivatives IVa,b,c, both as racemates and the (+)-isomers.[15] They were pentazocine-like analgesics with weak or no antagonist actions, and showed no PDC in monkeys. Thus, potent antagonists cannot be obtained in this series by replacing the N-methyl group of a potent agonist with classical antagonist substituents (IVd=3 x morphine; high PDC).

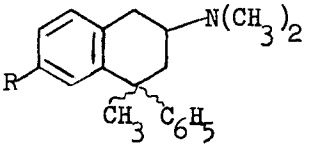
6,7-Benzomorphans - Very little new structure-activity information concerning benzomorphans appeared during 1973. The sec-amine 8β-hydroxy-6,7-benzomorphan had analgesic activity about ½ that of codeine (mouse hot-plate) while the corresponding N-methyl derivative was inactive, the reverse of the usual relative potency.[16] Pentazocine underwent a chemical change in aqueous acid conditions used to hydrolize glucuronides.[17] The mass spectral fragmentation of 9α- and 9β-alkyl-6,7-benzomorphans, including pentazocine, indicated that the configuration at C-9 could be assigned even when only one isomer of a diastereomeric pair was

available.[18]

Aminotetralins - Additional details of the synthesis of 49 1,3-bridged-2-aminotetralins (V) were published;[19] several were equal to or more potent than morphine (rat tail-flick). V was the longest acting antagonist tested in the Univ. of Michigan morphine-dependent monkeys.[10] It displayed twice the duration of cyclazocine and was about 1/5 as potent as nalorphine as an antagonist. In mice it was equivalent to morphine (hot-plate).



V

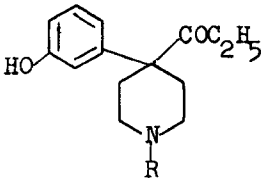


VI

a, -H
b, -OCH_3
c, -OH

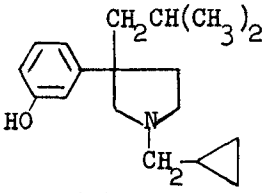
Three diastereomeric pairs of tetralins VI were synthesized as methadone analogs.[20] VIa were analgesics comparable to meperidine (mouse hot-plate) with little differences in the potency of the diastereomers; substitution of hydroxyl for hydrogen increased potency somewhat. Among a series of potential antiparkinson agents, 2-methylamino-5-methoxytetralin showed analgesic activity in the codeine-propoxyphene range (mouse tail-pinch), but was more toxic.[21]

Piperidines and Pyrrolidines - New antagonist-analgesics were reported in the N-alkyl-norketobemidone series.[22] The N-amyl, hexyl and heptyl compounds VIIa,b,c were strong agonists with weak antagonist properties, and no PDC in monkeys. VIIa was an analgesic nearly 3 times more potent than the parent VIId (mouse hot-plate). This contrasts with the more traditional N-propyl, CPM and allyl analogs which were not antagonists but weak to medium strength agonists with high PDC.



VII

a, -Amyl
b, -Hexyl
c, -Heptyl
d, -Me

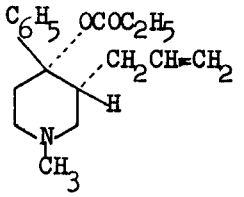


VIII, (-)-isomer

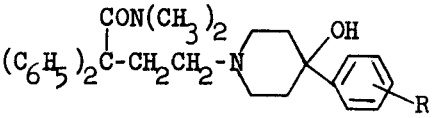
In the pyrrolidine series, additional details of the selective antagonist actions of N-CPM and N-allyl analogs of profadol appeared,[23,24] and CI-747 (VIII) was selected for detailed evaluation. Its analgesic potency was 40-50 times pentazocine (mouse antiwrithing) with antagonist properties 5-6 times pentazocine.

The absolute stereochemistry and analgesic properties of the diastereomeric 3-allyl-prodine analogs (IX) were studied in detail by two groups who deduced similar conclusions.[25-27] The α-(trans-Ph:allyl) diastereomer IX was 13-15 times more potent than morphine (mouse hot-plate), and its (+)-enantiomer was 40 times morphine and 260 times its (-)-isomer. It was suggested that the equatorial allyl group enhanced receptor association.

Similar absolute stereochemical studies of other prodines were also described.[28,29]



IX



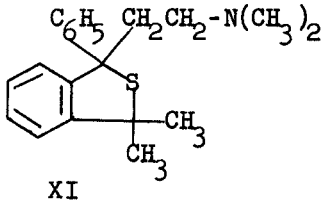
X

a, R = p-Cl; b, R = 3-CF_3-4-Cl

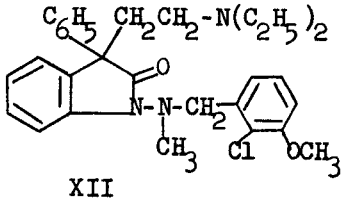
A series of 2,2-diphenyl-4-piperidinobutyramides (X) were studied in which the goal was separation of analgesic and antidiarrheal (constipating) activities.[30] Loperamide (Xa) and fluperamide (Xb) were selected for further investigation each being more potent than diphenoxylate and codeine in protecting against castor-oil-induced diarrhea and very weak analgesics (rat, warm water tail-withdrawal). Closely related analogs combined strong analgesic properties with strong antidiarrheal activity. In this study, potent analgesic activity was an unwanted side effect.

Several additional piperidines were active analgesics. The 3-methyl derivative of the potent narcotic fentanyl was 10 times more potent than the parent drug.[31] N-[2-(2-Oxazolidinone-5-yl)ethyl]-4-phenyl-4-propionoxypiperidine showed morphine-like analgesic potency,[32] and an N-(2-indanyl)-analog had meperidine-like potency.[33]

Diphenylpropylamines and Miscellaneous Compounds - Among a series of thiophthalanes studied for analgesic activity,[34] Lu 6-062 (XI) was the most active. It was approximately equal to morphine (mice, hot-plate, tail-pinch and Nilsen tests), produced a Straub-tail reaction and was antagonized by nalorphine. Similar thiophthalanes with a propylamino side chain had been previously studied as antidepressants, but were not analgesics and did not produce a Straub-tail.



XI

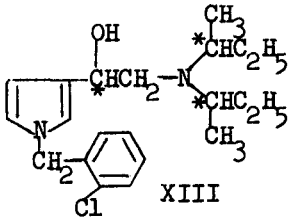


XII

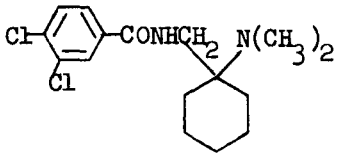
Another approach to the separation of antidiarrheal from narcotic analgesic activity was the comparison of Straub-tail vs. constipating effects.[35] CI-750 (XII), selected for evaluation,[36] did not produce a Straub-tail, showed only weak analgesic activity and had a constipating effect about equal to diphenoxylate. Piperidinoethyl and pyrrolidinoethyl analogs of XII with a 1-dimethylamino group had potent narcotic actions.

Agonist and antagonist properties of viminol stereoisomers (XIII, previously known as diviminol) were described.[37] This compound has 3 asymmetric centers and 6 stereoisomers. Analgesic activity resided in a

(-)-isomer (R_2; several times morphine, some PDC) and antagonist activity in a (+)-isomer (S_2; nalorphine-like) which were not enantiomers of each other. No other isomers had analgesic or antagonist activity. In the



XIII

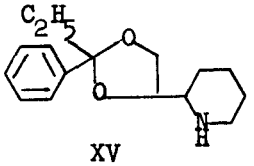


XIV

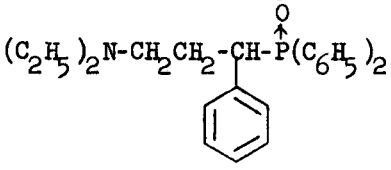
racemate, the agonistic activity of R_2 is apparently partially opposed by the antagonistic S_2 isomer. The result is a racemate with codeine-range analgesic activity and a low order of PDC.

Analgesic activity was found in a group of N-substituted cyclohexylmethylbenzamides, and AH 7921 (XIV) was equipotent to morphine (mice, antiwrithing and hot-plate) and showed high PDC in monkeys. This represents a structure distinctly different from previous high PDC narcotic-like compounds.[38]

The dissociative anesthetic etoxadrol (XV) produced analgesia at low subanesthetic doses (0.5-1 mg), measured by elevation of the electrical stimulation threshold in monkeys.[39] It was comparable to morphine but shorter in duration. XV was not active in mice (antiwrithing), perhaps since dissociative anesthetics produce different effects in rodents vs. higher species. Further studies as a parenteral analgesic were suggested.



XV



XVI

A study of 24 monoaryl- and diarylphosphorus analogs of methadone revealed moderate analgesic activity in several compounds; XVI was 1/4-1/5 the potency of morphine (mice, hot-plate and antiwrithing).[40] Unfortunately, the toxicity was highest in compounds with the best analgesic activity. Pharmacological implications of the conformations of methadone and isomethadone were analyzed.[41,42]

Aniinflammatory Analgesics - This class of compounds is discussed in detail in Chapter 20. Studies of arylalkanoic acids and derivatives continue unabated. Details of analgesic testing of these agents are frequently incomplete.

Pharmacology, Biochemistry and Metabolism - The variety of studies covering methods, mechanism of action, biochemistry, etc. continues to increase. Detailed discussion of these is outside the scope of this review; however, the following selected examples are illustrative of current trends.

A full paper on biodegradable poly-lactic acid long-acting delivery

systems for narcotic antagonists appeared,[43] following up the initial abstracts. The use of chronic spinal dogs dependent on morphine was described for quantitative assays of antagonists.[44] Methods for producing antibodies to morphine for radioimmunoassay were reviewed.[45]

Further studies of specific opiate receptor sites based on stereospecific binding in nervous tissue appeared.[46] The initial results were confirmed by other investigators.[47,48] The role of sodium in enhancing antagonist binding but decreasing agonist binding in vitro was found as an important qualitative difference that may be relevant to the divergent pharmacological properties of antagonists and agonists.[46]

Several additional metabolic findings are of interest. The formation of codeine from morphine (or heroin) was observed in man;[49] a significant increase of O-methyl transferase activity leading to increased codeine in addict urine could be a specific marker for the addiction state. The long-sought primary amine metabolite from levomethadyl acetate was identified by combined gas chromatography-mass spectrometry (gc-ms) of rat tissue and plasma samples, and was found to be a highly potent analgesic (1.5 mg/kg sc, mouse antiwrithing).[50] Thus, the general tendency of mono- and di-demethyl congeners to have a low order of activity does not apply in this series. Both mono- and di-demethyl metabolites of d-propoxyphene were observed in man, using combined gc-ms and deuterium-labelled propoxyphenes.[51] α-(+)-Norpropoxyphene was the major metabolite, and was about 1/10 as active as the parent drug (mouse antiwrithing, and rat tail-flick).[52]

The role of biogenic amines as related to narcotic effects continues to be an active field. Further studies appeared concerning enhanced cerebral serotonin turnover,[53] balance with the catecholamine system,[54] dopamine turnover in the striatum,[55] and homovanillic acid content in the caudate nucleus.[56] Additional details of the cyclic-AMP antagonism of morphine analgesia,[57] and its effect on tolerance and physical dependence[58] were also published.

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Chapter 3. Antiparkinsonism Drugs

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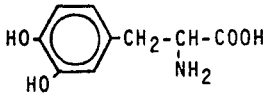
General - Knowledge of the nature of the biochemical lesion of Parkinson's disease and the availability of rational corrective therapy continues to spur activity to find improved and novel entities with existing and different modes of action. Many useful reviews are available in this field. This chapter updates the compilation by Vernier[1]. Levodopa is reviewed by McDowell[2] and it and other developments are covered by Calne[3]. The large and growing amantadine literature is well analyzed by Parkes[4].

This review will cover therapeutic agents and leads under four mechanisms of action: 1. dopamine replacement, 2. dopamine release, 3. dopamine receptor stimulants and 4. anticholinergics. Each agent will be discussed under its presumed major mode of action even though the evidence for it may not be definitive or the agent may act by several mechanisms.

Dopamine replacement - This most effective treatment for Parkinson's disease is supplied by the naturally-occurring amino acid levodopa (I, L-3,4-dihydroxyphenylalanine[5-7]). In the usual oral doses of 2.5 to 6 g daily it enters the brain and is decarboxylated to form dopamine (II)



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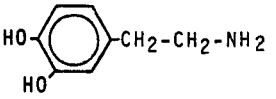


II

which presumably replenishes the deficient neurotransmitter stores in the basal ganglia resulting from midbrain substantia nigra lesions.

In 60 to 80% of cases akinesia, rigidity and to a lesser extent tremor are moderately to markedly improved, but management is difficult. This is due to numerous side effects, the necessity of giving many doses daily, and the slowly increasing dose titration, leading to a long induction time for optimal effects. Some patients have a short and/or variable time of symptomatic relief following each dose. Sudden freezing, the "on-off effect," is often noted[8]. Its causes are under study and several approaches to its alleviation are in development (determinations of levodopa plasma levels for optimal time and dose titration, sustained release formulations, etc.).

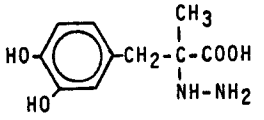
Principal levodopa side effects are in decreasing order of incidence: 1. Nausea and vomiting, 2. postural hypotension, 3. cardiac arrhythmias, 4. abnormal involuntary movements and 5. psychiatric disturbances.

Considerable research effort is directed toward the determination of how much of the original treatment rationale can be substantiated. Although generally a greater than originally expected complexity is being

found, most results are consistent with original expectations.

Carbidopa and benserazide, two agents which are potent aromatic amino acid decarboxylase inhibitors *in vitro* and *in vivo*, have been extensively studied in patients and have been found effective in lowering the clinically required levodopa dose and are nearing approval for broad availability. They do not enter the brain and thus selectively decrease the peripheral destruction of levodopa, decreasing exposure to some of the adverse effects of dopamine, and proportionately enhance the levodopa-induced dopamine increase in the brain, especially in the extrapyramidal centers[9,10].

Early reports of the biochemistry and pharmacology of carbidopa (III, MK 486, the L form of MK 485, also referred to as HMD) indicated its



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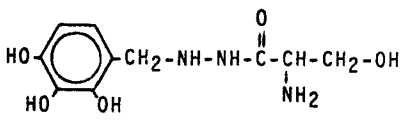


IV

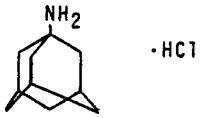
extracerebral distribution and potent *in vitro* and *in vivo* inhibition of aromatic amino acid decarboxylase[11]. Its interactions with levodopa in animals were characterized by Lotti and Porter[12]. Carbidopa enhanced the ability of levodopa to increase motor activity of mice and rats and to reverse reserpine-induced hypothermia and suppression of locomotion and ptosis. Brain dopamine levels were concomitantly enhanced. In contrast levodopa emesis in dogs and pigeons was attenuated.

Clinical experience with carbidopa and levodopa has been extensive and favorable[13,14]. Carbidopa alone had no influence on Parkinsonian patients and is reported to be quite non-toxic. It raised plasma levels of levodopa about five-fold, thus indicating about an 80% reduction of the required levodopa alone dose. Carbidopa doses of 100-300 mg/day combined with levodopa in controlled trials resulted in a rapid onset of therapeutic effect (a few days or weeks compared with months with levodopa alone), and strikingly reduced the incidence of nausea and vomiting. Patients on long term combined therapy achieved superior relief. Combined treatment may reduce cardiac arrhythmias, but the incidence of involuntary movements is increased. Several groups found a 1:10 combination most useful with a daily dose of carbidopa 100 mg: levodopa 1000 mg. Marsden[14] suggests that it may become the treatment of choice.

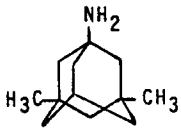
The pharmacology of and clinical experience with benserazide (IV, Ro 4-4602) is generally similar to that of carbidopa[10]. It is somewhat less potent *in vitro* as a decarboxylase inhibitor but *in vivo* and in the clinic its potency is similar to that of carbidopa[9,10,12]. It differs in penetrating the brain in animals at high doses. Benserazide administration, like carbidopa, increases absorption of levodopa from the gut, increasing plasma levels, and blocks peripheral conversion to dopamine. In the brain the inhibitors increase levodopa penetration and catecholamine (dopamine and norepinephrine) levels.

Clinically benserazide+levodopa treatment improved the therapeutic results, decreased the required levodopa dose (to about 1/5) and decreased the incidence of nausea and vomiting. It generally did not decrease the involuntary movements but increased them. Benserazide seems to be well tolerated on a long term administration at the 100-400 mg/day levels, with few transient changes in liver biopsies and enzyme levels[15].

Dopamine releasers - Schwab noted in 1968 the antiparkinsonism effect of amantadine (V), an antiviral agent which has since been extensively



V



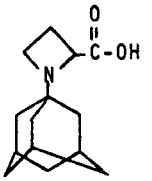
VI

tested[17] and approved for that additional indication. Amantadine affects catecholamines, primarily dopamine, and had no anticholinergic effects[18]. It caused pressor responses in the dopamine-primed dog with much less effect upon norepinephrine, suggesting predominately dopamine release[18,19]. Amantadine antagonized effects of major tranquilizers on several kinds of behavior, including the conditioned avoidance response[22], affected the rotational activity of striatal-lesioned rats and mice[23] and interacted with apomorphine[24,25]. This and considerable other biochemical and pharmacological evidence is generally consistent with the release of stored dopamine as a major element in its action together with facilitation of neural release[26-28]. Amantadine also increased synthesis of levodopa and presumably dopamine in rat striatum[29]. Although many recent reports deal with these aspects of the animal pharmacology, the exact mechanism is not yet fully established.

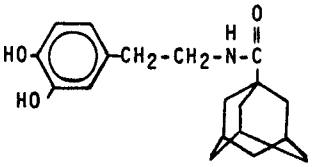
In patients the onset of action is prompt, faster than levodopa but of less intensity, with fewer side effects than levodopa, particularly nausea and vomiting and dyskinesias. At doses of 200-300 mg/day maximum benefit is achieved soon after beginning drug and this effect may be sustained over long periods, although some reports indicate a decline in effect.

Although no clinical reports on D 145 (VI, DMAA) have been found, it is a close congener of amantadine which shows catecholamine interactions generally similar to it, suggesting capacity for dopamine release. It may also have some capacity for direct stimulation of dopamine receptors[30]. Moderate to high i.v. doses of D 145 decreased decerebrate rigidity in cats[31]. Other biochemical findings have been noted[32].

Carmantadine (VII, Sch 15427) is structurally related to amantadine[33]. It shares some of its pharmacological actions, was effective in a



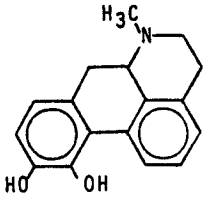
VII



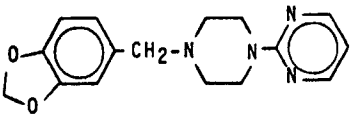
VIII

head-turning test[34], and is in early clinical trials. Dopamantine (VIII) combined elements of both amantadine and dopamine in its structure, shares some pharmacological effects of amantadine and is in early clinical trials[35].

Dopamine receptor stimulants - A chemical rearrangement of morphine yields apomorphine (IX) which is now known to stimulate dopaminergic receptors.



IX



X

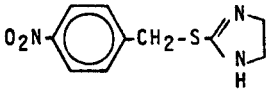
Schwab[36] discovered in 1951 that it relieved tremor and rigidity of parkinsonism. This has been confirmed by Cotzias[37], Castaigne[38] and others. It markedly diminished tremor in monkeys[39]. Apomorphine is not a practical therapeutic agent (poor oral absorption requiring parenteral administration, short duration of action, high incidence of nausea and emesis, instability) but is an important synthetic and biological starting point in the search for new agents, since it contains common elements of the dopamine structure. Experiments with apomorphine have shed light on the action of levodopa and dopamine in the treatment of parkinsonism and also the nature of drug-induced parkinsonism which accompanied the use of major tranquilizers which antagonize apomorphine emesis and stereotypy[40].

Originally tested in cardiovascular disease, trivastal (X, ET 495) has been proposed as a dopamine receptor stimulant with better oral absorption and longer duration of action than apomorphine[41]. In addition to its direct effect on the receptors the data suggested a weak dopamine and norepinephrine releasing effect. Trivastal blocked caudate EEG spindles as did apomorphine[42] and reduced tremor in monkeys with midbrain lesions[43]. Others have reported its drug dynamics and pharmacological actions[44,45].

Reports of clinical trials[46] in 125 patients at doses of 20 to 240 mg/day led to positive conclusion of benefit in 4 studies (involving 83 patients) and negative assessment in 3 studies (involving 42 patients). Side effects were striking and seen in most patients but could be reduced with lowered doses. Dyskinesias, like those with levodopa, were most frequent, with nausea and vomiting, daytime drowsiness (with insomnia at

night) also found frequently. Less common were confusion, headaches and dizziness. Most trivastal trials continued patients on previous medication including levodopa so that the therapeutic role of trivastal is not yet clear.

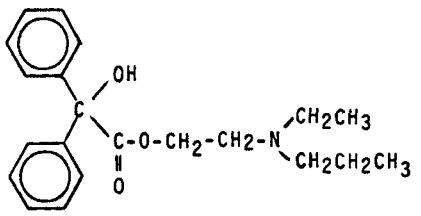
Preclinical data suggest that NBTI (XI) at fairly high doses (40 mg/kg i.p.) caused tremors and stereotypy in rodents reminiscent of apomor-



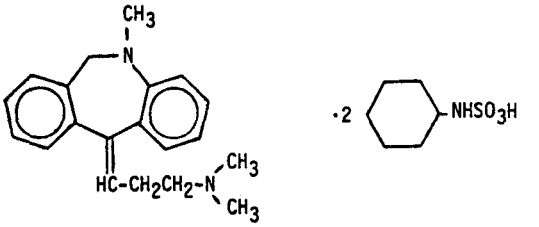
XI

phine, which was blocked by chlorpromazine and other major tranquilizers but not by anticholinergics. It restored locomotor activity in reserpine-depleted rats like levodopa[42,47]. It may stimulate dopaminergic receptors[48]. Reports of its clinical status have not appeared.

Anticholinergics - The peripheral anticholinergic effects of benapryzine (XII, BRL 1288) were considerably weaker than those of trihexyphenidyl



XII



XIII

but its central effects were nearly equivalent in animals and man[49]. In a pilot trial benapryzine at higher doses than those of trihexyphenidyl was found to be equivalent to it, but with possibly fewer side effects[50]. Two other partially controlled trials (13 and 28 patients) indicated effectiveness and safety at 200 mg/day[51]. Benapryzine may represent at least partial success in separating central from peripheral anticholinergic effects, possibly as a result of difference in pharmacokinetics[53]. Direct effects have been demonstrated by iontophoretic application to cortical neurons[54].

The morphanthridine elantrine (XIII, Ex 10-029) has been tested in man and its pharmacological action appears to be mainly, but not exclusively, related to its anticholinergic action[55]. Two clinical trials (25 and 13 patients) report efficacy alone at doses of 30-60 mg/day and with levodopa[56,57]. Side effects were predominately of the anticholinergic type. A recent substantial multiclinic controlled study[58] in 89 patients found that elantrine, 40 mg daily, effectively reduced tremor and possibly rigidity more than the same dosage of trihexyphenidyl. Elantrine caused more dry mouth. Thus elantrine was effective but not clearly more selective.

Comment - Earlier research interest has shifted from anticholinergic to catecholamine actions. Is the formation of 6-hydroxydopamine, which causes adrenergic nerve terminal destruction, the cause of the biochemical lesion of Parkinson's disease? If true, new possibilities for cure or prevention through influencing the cause could emerge from the intensive catecholamine research effort.

Additional dopamine releasers will probably be tested since this mode of action has been identified. Effective and selective dopamine reuptake blockers should be explored as another therapeutic approach. Direct dopamine receptor stimulation is probably the most active present area. In view of the complex catecholamine physiology other neurological target uses for these leads will be sought and undoubtedly found. Combinations of the last 3 approaches will be primarily initiated by clinicians.

New small animal methods, supplementing oxotremorine tremor, etc., have made possible study of adrenergic actions based mainly upon the changes in locomotor activity and stereotypy exerted by amphetamine and apomorphine and by drug effects upon turning behavior after striatal lesions caused by 6-hydroxydopamine, electrolysis and suction.

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Chapter 4. Psychotomimetic Agents

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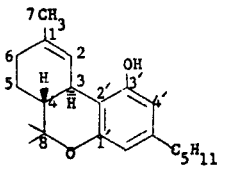
General - Comprehensive surveys on the chemistry,[1] pharmacology[2] and metabolism of cannabinoids have appeared.[3] The proceedings of conferences dealing with marihuana have been published.[4-7] Governmental publications which cover the biological, social and legal aspects of marihuana have also appeared.[8-11] An excellent account of ergot has been published in which the taxonomy and distribution of the Claviceps species, the constituents of ergot and their biosynthetic origin, and the production of ergot alkaloids all have been reviewed.[12] Extensive reviews on the chemistry[13-4] and biosynthesis[15] of peyote alkaloids have appeared. The biochemistry of psychoses[16] as well as the role of adrenochrome[17] as a psychotomimetic agent have been the subjects of reviews. A review of the chemistry and pharmacology of Piper methysticum, the pepper plant from which the narcotic drink kava-kava is prepared, has been published.[18]

I. Chemistry A. Cannabinoids - A definitive paper appeared on the isolation and structural elucidation of Δ^1-tetrahydrocannabinol (Δ^1-THC) (1), cannabigerol (2), cannabichromene (3), and cannabicyclol (4). A description of the absolute configuration of both chiral centers (C-3 and C-4) in Δ^1-THC as R was also presented.[19] Full details on the total syntheses of (±)-cannabidiol (5), (±)-Δ^5-THC, (-)-Δ^1-THC, (-)-$\Delta^{1(6)}$-THC, as well as the racemic and (+)-modifications of the latter two THC's, were



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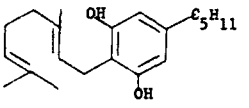

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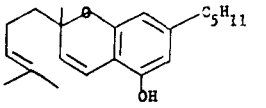

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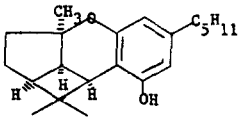

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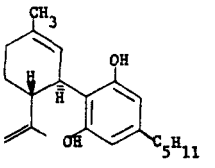

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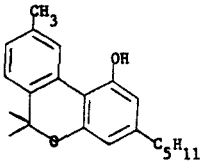
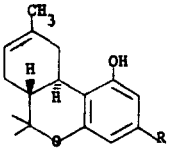
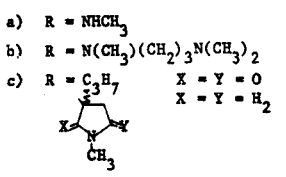


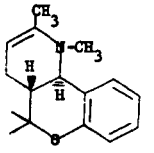
6

published.[20-1] Other cannabinoid preparations involving the condensation of terpenoid moieties were also described.[22-3, 232] Propyl and methyl analogs of cannabinoids 1, 5, and 6 were isolated and characterized[24-6] from natural sources. A propyl analog of 3 was also reported.[27]

Synthetic cannabinoids with nitrogen containing side chains such as structures 7a-c were prepared in analogous fashion according to previously described synthetic routes[28-9] A unique approach to the trans-aza structure 8 was also reported.[30]







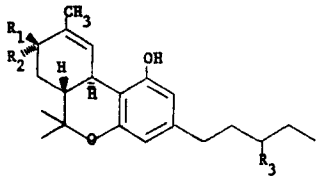
7 8

Unfortunately, biological data were not reported for these interesting structures.

The ready availability of various cannabinoids by isolation and synthesis led to an intensified study of their metabolism in various animal species.[31-3] In general, hydroxylation occurred in any available allylic or benzylic position of the THC framework with the potential for further metabolic conversion.[34-8] In man, an important

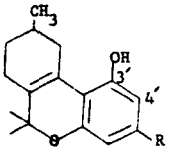
metabolite of Δ^1-THC was the corresponding 7-OH derivative which also elicited potent cannabinoid-like effects.[39-42] Synthetic developments kept pace with the isolation and identification of the various metabolic products. Thus, procedures for the synthesis of 7-OH-$\Delta^{1(6)}$-THC[43-5] and 7-OH-Δ^1-THC[46-7] were described.[58]

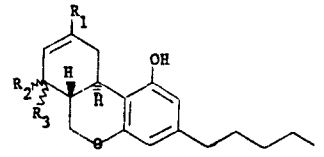
Other oxygenated metabolites such as 9 and 10 were also derived by synthetic routes.[32,35,46,48-9]



a) R_1=OH; R_2=H; R_3=H
b) R_1=H; R_2=OH; R_3=H
c) R_1, R_2=carbonyl; R_3=H
d) R_1=R_2=H; 1,2-α-epoxy; R_3=H
e) R_1=R_2=H; R_3=OH

9





a) R_1=CH_3; R_2R_3=carbonyl
b) R_1=CH_3; R_2R_3=H, OH
c) R_1=CO_2H; R_2=R_3=H

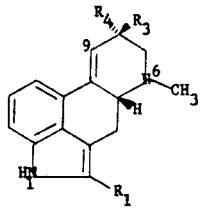
10

a) R=$OCH(CH_3)C_5H_{11}$
b) R=$C(CH_3)$=$C(CH_3)C_5H_{11}$
c) R=$C(CH_3)_2C_6H_{13}$
d) R=$CH(CH_3)CH(CH_3)C_5H_{11}$

11

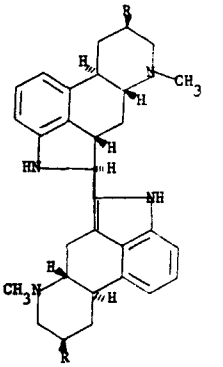
A variety of new Δ^3-THC derivatives (11a-c) was prepared including Mannich condensation products in the 4' position.[50]

B. Lysergic Acid Structures. - Several unsuccessful attempts at a new synthesis of lysergic acid (12a) were summarized.[51] A review on analogs of 12a, most of which are partial structures, appeared.[52] An efficient process for the preparation of various secondary and tertiary lysergamides and the corresponding di- and tetrahydro derivatives was described.[53] N^6-Demethylation of lysergic acid diethylamide (LSD) was accomplished[54] as well as the dimerization of a wide variety of ergot alkaloid derivatives to structures of type 13.[55] A convenient preparation of the $\Delta^{7,8}$ lysergic acid ester 15 from the corresponding N-oxide 14 was disclosed.[56] Cyclic orthothioesters were used in a novel Friedel-Crafts type approach to the preparation of various 2-substituted 9,10-dihydrolysergic acid derivatives 12b including 12a (R_1=CH_3).[57] A total synthesis of (±)-isosetoclavine (12c) was described.



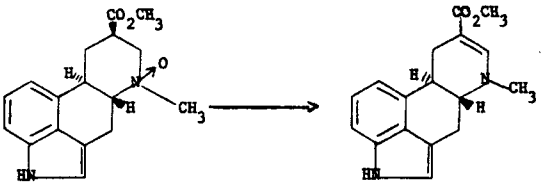
a) R_1=H; R_3=CO_2H; R_4=H
b) R_1=CH_2CF_3, CHO
R_3=CO_2CH_3; R_4=H
c) R_1=H; R_3=OH; R_4=CH_3
d) R_1=H; R_3R_4= ═CHOAc

12



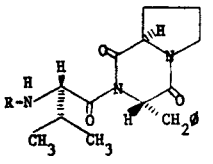
R=CH_2Cl, CO_2CH_3, CH_2CN

13



14 15

Studies involving the unraveling of the complex biosynthetic sequence of the ergot alkaloids continue to be reported.[59-61] A comprehensive review on the biosynthesis of ergot alkaloids was published.[62] Of particular interest was the disclosure that the enol acetate 12d was an efficient lysergic acid precursor.[63] A new alkaloid 16 (R=(+)-lysergyl) from a Claviceps purpurea strain was isolated and characterized.[64]



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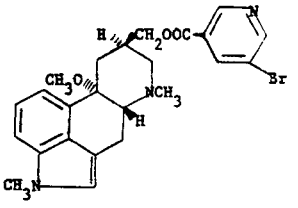
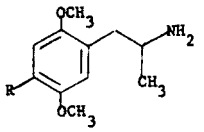
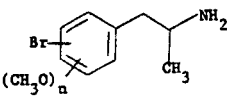


17

C. Phenylalkylamine Types. - Analogs of DOM 18a (R=Cl, Br, I, NO_2, NH_2)[65] as well as the major metabolites of DOM in the rat (18, R=CH_2OH, CO_2H)[66] were prepared. A series of bromomethoxyphenylisopropylamines 19 (n=1, 2) was also described, with brief references to pharmacological activity.[67-8] A highly useful method for the asymmetric synthesis of phenylisopropylamines as well as the methodology for the determination of their enantiomeric purity were published.[69-70] Numerous analogs of the psychotomimetic phenylisopropylamines were prepared wherein the structural features were incorporated in the more constrained framework of a 2-amino-1, 2, 3, 4-tetrahydronaphthalene (aminotetralin) system 21 (R=CH_3, OH, OMe, H, $-OCH_2O-$).[71-3] The aminoindan 20a and amino-





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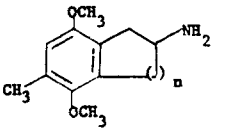
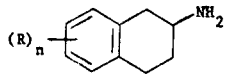


20 a) n=1 b) n=2

a) R=CH_3 (DOM)
b) R=CH_2CH_3 (DOET)
c) R=Br (DOB)

18



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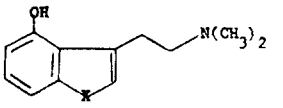
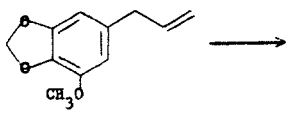


22 a) X=NH b) X=S

tetralin 20b analogs of DOM were also prepared.[74]

The preparation of the sulfur analog 22b of psilocin (22a) was described.[75-6] Weak sedative and vasopressor activity was found.

In vitro experiments provided evidence for the bioconversion of myristicin (23) to the corresponding phenylisopropylamine derivative, MMDA 24.[77] Other workers, using the intact animal, demonstrated the presence of structures 25a, b as major metabolites



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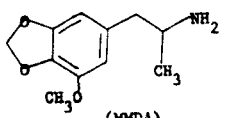
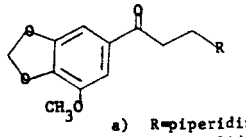


(MMDA)

24



a) R=piperidino
25 b) R=pyrrolidino

of the rat and guinea pig, respectively.[78] The full implication of these bioconversions with respect to behavioral observations in animals[79] as well as in man remains to be determined. A review on nutmeg was published.[80]

D. Other- Studies continued on the measurement of physical chemical parameters in order to elucidate structure activity patterns in psychotomimetic compounds. Thus X-ray,[81-2] nmr[83] and total orbital energies[84] were used to study molecular conformation and its relationship to psychotomimetic activity in the lysergic acid series. The conformations of psilocin and mescaline were examined by molecular orbital and classical potential function calculations.[85] Correlations of uv spectra,[86] native fluorescence[87] and association constants[88] with the psychotomimetic activity of various methoxylated phenylisopropylamines were made. Quantum mechanical studies of

the relationship between psychotomimetic activity and the structures of various anticholinergic agents were also published.[89-90]

II. Pharmacology. A. Cannabinoids. - Acute and chronic toxicity, teratology, and reproduction studies on crude marihuana extract, Δ^1-THC and other cannabinoids were performed in animals.[4,10,91-4] Controlled human studies failed to reveal any drug related permanent effects[2,11,95,96] although chronic marihuana smokers had impaired cellular mediated immunity.

In animals, Δ^1-THC showed signs of analgetic, antipyretic, antiinflammatory,[97-100] and anticonvulsant properties,[101-2] but no antidepressant effects.[103] In man, Δ^1-THC was inactive as an analgetic in experimental pain.[104] Δ^1-THC caused hypothermia in several animal species,[105-7] while complex and biphasic effects were seen on rodent aggressive behavior.[108-111] Δ^1-THC potentiated the effects of depressant compounds,[112] but exhibited no clear cut antagonism towards amphetamine.[113] Similar findings were also reported for synthetic cannabinoids.[7] Cannabidiol and cannabinol, which were considered to be biologically inactive, exhibited anticonvulsant activity[114] and interacted with Δ^1-THC both pharmacologically and metabolically in animals.[115-6] Furthermore, intravenously, cannabinol was found to be weakly active in man.[117] The pharmacological properties of several synthetic Δ^1-THC derivatives have been described in animals.[7,50]

In animal operant procedures, Δ^1-THC impaired performance mainly affecting temporal discrimination and short term memory.[2,5] However, there were reports that Δ^1-THC might facilitate acquisition of avoidance performance.[118-120]

Tolerance to the effects of Δ^1-THC developed rapidly, was long lasting, and did not appear to be metabolic in origin.[5] However, there were several behavioral parameters which failed to exhibit tolerance.[121-4] Thus far, only the rhesus monkey exhibited signs resembling physical dependence upon withdrawal of Δ^1-THC.[6] In man, tolerance to cannabis preparations was less apparent and no true physical dependence was seen.[8,10,125] The so-called "reverse" tolerance postulated in man could not be recently confirmed.[125-6] Δ^1-THC decreased sleep induction time in man, however, a "hangover" phenomenon was observed the next day.[127] Extensive human studies on cannabinoids dealing with subjective effects, mental function, memory, motor and driving performance were published.[5,11,128-31]

The similarity of cannabinoids to other known psychotomimetic compounds was investigated. Δ^1-THC appeared to be different from LSD in terms of physiological and psychotomimetic profiles in man (ref. 4, p. 196). However, certain similarities and differences were seen between marihuana and ethanol[132-4] with an indication of cross-tolerance.[11,135] No cross-tolerance could be demonstrated between Δ^1-THC and morphine, mescaline or LSD.[5,136]

In animals, Δ^1-THC produced hypotensive responses, bradycardia, reduced venous return[137] and antihypertensive effects in certain laboratory models.[138-9] In man, Δ^1-THC caused tachycardia, conjunctival reddening, pressor effects, impairment of vasoreflexes and ECG changes.[140-2] Some recent evidence indicated that Δ^1-THC might reduce intraocular pressure and pulmonary air resistance in man.[11]

Studies on absorption, plasma levels, organ distribution,[143-7] urinary and fecal excretion, and metabolic conversions[35,39,148-9] of Δ^1-THC and/or $\Delta^{1(6)}$-THC in animals and man were published.[31,33] The question of whether Δ^1-THC or 7-OH-Δ^1-THC was mainly responsible for the effects observed with Δ^1-THC has not been resolved.[106,150] However, it has been established that 7-OH-Δ^1-THC was present in the mouse brain after Δ^1-THC administration, and was found to be more potent than Δ^1-THC based on brain concentrations.[151-2]. On the basis of plasma levels, excretory patterns, and concomitant drug effects, it appeared that 7-OH-Δ^1-THC most likely was responsible for the activity in man.[39,41,153] Other hydroxylated metabolites, such as structures 9a,b[154] and 10c proved to be inactive.[35] The metabolism of DMHP 11d was studied in several animal species.[155]

The structure-activity relationships and relative potencies of various

cannabinoids were presented.[33,156] It should be noted that differences in the potency of Δ^1-THC and $\Delta^{1(6)}$-THC, as well as various Δ^3-THC derivatives and analogs, existed depending on the test animal and procedure used.[50,157]

Despite increased knowledge, the pharmacological classification of cannabinoids remains elusive. Any labeling such as "depressant" or "hallucinogenic" should be avoided because of the complex and unique properties of this class of compounds.

B. Lysergic Acid and Tryptamine Types. - Important contributions towards the understanding of the actions of psychotomimetic substances, e.g. the role of serotonergic neurones, sites of central action, and development of tolerance and cross-tolerance were reviewed.[16,158-62] The influence of ergot alkaloids on pituitary prolactin and prolactin-dependent processes was also reviewed.[163] The metabolism of nicergoline (17) was studied in various animal species, including man.[164]

LSD appeared to exert selective effects on serotonergic neurones in the brain stem raphe nuclei, however, it is undetermined whether it acts by blocking or mimicking 5-hydroxytryptamine (5-HT).[160-1,165-9] Bufotenin and N,N-dimethyltryptamine (DMT) appeared to act similarly to LSD on the raphe nuclei, while mescaline and DOM appeared to be different. Atropine, 2-Br-LSD, and chloropromazine were inactive on raphe neurones.[170-2] The possible involvement of acetylcholine, γ-aminobutyric acid (GABA) and tryptamine in the central action of psychotomimetic agents was implicated.[173-5]

The phenomena of tolerance and cross-tolerance among psychotomimetic agents were intensively studied. A cross-tolerance was observed between LSD and tryptamine or mescaline,[174,176-7] but not between LSD or mescaline and amphetamine or LSD and DMT.[177-9] Interoceptive effects induced by mescaline, psilocybin and LSD were poorly differentiated by the rat.[180-1]

Despite structural heterogeneity, the psychotomimetic agents in general caused an increase in brain 5-HT levels and a decrease in its turnover rate.[159,184-5] The effects on brain norepinephrine (NE) and dopamine were less clearly defined, but a lowering of brain levels and higher turnover rates of NE have been reported.[182-5]

Specific and sensitive radioimmune assays were developed for analysis of LSD and mescaline in biological fluids.[186-8] Furthermore, antibodies were obtained which exhibited high specificity to LSD and cross-reactivity to other psychotomimetics related to tryptamine and phenethylamine.[189]

The distribution of LSD in the rat brain exhibited patterns mimicking regional localization of 5-HT.[190] The central effects of LSD were antagonized by several steroidal agents[191] and N,N-diethyl-1-methyl-1,2,5,6-tetrahydronicotinamide.[192] Furthermore, an active immunization by protein conjugates containing lysergic acid or tryptophan prevented certain central effects of LSD in mice.[193] LSD facilitated acquisition of learned behavior and improved light discrimination and recent memory in rats and monkeys.[194-7] There was little well documented evidence to implicate LSD for causing chromosomal damage and teratologic effects both in man and animals.[198]

The determination of a N-methyltransferase in human blood[199] and brain[200] that was capable of converting tryptamine into DMT was described. The presence of an endogenous substance capable of metabolizing DMT in normal subjects was postulated.[199] Chronic schizophrenics excreted DMT and bufotenin in their urine, while normal subjects did not.[201-2]

C. Phenylalkylamine Types. - The human pharmacology of mescaline, its congeners and phenylisopropylamine analogs was reviewed.[203] A comprehensive study on the acute toxicities of mescaline and its derivatives revealed that the dog was more sensitive than the rhesus monkey to the toxic effects of these compounds.[204] Rats failed to differentiate 2,3,4-trimethoxyphenylethylamine, a non-psychotomimetic analog of mescaline, from mescaline itself.[205]

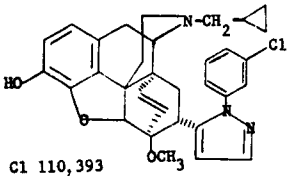
A stereoselectivity of LSD, DOM 18a, DOB 18c, and DOET 18b for central effects[206-8] was demonstrated in that the R-configuration elicited the more potent effect, while peripherally such a differentiation was not shown.[209-10] No such clear

differentiation with respect to psychotomimetic effects could be shown with R-(-) or S-(+)-amphetamine, although the S-isomer was more potent as a stimulant than the R-isomer.[211]

Structure-activity requirements in the phenylisopropylamine series were presented.[203] Relative potency relationships in various experimental procedures for phenylisopropylamine and DMT analogs were discussed.[212-3] A transoid conformation for mescaline at the receptor level was supported by the greater behavioral effects of 28a over 28b in the rat.[214]

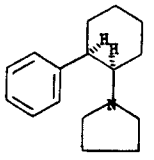
DOM caused aberrant gross behavioral patterns with a concomitant hypersynchronous EEG in the cat.[215] It also produced a lethal hyperthermia in rabbits.[216] The cardiovascular activity of DOM was characterized by a pressor response, tachyphylaxis and peripheral vasodilation followed by vasoconstriction.[217] The central and peripheral effects induced by DOM were susceptible to 5-HT antagonists.[215-7] Organ distribution, metabolic conversions and the excretory pattern of DOM were studied in laboratory animals.[218-220] The unchanged compound appeared to be responsible for the activity observed.[219] In man, DOET,[220] DOB[221] and MMDA[222] caused a mild euphoria along with a feeling of enhanced awareness at relatively low doses. Various aminotetralins produced smooth muscle contractions in vitro probably through 5-HT receptors.[223] 2-Amino-7-hydroxytetralin caused central effects similar to those seen with mescaline and LSD in rats.[224]

D. Other - A new thebaine derivative 26 proved to be an extremely potent psychotomimetic agent, qualitatively mimicking cyclazocine in man.[225] A novel cholinesterase inhibitor 27 caused severe sensory disturbances in man.[226] Low doses of N,N-dipropyltryptamine were evaluated as a potential adjunct in psychotherapy.[227] The biochemical effects of psychotomimetic anticholinergic drugs were reviewed.[228] In man, sensory disruptive effects produced by atropine, scopolamine and Ditran were similar and could



Cl 110,393

26



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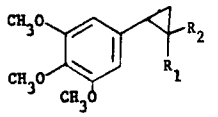

a) R_1=H; R_2=NH_2
b) R_1=NH_2; R_2=H

28

be classified as simple delirium rather than as psychotomimetic syndromes.[229] In rats, cyclazocine[230] and levallorphan[231] produced similar effects to those seen with LSD.

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Chapter 5. Abuse of CNS Agents

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Introduction. The past few years have seen a marked increase in the abuse of CNS agents around the world. It has also seen the passing of Nathan Browne Eddy, who for thirty years had done so much to put a scientific pharmacological basis [54,55,233](see also [4,8,17,45,58,87,88,131,140,172,185] under much of the heroin abuse research being done today.

Abuse of drugs, both licit and illicit has been a worldwide epidemic, and has involved agents as diverse as opiates [2,21,31,73,78,83,84,105,126,187,188,190,191,215], amphetamine [5,117,178,207], LSD [16,189,201,202], barbiturates [59,60,61,96,208], other hallucinogens [50,52,93,95,182,183,184,193,206] marihuana [20,39,68,85,93,95,109,110,111,141,142,198,211], cocaine [71], etc., in addition to a spate of glue sniffing, abuse of nutmeg, snuff, oregano and, of course, the ancient scourge alcohol [6].

The hallucinogens are covered in chapter 4 of this volume. The literature of alcoholism is so vast that no attempt can be made in this limited space to cover it. Furthermore there is a real question in our society whether we consider it to be a drug since, for example, its use is not regulated by the Food and Drug Administration. Sedative abuse (hypnotics, tranquilizers) is a serious, much studied problem. However, not even suggestions of a solution are at hand, so this class of drugs too will not be reviewed.

Opiate Abuse. The opiate area of abuse qualifies for review in these pages on two counts. First, it is one of the most serious medical/sociological/legal/economic problems of our times, and secondly, approaches to the treatment or prevention of opiate abuse have yielded contributions to medicinal chemistry of the classical kind, and thus a review of these approaches is appropriate to the readership of this volume.

Economic Aspects of Opiate Abuse. One of the driving forces behind the rapid expansion of heroin abuse has been the enormous profit [92,128,132] to be made from heroin processing, smuggling [9,99], and sale. This profit motive, when added to the ease of contagion of the heroin habit among susceptible populations (e.g. peer groups), has made the drug abuse battle a difficult one for all concerned. Only now, after extensive efforts to interdict heroin traffic, to arrest and convict traffickers, and to enroll as many addicts as possible into treatment programs, has the explosive growth of heroin abuse shown signs of leveling off. Regrettably the total number of addicts is still rising, albeit more slowly, and estimates of the number of heroin addicts in the U.S. range as high as 800,000 [38], although estimates of 300,000 to 400,000 addicts are more commonly cited.

Opium harvesting is quite labor intensive (about 200 man-hours per kg opium [92] and hence opium tends to be grown commercially in areas where low-cost, short-term labor is available. Climatic requirements for the opium poppy are not great, and Papaver somniferum grows in areas as diverse as Bulgaria, China, Greece, India, Iran, Laos, Mexico, North Africa, Pakistan, Thailand, Turkey, and the U.S.S.R.

Most illegal opium products smuggled into the U.S. in recent years have come from Turkey via France, where morphine was converted to heroin. However, Turkey has only accounted for about 5% of the world production, so closing out opium cultivation in Turkey (subsidized by the U.S.) had little or no effect on illicit heroin supplies in the U.S., although it did contribute to a shortage of medicinal opium exacerbated by crop failures in India. Furthermore, legal cultivation of opium in Turkey is about to be reinstituted for economic reasons.

Recent prices paid to Turkish farmers for legal opium have been about $7.00 per kilogram, but black market prices have been as high as $250,000 per kilogram. At this price of crude opium, the cost of the heroin in a $5.00 "bag" in the U.S. is less than one cent. Hence, because of the profits made by farmers, it was unlikely that authorities would succeed in completely choking off Turkish black market supplies, and even if they did, the world black market has access to even less expensive Southeast Asian opium. The latter source developed to a high degree during the U.S. involvement in the Viet Nam war, and enormous amounts of heroin were smuggled into the U.S. using military postal systems. With the phasing out of U.S. military activities in Southeast Asia, alternative delivery systems will doubtless be developed.

An additional problem in dealing with opiate abuse is that if opium prices should rise prohibitively, synthetic alternatives like methadone would be made available to sophisticated black market organizations. Methadone has been found by addicts to be indistinguishable from heroin on injection in double-blind experiments [103,216,217,218,219].

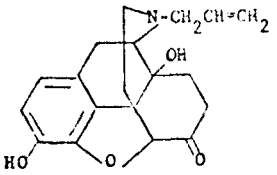
The extraction of morphine from crude opium and its conversion to heroin are relatively simple processes that require only rudimentary equipment or training. The synthesis of opiates de novo requires more equipment and training, but should be quite available to the black market considering the economic rewards. Bearing in mind the $25.00/kg price paid for Turkish crude opium (which represents a cost of about $2.50/kg of morphine), it has been reported [128,132] that morphine base sells for up to $2000/kg, that 40% pure heroin sells for $20,000/kg, and that 1-3% pure heroin sells for $400,000/kg on the 'street'. The large profits from heroin production and distribution underline the difficulty in stopping smuggling of heroin. A look at the logistics of international movement of freight provides further discouragement since the entire U.S. heroin requirements are less than five tons a year, which can easily be lost amid the more than 100,000,000 tons of cargo that enter the U.S. by sea each year [221] or aboard the thousands of flights that enter the U.S. from abroad each year carrying about 200,000,000 persons through customs. Since the daily heroin requirement is only about 12 kg, and considering that a single person can readily carry $500,000 worth of heroin on one trip, the problem of interdicting heroin smuggling can be appreciated.

Government Efforts. The epidemic of heroin abuse in the U.S. has led to considerable activity by Federal, State and Local Governments [144] to deal with the problem. In the White House a Special Action Office for Drug Abuse Prevention was established. The FDA has exerted considerable effort in the area of policing drug treatment programs[56,57,204]. The National

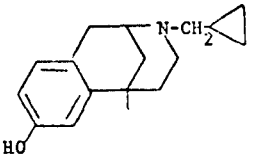
Institutes of Health mounted and funded considerable research effort in the area of opiate abuse [24,25,26,36,49,76,134,135,136,138,143,160,166,167,170,177,181,212]. The Congress has contributed money and focussed attention on the problem [28,33,80,94,111,118,129,144,157,159,174,210], the Department of Justice has increased its efforts [97], and the courts have made use of devices like civil commitment to encourage treatment [29,108]. On the international scene, the UN has likewise addressed the problem of opiate abuse [98,150,203].

Geographic Distribution of Heroin Abuse. Although opiate abuse originated in China and Southeast Asia [214], it has first of all spread to the major population centers of the U.S. [3,12,37,38,40,41,54,55,69,121,180], particularly New York [165], Puerto Rico [148], and California, where the Haight-Ashbury scene became infamous [195,190,191]. From the U.S. the heroin epidemic in the past decade spread to England [13], the Continent [53,113,120], especially Sweden [79], and to Japan [106], and the rest of the more affluent parts of the world like Australia, South Africa, etc.

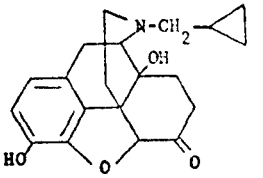
Opiate Antagonists. Martin et al [133] have shown that an injection of a narcotic antagonist into a non-dependent subject will block the subjective opiate-like effects of a subsequent injection of heroin or other opiate. Thus, he postulated that a long-acting antagonist could "immunize" an addict from the subjective effects of opiate abuse. The existing antagonists like naloxone, cyclazocine and naltrexone have certain drawbacks which may limit their usefulness as blocking agents in treating heroin abuse [230].



Naloxone



Cyclazocine



Naltrexone

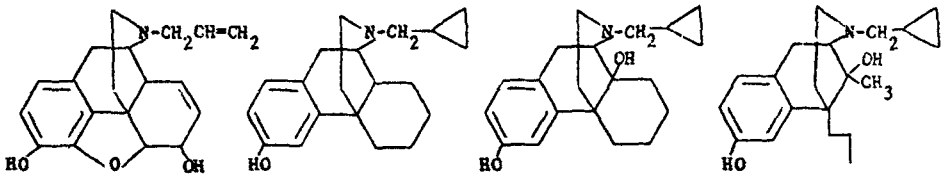
Naloxone, although a "pure" antagonist, has a short duration and poor oral activity. Hence, a 24-hour oral blocking dose must be measured in grams. Thus, considerations of cost and palatability would tend to rule out this substance. Cyclazocine has a desirable duration of action (about 24 hours) and good oral activity. However, its hallucinogenic liability may prevent its acceptability and widespread use. For example, an "induction" period of two weeks or longer is required to build up to a 24-hour oral blocking dose of 4 mg. Naltrexone has a duration of action intermediate between naloxone and cyclazocine and is relatively devoid of "agonist" side effects. However, although it has the most promising profile of the three agents, its relatively short duration of effect and its rather high oral dose requirement (approximately 60-100 mg for 24-hour blocking activity) are disadvantages, especially in considering it for incorporation into sustained release implants. Both naloxone and naltrexone suffer from the drawback that they provide no "reinforcement" to the heroin addict being treated with them. Cyclazocine suffers from the serious disadvantage of dysphoric activity at effective dose levels. Thus, new agents which are devoid of these problems are desirable. Antagonists

are discussed in more detail in chapter 2 [64,103,104,125,133,146,147].

Oxilorphan, a new totally synthetic antagonist synthesized by Monkovic et al [146,147], has many of the properties of the ideal antagonist agent. The studies of Jasinski et al comparing cyclazocine to oxilorphan [234] showed that oxilorphan had an oral potency about equal to that of cyclazocine and a duration of effect at least equal to that of cyclazocine, thus projecting 24-hour heroin blocking activity for a 4 mg dose.

A relative lack of hallucinogenic side effects at 4 mg doses were seen in former addicts [230]. Thus, a projected 24-hour blocking dose of 4 mg can be given orally to the post addict without any induction period and without any hallucinogenic effect. Additionally, oxilorphan produces some "euphoric" component which may assist in keeping post addicts in an antagonist program [230].

It should be mentioned that oxilorphan differs from cyclorphan only in the presence of the 14-hydroxy group. Cyclorphan is a potent analgesic as well as a potent hallucinogenic agent in man, resembling cyclazocine. So it will be seen that introduction of a 14-hydroxy group into cyclorphan [46,147] reduces its agonist and hallucinogenic effects, much as is seen with naloxone in comparison with, for example, nalorphine.



Nalorphine Cyclorphan Oxilorphan levo-BC-2910

Oxilorphan has been subjected to parenteral chronic toxicity and Phase I oral and parenteral testing. In a double-blind comparison of oxilorphan with cyclazocine [234] conducted at the Addiction Research Center at Lexington, Kentucky, oxilorphan was found to have an hallucinogenic potential one-fourth that of cyclazocine.

Levo-BC-2910 an analog of cyclazocine, has been evaluated in sub-acute toxicological testing by the NIMH in preparation for Phase I clinical testing.

The findings comparing the antagonist activity of oxilorphan, levo-BC-2910 and several reference agents are summarized in Table I below [230].

The most useful modality for dealing with the societal aspects of opiate abuse (as well as some of the medical and personal aspects) has been methadone maintenance. In consideration of the pharmacology of opiate dependence [4,17,45,58,87,88,131,140,172,185], Dole and Nyswander [51] proposed that maintenance of opiate addicts on relatively large doses of oral methadone would inhibit drug seeking behavior, "block" the subjective effects of parenteral heroin injection because of the tolerance induced, break the needle habit because of lack of reinforcement by this route, and make the addict more available for psychiatric treatment and rehabilitation, breaking the cycle of crime and punishment. Methadone maintenance has been

Table I

Narcotic Antagonist and Agonist (Analgesic) Activity of Oxilorphan, levo-BC-2910 and Reference Agents

ED_{50} mg/kg s.c.

Compound	Narcotic Antagonist Activity: Oxymorphone Straub Tail	Oxymorphone Narcosis	Morphine Antag.-RTF	Agonist (Analgesic) Activity: Mouse Writhing	Rat Writhing
Oxilorphan	0.19	0.03	0.012	12.8	8.0
levo-BC-2910	0.074	0.013	0.008	44	30
Naltrexone	0.027	0.006	0.003	>80	35.6
Cyclazocine	0.81	0.12	0.040	0.046	0.029
Cyclorphan	0.32	0.10	0.032	0.031	0.028
Levallorphan	0.29	0.32	0.086	26.3	11.8
Nalorphine	1.14	0.58	0.38	0.77	0.70
Pentazocine	12.0	10.1	12.2	3.7	0.95
Naloxone HCl	0.09	0.02	0.010	>80	>80
Morphine sulfate	-	-	-	0.70	0.16

widely employed in the U.S. [15,18,22,23,27,32,36,42,43,47,48,51,62,66,70,72,73,75,78,82,91,100,101,102,107,119,122,123,127,145,149,151,152,154,162,163,164,168,186,192,196] and elsewhere [14,19].

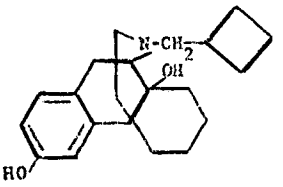
Addicts entering a methadone maintenance program commonly go through a detoxification program [34] and preinduction physical examination. Treatment is commonly started at low daily doses building up to a maintenance level of 40-120 mg/day, tailored to each individual. Most methadone is dispensed and ingested in clinic, since diversion of take-home supplies presents a problem discussed later in this review. Dose adjustment is important since too low a dose will permit heroin abuse, whereas too high a dose can lead to dangers while driving [112] or operating machinery, and can interfere with the rehabilitative process as well [62,65,196,197]. Most methadone treatment centers belong to city or state agencies and are federally funded, although private methadone maintenance clinics have been operated as well [15,156,213]. A number of drug free treatment programs also exist [46,161,162,163,164,170,192,196] under names like Synanon, Phoenix,etc. Complications arise due to multiple drug abuse [26,35,67,123], and these are countered by multi-modality treatment and community participation and preventive [62] and education programs [1,10,30,86,126,199,200,205]. The drug abuse of addicts while in treatment programs can usefully be monitored by urinalysis [8,158,176,186] at frequent but irregular intervals, and by following deaths or serious injuries that are reported on addicts by official sources [7,89,90,115,116,124,130,137,155,179]. Monitoring of crimes, especially burglaries, shoplifting and robberies, also provides a good quantitative index of heroin addict activity, and large scale treatment in centers like New York or Washington, D.C. has resulted in marked drops in arrest incidence, both in the case of individuals in treatment and for the community as a whole [44,153]. Inevitably the epidemic of drug abuse has given rise to unorthodox approaches ranging from transcendental meditation [11] to heroin maintenance [63,91,173] which we deplore.

Research on Less Abusable Forms of Methadone and Other Oral Opiates. As mentioned earlier, the diversion of methadone to street use from maintenance programs is a serious problem since Jasinski et al [103] have shown in double blind trials in post addicts that methadone and heroin are indis-

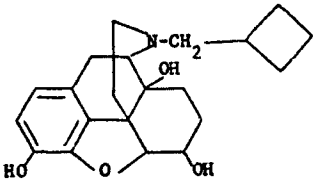
tinguishable on injection. It has been found [32,103, 230, 224] that a 20:1 mixture of methadone-naloxone is indistinguishable from methadone alone when taken orally for maintenance (or analgesia), but taken parenterally by the opium dependent subject it will precipitate abstinence and taken by the non-dependent subject the opiate effect will be eliminated, or postponed for several hours only to appear in an attenuated form. This combination relies on the fact that naloxone given orally is only about 1/100th as effective as it is parenterally. The methadone-naloxone dosage form has been further refined by the development of a gum-formulated tablet that resists dissolution and injection (thus preventing precipitation of severe abstinence in highly dependent subjects like methadone maintenance patients), and by the development of a color coding system for "take-home" methadone-naloxone tablets which permits tracking diverted "street" methadone to its diversion source. The gum formulation mentioned above, although giving identical methadone blood levels to those obtained from oral ingestion of a solution, 24 hours after dosing of each, produces less sedation and fewer "highs" than are seen with solutions.

The addition of 1 mg of naloxone to 100 ml of paregoric [230] produces a product which is indistinguishable from paregoric alone when taken orally as intended. When boiled down, filtered, and injected, however, the combination will produce abstinence in the dependent subject, as does the methadone-naloxone. Other agonists are also being combined with naloxone to deter oral abuse (e.g. oxycodone).

Development of Totally Synthetic Analgesics and Antitussives. There has been a long standing interest in developing non-abusable analgetics and antitussives which do not depend on opium supplies [54,55]. As a result of limitations in cultivation of opium in Turkey, and crop failures in India, the U.S. has had to dip into opium stockpiles, and this has accentuated the interest in synthetic analgetics and antitussives. Two agents of current interest are butorphanol [146,147,230] and nalbuphine [17]. Butorphanol is approximately equivalent in analgesic potency to cyclazocine and approximately equal in antagonist potency to nalorphine. In animals, the analgesic activity of butorphanol is 4-10 times that of morphine, 25-70 times that of pentazocine and 1-2.5 times that of nalbuphine, which is chemically related to butorphanol. Butorphanol has antagonist activity which is approximately 30 times that of pentazocine and 4-6 times that of nalbuphine.



Butorphanol
(levo-BC-2627)



Nalbuphine

Butorphanol demonstrated less physical dependence liability than pentazocine as determined in the mouse jumping test [131,172]. In the Rhesus monkey [229] butorphanol did not suppress abstinence in the withdrawn mor-

phine dependent animal. In addition, exacerbation of abstinence was observed. Physical dependence liability testing is now in progress by Dr.D. R. Jasinski at the Addiction Research Center, Lexington, Kentucky. Nalbuphine showed mixed agonist/antagonist effects in dependence testing, with precipitation of abstinence in morphine dependent subjects, but also drug-seeking behavior in persons subjected to direct addiction experiments.

Clinically, butorphanol has been evaluated in normal volunteers for safety and tolerance both orally and parenterally. Single intramuscular doses ranging from 0.1 to 20 mg were studied. There was no indication of hallucinogenic activity even in one volunteer who received the 20 mg dose [230]. Butorphanol [230] and nalbuphine [231] have been tested clinically for analgesia parenterally and found to be approximately 10 times morphine and equipotent to morphine respectively.

In summary, then, we see that progress has been made in the area of opiate abuse by application of classical medicinal chemical approaches of modifying various components of biological activity by structural modification, finding synthetic substitutes for natural products which improve on nature, optimizing agonist and antagonist properties, etc. It is hoped that these results will encourage future researchers to make similar progress in difficult areas like barbiturate abuse, alcoholism, etc.

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Section II - Pharmacodynamic Agents

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Chapter 6. The Etiology of Hypertension

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Hypertension is one of the greatest medical challenges of our time. It is estimated that upwards of 40 million Americans are afflicted by this "silent disease".[1] Although great progress in the treatment of hypertensive disease unquestionably has been made in recent years, the ultimate therapy remains to be discovered. The development of drugs which act specifically upon various systems to ameliorate the symptom (elevated arterial pressure) of this disease have, indeed, contributed a great deal to clinical management. They have enabled clinicians to effectively lower blood pressure in the majority of patients, and minimize many of the adverse side effects which were associated with earlier forms of therapy. There is little doubt that still better drugs can be developed. For example, a diuretic which does not alter potassium and uric acid excretion or glucose metabolism, a centrally acting suppressant of sympathetic vasoconstrictor nerve function which is devoid of sedative activity, a peripheral vasodilator which does not cause fluid retention, a beta adrenergic blocking agent which does not affect bronchial smooth muscle function, or an antagonist of peripheral adrenergic neurones which is specific to those neurones innervating blood vessels. The list of potential improvements in existing agents is virtually endless, and many would very significantly improve clinical therapy. The point remains, however, that regardless of how specific these agents become they still provide only palliative therapy. The real challenge and hope is to discover and develop agents which correct the underlying cause of the disease.

Before one can design a rational program to discover agents which will "cure" hypertension, the cause must be defined. While the cause of high blood pressure can be defined and corrected surgically or therapeutically in a small percentage of the hypertensive population, for the vast majority it cannot. This large group of patients with hypertension of unknown etiology are categorized as having "essential" hypertension. It is this group of patients which dictates the need for a major research effort to uncover the cause or causes of the disease process and to discover and develop agents which will afford optimal therapeutic control. Interestingly, it has been possible to induce hypertension in laboratory animals at will by a variety of methods for nearly forty years[2], yet the primary disturbance which promotes the high blood pressure in these animals remains an enigma.

Vascular Resistance - The major hemodynamic abnormality in hypertensive patients and laboratory animals is an elevated peripheral vascular resistance. Suggested etiologic factors which may contribute to the increased vascular resistance are enhanced humoral vasoconstrictor substances, decreased humoral vasodilator substances, enhanced neurogenic vasoconstrictor activity, expanded extracellular fluid volume, "auto-

regulatory" vasoconstriction and structural changes in the arterial walls. There is little question that the composition of arteriolar walls in hypertensive animals is modified. The wall is thickened in relation to the lumen[3-7]. Furthermore, resistance to flow in hypertensive vessels is increased even after maximal vasodilatation[8,9], and reactivity of hypertensive vessels to vasoconstrictor stimuli is enhanced[8,10]. Although most investigators believe the structural changes in the vessel walls are secondary to the increased intravascular pressure, some question remains as to whether the functional changes represent primary or secondary events[11].

Renin-Angiotensin-Aldosterone System - The major unresolved problem is the mechanism responsible for initiation of the abnormal vascular resistance. Humoral vasoconstrictor substances released from the kidney have been prime suspects since it was through restriction of renal blood flow that Dr. Goldblatt first induced hypertension in laboratory animals[2]. The subsequent discovery and elucidation of the renin-angiotensin-aldosterone system, and the participation of this system in the hypertensive process has occupied the minds and efforts of many researchers and has been reviewed frequently[12-14]. Recent studies which employed immunologic techniques to render animals insensitive to the vasoconstrictor action of angiotensin or specific angiotensin receptor blocking agents have demonstrated that the renin-angiotensin system is involved in the development of some but not all forms of experimental hypertension. Animals made hypertensive by restricting blood flow to one kidney and leaving the contralateral kidney intact generally exhibit a lowered blood pressure in response to angiotensin II blockade[15-18]. This is particularly true during the first weeks after onset of the hypertensive process. After the hypertension is well established, however, the blood pressure response to angiotensin II antagonism is much less consistent. On the other hand spontaneously hypertensive animals, metacorticoid hypertensive animals or animals rendered hypertensive by unilateral renal artery constriction and contralateral nephrectomy generally fail to demonstrate a decreased blood pressure in response to angiotensin II blockade[15-19]. Interestingly, immunization with a crude renal cortical extract markedly lowered the blood pressure of dogs with renal hypertension (bilateral renal artery constriction or unilateral renal artery constriction and contralateral nephrectomy) of several years duration[20-22]. This observation, coupled with those in which a specific angiotensin II antagonist was employed, suggests the possibility that the animals were immunized against a substance of renal cortical origin other than renin. This possibility is supported by more recent work which demonstrated that passive transfer of renin immunity to dogs with chronic renal hypertension did not restore blood pressure to normal[23]. Furthermore, active immunization of rabbits against angiotensin II failed to prevent the development of either perinephritic hypertension or single kidney Goldblatt hypertension[24,25]. It is apparent that the results obtained with immunization procedures have varied considerably depending upon the animal species, the specific immunization technique, and the type of experimental hypertension employed. The causative role of the renin-angiotensin-aldosterone system in the genesis of hypertension, therefore, remains in question.

Other Renal Prohypertensive Factors - The lack of agreement between investigators as to the etiologic significance of the renin-angiotensin system in experimental hypertension has prompted some to search for other renal factors which might be involved. Grollman has described a substance designated nephrotensin which was isolated from renal venous blood of patients with surgically remediable hypertension and from animals with renal ischemia hypertension[26-27]. Nephrotensin is presumably a polypeptide different from angiotensin I or II which exerts a potent vasoconstrictor action, and appears to be causally related to the hypertension associated with renal ischemia. The participation of this substance in human essential hypertension remains to be determined.

Several investigators have suggested that the increase in vascular resistance associated with hypertension is a secondary, "autoregulatory" response triggered by an increase in cardiac output[28-31]. If this is true, then the factors responsible for the increased cardiac output could become of greater etiologic importance than those which increase only vascular resistance. Cardiac output may be increased because of a change in renal function which promotes fluid retention and thereby enhances venous return to the heart[32]. Alternatively, venous return and cardiac output may be enhanced in response to increased venous smooth muscle tone[33]. A vasoconstrictor material of renal origin different from angiotensin II which markedly contracts vascular capacity has been demonstrated in dogs subjected to severe hypovolemic hypotension[34,35]. The chemical nature of this material and its role in the pathogenesis of hypertension remain to be determined.

Kallidin-Kinin System - Recent work has also suggested a role for the kallidin-kinin system in the pathogenesis of hypertension[36]. Significant increases in urinary excretion of kallikrein were found in spontaneously hypertensive and DOCA-salt hypertensive rats, while levels found in rats with renal hypertension and patients with essential hypertension were lower than those in respective controls. Additional data suggests that the kallidin-kinin system may be intimately involved in the regulation of salt and water excretion by the kidney[36,37].

Renal Antihypertensive Factors - In addition to a prohypertensive mechanism it has been recognized for many years that the kidney possesses an antihypertensive mechanism. Loss of the antihypertensive function may be as important to the pathogenesis of hypertension as activation of the hypertensive mechanism. Work in this area has been kindled in recent years by the demonstration that explants of renomedullary tissue will prevent the onset of renoprival hypertension or lower the blood pressure of animals with renal hypertension[38-41]. Extracts of renal medullary tissue have been shown to lower the blood pressure of animals with experimental hypertension by a number of investigators. Extracts possess both an acute transient hypotensive action and a slow onset, protracted effect. The acute effect is due to the presence of prostaglandins[42,43]. The substance responsible for the delayed response is also lipid in character, however, it appears to be a neutral lipid and not in the prostaglandin family[44-46].

The source of the renal medullary antihypertensive substances appears to be the interstitial cells located primarily in the papilla. Granulation of these cells and presumably secretion has been demonstrated to reflect changes in blood pressure of animals subjected to experimental hypertension[47,48]. Recently, rabbit interstitial cells have been grown in tissue culture[49]. The cells were obtained from autotransplants of renal medulla which prevented renovascular and renoprival types of hypertension. Extracts of the cultured cells were shown to contain the three renal medullary prostaglandins PGE_2, PGA_2 and $PGF_{2\alpha}$.

The possible role of prostaglandins in the pathogenesis of arterial hypertension is of great interest, since some prostaglandins not only dilate peripheral resistance vessels but also possess natriuretic activity[50]. It is therefore tempting to speculate that a deficiency of renal prostaglandins might lead to arterial hypertension. Release of prostaglandins into the renal venous blood has been demonstrated in response to vasoconstrictor stimuli or renal ischemia[51-54]. Most studies have suggested that the primary vasodilator prostaglandin released from the kidney is PGE_2, but because of its rapid metabolism by the lungs PGE_2 could not function as a general vasodilator hormone. Alternatively, renal prostaglandins may exert an antihypertensive affect through an action within the kidney to maintain normal renal resistance and prevent sodium and water accumulation.

Lee has suggested that PGAs, on the other hand, may be circulating antihypertensive hormones, since they are much more resistant to metabolic degradation on passing through the lungs[55]. There is controversy, however, pertaining to the ability of the kidney to form A prostaglandins[50]. Nonetheless, two groups have reported that circulating levels of PGA are lower in hypertensive than normotensive individuals, and are virtually absent in the blood of anephric subjects[56,57]. While the hypothesis that prostaglandins function as antihypertensive hormones is certainly attractive, it is obvious that much additional work is necessary for verification.

Autonomic Nervous System - The autonomic nervous system also is implicated in the pathogenesis of hypertension. Several investigators have suggested that heightened sympathetic nerve activity may underlie the initial labile phase of hypertension[58-60]. This hypothesis is supported by experimental studies which demonstrated that removal of the sympathetic nervous system chemically or immunologically prevented the onset of genetic or mineralocorticoid hypertension[60,61]. On the other hand, these procedures were ineffective in reducing the blood pressure of animals in which the hypertension was established prior to treatment[60-63]. Conversely, the onset of renal hypertension is not prevented by sympathectomy, but maintenance of this form of experimental hypertension appears to be dependent upon an intact, functional sympathetic nervous system[64]. Involvement of the sympathetic nervous system in hypertensive disease is also suggested by the antihypertensive efficacy of the many sympathetic suppressant drugs. Many clinical studies have been conducted in an attempt to quantitate the degree of sympathetic neuronal involvement in various forms of human hypertension. These studies have shown that neurally mediated vasoconstrictor tone is en-

hanced in most hypertensive subjects, but its role in the maintenance of the hypertensive state is controversial. The reader is referred to two recent articles which address this question in greater detail[65,66]. There would seem to be little doubt that the autonomic nervous system is involved in the hypertensive process. Whether it plays a primary or permissive role in the pathogenesis of the disease, however, is in question.

Genetic Factors - The fact that three independent groups have successfully bred strains of hypertensive rats indicates that genetic factors are also extensively involved in the genesis of hypertension[67-69]. Many investigators believe that the spontaneously hypertensive rat (SHR) is the model of experimental hypertension which most closely resembles essential human hypertension[67,70]. The etiology of the hypertension which occurs in these strains, however, has not been defined. The results of many studies which have been conducted with SH rats are highly questionable, because appropriate control animals are unavailable. Normotensive rats of even the same strain obviously are genetically different from their hypertensive relatives, and, therefore, are questionable controls. The one exception appears to be the Dahl strain. These animals require administration of salt to induce the hypertension. Therefore, one can have genetically similar animals with and without hypertension. Renal transplantation experiments with the Dahl SH rats have shown that the kidney is definitely involved in the pathogenesis of the hypertension[71-73]. The specific etiologic factors, however, have not been defined.

Although many factors undoubtedly modify the pathogenesis of hypertension, the bulk of evidence points toward the kidney as the focal point. Future studies directed toward a careful definition of renal prohypertensive and antihypertensive factors coupled with renal excretory function and autonomic nerve activity would appear to be most likely to yield the ultimate solution to this complex problem.

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Chapter 7. Antihypertensive Agents

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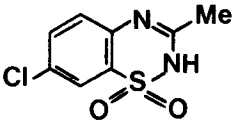
New Products - In February 1973, Schering Corporation introduced diazoxide (I) as Hyperstat I.V.[1], the first antihypertensive agent to reach the U. S. market in ten years. It was also marketed this year in Switzerland and Germany[2]. Suggested use is for emergency intravenous administration to hospitalized patients with malignant hypertension, for which it is claimed to be a welcome and effective treatment[3]. In a recent clinical study it was orally effective, sometimes combined with a β-adrenergic blocker, in some patients with severe and refractory forms of hypertension[4]. Debrisoquin (Declinax, II) was introduced in October in Switzerland by Hoffmann-LaRoche[2]. Long known for its utility in severe hypertension, this drug gave good results in a recent study of mild to moderately severe hypertensives[5]. Propranolol (Inderal, III) the only β-blocker available on the U. S. market[6], is not yet indicated for the treatment of hypertension in the U.S.A[7].



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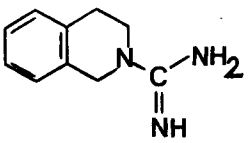

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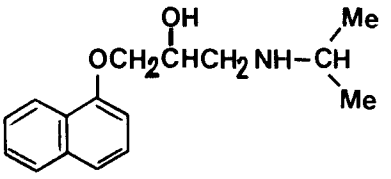
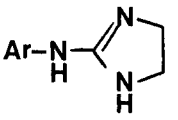


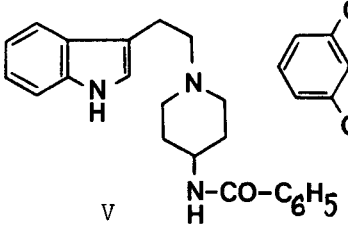
III

Drugs in Clinical Investigation - Although many single drugs were evaluated, clinical studies in 1973 reflected a growing trend to combination therapy. Clonidine (Catapres, ST-155, IVa), marketed in Europe, was found to be effective at low doses without postural hypotension or lasting sedative side effects in two long term studies[8,9]. A comparison with reserpine illustrated clonidine's lack of depressive reactions[10] and the combination with chlorthalidone (Combipres) was even more effective than clonidine alone[11]. The danger of hypertensive rebound and other side effects on sudden withdrawal from clonidine was noted[12,13]. One study described the drug as safe and effective for emergency treatment[14] whereas another reported serious pressor effects when patients with accelerated hypertension were treated intravenously[15]. New pharmacological studies[16-21] on the drug and its major metabolite, 4-hydroxy-clonidine, support a mode of action involving stimulation of central α-receptors. These receptors may be connected to an inhibitory neuron, the activation of which causes a depression of peripheral sympathetic action and a blood pressure fall[22]. The related compound IVb, in phase I of clinical trials in Europe, is active at 0.2-0.4 mg./day in man with maximal hypotension at 4-5 hours[23].

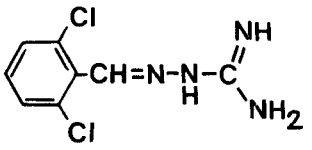
The mechanism of action of indoramine (V) has been reviewed.[24] A recent clinical trial, however, was disappointing due to the side effects observed.[25] Guanabenz (WY 8678, BR 750, FLA 137, VI) in animals shows a potency less than clonidine but greater than guanethidine with a mechanism showing similarities to both drugs.[26,27] Initial clinical trials showed the same potency ranking.[28,29] The drug was effective and further improved by hydrochlorthiazide addition.[28] The principal side effect was drowsiness.



IVa: Ar=2,6-dichlorophenyl
IVb: Ar=3-(2-chloro-4-methyl) thienyl

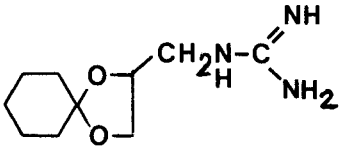


V

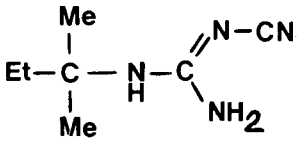


VI

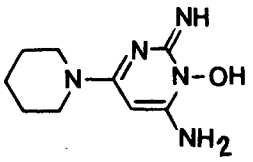
Guanadrel (U-28288D, VII), equipotent to guanethidine in the clinic, appears to offer more stable blood pressure control during the day with less frequent diarrhea.[30] Studies on guancydine (VIII) continued to support the mechanism of a direct vascular effect[31] with partial depletion of norepinephrine stores observed only after high chronic dosing under certain conditions.[32]



VII



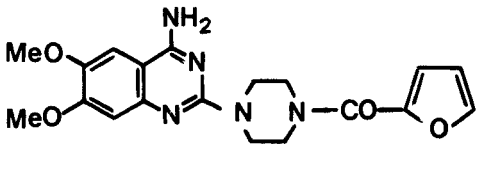
VIII



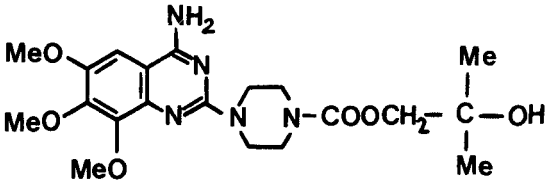
IX

Minoxidil (U-10,858, IX) is a useful hypotensive agent in uremic patients with severe hypertension[33] or those with malignant or accelerated hypertension refractory to conventional drugs.[34] Compared to hydralazine,[35] the drug was more potent, longer acting and showed no signs of tolerance development. It causes a marked increase in serum renin activity,[36,37] cardiac hyperactivity,[35] and sodium retention[34,35] but co-administration of a β-blocker and a diuretic counteracts these side effects.[4,34-38] The pharmacological profile is that of a potent, orally effective, long acting hypotensive in rats, dogs and monkeys which works primarily by a peripheral vasodilator mechanism.[39] Minoxidil is non-toxic in rats, monkeys and minipigs but causes a degenerative lesion in the right atrium of dogs after prolonged daily dosing. Discrepancy between the prolonged action and rapid clearance from plasma was explained by specific retention of the drug in vascular smooth muscle. It may interfere with some cellular component (calcium?) of the contractile process.[40] Another peripheral dilator, prazosin (CP-12299-1, X) gave good results in a recent trial (1.5 to 15 mg./day) even without a diuretic[41] in moderately severe hypertensives. Another member of this structural type, trimazosin (CP-19106,XI)

showed a statistically significant antihypertensive effect at 50-150 mg/day in a group of patients with essential hypertension[42] The combination drug Brinerdine (dihydroergocristine-clopamide-reserpine) is clinically effective[43,44] and more active than any of its separate ingredients or combination of two.

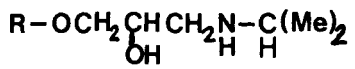


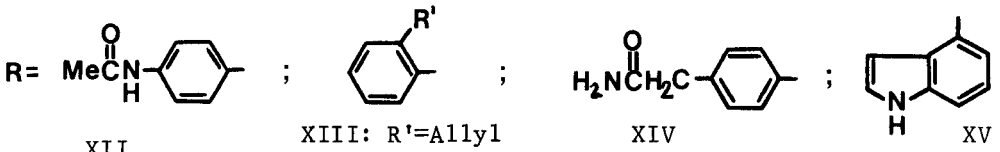
X



XI

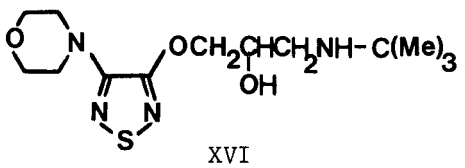
Recent clinical trials of propranolol (III) continue to illustrate its utility as a hypotensive[45] with activity improved by addition of a vasodilator such as hydralazine in severe hypertension[46] or with hydrochlorthiazide in mild to moderate hypertension[47] There is increasing evidence that the drug reduces plasma renin[48-51] Combining propranolol with adrenergic blockers and a diuretic has also been recommended[52] Intracerebroventricular (icv) studies of propranolol in animals by a number of investigators[53-57] have led to the view that its hypotensive activity is centrally mediated and depends on the integrity of noradrenergic neurons. Blockade of β-adrenoceptors in the brain is claimed to cause hypotension and bradycardia[55] However, it is noteworthy that practolol (Eraldin, ICI 50172, XII), a cardioselective β-blocker[58] which does not cross the blood-brain barrier[59] lowers blood pressure very well in man[60-61] This is also true for alprenolol (Aptin, XIII)[62-65] and I.C.I. 66082 (XIV)[66,67] Other β-blockers shown to lower blood pressure in clinical trials this year were pindolol (Visken, LB-46, XV)[68-71] timolol (MK-950,



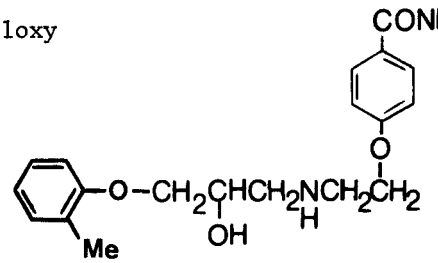


XII XIII: R'=Allyl XIV XV

XVII: R'=Allyloxy



XVI



XVIII

XVI);[72-74] which was considerably more potent than propranolol in animals[75,76] and man, and oxprenolol (Trasicor, XVII);[77-79] which was also very successful when combined with cyclopenthiazide[80] in a large scale study. Tolamolol (UK 6558, XVIII) caused a pronounced blood pressure fall in 3 of 4 normotensive humans.[81] Many analogs have been described.[82] Though cardioselective β-blockers are an improvement over non-selective β-blockers, for example, in patients with asthma, each reduces contractile force along with heart rate. A "chronoselective" β-blocker is still to be found.[83]

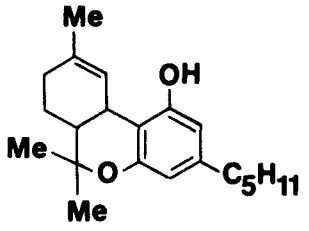
Natural Products and Related Compounds - A vasodepressor factor (VDF) was isolated from canine plasma and aortic tissue and human plasma.[84] The polypeptide, molecular weight <2000, containing glutamic acid, proline and glycine as principal components, rapidly lowers blood pressure in rats when given i.v. VDF levels increase in dogs during induced hemorrhagic shock and high levels are seen in patients with different types of shock. A tridecapeptide called neurotensin isolated from bovine hypothalami is composed of Lys, Arg_2, Asx, Glx_2, Pro_2, Ileu, Leu_2, Tyr_2[85] and is active in rats at 100 pmoles/kg. Synthetic peptides originally isolated from Bothrops jararaca venom, specific inhibitors of angiotensin-converting enzyme, continue to appear promising as diagnostic tools and perhaps in treatment of renal hypertension.[86-88] A study of 57 related peptides indicates that the C-terminal sequence Pro-Gln-Ileu-Pro-Pro is required for significant inhibition.[89] The angiotensin II antagonist effect of Sar^1-ala^8-angiotensin II was confirmed in dog models[90,91] and in man.[92] Hypotension was achieved in hypertensive patients with high renin levels but not in those with low or normal levels. In vitro studies indicate ($Ileu^8$)-angiotensin II to be the most potent antagonist yet developed, but substitution of sarcosine for aspartic acid in the one position increases in vivo activity.[93] The peptide from the native renin substrate Asp-Arg-Val-Tyr-Ileu-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser has been modified slightly to produce competitive inhibitors of renin.[94] The shorter peptide His-Pro-Phe-His-Leu-D-Leu-Val-Tyr is also a potent inhibitor.[95]

Arachidonic acid is hypotensive in spontaneous hypertensive rats likely due to conversion to PGE_2 since activity is inhibited by antiinflammatory agents.[96] The antihypertensive effect of PGA_2 in man is thought mainly due to its effect on renal circulation where it causes vasodilatation and excretion of water and sodium. It also inhibits angiotensin.[97]

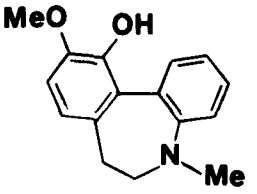
The hypotensive activity of Δ^9-tetrahydrocannabinol (XIX) in animals suggests it as a new lead.[98,99] Development of tolerance to this effect may involve an immunogenic reaction.[100]

Methylapogalanthamine (XX), hypotensive in cats, dogs and rabbits, has adrenolytic, spasmolytic and sedative properties. It has been approved for wide clinical use in the U.S.S.R.[101] A naphthoquinone (XXI) from the fungus Corynespora cassicola inhibits catechol-o-methyltransferase in vitro and is a hypotensive of long duration (24 hrs.) in spontaneous hypertensive rats after 12.5 mg/kg i.p.[102] Fusaric acid (5-n-butylpicolinic acid) analogs with chlorine or bromine in the 3- and/or 4-position of the side

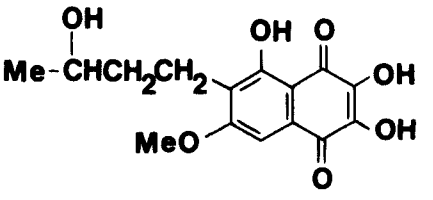
chain are more potent dopamine-β-hydroxylase inhibitors than the parent compound.[103,104] The 4'-chloro analog (FD-008) causes marked sustained hypotension in spontaneous hypertensive rats at 12.5-25 mg/kg. p.o.[105]



XIX



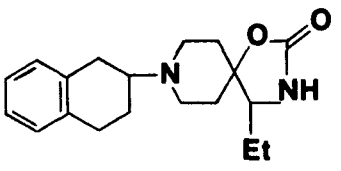
XX



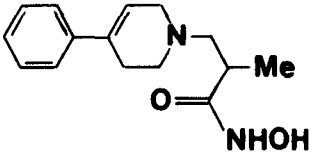
XXI

Other Compounds Affecting Adrenergic Transmission - The predominantly central mode of action of α-methyldopa has received strong support[106-110] and the mechanism has been more precisely defined. The drug is metabolized in the CNS to α-methylnorepinephrine which may act on brain α-adrenoceptors to cause hypotension, as do other potent α-stimulants given icv,[22,111] or may act indirectly by releasing norepinephrine from adrenergic neurons. The hypotension caused by p-hydroxynorephedrine (PHN) in man is more likely due mainly to a peripheral false transmitter mechanism since this compound does not penetrate the blood-brain barrier.[112]

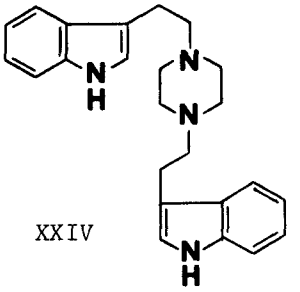
The potent α-blocker 12473JL (XXII) has been selected for clinical trial from a number of oxazolidones with antihypertensive, antiarrhythmic and analgesic activity.[113,114] Compound XXIII is reported to be a potent α-blocker with prolonged action.[115] The piperazine XXIV, probably an



XXII



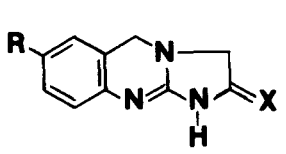
XXIII



XXIV

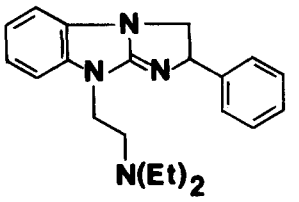
α-blocker, is active at 0.2 mg/kg s.c. in metacorticoid rats[116] but displays CNS depressant properties. Methoxyl substitution in the indole nuclei reduces activity. The tricyclic structure XXVa, orally active at low doses in hypertensive rats and neurogenic dogs, shows α-blocking and norepinephrine releasing properties.[117] Several related tricyclic compounds are quite active though none as active in all models as XXVa.[118,119] Linearity of the tricyclic system appears essential for activity.

Related compounds reported as active in animal models are XXVb[120] (also a platelet aggregation inhibitor) and XXVI.[121] MJ-9465-2 (XXVIIa), active in renal and DOCA hypertensive rats appears guanethidine-like,[122] but may also release antidiuretic hormone.[123] The most active member of a series of 48 analogs is XXVIIb.[124]

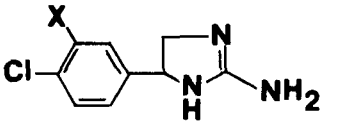


XXVa R=H, X=H_2

XXVb R=CH_3, X=O



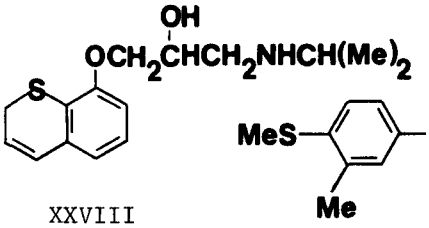
XXVI



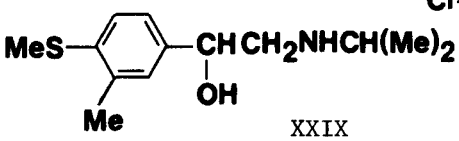
XXVIIa X=H

XXVIIb X=Cl

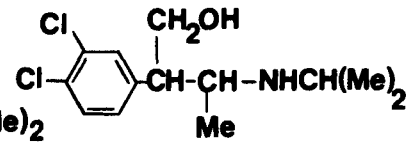
S-2395 (XXVIII), though a non-specific β-blocker, is much more potent than propranolol.[125] CP-240-S (XXIX) is more active than propranolol as a hypotensive in spontaneous hypertensive rats though less β-blocking.[126] The DCI variant XXX is the most potent in a series of β-blockers showing positive inotropic and hypotensive activity orally in rats and dogs.[127] A study of the α-methyl analogs of propranolol and practolol shows that the substitution reduces potency but increases cardioselectivity.[128]



XXVIII

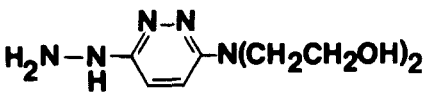


XXIX

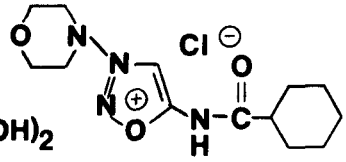


XXX

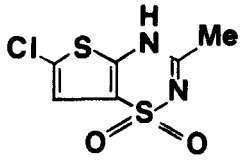
Peripheral Vasodilators and Other Antihypertensives - L-6150 (XXXI), which has hydralazine-like activity with less toxicity, shows no lupus erythematosus-like syndrome in Collie dog studies.[129,130] A group of 3-amino-4-halosydnone imines related to PR-G-138 (XXXII) show comparable oral activity to the parent compound in hypertensive rats (ca. 10 mg/kg).[131] Diazoxide analogs with the benzene ring replaced by thiophene, e.g. XXXIII, are active in DOCA rats and in dogs.[132] The nicotinic acid ester H-1 (XXXIV) is a hypotensive and vasodilator in spontaneous hypertensive rats.[133]



XXXI

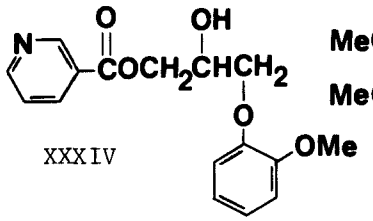


XXXII

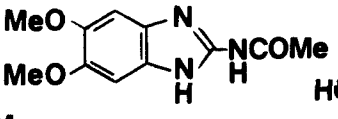


XXXIII

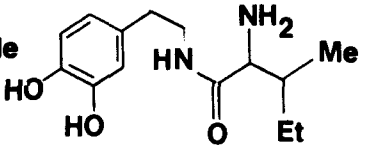
XXXV lowers blood pressure in hypertensive rats mainly by a peripheral mechanism.[134] Abbott 41596 (XXXVI), the best of a series of amino acid amides of dopamine, causes renal vasodilation of 3 hours duration at 12 mg/kg p.o. in dogs.[135] It will be tested clinically for potential prophylactic use in hypertension and in renal diseases. A series of phosphodiesterase inhibitors from Merck show cardiostimulant, bronchodilator and hypotensive properties in animals. In this series, compound XXXVII shows good hypotension at doses that show no cardiac stimulation.[136] The clonidine analog XXXVIII is active at 0.5 mg/kg p.o. in renal hypertensive



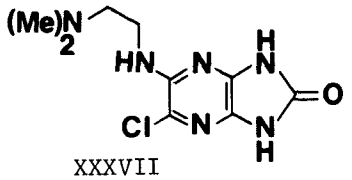
XXXIV



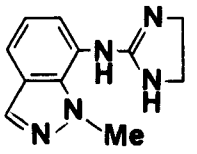
XXXV



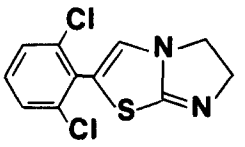
XXXVI



XXXVII

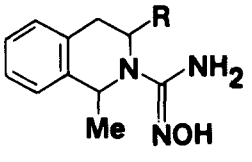


XXXVIII

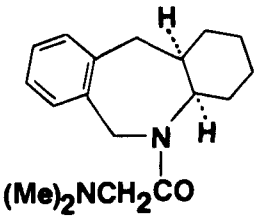


XXXIX

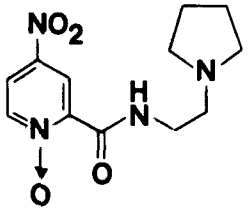
rats.[137] Sandoz 44-549 (XXXIX) causes sustained hypotension and bradycardia in normotensive and DOCA rats at very low doses but slight structure modifications decrease activity markedly.[138] The hydroxyguanidines XL, active in low doses in renal hypertensive rats and hypertensive dogs, appear to act by a direct central effect.[139] W-1984 (HCl salt: W-2587) (XLI) is orally active in hypertensive rats, dogs and monkeys and it, too, is thought to act centrally.[140] Certain 2-carbamoyl-4-nitropyridine-N-oxides are hypotensive in rats, dogs and humans. The best of these appears to be XLII.[141]



XL



XLI



XLII

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Chapter 8. Antiarrhythmic and Antianginal Agents

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ANTIARRHYTHMIC AGENTS

Introduction - Cardiac arrhythmias generally result from enhanced automaticity or disturbances in conduction. These disturbances cause alterations in cardiac rate, regularity, origin of cardiac impulse or sequence of activation of the atria and ventricles.[1-6]

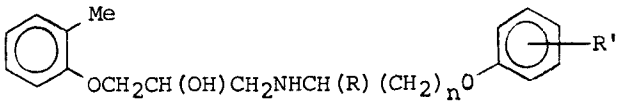
β-Receptor Blocking Agents - Current synthetic efforts in β-blockers has focused mainly on compounds of generic structure 1. By alteration of the

$$ArOCH_2CH(OH)CH_2NHCH(CH_3)_2$$

1

Ar = a) 3,4-dihydro-1(2H)-naphthalenon-5-yl
b) 4-$AcNHC_6H_4$-
c) 4-$MeOCH_2CH_2C_6H_4$-
d) 4-$MeOCH_2CH_2OC_6H_4$-
e) 2-CH_2=$CHCH_2C_6H_4$-
f) 8-thiochromanyl
g) 1,2,3,4-tetrahydro-1,4-ethano-5-naphthyl
h) 2-acetyl-7-benzofuranyl
i) 7-indenyl
j) 5-methyl-8-coumarinyl
k) 2,3-$Me_2C_6H_3$-
l) 4-$C_6H_4CONH_2$
m) 2,3,4,5-tetrahydro-1H-2-benzazepin-1-on-7-yl
n) 4-indolyl
o) 4-CH_2=$CHCH_2C_6H_4$-

nature of Ar-, compounds of greater cardioselectivity and lesser cardiac depressant activity have been sought. Schwender and co-workers studied the effects of aromatic substitution[7] and carbonyl-containing analogs[8] on the β-blocking properties of bunalol (1a) and concluded that no enhancement of cardioselectivity could be obtained. Tolamolol (2) was the most cardio-



2 (R=H, n=1, R'=4-$CONH_2$)

selective member of a series of β-blockers and clinical studies indicated negligible myocardial depression[9]. Practolol (1b) was effective in treating supraventricular tachyarrhythmias in patients who could not tolerate

propranolol because of its bronchoconstrictor activity.[10] The (-)- isomer of practolol retains the β-blocking activity and, unlike propranolol, little anti-arrhythmic activity is retained in the (+)-enantiomer.[11] The absolute configuration of (+)- practolol was determined to be (R) by total synthesis.[12]

H 93/26 (1c) and H 87/07 (1d) were reported to possess considerable cardioselectivity and H 87/07 had intrinsic activity similar to alprenolol (1e) and practolol.[13] No improvement in anti-arrhythmic activity over propranolol was seen for S-2395 (1f)[14], K-4423 (1g)[15], BFE-60 (1h)[16], or YB-2 (1i)[17]. Greater potency and less cardiac depressant properties than propranolol were ascribed to CS-359 (1j)[18] and D-32 (1k).[19]

No improvement over practolol could be obtained for a reverse amide (1l) or cyclized reverse amide (1m).[20]

A series of N-substituted-2-amino-1-(thienyl)ethanols were evaluated as β-blockers and found to be less potent than propranolol as inhibitors of the tachycardic response to isoproterenol.[21] A series of heterocyclic propanolamines had β-blocking activity, the most potent (3) having ten times the activity of propranolol on the guinea pig atrial strip.[22]

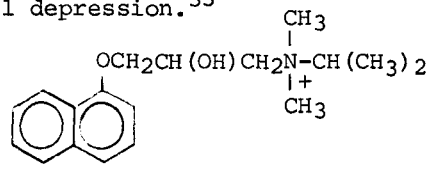
$$ArCH_2CH(OH)CH_2NHC(CH_3)_3$$

3 (Ar=6-phenanthridinyl)

Clinical evaluation of bunitrolol (Koe 1366)[23] and timolol[24] shows no clear advantages over propranolol for increasing exercise tolerance or treating arrhythmias. In dogs, pindolol (LB 46, 1n) produced no depression of ventricular function and increased myocardial performance, while K8 592 depressed myocardial performance and propranolol produced an even greater depression in ventricular function.[25] Thus, subtle changes in structure confer subtle changes in pharmacological profile whose significance is not readily understood. Vaughan-Williams has suggested that p-substituted phenyl groups tend to make a β-blocker more cardioselective, since the ortho-substituted isomer of practolol has no cardioselectivity, whereas p-oxprenolol (1o) was highly cardioselective and more potent than practolol.[26] The role of metabolism in delineating the profile of activity of a β-blocking agent is not clear, but evidence for separation of β-blocking activity and anti-arrhythmic activity via metabolites exists.[19]

Quaternary Ammonium Derivatives - Quaternization of amine-containing anti-arrhythmic agents has sometimes resulted in improved activity with a reduction in certain side effects. UM-272 (4), the dimethyl derivative of propranolol, exhibits neither the β-blocking properties nor the local anesthetic properties of propranolol. UM-272 is effective against digitalis and myocardial infarction arrhythmias in the dog.[27,28] In addition, UM-272 was shown to significantly increase ventricular fibrillation threshold[29,30] and reduce the occurrence of ventricular fibrillation following acute occlusion of the left anterior descending coronary artery and its subsequent de-occlusion. Miura and co-workers have found that this compound exhibits electrophysiological effects similar to propranolol on isolated canine

Purkinje fibers and rabbit atria.[31] Quaternized lidocaine (methyl lidocaine) exhibited the therapeutic efficacy of lidocaine without the CNS side effects of the latter.[32] On the other hand, the dimethyl ammonium quaternary salt of bunalol exhibited only weak anti-arrhythmic activity with pronounced myocardial depression.[33]



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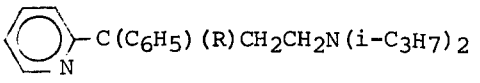
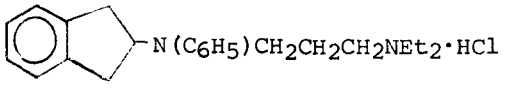
Bretylium tosylate, a neuronal blocking agent, has generated interest as an anti-fibrillatory agent.[34] Counsell and co-workers have synthesized a series of bretylium analogs and found that o-iodobenzyltrimethylammonium iodide showed high uptake by myocardial tissue.[35] Ring-labeled ^{131}I bretylium analogs (RIBA) were useful as myocardial scanning agents.[36]

Miscellaneous Agents - Clinical studies indicate that disopyramide phosphate (5a, R=$CONH_2$, Norpace®) was effective in treating both atrial and ventricular arrhythmias.[37-40] Adelstein found that replacement of the amide by heterocyclic rings (5b, 5c) did not significantly enhance activity.[41] Cusic and co-workers found that substitutions on phenyl or pyridyl groups did not improve the therapeutic index of disopyramide.[42]

Aprindine (6) is a new anti-arrhythmic agent that is 20 times more potent than lidocaine as a local anesthetic. Clinical studies have shown that aprindine is a long-acting, orally effective anti-arrhythmic agent which compares favorably with intravenous lidocaine in preventing ventricular arrhythmias after acute myocardial infarction.[43]



5 a) R=$CONH_2$
b) R=5-tetrazolyl
c) R=2-methyl-1,3,4-oxadiazol-5-yl



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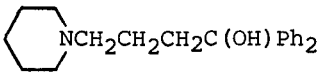
Diphenidol (7) was recently shown to effectively protect dogs against digitalis intoxication.[44] Its mechanism of action was found to result from a shortening of the atrium-to-His bundle conduction time and a suppression of ventricular automaticity. Studies on a group of patients exhibiting digitalis-induced arrhythmias confirmed the animal data and strongly suggest a clinical role for diphenidol.[45]

A similar type of action has been suggested for the steroidal anti-aldosterone agent, potassium canrenoate.[46]

DCAA (17,21-di-monochloroacetyl ajmaline) abolished digitalis-induced arrhythmias in the majority of dogs and significantly reduced mortality rate. No adverse effect on myocardial function was observed.[47]



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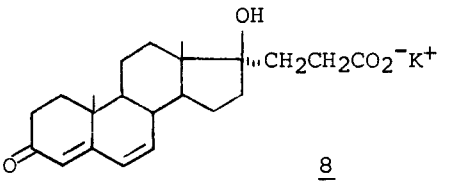

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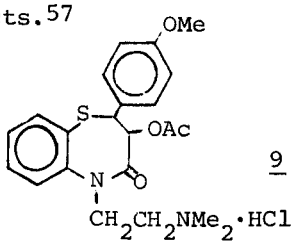
Verapamil (iproveratril) was shown to be an anti-arrhythmic agent several years ago[48,49] and Singh and Vaughan-Williams postulated that this drug acts by a unique mechanism of action; namely, blockade of the calcium-carrying depolarizing current.[50] Recent studies have confirmed the electrophysiological characteristics and effectiveness of verapamil both in vitro and in ouabain intoxicated dogs. In each case, the effect of verapamil was readily reversed by administration of calcium, thus lending support to Singh and Vaughan-Williams' hypothesis.[51,52]

In isolated guinea pig atria, BL-3676 and BL-3677 (isomers of 5-endo-benzoyloxy-N-(dimethylaminopropyl)-bicyclo(2.2.1) heptane-2,3-endo-dicarboxylic acid imide HCl) increased stimulus threshold but did not alter the resting membrane potential.[53] In dogs, W36095 (2-amino-2',6'-propionoxylidide) was an orally effective, long-acting anti-arrhythmic agent with little depressant effect on the cardiovascular system.[54]

CRD-401, the benzothiazepine derivative 9, reversed epinephrine-induced arrhythmias in guinea pigs, but failed to reverse arrhythmias due to two-stage coronary ligation.[55] LL-1530 (10), whose structure is reminiscent of propranolol, was not a β-blocker and was found to have a better therapeutic index and anti-arrhythmic activity of longer duration than quinidine in dogs[56] and was effective clinically with minimal myocardial depressant effects.[57]



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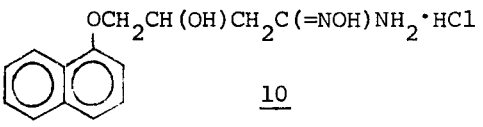


10

$PGF_{2\alpha}$ is reportedly effective in reversing ouabain-induced arrhythmias in cats[58] and a clinical study verified the therapeutic effects.[59] The mechanism of action has not been studied.

ANTIANGINAL AGENTS

Angina pectoris is the result of a disparity between myocardial oxygen demand and oxygen supply. Factors which determine oxygen demand include heart rate, arterial pressure, preload, ventricular volume and mass, and contractility.[60] Oxygen supply is determined by the caliber of the coronary arteries, arterial pO_2, oxygen carrying capacity of the blood, affinity of hemoglobin for oxygen and viscosity of the blood.[61]

Current antianginal therapy is aimed at reducing oxygen demand and/or increasing oxygen supply. The nitrites and organic intrates reduce preload by causing venous pooling of blood. These agents have also been shown to redistribute myocardial blood flow, thereby improving oxygen delivery to ischmic areas even though total coronary blood flow is not increased.[62,63]

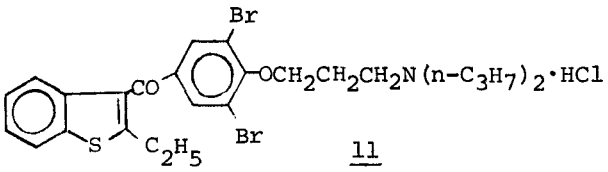
Nitrates continue to be drugs of choice in alleviating anginal pain, but controversies exist as to the efficacy of long-acting nitrates.[64] A recent study indicated that cutaneous absorption of nitroglycerin from an ointment base may be distinctly superior to that of sublingual or oral nitrates in angina prophylaxis,[65] suggesting that the search for more effective nitrates might be better served by exploring new routes of administration for nitroglycerin.

Propranolol reduces oxygen needs through a β-adrenergic blocking action which results in reduced heart rate, blood pressure and contractility.[66,67] Propranolol also decreases the affinity of hemoglobin for oxygen at low oxygen tension.[68] This increased P_{50} results in a greater release of oxygen in hypoxic tissues.

A study comparing the effectiveness of propranolol, oxprenolol, and practolol in anginal patients suggests similar antianginal activity for all three drugs.[69]

The previously described dimethyl quaternary analog of propranolol, UM-272, is not a β-adrenergic blocker. Like propranolol, UM-272 was recently shown to reduce the ST segment shifts observed in canine electrocardiograms during cardiac ischemia.[70] This finding indicates that β-adrenergic blockade *per se* may not be necessary for effective antianginal action. The effect of UM-272 on the hemoglobin-oxygen dissociation curve has not been studied.

L 8040 (11), an analog of the coronary vasodilator amiodarone, was shown to be effective in decreasing myocardial oxygen consumption by an adrenergic blocking effect, but not by competitive β-blockade.[71] Cardiac output was not reduced.



11

Chlorthalidone (Hygroton®), a diuretic, has an antianginal effect unrelated to its hypotensive effect.[72]

A double-blind study of perhexilene (Pexid®) in angina patients showed improved exercise tolerance.[73]

Although the search for better antianginal drugs continues, there is no consensus of opinion regarding the most desirable mechanism of action.

The controversy over the actions of nitroglycerin and propranolol continues and, therefore, the relative importance of selective dilatation of collateral coronary arteries compared to reduction in myocardial oxygen needs *per se* has not been established. While propranolol appears effective in the prophylactic therapy of angina pectoris, concern has been raised over the apparent "rebound" increase in anginal attacks after cessation of therapy. Therefore, further investigation is needed before propranolol is accepted as the *sine qua non* of anti-anginal drugs.

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Chapter 9. Antithrombotic Agents

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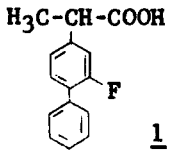
The medical problem of thrombosis encompasses three distinct physiological processes, platelet aggregation, coagulation and fibrinolysis. Drugs capable of influencing one or more of these processes are potentially useful from either a prophylactic or a therapeutic standpoint in various clinical situations. This review will attempt to highlight developments during the past year in this very active field of medicinal research.

Platelet Aggregation Inhibitors

A large number of compounds capable of inhibiting *in vitro* platelet aggregation (PA) have been reported in recent years.[1-4] Some of these compounds, such as aspirin, dipyridamole and sulfinpyrazone were being used clinically for other purposes. Others are new compounds which were either developed particularly for their effect on PA or which possess this potential in association with another pharmacologic activity, e.g. the nonsteroidal anti-inflammatory agents. In either case, the demonstration of *in vitro* activity cannot be considered sufficient to qualify the compound as an antithrombotic agent. It should also be capable of inhibiting thrombosis *in vivo* in one or more of the available laboratory models. Many compounds possess potent *in vitro* activity but fail to be effective *in vivo*, either due to poor absorption, rapid metabolism or the presence of undesirable side effects.

Aspirin and Other Non-Steroidal Anti-Inflammatory Agents - Aspirin continues to receive the greatest attention of the compounds in this category. In 1971, Smith and Willis reported that aspirin interferes with prostaglandin production in human platelets.[5] Recently, it has been reported that both aspirin and indomethacin administered to human subjects abolished platelet prostaglandin production as well as the second wave of epinephrine induced PA.[6] Furthermore, the duration of the effect on prostaglandin production matched the duration of the effect on PA. It was suggested that the nonsteroidal anti-inflammatory agents exert their antihemostatic or antithrombotic effects through disruption of platelet prostaglandin production. It has also been demonstrated that aspirin and PGE_1 produce supra-additive effects on PA and experimental thrombosis,[7,8] although conflicting evidence exists concerning *in vivo* potentiation.[9] The implication is that platelet levels of two or more prostaglandins may act to modulate platelet adhesiveness. Additional experimental evidence has also been reported which substantiates the effectiveness of aspirin in preventing arterial thrombosis. The compound was active in a variety of animal models where mechanical, chemical, electrical or laser injury was used to induce arterial thrombosis.[10-14] In addition, studies in cats have demonstrated aspirin to be effective in reducing the incidence of thrombosis on catheters placed in the inferior venae cavae.[15]

Three new synthetic anti-inflammatory agents have been reported which possess activity against collagen induced PA. Flurbiprofen (1) was effective *in vitro* in human, rat and rabbit platelet rich plasma (PRP) and in rats and rabbits following *in vivo* dosing.[16] It produced significant inhibition of collagen induced PA in human PRP following a single oral dose of 10 mg/kg and had a duration of action in rats of nearly two days. Flurbiprofen did not affect platelet cyclic AMP levels but was capable of inhibiting platelet release of serotonin. It was effective in several other *in vivo* animal models which included the protection of mice from lethal thromboembolism resulting from collagen infusion. Sudoxicam (2) was also found to be a potent inhibitor of collagen induced PA both *in vitro* and *in vivo*.[17] This compound demonstrated activity in humans following oral administration of 50 mg/kg and in animal models of thrombosis. The third agent, ditazol (3) was somewhat less potent in rabbit PRP but was also reported to produce some inhibition of ADP induced PA at high concentrations.[18] Bleeding time in mice was prolonged at daily doses of 100 mg/kg p.o., but PA was only slightly inhibited in human PRP after subjects had received the compound for two days at a dose of 800 mg/day.



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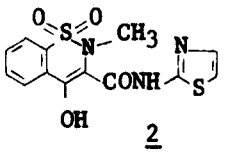

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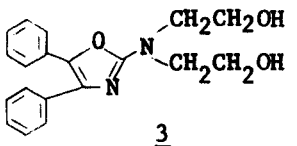

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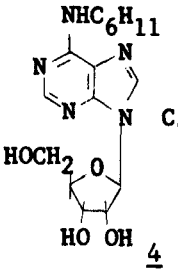
Dipyridamole and Related compounds - A variety of pyrimidopyrimidines and thienopyrimidines have previously been shown to inhibit the primary phase of ADP induced PA and to demonstrate activity in animal models of thrombosis. In addition, dipyridamole has been shown to normalize enhanced platelet consumption associated with a variety of clinical conditions, and it may be more effective when used in combination with an anticoagulant or another inhibitor of PA such as aspirin.[19] There have been several attempts to clarify the mechanism of action of these agents but no clear cut theory has yet evolved.[20,21] It would appear that these agents have some degree of phosphodiesterase inhibitory activity and that they are capable of potentiating the inhibitory activity of adenosine on PA. In view of the rather weak activity of dipyridamole, the conflicting reports of its clinical effects and the limiting side effects of more potent analogs, a good candidate for extensive clinical evaluation probably has not yet emerged from this series of compounds.

Adenosine Derivatives - Adenosine and certain of its derivatives were among the first compounds described which were capable of inhibiting PA. Adenosine is not useful clinically due to its severe cardiovascular side effects and the fact that it is rapidly inactivated *in vivo*. 2-Chloroadenosine was found to be a powerful inhibitor of PA but was very toxic.[22] 2-Methylthioadenosine 5'-monophosphate was shown to be less toxic but also was considerably less potent.[23] In searching for potentially useful analogs, a series of N^6-substituted adenosines and N^6-substituted adenosine 5'-monophosphates have recently been synthesized.[24]

Compound 4 is an example of one of the more active members of this series. Studies in rabbits demonstrated that this agent was also active following an intravenous infusion of 8 mg/kg. Additional 2-thioadenosine compounds were reported which also were effective in vitro.[25] Compound 5 is an example of one of the more active members of this series. However, no in vivo data were reported for these compounds and it is not known if either series possesses activity in animal models of thrombosis.



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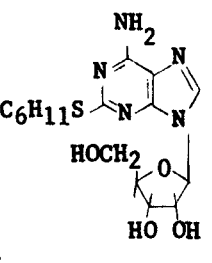

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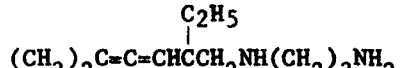
Miscellaneous Agents - It has been reported that high concentrations of penicillin G are capable of inhibiting ADP, collagen and thrombin induced PA as well as the platelet release reaction in human PRP and in suspensions of washed platelets from rabbits or pigs.[26]

Clofibrate and related compounds have been used extensively as lipid lowering agents in patients with various forms of hyperlipemia and atherosclerosis. In addition to lowering serum lipid levels, these compounds have been reported to affect all three aspects of the problem of thrombosis being considered in this review. Two recent reports further describe the ability of these compounds to decrease PA in hyperlipemic patients.[27,28]

A series of substituted 3,4-pentadienyldiamines was reported to inhibit ADP induced PA both in vitro and in vivo.[29] Compound 6 was one of the more potent members of the series and was effective in guinea pigs administered 30 mg/kg p.o. for four days. However, 100 mg/kg for four days was lethal. The lower dose also had a slight effect on ADP induced fall in guinea pig platelet count. A series of N-diethylaminoethylaryloxyacetamides were also reported. Compound 7 was one of the more active members of the series and inhibited both collagen and ADP induced PA in vitro.[30] However, this agent did not demonstrate activity in rabbits after an intravenous dose of 10 mg/kg. The compound β-(2-p-chlorophenylthiazole-4yl)acrylic acid (WY-23,049) (8) has been reported to inhibit ADP-induced thrombocytopenia in rats and to significantly prolong clotting time.[31] The compound AY-16,804 (9) was found to have in vitro activity in rat PRP and was nearly equipotent to sulfinpyrazone but markedly less active than PGE_1 in vivo.[32]



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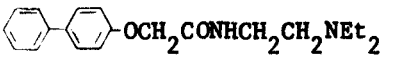

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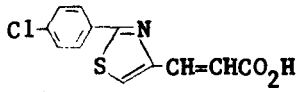

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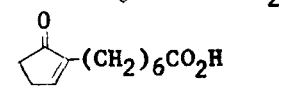

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General Considerations - The spectrum of potential clinical uses for an effective compound in this area is continuing to broaden. During the past year a number of reports have appeared which tend to substantiate the role of PA in the hyperacute rejection of tissue transplants. It has been known for some time that antigen-antibody complexes are capable of aggre-

gating blood platelets and that intravascular coagulation has been shown to play a major role during the hyperacute rejection of allografts. Recently Claes has shown that during such a rejection process involving dog kidney, labelled platelets aggregate and are removed as blood perfuses the transplanted organ.[33] Furthermore, it was demonstrated that this platelet accumulation in the allograft could be abolished with the use of platelet anti-aggregation substances such as cyproheptadine or dextran and that transplant rejection was postponed in treated animals.[34] Similarly, it has been shown that PA plays a role in hyperacute rejection of heterotropic cardiac allografts in presensitized dogs.[35] Once again, an anti-aggregation agent, sulfinpyrazone, proved effective in postponing rejection. A novel model for the study of allograft rejection was reported in which the rejection process was followed microscopically in a segment of kidney allografted into the ear chamber of a rabbit.[36] When a whole kidney was allografted into an animal with an established ear chamber allograft, both grafts rejected simultaneously within seven days. It has been suggested that although platelet participation is dramatic in the case of hyperacute rejection, PA may play a more subtle, though important, role in more chronic forms of rejection. It was found that cyproheptadine added to the therapeutic regimen had a beneficial effect on the post-transplant course of rejection crisis in human renal transplant patients.[37] It was also reported that heterologous antithymocyte globulin and antilymphocyte globulin, which are often used as part of the therapeutic regimen in transplant patients, have platelet activating activity *in vitro* and may have thrombogenic properties when administered *in vivo*.[38] Thus, an anti-aggregation agent may be useful in post-transplant therapy in counteracting the thrombogenic properties of these globulins, as well as preventing both antigen-antibody-induced hyperacute rejection and more subtle platelet involvement leading to the endothelial thickening associated with chronic forms of rejection.

A number of rather limited trials have been reported with agents such as aspirin, dipyridamole, sulfinpyrazone and cyproheptadine in a variety of clinical conditions. An example is a rather dramatic effect seen with aspirin and dipyridamole in a case of thrombotic thrombocytopenic purpura.[39] Variable results with these agents have also been previously reported in transient cerebral ischemia,[40,41] platelet consumption following prosthetic heart valve surgery,[42,43] and postsurgical venous thrombosis.[44] Certainly results from clinical studies of a larger scale, such as those currently underway in the United States and Canada, will have to be completed before a valid assessment of the potential clinical utility of these agents can be made.[45,46]

Anticoagulants

Anticoagulant therapy in recent years has involved the use of either heparin or the oral anticoagulants, coumarin and indandione derivatives, on venous thrombosis. The only newer agents with clinical potential appear to be several snake venoms or venom fractions which act either through defibrinogenation or inhibition of prothrombin activation.

In view of the growing body of laboratory and clinical evidence

which implicates platelet aggregation as the initiating event in most forms of arterial thrombosis, the emphasis of anticoagulant therapy is focusing more and more on conditions such as postoperative venous thrombosis and pulmonary embolism.

Currently, the use of subcutaneous heparin in low doses is receiving a great deal of attention. Numerous studies and opinions have been published in the past two years regarding this form of therapy. Gallus et al. reported a prospective study of 350 high risk surgical and medical patients.[47] The thrombosis rate was lower in those patients treated with the low-dose heparin regimen, regardless of whether they had elective surgery, emergency hip surgery or were suspected of having myocardial infarction. Overall, the rate of thrombosis in heparin-treated patients was 3.6 % as compared to 21.5 % in the untreated group. While there have been some studies which have not demonstrated a beneficial effect of subcutaneous heparin in low doses, the predominant opinion is that venous thrombosis and pulmonary embolism can be delt with effectively by combining the latest diagnostic techniques such as I^{125} fibrinogen scans with the proper use of currently available anticoagulant agents.[48]

The anticoagulant properties of several snake venoms have previously been reported.[4] The venom of Agkistrodon acutus contains a procoagulant principle as well as an anticoagulant fraction, both of which have been purified and studied. It has recently been found that the anticoagulant factor inhibits prothrombin activation by interfering with the interaction between prothrombin and its activation factors.[49]

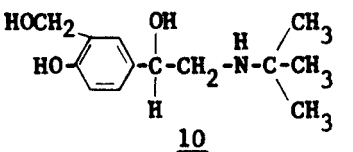
Fibrinolytic Agents

Potential for the application of fibrinolytic therapy lies in two distinct areas. One area involves the concept of curative therapy directed primarily toward dissolution of thrombi formed in cases of vascular obstruction. The other area is based on the concept that the endogenous fibrinolytic system of the body is responsible for clearing fibrinous deposits from the circulation as soon as they are formed and this concept involves prophylactic therapy as accomplished by chronic enhancement of the endogenous fibrinolytic activity. Evidence for diminished fibrinolytic activity in patients suffering from diseases which have a thrombotic component has been recently reviewed.[50]

With respect to clinical effectiveness, success has been restricted to the curative aspect and has involved the intravenous infusion of direct activators which convert plasminogen to plasmin.[51] The greatest success has been achieved with streptokinase, a potent activator which is believed to exert its effect through activation of both circulating plasminogen and intrinsic thrombus plasminogen by formation of an activator complex with the plasminogen molecule itself. The current literature contains an excellent comprehensive review of the clinical pharmacology, mechanism of action and therapeutic uses of streptokinase.[52] Streptokinase, however, has been faulted on three major points: (A) its induction of pyrogenic side effects, (B) its induction of a refractory state with respect to fibrinolysis because of the ability of this agent to act as an antigen and (C) its low se-

lectivity in causing activation of gel phase plasminogen within thrombi in preference to soluble phase plasminogen in the general circulation which often leads to hyperplasminemia and a hemorrhagic state.[52] New developments include the synthesis of compounds, notably substituted benzylamines for the purpose of potentiation of urokinase fibrinolytic activity,[53] and the development of a streptokinase-plasminogen mixture (molar ratio 2:1 to 1:2) as an activator of the fibrinolytic system which is not affected by natural inhibitors in the blood.[54] The principle behind the latter concept is that the plasminogen masks the antigenic groups of streptokinase, consequently the mixture can be injected without risk of reaction even if the patient has antibodies against streptokinase resulting from prior contact with streptococci or prior injection with streptokinase. Clinical success in clot dissolution without the concomitant problems of pyrogenicity and antibody formation with associated development of resistance and titration problems has been achieved with urokinase. The high cost of urokinase, which is now approximately $ 1000 per patient per day, precludes this agent from becoming the drug of choice. Unless current attempts to reduce the cost of urokinase production are successful, the future for acute fibrinolytic therapy lies in the preparation of a synthetic plasminogen activator. However, because of the enzymatic nature of the conversion of plasminogen to plasmin, development of a low molecular weight direct activator of a nonenzymatic nature is essentially precluded. Although the search for agents which might reduce the level or block the action of natural inhibitors of plasmin or of plasminogen activation continues, as yet no agent with an effective efficacy/toxicity ratio has been found.[55] The greatest efforts have been directed towards the search for low molecular weight agents which induce the release of endogenous plasminogen activator. The goal is to obtain a non-toxic, orally effective agent capable of producing either a modest but prolonged increase in the level of circulating plasminogen activator for prophylactic purposes or a strong, relatively prolonged fibrinolytic response of sufficient magnitude to be effective acutely in dissolving preformed thrombi. Since no concrete evidence presently exists that such indirect activation of the endogenous fibrinolytic system is either powerful enough or prolonged enough to dissolve preformed thrombi, the greatest potential for indirect activators of fibrinolysis undoubtedly involves the prophylactic approach to the problem.

It has been suggested that a β-type of adrenergic receptor may be wholly[56-58] or partially[59] involved in the fibrinolytic response to infusion of such agents as epinephrine. However, substantial evidence also exists that this is not the case and that α- and β-blocking drugs do not inhibit the fibrinolytic response to agents such as serotonin, bisobrin and epinephrine,[60-62] to exercise-induced fibrinolytic responses[63] or to fibrinolytic responses induced by venous stasis.[64] Fibrinolytic responses induced in humans by oral or parenteral administration of the β-receptor agonist salbutamol (10) have been reported.[58,65,66] This not only suggests the possibility of a new lead for orally active non-toxic fibrinolytic agents but also may serve to shed some light on the implication of β-receptors in the endogenous fibrinolytic sequence. Salbutamol, a synthetic saligenin



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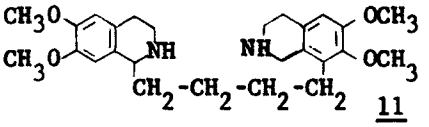
analogue of isoprenaline, is a selective β_2 receptor stimulant, which acts selectively on smooth muscle of bronchi, uterus and arteries in preference to heart and intestinal muscle. Consequently, salbutamol produced a fibrinolytic response without the problems of tachycardia associated with the administration of β_1 receptor stimulants such as epinephrine and isoprenaline[58,66] and provides evidence that plasminogen activator responses may be β_2 receptor mediated.[58] However, it has been recently reported in humans treated chronically with orally administered salbutamol (4 mg/kg qid) that a profound depression of the plasminogen activator, factor VIII and pulse rate responses to epinephrine infusion occurred.[66] In addition, there was no evidence of significant change in the resting levels of these parameters indicating a lack of prophylactic fibrinolytic potential for prolonged salbutamol administration at this treatment level.



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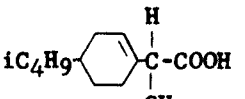
Promising results have been reported concerning the induction of fibrinolytic activity in humans and experimental animals by the bis-tetrahydroisoquinoline EN-1661 (bisobrin) (11) and related compounds.[67,69] These investigations have led to the synthesis of other isoquinoline derivatives[70] and a series of 1,ω-diphenyl-1,ω-alkane diamines of comparable general activity.[71] However, it has been demonstrated that the fibrinolytic activity induced by bis-6,7-dimethoxy isoquinoline derivatives is reduced significantly with prior administration of the antihistaminic promethazine.[70] It is postulated that this type of fibrinolytic agent acts by inducing the release of histamine which in turn is able to activate the fibrinolytic system by mediating the release of plasminogen activators localized in the vascular endothelium.[70,72-74] The fibrinolytic activity elicited by dimethoxyisoquinoline derivatives and related compounds appears to be limited in duration and effect and not adequate for an effective thrombolytic action in cases of massive vascular occlusions.[55]

The well known association of anti-inflammatory and fibrinolytic activities continues to be reported as indicated by evidence of comparisons of fibrinolytic activity of flufenamic acid with niflumic acid, mefenamic acid, azapropazone dihydrate and some trifluromethyl analogues of azapropazone in the hanging plasma clot test.[75] A number of dl-α-4-isobutyl(cyclohexen-1-yl)alkanoic acids and their derivatives, some of which have anti-inflammatory properties, were reported to have fibrinolytic activity in the standard hanging plasma clot test.[76] The most active compound (12) was reported to decrease fibrin monomer levels after intravenous infusion into rabbits and to decrease the euglobulin clot-lysis time in rat blood after daily oral administration for prolonged periods with no evidence of tachyphylaxis. Results in this area are sometimes confounded by the fact that elevated plasma fibrinogen levels observed in the inflammatory state can exert a marked influence on the fibrinolysis values obtained by standard clot-lysis methods[77,78] and may mask the true fibrinolytic activity. This frequently makes results difficult to interpret as observations of increased fibrinolytic activity may be secondary to decreases in plasma fibrinogen content. It should also be emphasized here that Baillie and Sim[79] maintain



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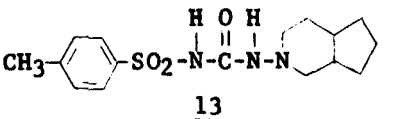
that the effects of acidic anti-inflammatory agents (such as those of Von Kaulla) on enzyme systems are accomplished as a result of their combination with substrate protein rather than by the enzyme itself. Consequently, the enzyme activating effect of such compounds in the hanging clot system should be interpreted with caution since the possibility exists that neither plasmin nor plasminogen are directly involved. These authors reported that flufenamic acid and 3-bromo-4-tertiarybutyl benzoic acid were not only highly active in the hanging clot test but also were both potent inhibitors of plasmin. They also found a positive linear correlation between fibrinolytic and antiplasmin activities ($r=0.81$, $p<.05$) using this test.

The literature contains equivocal results concerning the beneficial effects of prolonged clofibrate treatment of fibrinolysis.[80-83] The most recent study involved treatment of elderly arteriosclerotic patients with a combination of two clofibrate derivatives, diisopropylamine p-chlorophenoxyisobutyrate and 2-dimethylaminoethanol p-chloro-phenoxyisobutyrate (Gerobrek^R) for a period of 30 days. A statistically significant increase in fibrinolytic activity was observed along with a significant reduction of platelet aggregation and a potentiation of the anticoagulant activity produced in patients receiving an indirect anticoagulant.[28] Although studies have suggested that clofibrate exerts a protective effect against sudden death and myocardial infarction unrelated to its lipid-lowering action,[84] the effect of Gerobrek^R in elevating fibrinolytic activity is in all probability secondary to hypolipemic effects. It is well documented that in humans, low density of lipoproteins and chylomicrons inhibit the fibrinolysis of plasma euglobulin *in vivo*.[85-87] In the same context, treatment of hyperlipemic patients orally with a combination of nicotinic acid and the synthetic heparin-like polyanion SP54^R has been reported to reduce the hypercoagulable state by increasing fibrinolytic activity, reducing platelet aggregation and reducing both cholesterol and triglyceride levels in the blood over a 9-week period.[87] Increased fibrinolytic activity associated with decreased blood lipids in humans was also reported subsequent to oral diethanolamine treatment.[89]

Compounds causing enhancement of fibrinolytic activity over extended periods of time by increasing concentrations of circulating plasminogen activator still fall mainly into the group of anabolic steroids such as ethylestrenol and stanozolol and the glucose-lowering agents such as tolbutamide and phenformin. Treatment with orally administered phenformin in conjunction with either ethylestrenol or stanozolol continues to give evidence of prolonged improvement of overall fibrinolytic activity as evidenced by the results of a nine month study in patients with cutaneous vasculitis having impaired fibrinolytic activity.[90] The oral antidiabetic drug gliclazide (S1702) (13) was reported to induce fibrinolytic activity in dogs and rats after both oral and parenteral administration.[91] Infusion of prostaglandin E_2 into female patients for the termination of pregnancy was reported to increase plasma antithrombin concentration and increase fibrinolytic activity.[92]



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Heparin-serotonin complexation to form an entity with anticoagulant activity and lytic activity different from that of plasmin with respect to the fact that it lysed unstabilized fibrin clots in the presence of EACA has been reported.[93] Evidence of a protective action of vitamin C in deep vein thrombosis has also been reported.[94]

A potential area of interest which has as yet had no clinical application is the approach of rendering a thrombus fibrin more susceptible to fibrinolysis, and is treated in a comprehensive review on factor XIII inhibition.[95]

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Chapter 10: Pulmonary and Antiallergy Drugs

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This review covers papers published in the period 1971-1973 with a few older citations when they were needed for the appropriate discussion of a concept. The main emphasis of the review is the present or potential therapy of asthma or reversible airways disease. A section is devoted to emphysema, an "irreversible" airways disorder.

Asthma is a type of reversible, obstructive pulmonary disease due to widespread narrowing of the bronchial airways and edema of the bronchial mucosa, caused by specific allergic and/or nonspecific irritative stimuli [1,2]. A specific immunoglobulin, IgE, is produced in the atopic or allergic individual, in response to the appropriate exposure to antigens. An important predisposing factor to the asthmatic attack is the acute sensitivity of the bronchial tree to pharmacologic agents, irritants and specific allergens.

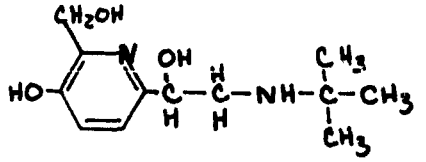
Therapy of reversible airways disease includes symptomatic relief of the attack, control of specific causative factors as well as generalized care of the patient. Drugs are employed to relax and dilate bronchioles (beta-sympathomimetics and phosphodiesterase [PDE] inhibitors), reduce inflammation (steroids), liquefy mucus (acetylcysteine), decrease mucous membrane congestion (alpha-sympathomimetics) and prevent mediator release (cromolyn sodium). Initial clinical studies of anticholinergics in asthmatics and bronchitics are under way.[3-5]

Beta Adrenoceptor Agonists - Beta adrenoceptors have been divided into subclasses based on the relative activities of various sympathomimetic amines on different target tissues.[6] For example, beta-1 receptors are found in the heart and beta-2 receptors are found in bronchiolar and other smooth muscle. The potent beta adrenoceptor stimulant isoproterenol, a common agent for the treatment of asthma, is nonselective and may cause undesirable cardiac stimulation at doses which relax bronchiolar smooth muscle. Besides direct effects on bronchial smooth muscle, these compounds inhibit mediator release from skin[7] or passively sensitized human lung[8] and also inhibit antigen-stimulated histamine synthesis in human leucocytes.[9]

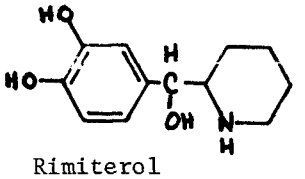
Chemical modifications of the catecholamine structure by a) replacement of the 3-hydroxyl group, b) replacement of the 3,4-dihydroxy configuration by a 3,5-dihydroxy configuration, c) attachment of a larger substituent on the alkyl nitrogen and d) alteration of the ethanolamine side chain have yielded compounds with greater bronchoselectivity, longer duration of action and greater oral activity than isoproterenol. Differences in duration of activity and oral potency are probably related to alterations of the 3,4-dihydroxy configuration; lack of the 3,4-dihydroxy configuration precludes metabolism by catechol-O-methyl transferase (COMT)

and appears to interfere with sulphate conjugation in the gut[10-12], both major metabolic pathways for isoproterenol.

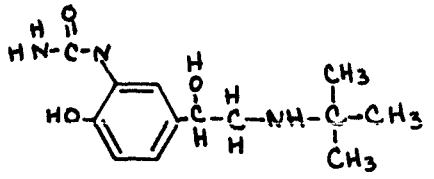
Compounds reported to be more selective and longer acting than isoproterenol include salbutamol,[13-14] terbutaline,[15-16] Th 1165a,[17-19] salmefamol,[20] hexoprenaline,[21] trimetoquinol,[22-23] soterenol,[24] rimiterol,[26] carbuterol,[27-29] and pyrbuterol.[30] Isoetharine, also more bronchoselective than isoproterenol, has been tested using a sustained release system to prolong duration.[31] The bronchodilator activity of a group of monofluoromethanesulfonanilides was recently reported;[32] unfortunately the best agent in this series caused irreversible eye changes in canines.



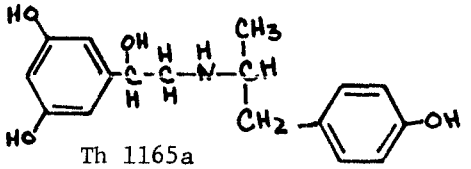
Pyrbuterol



Rimiterol



Carbuterol



Th 1165a

A common clinical problem of the oral or parenteral route of administration of the newer beta-stimulants, such as salbutamol and terbutaline, is digital tremor.[16,33] This effect is mediated via adrenoceptors of the beta-2 subclass. Incidence of tremor may be as high as 40%.[34] No substantial separation between bronchodilator and tremorogenic activity has been reported.[33] A decrease in the tension and degree of fusion of incomplete tetanic contractions of the slow contracting soleus muscle of the cat is used as a laboratory model predictive of tremor in man.[35]

Use of pressurized aerosols containing beta adrenoceptor agonists was implicated in increased deaths among asthmatics in England.[36] However, Stolley recently showed a strong correlation between countries having an elevated death rate in asthmatics and countries marketing a strong preparation of isoproterenol.[37] However, in the U.S., where the strong preparation was not marketed, the death rate from asthma decreased during the years 1958-1969 while the number of pressurized aerosols sold increased 60% between 1964 and 1968.[38]

Prostaglandins - Prostaglandins are found in nearly all tissues; PGE_2 is the dominant prostaglandin in bronchial tissue, while $PGF_{2\alpha}$ predominates in lung parenchymal tissue.[39-40] However, prostaglandins are not stored as such in tissue. A large number of stimuli can trigger phospholipase A which cleaves arachidonic acid from cell membrane phospholipids.[41-42] Arachidonic acid is rapidly converted to prostaglandins by the enzyme prostaglandin synthetase. Thus measurement of tissue levels of prostaglandins may be indicative of synthetic ability at the time of measurement rather than basal tissue values.

Aerosol administration of PGE_1 or PGE_2 produces bronchodilatation in animals[43] and man[44] with minimal cardiovascular side effects.[45-46] By this route PGE_1 or PGE_2 was 10 times more potent on a weight basis than isoproterenol, but had a slower onset of action;[47] duration of activity was in the range of isoproterenol and therefore considerably less than the long acting beta-2 stimulants. A limiting side-effect of the aerosol administration of PGE_1 or PGE_2 is laryngeal irritation and coughing.[46] Furthermore, some asthmatics have developed an attack of asthma after inhalation of PGE_1, possibly due to irritant effects in the absence of a bronchodilator response.[48] While PGE_1 has been reported to increase cAMP levels in isolated lung tissue, further mechanism studies are in order.[49]

$PGF_{2\beta}$ caused bronchodilatation in the cat and guinea pig.[50] $PGF_{2\alpha}$ produced bronchoconstriction in man.[51] Human asthmatics were 8000 times more sensitive to the bronchoconstrictor effects of $PGF_{2\alpha}$ than were normals and only 10 times more sensitive to histamine-induced bronchospasm.[52] It has been suggested that the endogenous PGF/PGE ratio may be critical in asthmatics, but this awaits definition.[53]

Certain analgesic, antiinflammatory drugs such as aspirin, indomethacin, flufenamic acid and mefenamic acid inhibit prostaglandin synthesis.[54] Aspirin and the fenamates also antagonized the direct constrictor effects of $PGF_{2\alpha}$, but not the relaxant effects of PGE_2,[55] on isolated human bronchial tissue.[54] Indomethacin and aspirin reduced the basal tone of isolated trachea, decreased the effect of small doses and increased the effect of large doses of histamine or acetylcholine.[56] These latter studies suggest a modulating influence for the prostaglandins in tracheal smooth muscle.

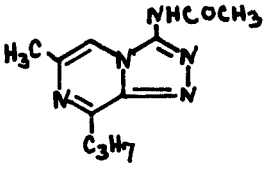
High molecular weight anionic polyesters of phosphoric acid and phloretin, called polyphloretin phosphate (PPP) have antagonized the constrictor effects of $PGF_{2\alpha}$ on human bronchi. Yet neither the dilator response to PGE_2[57] nor the constrictor response to carbachol[58] were altered, suggesting selective antagonism. PPP also inhibits alkaline phosphatase, hyaluronidase and urease. The different molecular weight constituents of the mixture of PPP polymers have been separated to yield the PG-inhibitory component in the low molecular weight fractions and the enzyme inhibitory activity in the high molecular weight fractions.[59] Asthmatics have failed to show any improvement in airway resistance after 5 mg doses of PPP were inhaled.[48]

Prostaglandins inhibited immunologic release of histamine from human leucocytes[60] and human lung fragments;[61] this inhibition was associated with increased cAMP levels.

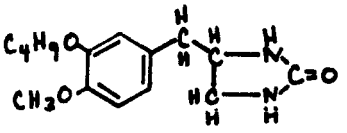
PDE Inhibitors - cAMP is rapidly metabolized by a PDE which hydrolytically cleaves the 3'phosphate bond to yield a 5' phosphate ester of adenosine (AMP). A similar reaction occurs for cGMP. cAMP relaxes smooth muscle such as isolated trachea.[62] The demonstration of a Ca^{++}-dependent increase in cGMP in smooth muscle in response to cholinergic agents, high K^+ concentrations, and histamine suggests a role of this nucleotide in smooth muscle control.[63] However, dibutyrl cGMP and 8-bromo-cGMP relaxed tracheal smooth muscle.[64] A biphasic contraction and relaxation have also been reported.[65]

Theophylline and other methylxanthines are PDE inhibitors and are frequently used to treat asthma. In a comparative study using frog erythrocyte cyclic nucleotide PDE, theophylline ($4x10^{-4}$M) was a potent inhibitor of the hydrolysis of cAMP but was a much less effective inhibitor of the hydrolysis of cGMP.[66]

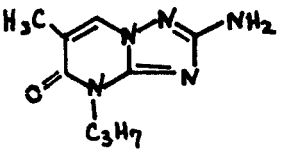
Compounds capable of inhibiting PDE's from various sources have been tested for bronchodilator efficacy. A triazolopyrazine (ICI 58,301) and a triazolopyrimidine (ICI 63,197) protected guinea pigs from the lethal effects of histamine (respective ED_{50} values were 0.5 and 0.05 mg/kg).[67] Propranolol did not block the antibronchoconstrictor effects in vitro but did in vivo. The apparent blockade in vivo could be due to antagonism of reflexly-released catecholamines which contribute to the protective response.



ICI 58,301



I_{BM}



ICI 63, 197

Studies utilizing rat erythrocyte PDE led to the discovery of 4(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (RO 20-1724; I_{BM}).[68] This agent was 5000 times more potent than theophylline in the erythrocyte preparation, with an I_{50} of 0.1 μM. I_{BM} was about 400 times more potent than theophylline in relaxing the isolated guinea pig trachea and its effect was not blocked by propranolol. Similar results were noted using isolated human trachea and bronchi. Interestingly, I_{BM} was a very poor PDE inhibitor in dog tracheal smooth muscle or in whole guinea pig trachea. Thus its mechanism of tracheal relaxation cannot be explained in terms of

PDE inhibition. The purine compound inosine has also been reported to inhibit PDE and inhibit bronchospastic responses in guinea pigs.[69]

Anticholinergics - In both animals and man, stimulation of epithalial irritant receptors in the airways by antigenic and nonantigenic physical or pharmacological agents resulted in reflex bronchoconstriction.[70] This bronchoconstriction could be blocked by atropine in humans[71] and by atropine or vagotomy in animals,[72] indicating a parasympathetic mediation. Vagal blockade reduced the increase in pulmonary resistance caused by histamine (injected into the pulmonary circulation) from 800% to 100%.[73] Bronchoconstriction caused by antigen inhalation in asthmatics has been blocked by atropine.[3] These experiments have challenged the traditional concept of mediator-induced direct muscle contraction as the primary event in bronchospasm. Atropine has historically been viewed as dangerous to asthmatics because of the potential of drying effects and mucous plugs. In one acute trial, isopropyl atropine (Sch 1000) was found superior to salbutamol,[4] while in another the reverse was reported.[5] Acute studies may not be the most appropriate means to evaluate the problem of mucous plugs. It has recently been reported that a few selected patients were treated with aerosolized atropine for up to 6 months without observing mucous problems.[74]

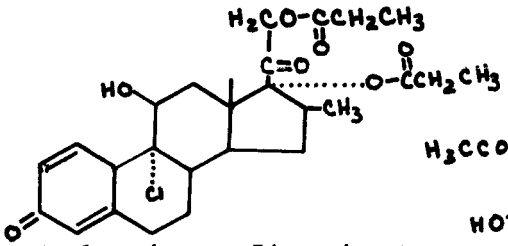
Emphysema - Emphysema is an anatomic alteration of the lung characterized by an abnormal enlargement of the air spaces distal to the terminal, nonrespiratory bronchiole, accompanied by destructive changes of alveolar walls. Since Brown-Sequard[75] first discussed the production of experimental emphysema in the late nineteenth century, reports of various experimental procedures to induce this condition have appeared in the literature. Emphysema-like conditions have been produced by phosgene,[76] cigarette smoke,[77] tracheal ligation and phytohemagglutinin,[78] papain, administered intratracheally[79] or by aerosol,[80] polymorphonuclear-enriched leukocyte homogenates,[81] and cadmium chloride aerosol.[82]

Utilization of proteolytic materials, such as papain, to produce an experimental emphysema-like state may be of pertinent value in light of recent evidence that a deficiency of an antiproteolytic factor, α_1-antitrypsin, is associated with panlobular emphysema.[81] Many investigators have indicated that papain-induced emphysema is similar morphologically, pathologically and physiologically to human emphysema.[79,80,84-87] Cadmium is of special interest because of its presence in cigarette smoke. Progesterone antagonized the development of emphysema induced by intratracheally-administered papain and tracheal ligation[88] and by papain aerosol in male rats,[89,90] as well as the development of an emphysema-like condition produced by phytohemagglutinin and tracheal ligation.[78] Dimethisterone and megestrol acetate were effective against an emphysema-like condition produced by papain aerosol and tracheal ligation.[88] Medroxyprogesterone acetate prevented the development of an emphysema-like lesion caused by the microbial protease, thermolysin.[91] Reasons for initially studying progesterone in experimental emphysema included the observations that a) there was a lower incidence of pulmonary emphysema in

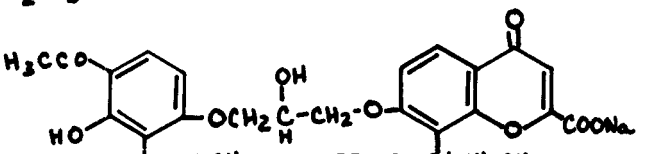
females than in males,[92] b) female emphysematous patients improve during pregnancy when the blood titer of progestational hormones is elevated.[93]

The mechanism of the beneficial effect of progesterone is unknown. Since progesterone and other progestagens are effective in several types of emphysema-like conditions, it seems unlikely that the beneficial effect is due to antagonism of papain-induced proteolysis. Studies to a) define the dose response relationship of progesterone in experimental emphysema and b) evaluate the relationship between progestational activity and "antiemphysema" activity seem appropriate.

Corticosteroids - Corticosteroids have a number of side effects that can restrict their use in asthma. The most troublesome of these is the suppression of normal adrenal function and the development of Cushingoid symptoms. Recently beclomethasone-17,21-dipropionate has been used clinically with rather good results as a replacement for oral steroid therapy.[94-97] In patients with moderately severe chronic bronchial asthma that were not on oral steroids, beclomethasone aerosol (100 μg, 4 times a day) improved lung function tests and reduced bronchodilator usage without a significant effect on plasma cortisol levels.[98] The lack of suppression seen with the aerosol may be due to the localized application and lack of absorption. In patients already on maintenance oral steroids, aerosol beclomethasone reduced but did not replace prednisolone.[96] This limited response may be due to a) too low a dose of aerosol steroid or b) other necessary systemic activities of corticoids discussed below that cannot be replaced by topical application.



Beclomethasone Dipropionate



FPL 55712

Although part of the effect of steroids in asthma may be due to the well-known antiinflammatory action and stabilization of lysozomal membranes, there also appear to be effects of these compounds on the immune system. The stimulation of *in vitro* immunoglobulin synthesis by epinephrine on peripheral leucocytes from normal humans could be augmented by 10^{-6}M hydrocortisone, while lymphocytes from asthmatics did not respond to epinephrine or to augmentation with hydrocortisone.[99,100]

Corticosteroids have also been shown to augment the effect of catecholamines on bronchial tissue, such that guinea pigs, chronically treated with hydrocortisone, were more sensitive to isoproterenol. Lungs from such guinea pigs took up less epinephrine and norepinephrine *in vitro* without changing catecholamine metabolism.[101,102]

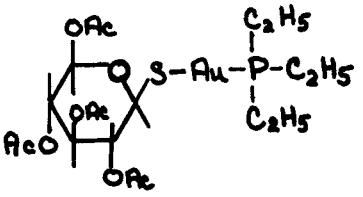
Specific Inhibitors of Anaphylactic Mediators - Slow reacting substance of anaphylaxis (SRS-A), a constrictor of bronchial smooth muscle, has been shown to differ from any of the other known physiologically active agents (MW of 350-450; pI=4.7).[103] Diethylcarbamazine inhibits the release of SRS-A from rat and human tissues but it has not been effective in ameliorating bronchial constriction in asthmatics, perhaps because of toxic limitations. FPL 55712 acts in vitro as a highly specific antagonist of SRS-A-induced contractions of guinea pig ileum, being several times less active against other contractors.[104] FPL 55712 also reversed an ongoing SRS-A-induced contraction of guinea pig ileum. The clinical utility of this compound is unknown.

Antihistamines, which usually exert their effects by blocking histamine at end-organ receptors, are effective in urticaria and allergic rhinitis but are of questionable efficacy in asthma. Troublesome side effects of the clinically useful antihistamines include both CNS stimulation and depression. RMI 9918 has been reported to be an effective H_1-specific antihistamine in rats, mice and guinea pigs with no observable CNS activity.[105]

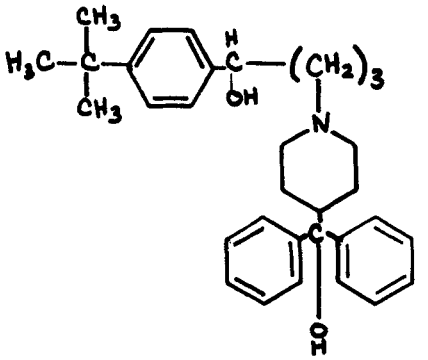
Anaphylactic Inhibitors - Cromolyn sodium inhibits the release of allergic mediators such as histamine and SRS-A from sensitized tissues, but does not interfere with the combination of antigen and antibody. Like its precursor azatadine, azanator maleate (Sch 15,280) has significant antimediator activity.[106] It also inhibits rat reaginic passive cutaneous anaphylaxis (ID_{50}=3 mg/kg p.o.) and in vitro anaphylactic histamine release from rat peritoneal mast cells ($IC_{50} = 10^{-5}$M). This drug also relaxes guinea pig tracheal smooth muscle (not mediated via beta adrenoceptors) and prevents anaphylactic bronchoconstriction in sensitized rats. Other "anti-allergy" compounds shown experimentally to inhibit allergic reactions in a wide range of in vitro test models include three xanthones, (AH 6556) (AH 7079) (AH 7725).[107] A series of xanthone-2 carboxylic acids antagonized passive cutaneous anaphylaxis in the rat.[108]

There has been great interest in studying mechanisms for preventing allergic reactions at the level of the antigen-antibody target cell. When incubated with cromolyn sodium in vitro rat peritoneal mast cells and human lung tissues become stoichiometrically autodesensitized to cromolyn.[109-111] The clinical implications of this observation are unknown.

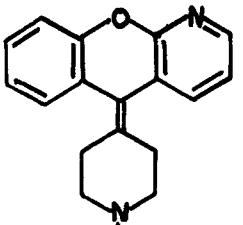
Chrysotherapy has been used for arthritis and some related diseases, and reports exist of some value in asthma. SK&F-39162 appears to be a specific inhibitor of anaphylactic histamine release in reaginically sensitized animals. Yet the compound has no direct antagonism to known mediators.[112] It is not known whether this compound has the same liabilities of other gold salts in humans, such as kidney and skin lesions.



SK&F - D39162



RMI 9918



Sch 15280

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Section III - Chemotherapeutic Agents

Editor: George H. Warren, Wyeth Laboratories, Inc., Philadelphia, Pa.

Chapter 11. Antibiotics

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General - The molecular basis of antibiotic action was discussed in a monograph[1] and at a symposium.[2] The proceedings of several symposia were published, each devoted to a single antibiotic[3-5] or drug combination.[6] A collection of papers on the clinical evaluation of amoxicillin[7] and a preliminary report on the pharmacokinetic properties and therapeutic efficacy of pivampicillin[8] appeared. An issue of "Seminars in Drug Treatment"[9] contains reviews on adverse effects of antibiotics[9a] and the treatment of various bacterial infections.[9b-e] A therapeutic guide to selection of systemic antibacterial agents was prepared.[10] A review appeared on the nephrotoxicity of antibiotics and the therapy of infections in patients with impaired renal function.[11] The synthesis of β-lactams[12], the allergenicity of penicillins[13], the biological properties of semisynthetic penicillins[14], and the chemical and biological properties of ansamycins[15] were reviewed. Several antibiotics[16a-d] and antibacterial agents[16e,f] are included in the first two volumes of a new serial publication[16] on physical, chemical, and biological properties of selected drugs. The concentration of antibacterial drugs in interstitial fluid was determined by a new experimental technique.[17] The molecular properties of R-factors[18-20] and the biochemical mechanisms of resistance to antibiotics[21] were discussed.

Infectious resistance to antibiotics - Plasmid-borne antibiotic resistance was demonstrated in *Streptococcus faecalis*[22-24] and *Bordetella bronchiseptica*[25] and evidence for its conjugal transfer was adduced.[24,25] The curing of an R-factor from *Escherichia coli* by trimethoprim was achieved.[26] R-factors carrying multiple copies of a penicillinase gene were identified.[27] An R-factor mediated resistance to penicillins not involving β-lactamase was described.[28] R-factor transfer in *E. coli* occurred under anaerobic conditions, simulating those prevailing in the mammalian gastrointestinal tract.[29] Transfer of R-factor in the human gut from an ingested donor strain to resident organisms was detected, but only when the host was treated with therapeutic doses of a relevant antibiotic.[30] The authors confirmed the identity of the plasmid in the donor and recipient strains by DNA/DNA hybridization.[31] The transfer of a plasmid carrying a β-lactamase marker from *Klebsiella aerogenes* to *Pseudomonas aeruginosa* occurred in the burns of a patient.[32]

Infections caused by obligate anaerobes - An intense effort of late to develop anaerobic methodology for use in the clinical laboratory resulted in numerous publications on the collection[33-35] and transportation[33-36] of specimens, the culture[35-40] and identification[40,41] of organisms, and the determination of their antibiotic susceptibility profile.[42-47] Several lab-

oratory manuals[48-50] appeared on this subject and part of the proceedings of the Second International Symposium of Microecology[51] was devoted to the discussion of anaerobic techniques.

Two drugs were tested against infections with anaerobes. Clindamycin 2-phosphate, recently approved for parenteral use, was highly effective against various infections caused by Bacteroides, such as septicemia, peritonitis and abscesses, including a brain abscess.[52-54] During acute meningitis, the drug reached bactericidal concentrations in the CSF.[54] Metronidazole was bactericidal for all 77 strains of Bacteroides fragilis against which it was tested, at concentrations obtainable in the serum following normal therapeutic doses.[55,56] Initial clinical experience with this drug in treating deep-seated anaerobic infections showed promise.[57]

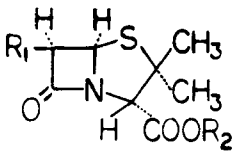
β-Lactams - The effect of FL 1060 (1a), a 6β-amidinopenicillanic acid, on E. coli was intensely investigated. The morphological changes elicited by this compound differ from the classical penicillin-induced response.[58,59] The three enzymes sensitive to penicillins, i.e., peptidoglycan transpeptidase, D-alanine carboxypeptidase, and endopeptidase, were not inhibited at bactericidal concentrations of FL 1060[60,61], but the formation of peptidoglycans covalently bound to the cell wall or to lipoproteins was impaired.[60] A number of pivaloyloxymethyl 6-N'-cyanoamidinopenicillanates (1b) were prepared.[62] The antibacterial activity of the corresponding free acids, obtained by enzymatic hydrolysis of the esters, resembled that of structurally analogous penicillins.

A carbon analogue of penicillin V (1c) was resistant to Bacillus cereus β-lactamase and moderately active against gram-positive organisms.[63] Several 2-acetoxymethyl penicillins (2a,b)[64] and 2-spirocyclopropyl cephalosporins (3)[65] were prepared. Various chemical procedures were developed for the 7(6)α-methoxylation of β-lactam antibiotics.[66-72] Conversion of penicillins to cephalosporins was achieved by new synthetic routes.[73-76] Some [2,3]-fused tricyclic cephem derivatives (4a-c) were synthesized.[77,78] Compound 4c displayed significant antibiotic activity.[78] Racemic cephalothin, 7-methoxycephalothin, and cefoxitin were obtained by total synthesis of a novel type.[79-81] A nuclear analogue of a 7-methylcephalosporin (5), prepared by total synthesis, lacked antibiotic activity.[82]

The asymmetric incorporation of valine into penicillins N and V and cephalosporin C was demonstrated by incubating the producing cultures with 3-chiral valine.[83,84] The 2α-methyl of the penicillins and the cephalosporin 3-methylene derive from the same methyl group of the amino acid.

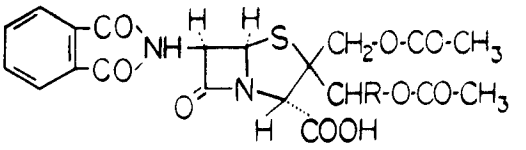
The activity of selected cephalosporins against Mycobacterium tuberculosis H37Rv was correlated with their structure.[85] Two other studies relating activity to structure were undertaken, one on 7(R)-mandelamidocephalosporanic acids[86], the other on α-sulfopenicillins.[87]

Amoxicillin was effective and well tolerated in a variety of infections: chronic respiratory diseases caused by Haemophilus influenzae in children[88]; urinary tract infections due to gram-negative bacilli or S.



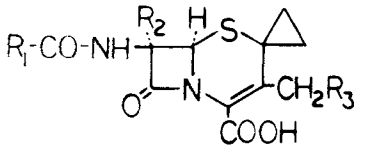
1

	R_1	R_2
a	$(CH_2)_6N\text{-}CH{=}N\text{-}$	-H
b	$NC\text{-}N{=}CR\text{-}NH\text{-}$	$-CH_2\text{-}O\text{-}CO\text{-}C(CH_3)_3$
c	$C_6H_5\text{-}O\text{-}CH_2\text{-}CO\text{-}CH_2\text{-}$	-H



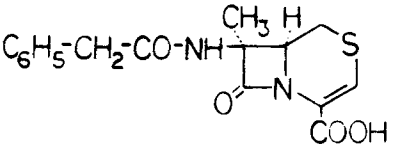
2

	R
a	-H
b	$-O\text{-}CO\text{-}CH_3$

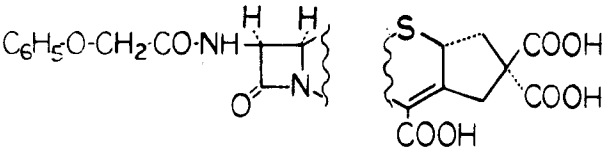


R_2 = -H or $-OCH_3$

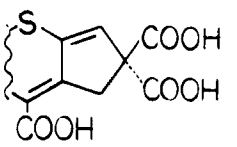
3



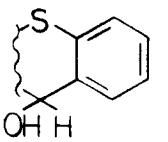
5



4a



4b



4c

faecalis[89,90]; uncomplicated gonorrhoea (when administered in combination with probenecid).[91] The serum concentrations of ticarcillin after parenteral administration to patients suffering from neoplastic diseases were comparable to those obtained with carbenicillin.[92] However, the serum inhibitory activity against P. aeruginosa in vitro was greater with ticarcillin. Given by iv infusion, ticarcillin was effective in 18 of 20 patients with Pseudomonas infections, even in the presence of severe granulocytopenia.[93]

Cefamandole was more active in vitro than cephalothin, cephaloridine or cephalexin against Haemophilus and gram-negative bacilli. Moreover, its spectrum covered many organisms resistant to the other cephalosporins, such as strains of Enterobacter and indole-positive Proteus species.[94] Cefoxitin is highly resistant to degradation by most β-lactamases from gram-negative organisms.[95,96] In human volunteers the mean serum concentrations of cefoxitin reached peak values of 10.9 and 22.5 μg/ml after im administration of 0.5 and 1 g, respectively.[96] About 90% of the administered dose was recovered in the urine. Cefazolin was approved for use in the U.S. Recent reports confirm the high efficacy and tolerance of this compound in the treatment of a variety of severe infections, including staphylococcal and bacillary endocarditis.[97-99]

Aminoglycosides - Amikacin (BB-K 8), when compared to other aminoglycosides, shows a broad spectrum of activity[100] that includes a number of strains of E. coli, Klebsiella, Enterobacter, Pseudomonas and Serratia resistant to tobramycin, gentamicin and kanamycin.[101] It may present no advantage in vitro over gentamicin.[102] However, in a study involving amikacin, gentamicin, tobramycin, sisomicin and butirosin, determination of an inhibitory index (ratio between mean peak serum levels and concentration required to inhibit 2/3 of each species) showed that the index for amikacin was the largest (with the exception of P. aeruginosa, where all agents were equally effective).[103] Spectinomycin was the least active when compared to these aminoglycosides[104] and its major value may be in the treatment of gonorrhoea in patients allergic to penicillin.[105] Pharmacokinetic studies on amikacin and tobramycin were reported.[106-108]

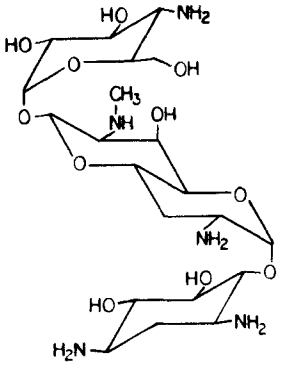
Mixtures of a penicillin with tobramycin or gentamicin were synergistic in vitro against enterococci[109,110] and a spectrum of gram-negative clinical isolates.[111] A similar combination therapy with a penicillin or cephalosporin and these aminoglycosides has been reported efficacious in gram-negative infections of cancer patients.[112] However, there were two reports of acute renal failure associated with combined gentamicin-cephalothin therapy in patients with normal renal function.[113,114]

The chemical procedures for the synthesis of 4"-deoxygentamicin C[115], configurational and positional isomers of amikacin[116], butirosin B[117], 5"-amino-5"-deoxybutirosin[118], 3',4'-dideoxybutirosin B[119], 3'-deoxykanamycin B (tobramycin)[120], 6'-amino-6'-deoxylividomycin B, 6'-deoxy-6'-methylamino-lividomycin B and 6'-deoxy-6'-(2-hydroxyethylamino)lividomycin B[121] have been described. 1-N-(L-γ-Amino-α-hydroxybutyryl) derivatives of kanamycin B, 3',4'-dideoxykanamycin B[122], lividomycin A[123] and 3',4'-dideoxyneamine[124] were also synthesized. The total syntheses of dihydrostreptomycin[125] and of kasugamycin[126], the latter through the methyl N,N'-diacetyl-α-d-kasugaminide, were described. Degradation of butirosin yielded 1-N-aminohydroxybutyrylneamine, a compound having antimicrobial activity. Substitution of the aminohydroxybutyric acid in butirosin with other acyl groups produced a new series of active compounds.[127]

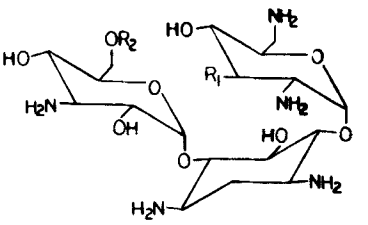
New active aminoglycosides were produced biosynthetically from mutant cultures of Bacillus circulans (butirosin)[128] and Streptomyces ribosidificus

(ribostamycin).[129] These mutants could form antibiotic only when 2-deoxystreptamine (2-DOS) was added exogenously. Substitution of the 2-DOS with streptamine and streptidine in the former culture and with 1-N-methyldeoxystreptamine, streptamine and 2-epi-streptamine in the latter culture yielded new antibiotics. A similar mutant of Streptomyces kanamyceticus (kanamycin) yielded two new kanamycin analogues by the addition of 1-N-methyldeoxystreptamine and 2-epi-streptamine.[129] The successful incorporation of streptidine by the B. circulans mutant and 1-N-methyldeoxystreptamine by S. ribosidificus and S. kanamyceticus is in contrast to the production of hybrimycins, where a rigid requirement for an unsubstituted 1,3-diamino function on the aminocyclitol was reported.[130] The scope of enzymes which inactivate aminoglycosides has been widened with the reports that neomycin-kanamycin phosphotransferase I (from E. coli), which phosphorylates the 3'-hydroxyl group of the kanamycins, also phosphorylates the 5"-hydroxyl group of lividomycin A.[131] An enzyme in P. aeruginosa was found to phosphorylate only the 5"-hydroxyl group of ribostamycin.[132] 3-N-Carboxymethylation of ribostamycin was induced by the producing culture (S. ribosidificus)[133], where the acetic acid is introduced via the methyl group, the first biochemical report of this reaction. Gentamicin acetyltransferase II catalyzes the acetylation of the 2'-amino group of the aminohexose ring of gentamicin, tobramycin, kanamycins and related antibiotics.[134] Another new enzyme, discovered in E. coli resistant to gentamicin, acetylates the 3-amino group of the 2-deoxystreptamine moiety of gentamicin C_1.[135] Antibiotic inactivating enzymes have also been discovered in actinomycete cultures.[136] Two antibiotics related to the butirosins were reported as minor components in that fermentation.[137]

The elucidation of the structures of the members of the nebramycin complex has continued. Factor 2 (now called apramycin) has the unique structure (6)[138], factor 4 (7a) is 6"-O-carbamoyl kanamycin B, factor 5 is kanamycin B (7b), factor 5' is 6"-O-carbamoyl tobramycin (7c) and factor 6 (7d) is tobramycin. Factor 5 and tobramycin are not normally detected in fermentation broths but arise from the acid- or base-catalyzed hydrolysis of their carbamoyl derivatives.[139]



6



7

	R_1	R_2
a	-OH	$-CO-NH_2$
b	-OH	-H
c	-H	$-CO-NH_2$
d	-H	-H

The structure-activity relationships among the aminoglycoside antibiotics, based on bacterial inhibition and misreading of polypeptide synthesis, was discussed.[140]

A thin layer chromatographic assay for the separation and quantitation of the members of the gentamicin complex was detailed.[141] In addition, biological[142,143] and radioimmune assays[144] for the measurement of members of this group were reported. A rapid assay for serum levels of amikacin and all aminoglycosides with a free 6'-amino group using the kanamycin acetyltransferase from an E. coli strain carrying the R-factor for this enzyme was described.[145]

Ansamycins - Rifampicin appears to have immunosuppressive effects in some tubercular patients when used in conventional doses.[146] However, it is still a highly useful drug in the treatment of tuberculosis in combination with ethambutol[147] or streptomycin plus isoniazid[148], but long term treatment of chronic infections such as trachoma with rifampicin as the sole drug appears undesirable because of the emergence of resistant mutants.[149] Rifampicin action was shown to be caused by its tight binding to RNA polymerase[150], but no genetic effects were shown in Drosophila or in human leukocyte chromosomes in vitro.[151] Rifamycin biogenesis probably occurs through a condensation of propionate and acetate units.[152]

Chloramphenicol - Chloramphenicol is considered the drug of choice for treatment of typhoid fever, but recently R-factor mediated resistant strains of Salmonella typhi have appeared in epidemics in Mexico[153], Los Angeles[154] and Viet Nam.[155] In staphylococci, chloramphenicol resistance has been shown to be caused by acetyltransferases of 4 distinct types, each existing as a tetrameric protein with a molecular weight of 80,000 and an identical subunit size of 20,400.[156]

Studies on the biosynthesis of chloramphenicol by Streptomyces venezuelae, based on a carbon-by-carbon degradation of the antibiotic using labeled glucose, were consistent with the shikimate pathway of aromatic biosynthesis.[157] The structure-activity relationships of 37 chloramphenicol derivatives were studied. The trifluoro derivative is 1.7 times more active against E. coli than the parent.[158]

Lincomycin - A detailed pharmacokinetic study of clindamycin 2-phosphate in man was reported.[159] Both lincomycin[160] and clindamycin[161] were shown to cross the placental barrier in pregnant women in therapeutic concentrations. Intrathecal administration of lincomycin was successful in treatment of 86% of patients with meningitis of gram-positive and gram-negative origin.[162] Reports on the successful use of clindamycin 2-phosphate in a variety of infections continue to mount.[163-167] An oral dosage form, clindamycin 2-palmitate, was reported to be well tolerated by man[168] and useful in pediatric practice.[169,170]

2-Deoxylincomycin has the same spectrum as the parent but only 1% of the activity. Its β-anomer and 2-O-methyllincomycin were only marginally active.[171] However, 7-O-methyllincomycin has the same antibacterial spec-

trum as lincomycin and 1.3 to 1.8 times the in vitro activity. It showed equivalence to lincomycin in mouse protection tests against Staphylococcus aureus on subcutaneous injection.[172] A series of lincomycin 2-monoesters[173] and 2,7-di(alkyl carbonate) esters[174] was synthesized. Good antibacterial activity was observed with monoesters of chain lengths from C_4 to C_{16}. All of the 2,7-diesters were inactive in vitro although many were hydrolyzed by the esterase in rodent serum but not by human serum. N-Demethyllincomycin was produced by Streptomyces lincolnensis in fermentations to which sulfonamides were added.[175]

New celesticetins, members of a class of antibiotics related to the lincomycins, have been produced by mutants of Streptomyces caelestis. N-Demethylcelesticetin and N-demethyl-7-O-demethylcelesticetin were less active than celesticetin itself.[176] A procedure for separation by gas chromatography of closely related members of this family as the trimethylsilyl ethers was described.[177]

Macrolides - Chemical modifications of erythromycin A yielded the 8-hydroxy derivative, having one-half the antibacterial activity of the parent compound but 500-600 times greater acid stability.[178] A similar derivative of erythromycin B was also less active.[179] In vitro and in vivo antibacterial activities of 9(S)-erythromycylamine (the C9 amine counterpart of 9(S)-dihydroerythromycin), of a series of aliphatic and aromatic aldehyde condensation products of 9(S)-erythromycylamine, and of 9(R)-erythromycylamine were reported. N-Benzylidene-9(S)-erythromycylamine was absorbed better than erythromycin after oral administration in mice and dogs, but gave lower blood concentrations in human subjects.[180]

Chemical modifications of the 17-membered macrocyclic antibiotic lankacidin group showed that some of the lankacidin C monoesters (particularly the propionate) had little in vitro but good in vivo activity, with wide tissue distribution.[181,182] Maridomycin has also been described[183] and modified in a similar manner, with the 9-propionyl derivative being the most effective for in vivo use.[184] The structures of megalomicins A, B, C_1 and C_2 have been elucidated along with the description of a new dimethylamino sugar, 2,3,6-trideoxy-3-dimethylamino-D-lyxo-hexopyranose, common to all of them.[185-187] The biosynthetic pathway to the platenomycins was studied through the use of blocked mutants and cosynthesis experiments.[188]

Peptide antibiotics - The structures of amphomycin[189] and the enduracidins[190] were elucidated, and the complete chemical synthesis of etamycin was accomplished.[191] A new ring closure method involving diethyl phosphite was reported useful for the synthesis of valinomycin.[192] The 3-hydroxypicolinic acid fraction of this antibiotic was shown to originate biosynthetically from L-lysine.[193] The total cell-free biosynthesis of bacitracin A was accomplished in a reaction mixture containing purified enzyme from Bacillus licheniformis, ATP, Mg^{++}, and 10 amino acids.[194] This synthesis was not affected by blockers of ribosomal protein synthesis.

Edeine D, one of the complex of 4 edeine antibiotics, was shown to contain glycine, 2-hydroxy-3-aminopropionic acid, 2,6-diamino-7-hydroxy-

azelaic acid, 2,6-diamino-Δ^7-azelaic acid, 2,3-diaminopropionic acid, spermidine and β-phenyl-β-alanine, a rare amino acid.[195]

A new spectrophotometric assay more suitable than the microbiological assay for measurement of the gramicidin and tyrocidine components of tyrothricin was described.[196] A gas chromatographic procedure for the assay of colistin was outlined.[197]

Polymyxin given prophylactically by aerosol was found to reduce significantly the incidence of colonization of the upper respiratory tract by nosocomial gram-negative bacteria in post-operative patients and in patients with respiratory failure.[198] Enduracidin, administered im, was effective against urinary tract and dermatological infections, but not against chronic osseus infections, caused by methicillin-resistant S. aureus.[199]

Tetracyclines - Doxycycline was reported effective in treatment of urinary tract infections[200], chronic trachoma[201], typhus[202], and respiratory tract infections.[203] Minocycline, likewise, was useful in the treatment of tetracycline-resistant staphylococcal infections[204,205] and urinary tract infections.[206] It was proposed that minocycline is effective against tetracycline-resistant staphylococci because of its ability to penetrate the cells more readily and to reach inhibiting concentrations at sensitive reaction sites.[207] Increased incidence of tetracycline-resistant pneumococci has been reported.[208] A tetracycline-novobiocin combination proved synergistic in the treatment of melioidosis caused by Pseudomonas pseudomallei.[209] Doxycycline, and presumably other tetracyclines, were shown to interfere with the bactericidal effect of human complement in vivo.[210]

The total syntheses of anhydrochlorotetracycline[211], d,l-7-chloro-6-deoxytetracycline, d,l-7-chloro-6-demethyl-6-deoxytetracycline[212], and d,l-4-amino-7-chloro-2-N-methylcarbamyl-2-decarbamyl-4-dedimethylamino-6-deoxytetracycline[213] were reported. A new fluorometric assay for oxytetracycline in blood and plasma using thiol reagents to prevent decomposition was described.[214]

Miscellaneous - The structures of α- and β-lipomycin[215], oleficin[216], and manumycin[217], polyene antibiotics active against gram-positive organisms, were elucidated. Two pigments with growth inhibitory activity for gram-positive bacteria were isolated: roseoflavin from a Streptomyces[218] and AB-64 from a variant of Actinomadura roseoviolacea.[219] The structure of the former was fully and that of the latter partially determined. Oxamicetin, closely related to amicetin, was isolated from Arthrobacter oxamicetus sp. n. and its chemical and biological properties were described.[220,221] The compound is moderately active against a variety of organisms, including acid-fast bacteria. A study on kinamycins, correlating activity to structure, was done.[222] The total synthesis of nybomycin and deoxynybomycin was accomplished.[223]

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Chapter 12. Antifungal Agents

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Reviews - The changing clinical setting and etiology of fungal endocarditis was reviewed, with particular reference being made to the changing pattern of this disease as seen in narcotic addicts and following cardiac surgery.[1,2] The renal toxicity of antibiotics, including that of amphotericin B, also was reviewed.[3] The therapeutic indications, pharmacology, and mode of action of amphotericin B, nystatin and candicidin were the subject matter of a new text on fundamentals of chemotherapy.[4]

Methods - A standardized procedure for *in vitro* susceptibility testing with 5-fluorocytosine (Ancobon) which permitted both more accurate and more reproducible results was described.[5] Two new experimental *in vivo* models were reported; one dealt with cladosporiosis in mice treated with 5-fluorocytosine[6] and the second with experimental urinary tract infections in rats caused by *Candida albicans*.[7] In the former model, 5-fluorocytosine protected experimentally infected animals against cerebral disease caused by *Cladosporium trichoides* but did not clear the tissues of infection; in the latter model, infections due to *C. albicans* were treated successfully with amphotericin B, and the model appears useful in the study of anticandidal agents.

Improvements were reported in cultural techniques available for the detection of candidemia in man by use of a lysis-filtration procedure.[8] Improved serologic diagnosis of fungal infections was the subject of several reports. A slide latex agglutination test for use in sporotrichosis was described as being specific, sensitive and rapid.[9] Similarly, an immunodiffusion test capable of detecting approximately 80 per cent of proven cases of blastomycosis was reported.[10] Reevaluation of a previously reported medium for identification of dermatophytic fungi[11] revealed that several nondermatophytic fungal pathogens could be misidentified as dermatophytes, thus possibly resulting in the selection of totally inappropriate therapy.[12] A method for coupling antibiotics, including amphotericin B, to cellulose and cellulose carbonate to produce active, insoluble agents was described.[13]

Clinical Experience - Amphotericin B continued to be the drug of choice in treatment of serious or systemic fungal infections. It was reported earlier that while either 2-hydroxystilbamidine or amphotericin B was effective in treatment of noncavitary pulmonary blastomycosis, the latter drug was the more effective agent in treatment of this infection in those cases with cavitary disease or with systemic organ involvement.[14] Amphotericin B also was described as useful in the treatment of canine blastomycosis.[15] A brief comparative study of the pharmacology of amphotericin B failures in treatment of cryptococcal meningitis, histoplasmosis and blastomycosis was reported.[16] The authors of this study attribute the higher failure and relapse rates observed in patients with cryptococcal disease treated with am-

photericin B to the lower ratios of *in vitro* minimal inhibitory concentration (MIC) to serum drug level as compared with similar ratios recorded in the other, more successfully treated infections. Continuous bladder irrigation with amphotericin B was reported to be useful and without toxicity in management of candidal urinary bladder infections.[17] Low nontoxic doses of amphotericin B (10-355 mg given over 4-18 days) were reported as efficacious in treatment of mucocutaneous and systemic *Candida* infections exclusive of meningitis and endocarditis.[18]

There was continued interest in the clinical use of clotrimazole (Bay b 5097), a compound previously described as being broad-spectrum and effective *in vitro*, but without effect orally *in vivo* against systemic fungal pathogens.[19,20] In one study, topical clotrimazole was reported to be well tolerated and superior to a drug-free vehicle in treatment of dermatophytic infections.[21] In contrast, it also was reported as being ineffective and associated with gastrointestinal side effects when given orally in the treatment of mycetoma due to *Madurella mycetomi* and paranasal aspergilloma, although it was said to be effective topically in treatment of candidal paronychia.[22] Other investigations reported late in 1972 described limited but successful results with clotrimazole in treatment of urinary tract infections due to *Candida albicans*[23] and chronic mucocutaneous candidiosis[24] in infants and children.

Miconazole, a β-substituted 1-phenethyl-imidazole (R 14889, 1-{2,4-dichloro-β-[(2,4-dichlorobenzyl)oxy]phenethyl}imidazole) with broad spectrum antifungal activity[25] was evaluated both in normal individuals and clinically. It was reported as being effective topically in treatment of both dermatophytic infections[26] and locally in treatment of vaginal candidiasis.[26,27] Pharmacological studies showed that oral miconazole, 3 g/day, produced no hematologic, biochemical or cardiac abnormalities and was capable both of reducing the fungal flora of sputum and feces and of producing moderate blood levels; it was not absorbed through skin or mucous membranes.[28]

Studies continued with two other topical agents for use in treatment of dermatophytoses. Tolnaftate (Tinactin), as a 1 per cent powder, was found effective in both prevention and treatment of tinea pedis caused by *Candida*, *Trichophyton* and *Epidermophyton*.[29] Haloprogin (Halotex), used topically as either a 1 per cent solution or as a cream, was found to be more effective than a placebo[30] and comparable to tolnaftate[31] in the treatment of experimental dermatophytic infections (*Trichophyton rubrum*) and naturally occurring human infections.

Additional experience was gained both in the clinical use of and pharmacology of 5-fluorocytosine. Oral 5-fluorocytosine was found beneficial in treatment of both meningeal and pulmonary aspergillosis although relapses occurred in some patients with chronic cavitary disease and some resistance to the drug was observed.[32] It was reported to be effective in treatment of urinary tract *Candida* infections and without side effects at dosages of 100 mg/kg/day in children with congenital anatomical abnormali-

ties.[33] It also was reported effective in treatment of pulmonary Candida infections.[34] Pharmacological studies revealed a direct relationship between the status of renal function, as measured by serum creatinine clearance rates, and retention of the drug; guidelines for determination of appropriate dosages in patients with impaired renal function were presented.[35] Contrarily, hepatic function failed to influence 5-fluorocytosine serum levels, either in experimental animals with CCl_4-induced hepatic insufficiency or in a patient with postnecrotic cirrhosis.[36]

Preliminary data were reported on the successful use of combinations of amphotericin B and 5-fluorocytosine in treatment of human cryptococcosis.[37] This mode of therapy, based on the earlier demonstration of a synergistic action between the two drugs in vitro against yeasts,[38] was validated experimentally in animals. In one study in mice infected with Candida albicans, CD_{50} dosages of amphotericin B were reduced in the presence of an otherwise inactive dose of 5-fluorocytosine.[39] An additive effect for the two drugs was observed in mice infected with Cryptococcus neoformans.[40]

New Antifungal Agents - New sources of antifungal agents were investigated. One of these, the passion fruit, Passiflora spp., yielded an active agent following aqueous extraction of cut rind.[41] This agent, with UV absorption max. at 340, 358 and 375 to 377 nm, was active against a variety of fungi including both yeasts and dermatophytes.[42] Antifungal compounds also were detected in cowpea tissues following infection with tobacco necrosis virus[43] and in cuticle of the coffee berry.[44]

Polifungin, a complex of tetraene antibiotics similar to nystatin was isolated from Streptomyces noursei var. polifungini ATCC 21581.[45] This complex was best recovered by paper chromatography at pH 8.2 and was developed with a chloroform-methanol-ammonia solvent system; it appeared to consist of three fractions. Another heptaenic antibiotic, cryptomycin, was isolated from a new species of actinomycete, Actinomyces bulgaricus.[46] This antibiotic, consisting of a complex of 6-7 heptaene antibiotics, was highly active against yeasts and filamentous fungi including C. neoformans. Preliminary data were reported on U-42,126, an antifungal antibiotic produced by fermentation of Str. sviceus.[47] This compound was active in vitro against fungi and in vivo against L1210 leukemia in mice. Actinomyces helvoloviolaceus spp. nov., isolated from a soil sample, was found to produce a polyenic heptaene identical to component A3 of the levarin complex.[48]

Various synthetic antifungal compounds were examined. A series of polyhalophenyl esters of p-sulfamoylcarbanilic acid were tested against yeast and fungi and were found to be active against C. albicans, Penicillium notatum and Aspergillus fumigatus.[49] The most active compounds, in order, were the pentachloro, tribromo and triiodophenyl esters. N-trisubstituted methylimidazoles related to clotrimazole and 2 congeneric compounds with 2-pyrrylmethylidenimino and 2-pyrimidinyl amino groups substituted for the imidzaole nucleus were tested;[50] three compounds were found

active against a variety of pathogenic fungi in vitro and one was active in vivo against C. albicans, A. fumigatus and C. neoformans.

An alkaloid, 6,6'-dihydroxythiobinupharidine isolated from Nupar, was described.[51] It was reported as being active against several of a variety of human fungal pathogens, including Histoplasma capsulatum, Blastomyces dermatitidis, Trichophyton mentagrophytes, Microsporum gypseum, M. canis, C. neoformans and C. albicans.[51] Optimal conditions were reported for fermentation of A9145, a water soluble antifungal containing adenine.[52] Production was stimulated by cottonseed oil, adenine, various nucleosides, and tyrosine. The antifungal properties of 2-alkenoic acids and 2-bromoalkanoic acids were studied.[53] Antifungal activity of these compounds and of analogous alkanoic and 2-fluoroalkanoic acids were affected by chain length, pH and presence or absence of absorbents. The relative antifungal activity of the compounds was: 2-alkenoic acids > 2-fluoroalkanoic acids > 2-bromoalkanoic acids > alkanoic acids.

Biological Studies - One of the more important areas of study with antifungal agents involved the synergistic action of amphotericin B with other antifungal or even antibacterial compounds. This developed from earlier in vitro studies with amphotericin B and 5-fluorocytosine[54] in which combinations of the two drugs were found to be additive or synergistic. Amphtericin B was found to potentiate the antitumor activity of 5-fluorouracil and of chromomycin A3 on RNA synthesis in transformed fibroblastic cells while polymyxin B potentiated bleomycin 2 in its action on both DNA and RNA syntheses.[55] Combinations of amphotericin B and rifampin were found to be fungicidal for yeast-phase cells of H. capsulatum at low concentrations, whereas either drug alone was only fungistatic and only at higher concentrations.[56] RNA synthesis was inhibited by the combination of the two drugs. Combinations of amphotericin B and 5-fluorocytosine were additive or synergistic in vivo in mice infected with C. albicans and C. neoformans.[39,40]

The effect of polyenes of membranes of susceptible and resistant fungi was the subject of several investigations. Mutants of C. albicans resistant to nystatin, amphotericin B and filipin were isolated following exposure to N-methyl-N'-nitro-N-nitrosoguanidine; they apparently were stable and differed from the parent yeasts primarily in morphology[57]. Changes in cell membrane chemistry were studied in these organisms; new sterols were detected in membranes of nystatin-resistant mutants of C. albicans and Saccharomyces cerevisiae, and there also were increases in ergosterol levels [58] in the polyene-resistant mutants. Three compounds, SKF 525-A (β-diethylaminoethyldiphenylpropyl acetate), SKF 3301-A (2,2 diphenyl-1-[β-dimethylaminoethoxyl]-pentane) and SKF 16467-A (N-dimethylaminoethyl-α, α-diphenyl valeramide, HCl) which inhibited cholesterol biosynthesis were inhibitory for both the polyene-resistant mutants and the parent yeasts.[59] Electron micrographic studies of yeast phase cells of H. capsulatum and B. dermatitidis exposed to amphotericin B and hamycin revealed degenerative changes in both plasma membranes and mitochondria followed by plasmolysis within 6 hours of exposure.[60] Mutants of Neurospora crassa with blocks in

the earlier stages of ergosterol synthesis were resistant to various polyene antibiotics.[61] Lyposomes were used as models for the study of the action of polyene antibiotics.[62] It was noted that changes in permeability were related to the composition of membrane phospholipid fatty acid chains, and that the distribution of double bonds in the sterol nucleus determined the selective toxicity of the polyenes for natural sterol-containing membranes. These observations were confirmed in studies on natural membranes.[63] Similar differences in binding affinity of amphotericin B and another polyene, vitamin A, were measured in a study of the enhancement by these compounds of the specific actions of rifampin, fusidic acid and 1,3-bis (2-chloroethyl)-1-nitrosourea on mouse L cells.[64]

Additional data regarding 5-fluorocytosine were provided. In one study, it was noted that premature reading of inhibitory endpoints could be misleading in terms of missed resistant strains; percentages for primary resistance to the drug in several species of pathogenic yeasts also were reported.[65] Cytosine, uridine and adenine were found to be antagonistic for both 5-fluorocytosine and 5-fluorouracil,[66] thus supporting the suggested pathway and mode of action for 5-fluorocytosine; i.e. intracellular deamination of the former with incorporation of the latter in 5-fluorouridylic acid. Data regarding 5-fluorocytosine resistance in C. neoformans suggested the existence of a similar pathway in this latter organism.[67]

Several other studies were concerned with the effect of antibacterial and antifungal agents on nucleic acid synthesis. For example, nalidixic acid was noted to cause a transient inhibition of RNA, DNA and protein synthesis in cells of S. cerevisiae.[68] Lomofungin was found to have a specific inhibitory effect on synthesis of both ribosomal and messenger RNA precursors in protoplasts of Saccharomyces spp.[69] It also was inhibitory for purified Escherichia coli DNA-dependent RNA polymerases.[70]

The sterol binding activity of the polyene filipin was studied.[71] Two forms of the drug were demonstrated; an "active form" which binds with cholesterol and an "inactive form" which does not bind with cholesterol. Inactive filipin was converted to the active form by incubation at 50° for 2 hours. An alcoholic extract of bark of the walnut tree, Juglans regia, was found to contain a fungistatic agent active against many common dermatophytic pathogens.[72] The stabilities of nystatin and amphotericin B in the presence of phosphate buffers were studied and it was found that both drugs were stable at a pH of 5 to 8; it also was noted that nystatin was more active at a lower temperature (30-25°C) while amphotericin B was more active at a higher temperature (41°C).[73] Intravenous solutions of this drug in 5% dextrose were found to be stable for up to 24 hours when exposed to light at 25°C; addition of hydrocortisone or heparin had no effect on stability.[74] 5-Fluorocytosine was found to increase survival in immunosuppressed mice injected with C. albicans.[75]

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Chapter 13. Antiparasitic Agents

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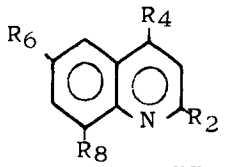
Antiprotozoal Agents - The chemotherapy of protozoan diseases was comprehensively reviewed.[1] The Abstracts of the Ninth International Congress on Tropical Medicine and Malaria[2] contain many articles on protozoan and helminthic diseases. Many aspects of parasite biochemistry and drug action were presented at a recent meeting and have been published.[3]

Antimalarial Drugs. General - The current state of malaria chemotherapy, with special emphasis on drug resistance, was reviewed by the WHO scientific group.[4] This survey contains a summary of the structures of 24 antimalarial drugs from 8 chemical classes in general use, of 16 experimental compounds receiving clinical trials, and of 9 experimental compounds so far used only in animal models. A general review on antimalarial drugs and their actions,[5] acridine antimalarials,[6] and diagnosis and treatment of malaria became available.[7]

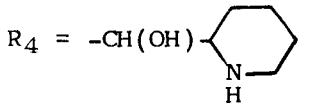
Clinical Data - The phenanthrene methanol WR33063 cured a high percentage of human volunteers infected with several Plasmodium falciparum strains, including chloroquine-resistant ones, without evidence of phototoxicity or other serious side effects, although occasionally a treatment failure occurred, even at the largest doses used in therapy; such failures might be due to drug resistance.[8] In another study, WR33063 did not protect volunteers from chloroquine- and pyrimethamine-resistant P. falciparum infection when given prophylactically.[9] The quinoline methanol WR30090 likewise demonstrated effective antimalarial action, with low toxicity, against multi-drug-resistant P. falciparum and P. vivax in human volunteers.[10] Contrary to expectation, the induced photosensitivity was judged by these investigators to be rare, of minor degree, and easily reversed by drug withdrawal. Prophylactically, this drug provided suppressive cures to 75% of volunteers exposed to P. falciparum, but not to P. vivax.[9] Although these two drugs exemplify a major advance in the cure of P. falciparum infections, they are only suppressive against P. vivax where the drug of choice still remains primaquine.[11] Amodiaquin appears curative against P. falciparum infections resistant to the structurally closely related chloroquine. This surprising observation was supported by new experiments showing no apparent difference in binding of ^{14}C-amodiaquin by erythrocytes of monkeys infected with chloroquine-susceptible and chloroquine-resistant P. falciparum, whereas erythrocytes of chloroquine-resistant infections have a deficiency of ^{14}C-chloroquine binding.[12] The triple combination of chloroquine, sulfamethoxydiazine, and pyrimethamine (ratio 30:10:1) showed a high degree of potentiation of the drug effects upon P. berghei infections in mice.[13]

Drug Modifications - The success of quinoline methanols and phenanthrene methanols in the cure of P. falciparum malaria caused the recent synthesis of many analogs intended not only to increase the activity but also to reduce the phototoxicity. The more active quinoline methanols are the adamantyl derivative I (curative in mice against P. berghei at 100-320 mg/kg),[14] the phenoxy derivative II (curative at 40-80 mg/kg),[15] and the 7,8-benzo derivative III (curative at 100 mg/kg).[16] A low phototoxicity was observed only for I. The bis-trifluoromethyl phenanthrene methanol IV (curative at 40 mg/kg)[17] and the naphthylthiophene V (curative at 100-320 mg/kg)[18] are interesting new versions of this lead. An analysis of 107 phenanthrene methanols for the contribution of polar and partition effects of substituents according to Hansch and of a smaller number according to the Free-Wilson Additivity Scheme was reported.[19] Two highly active pyridine methanols were VI (curative at 20-40 mg/kg)[20] and VII (curative at 20-80 mg/kg).[21] The quinacrine analog VIII (curative at 80 mg/kg) is more active and less toxic than quinacrine itself.[22] DHDS (X), a suspected metabolite of diaminodiphenylsulfone (DDS, IX), had no antimalarial activity but caused marked methemoglobinemia, a side effect also of DDS chemotherapy. The tetraacetyl derivative TAHDS (XI), however, had antimalarial activity (curative at 160 mg/kg) superior to DDS (TAHDS) without causing formation of methemoglobin. It is not known if TAHDS is metabolized to DDS or another active metabolite or is active in its own right, but it is clear that DHDS is not responsible for the antimalarial action of either DDS or TAHDS.[23] The acylaminonitrodiphenylsulfone XII had curative action on the order of DDS.[24] The pyrimidinylaminobenzimidazole XIII was selected from a group of 38 compounds with curative action against P. berghei; it is well tolerated in mice. This benzimidazole was synthesized as one of the hypothetical active metabolites of the antimalarial guanidinopyrimidine (XIV), which is toxic and less active than XIII.[25] The synthetic effort in the coenzyme Q antimetabolite area produced two new quinolinequinones with antimalarial activity: XV (curative at 320 mg/kg)[26] and XVI (prophylactic in chicks against P. gallinaceum at 120 mg/kg).[27] For inhibition of coenzyme Q enzymes by 6-hydroxy-7-alkyl quinolinequinone analogs of CoQ and for antimalarial activity, an alkyl chain length of 15 carbon atoms appears optimal.[28] An optimal range for the energy of the lowest empty molecular orbital as calculated by the Hückel technique as well as an appropriate partition coefficient, was determined for many of the antimalarial naphthoquinones.[29] A reexamination of 295 of these compounds showed that variation of the alkyl substituent according to Topliss' scheme (methyl, isopropyl, cyclopentyl, and cyclohexyl) would lead to some of the most active analogs after synthesis of only these four compounds.[30] A compound related to hetol and twice as active (curative of P. berghei in mice after a single s.c. dose of 320 mg/kg) is XVII.[31]

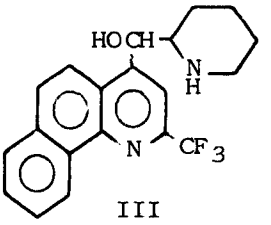


I-II

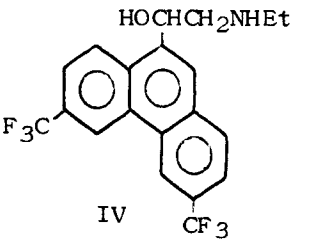
I R_2 = 1-adamantyl,
R_4 = $-CH(OH)CH_2N(N\text{-}Bu)_2$,
R_8 = CF_3
II R_2 = OPhCl-p,



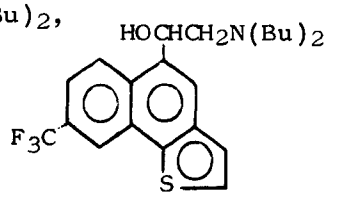
R_6 = R_8 = Cl



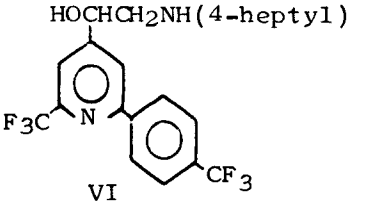
III



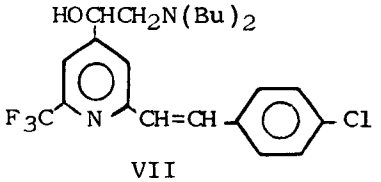
IV



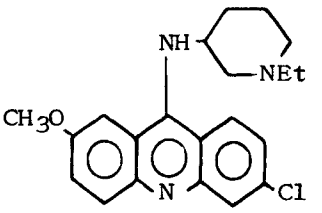
V



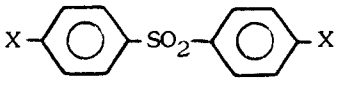
VI



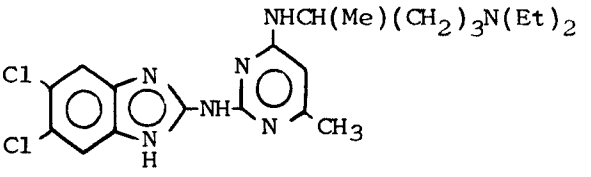
VII



VIII



IX, DDS, X_1=X_2=NH_2
X, DHDS, X_1=X_2=NHOH
XI, TAHDS, X_1=X_2=N(OAc)Ac
XII, X_1=NO_2,
X_2=$NHCOCH_2NH(CH_2)_6Me$



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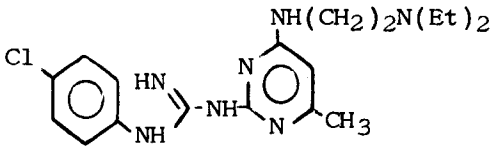
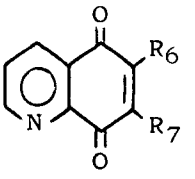
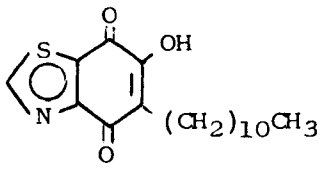


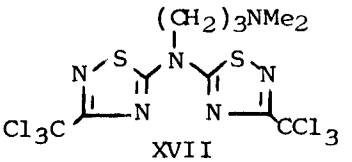
XIV



XV R_6 = OH,
R_7 = $S(CH_2)_{17}CH_3$



XVI



XVII

Coccidiosis - Two very comprehensive and up-to-date reviews of coccidiosis appeared.[32,33] The current commercial coccidiostats were classified into 12 groups according to their mode of action.[34] It

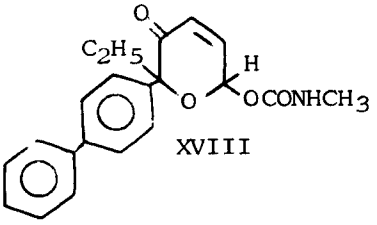
was pointed out that coccidia resistant to one drug of a group are also resistant to the other drugs of the same group and that a switch in medication must be to a different group to overcome this resistance. Tissue cultures of Eimeria tenella have been used to detect anticoccidial activity,[35] mode of drug action,[36] growth factor antagonism,[37] and efficacy of drug combinations,[38] and this exciting new technique was reviewed.[39] The now familiar assay of chicken embryos infected with E. tenella continues to give interesting insights into the activity and mode of action of coccidiostats.[40,41]

The continuing appearance of drug-tolerant coccidia strains and the concomitant problems for prophylactic chemotherapy were reflected in the high activity aimed at discovery of new anticoccidials, as well as in numerous studies of the sensitivity of newly isolated field strains to existing drugs. While meticlorpindol was efficacious against infections by E. tenella strains tolerant to other anticoccidial agents, a meticlorpindol-tolerant strain was not cross-resistant to 14 other coccidiostats.[42] An observation that a meticlorpindol-tolerant strain of E. acervulina showed greater than expected sensitivity to decoquinate[43] led to a synergistic drug combination of a 4-hydroxyquinoline coccidiostat plus meticlorpindol, which was effective at lower levels than those used for either coccidiostat alone. Although both classes of drugs are similar in structure and both inhibit the growth of sporozoites at the same stage of the life cycle, no cross-resistance is present between them and it was concluded that they probably act upon closely related but nonidentical metabolic pathways.[44] An E. tenella strain resistant to Novastat treatment was developed and shown to be cross-resistant to treatment with nihydrazone, nitrofurazone, Trithiadol, Unistat and zoalene, but not to 9 other coccidiostats.[45]

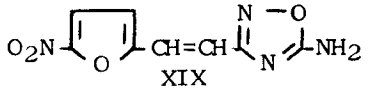
The structure of the ionophore antibiotic A204A was described.[46] The conformation of X537A, a polyether antibiotic, in complexes with metal salts and amines in different solvents was discussed;[47] the generic name lasalocid has been adopted for it, and the optimal dose against mixed Eimeria infections in chickens was determined to be 0.0075% in feed.[48]

The activity of monensin was studied in broilers[49] and calves.[50] Broilers raised on a monensin-medicated diet developed only a low immunity to Eimeria infections.[51] The biosynthesis of monensin was discussed.[52] Robenedine at 30 g/ton of feed protected chickens from coccidiosis caused by 8 Eimeria species.[53] A tetrahydro analog of robenedine is claimed in the patent literature for use of control of malaria and coccidiosis.[54] Residue and metabolism of robenedine in rats was studied.[55] It was shown that buquinolate does not interfere with the host's development of immunity against coccidial species,[56] although such interference could have been

expected because of the drug's mode of action upon the sporozoites in the very early part of the life cycle.[34] 3,5-Dinitrobenzamides substituted in the 2-position with an amino or ethoxy group appeared to be active in experimental chicken coccidiosis.[57] The 6-hydroxy-2H-pyran-3(6H)-one derivative XVIII was active against E. tenella infections in chicks at 0.05% in the feed.[58] The recent patent literature mentions 4-nitro-5-cyanoimidazole derivatives further substituted at the 1- and 2-positions,[59] cinnamylideneaminobenzylideneaminoguanidines,[60] 6-aminopurines and their N^1-oxides with further substitution at the 9-position,[61] aminoethylcarbazole,[62] indazolylphenylureas,[63] 3-amino-2,4-diarylthiophene-1,1-dioxides,[64] and a new antibiotic, salinomycin ($C_{39}H_{65}O_{10}Na$) as potential coccidiostats.[65]



XVIII



XIX

Trypanosomiasis - A review series on animal trypanosomiasis is being published.[66] A monograph on Chagas's disease[67] contains a description of the disease, its diagnosis, and the authors' extensive experience with nifurtimox therapy of acute and chronic cases in the clinic. With a 90- to 120-day treatment using nifurtimox, a high cure rate of Chagas's disease could be obtained. The 2-nitroimidazole derivative Ro-7-1051 showed high efficacy in laboratory studies against T. cruzi.[68] The nitrovinylfuran derivative SQ 18,506 (XIX) had curative action in mice infected with T. brucei and T. rhodesiense at a dosage of 200 mg/kg every 12 hours for 3 days.[69] Some tetracyclic diamidines were synthesized but did not approach the high trypanocidal activity of diminazene.[70]

Amoebiasis - Inhibition of Entamoeba histolytica protein synthesis was correlated with the amoebicidal activity of a number of alkaloids and antibiotics.[71]

Toxoplasmosis - This subject was reviewed.[72,73] The cat was implicated as the definitive host of Toxoplasma gondii with small mammals and birds as essential intermediate hosts.[74] The therapeutic effect of several sulfa drugs and DDS, alone and in combination with pyrimethamine or trimethoprim, in T. gondii-infected mice was reported. The effect can be reversed by p-aminobenzoic acid, and trimethoprim alone has no activity.[75] T. gondii infections in mice have been cured by clindamycin and N-demethyl-4'-pentyl clindamycin[76] and by sulfameter plus pyrimethamine or spiramycin.[77]

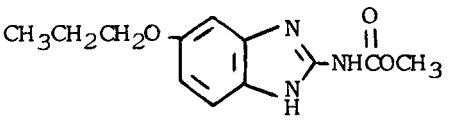
Antiprotozoal Nitrofurans and Nitroimidazoles - Ipronidazole at 0.00625% in feed had antihistomonal activity equivalent to that of dimetridazole and ronidazole at their recommended levels and was more effective than carbazone, nitarsone or 2-acetylamino-5-nitrothiazole in preventing mortality in turkeys.[78] 2-(4-Carbostyryl)-5-nitro-1-vinylimidazole, a urinary metabolite of 2-(4-methylstyryl)-5-nitro-1-vinylimidazole, was curative in calves against T. vivax

upon multiple dosing; this compound and several carboxylic acid derivatives also showed activity against T. vaginalis, E. histolytica, T. rhodesiense, T. cruzi, T. gambiense and T. congolense.[79]

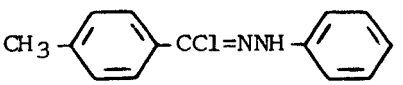
Antiprotozoal Antibiotics - Antibiotics with antiprotozoal activity that have been mentioned in the recent literature are G-418[80] (Entamoeba), dactylarin[81] (Leishmania and Entamoeba), and viomycin[82] (Trichomonas).

Drugs Active Against Nematodes, General - Three major reviews of this subject appeared.[83-85] Methodological contributions were made to the screening of anthelmintics[86,87] and to their evaluation in ruminants.[88,89]

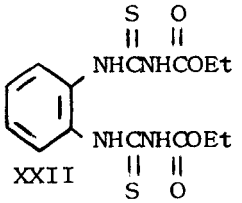
The role played by fumarate reductase inhibition in the mode of action of anthelmintics was further studied. Enzyme isolated from thiabendazole-sensitive Haemonchus was inhibited by thiabendazole, cambendazole, levamisole, morantel tartrate and disophenol, but not by mebendazole. Enzyme from a thiabendazole-resistant strain was similarly affected, except that the inhibitory effect of cambendazole was reduced and that of thiabendazole was not demonstrable.[90] In lambs, after 4 generations of Haemonchus strain BPL-2 had been exposed successively to single doses of cambendazole at 5, 5, 10 and 20 mg/kg, this parasite was less susceptible to cambendazole than it had been initially.[91] Studies on the effect of mebendazole on Trichinella larvae in vitro or in rats suggested that the mechanism of anthelmintic action is similar to that observed in certain other helminths, i.e., mebendazole reduced the parasite's uptake of exogenous glucose thus apparently depleting the endogenous energy resource (glycogen).[92] Studies with Ascaris also showed a primary inhibition of glucose uptake or transport, with secondary reduction in the uptake of other nutrients.[93] The physiological distribution of dichlorvos, when administered to pigs in polyvinyl chloride pellets, was studied. Radioisotope measurements indicated that certain components of the polyvinyl chloride were incorporated into the tissues, but no dichlorvos or its metabolites were found.[94] Dinitrophenol inhibited the uptake of arginine by Ascaris in vitro.[95]



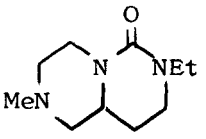
XX



XXI



XXII



XXIII

The structure-activity relationships of piperazine derivatives were studied by measuring their relative potentiation of the acetylcholine response in nerve-muscle preparations from Ascaris.

The results suggested that diethylcarbamazine inhibits cholinesterase.[96] Bephenium hydroxynaphthoate gave variable and often unsatisfactory results against Necator in man, even though a high dosage was used. It was suggested that the local strain of Necator may be partially resistant to the drug.[97] Diphetarsone (a pentavalent arsenical) was reported to be highly efficacious and well tolerated when used for the treatment of Trichuris infections in man.[98]

New Drugs - The latest of the benzimidazole anthelmintics, oxibendazole (XX), was reported to have a broad spectrum of activity and a good margin of safety. The drug is insoluble in water and only slightly soluble in most organic solvents. In sheep, single oxibendazole doses of 5-10 mg/kg were highly effective against immature and adult worms of the genera Haemonchus, Ostertagia, Trichostrongylus, Strongyloides, Nematodirus, Cooperia, Bunostomum, Oesophagostomum and Chabertia. In cattle, doses of 5-20 mg/kg were 80-100% effective in removing worms of the genera Haemonchus, Ostertagia, Trichostrongylus, Cooperia and Oesophagostomum. In swine exposed to Ascaris infection, the feeding of diets containing 0.05-0.10% oxibendazole provided protection against clinical signs of disease. Radioisotope studies in sheep showed that at 53 mg/kg, the peak plasma concentration was reached in 6 hours. Approximately 34% of the drug was recovered in the urine within 24 hours after dosing, and an additional 6% was recovered within 216 hours. In toxicologic studies, sheep tolerated a dose of 300 mg/kg; and a dose of 30 mg/kg, given at various intervals during gestation in sheep or rats, did not adversely affect fetal development.[99]

A new anthelmintic, p-toluoyl chloride phenylhydrazone (XXI), at 40 mg/kg p.o. was at least 97% effective against Haemonchus, Ostertagia, Trichostrongylus, Cooperia and Oesophagostomum in sheep. It was 87% effective against Nematodirus and inactive against Trichuris.[100]

Thiophanate (XXII), diethyl 4,4'-o-phenylene-bis-(3-thioallophanate), at 50 mg/kg, was at least 97% effective against Haemonchus, Ostertagia and Trichostrongylus in sheep and cattle. At 100 mg/kg it was highly active against Nematodirus and Cooperia, but variable against Dictyocaulus in sheep.[101] Synergistic action against Dirofilaria was claimed for a combination of 3,3'-[[[4-[(4,6-diamino-1,3,5-triazin-2-yl)amino]phenyl]arsinidine]bis(thio)]bis-D-valine (Fl51) and p-[5-[5-(4-methyl-1-piperazinyl)-2-benzimidazolyl]-2-benzimidazolyl)-phenol (HOE33258).[102] In rodents infected with Litomosoides, 3-ethyl-8-methyl-1,3,8-triazabicyclo[4,4,0]decan-2-one (XXIII) caused a transient reduction in microfilaremia but did not kill the adult worms.[103]

Drugs Active Against Trematodes - Major reviews of anti-trematode drugs appeared.[85,104] A new Technical Report from W.H.O. briefly reviewed the anti-schistosome drugs that are currently in use[105] (a much more comprehensive account of these drugs appeared in

1972).[106]

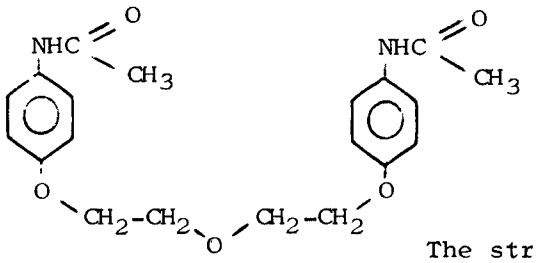
A method for screening compounds for fasciolicidal activity was described and was used to study the efficacy of many known fasciolicides.[107] A way of automatically measuring the movement of Schistosoma in response to drugs in vitro was reported and was proposed as a method for screening compounds for anti-schistosome activity.[108] Transfer of immature flukes from the parenchyma to the bile ducts of goats gave results which suggested that the drug susceptibility of Fasciola is more dependent on degree of maturation than on location.[109] The effect of bithionol on the glycolytic and oxidative metabolism of Paragonimus was studied.[110]

In a series of novel salicylanilides, fasciolicidal action required electron-withdrawing substituents on both the salicylic acid and aniline rings, together with a lipophilic group such as tert-butyl in the 3-position. However, not all such compounds were fasciolicidal; compounds with a plasma half-life of less than 36 hours in sheep were inactive against fluke; those with a half-life of 48-84 hours were active; and those with a half-life of 5-6 days were very active. The last group included rafoxanide and several di-substituted 3-tert-butyl-5-nitro-6-methylsalicylanilides.[111]

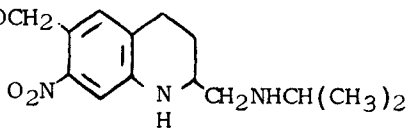
Fasciolicidal action was also reported for another series of salicylanilides in which an electronegative substituent was introduced in the 3-position. The choice of substituents in the aniline ring was restricted to halogens, methyl groups and tri-halogenated methyls (which improve lipid solubility). The most effective compounds were 3-nitro-2,6-dihydroxybenzoic acid anilides.[112]

The inhibition of succinate dehydrogenase from liver flukes in vitro by 25 substituted 2,6-dihydroxybenzanilides has been correlated with the Hansch parameter π, suggesting that the distribution of the compound between lipid and aqueous phase and not electronic or steric factors within the series is responsible for the activity.[113,114]

The evolution of diamphenethide (XXIV) from 4-tert-butoxyacetanilide was recounted. Efficacy and safety of diamphenethide appear to depend on deacylation (probably to the diamino analog) by liver deacylases. Diamphenethide is not active against liver flukes in vitro unless incubated in the presence of enzymatically functional liver cells. Further, the diamino analog is rapidly effective in vitro in the absence of such cells. It was suggested that the production of this metabolite in the liver parenchyma, and its rapid destruction in the liver and dilution in the bloodstream, account for the high activity observed against immature, tissue-dwelling flukes and the low activity against mature, duct-dwelling flukes.[115] The unique relationship between efficacy of diamphenethide and age of F. hepatica in sheep was described and related to pathogenesis and control measures.[116]



XXIV



XXV

The structure-activity relationships of fasciolicidal thiobisphenol derivatives were described.[117]

Drugs Active Against Schistosomes - The level of serotonin in Schistosoma mansoni was increased when hycanthone (or a benzothiopyranoindazole) was given to the host mouse.[118] Hycanthone caused morphological transformation of virus-infected rat cells in culture[119] and caused mutations in Salmonella.[120] However, no mutations were observed in thousands of offspring of male mice treated with a high dosage of hycanthone.[121] Successful clinical use of hycanthone continued.[122] The effect of the drug on liver enzymes of infected and noninfected hosts was studied.[123]

Oxamniquine (XXV) was highly active against S. mansoni in man and was well tolerated except for pain at the site of injection.[124]

The antischistosome activity of nitrofuran compounds was further investigated. Comparison of 3-(5-nitro-2-furyl)-substituted derivatives of propionic, acrylic and propiolic acids showed that a vinyl group is necessary for activity.[125] In derivatives of 3-(5-nitro-2-furyl) acrylic acid, the 5-nitro group was required for activity.[126] In a group of (5-nitro-2-furyl)-vinyl heterocycles containing a weakly basic N in a specific position, both the vinyl group and the N were essential for antischistosome activity.[127]

Tubericidin (a purine nucleoside) was tested against Schistosoma in monkeys. Erythrocytes, withdrawn from the monkeys, were allowed to absorb the drug and were then returned to the bloodstream of the donors. This procedure yielded efficacy against female schistosomes but not against males. Tubericidin is active against both sexes in vitro, and the differential in vivo is thought to be due to the greater ingestion of erythrocytes by females.[128]

In vitro, metrifonate (trichlorfon) was slightly more effective in inhibiting the cholinesterase of S. mansoni than of S. haematobium, whereas in man, the drug is more effective against the latter species.[129] Arylvinylsulfones were active against S. mansoni in laboratory animals and monkeys. The most active compound (p-tert-butylvinylsulfone) caused dermatitis.[130]

Drugs Active Against Cestodes, General - Major reviews of this

subject appeared.[83,85,104] At high dosage, cambendazole was highly active against Thysanosoma in sheep.[131] Taenia or Echinococcus eggs incubated in vitro in bunamidine hydrochloride solutions lost their infectivity for laboratory animals.[132] A study of the effect of drugs on the metacestodes of Echinococcus in vitro revealed that many were active, especially halogenated salicylanilides, halogenated bisphenols, bunamidine, and cyanine dyes.[133] In rodents, oxyclozanide at 4 mg/kg was effective against 13-day-old Hymenolepis microstoma.[134] Paromomycin was apparently effective against Diphyllobothrium in man.[135]

New Drugs - Axenomycins, a new group of macrolide antibiotics, were reported active against tapeworms in mice,dogs and sheep. The antibiotics were isolated from Streptomyces lisandri in three fractions one of which (axenomycin D) was more active than the others.[136] A series of 2,6-dihydroxybenzoic acid anilides related to resorantel, were highly active against tapeworms when halogens or methyl groups were substituted in the anilide ring. The compounds apparently disrupt the energy metabolism of the tapeworm by inhibiting glucolysis and decreasing the level of ATP.[112] p-Toluoyl chloride phenylhydrazone at 50 mg/kg was 100% effective against Moniezia in sheep.[100] Of 23 dithiodibenzanilides tested against Hymenolepis in vitro or in mice, none was as active as niclosamide.[137]

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Chapter 14. Antiviral Agents

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Introduction - This year, as in the past, reviews of antiviral chemotherapy have appeared in many forms, especially for those interested in clinical applicability.[1-3] More extensive discussions of selected areas of interest[4] and a broad overview of the types of viral agents involving the respiratory tract[5] have also appeared. Emphasis will be given to the current status of ascorbic acid as a clinically available therapeutic substance in relation to viral diseases. The clinical status of rifamycin SV derivatives, pyrimidine nucleosides, and interferon will also be commented upon. An exciting new class of bis-basic-substituted polycyclic aromatic compounds was the subject of additional evaluation during the past year.[6]

Ascorbic Acid - Following Dr. Pauling's initial anecdotal support for the efficacy of vitamin C against colds,[7] others have drawn conflicting conclusions.[8-12] The controversy has precipitated additional clinical study. An apparent dearth of respiratory tract infections during the scorbutic state has also been reported,[13,14] but investigations of the direct antiviral effects of ascorbic acid have been few. No direct viral inhibitory activity has been demonstrated *in vitro* against rhinoviruses, polio type 1, ECHO 11, Coxsackie A 21, influenza B, parainfluenza 3, respiratory syncytial virus, adenovirus 5, or herpes simplex virus.[15]

In the scorbutic state, bacterial phagocytosis is also impaired, and there is increased susceptibility to the lethal effects of endotoxin challenge.[16] It has also been noted that 0.01 M ascorbic acid inhibits the H_2O_2-myeloperoxidase-halide reaction of neutrophils.[17] This mechanism, presumably, is responsible for the intraleukocytic killing of bacteria, yet in the model systems studied there was no reduction (or augmentation) of leukocyte bactericidal activity even though the H_2O_2-myeloperoxidase-halide system was inhibited.

Tissue, leukocyte, and plasma levels of ascorbic acid are quite variable and tend to fall in a "stress" situation (even myocardial infarction), with a corresponding fall in urinary excretion of ascorbic acid in the unsupplemented state.[18-24] The amount of vitamin C within leukocytes on the 2nd or 3rd day after the onset of a cold is often at scorbutic levels,[19] although the mechanisms of ascorbic acid uptake by leukocytes are not imparied by the stress of infection.[23]

Supplementary ascorbic acid given to adults results in transient increases in plasma levels and increased excretion in the urine, with a degree of augmentation relating to dose, but not uniformly, in these substrates. Both leukocyte ascorbic acid (LAA) and serum ascorbic acid (SAA) are significantly elevated over unsupplemented levels at 1 g/day, rising proportionately to maximum uptakes, at about 6 g/day. It has been

observed that while suffering from a cold, those receiving supplemental vitamin C in doses of 1 g or greater do not exhibit the expected decline in LAA to scorbutic levels on the 2nd day.[19] The optimal dose for maintaining the LAA above scorbutic levels has not yet been clearly established. Several studies of the clinical efficacy of ascorbic acid have been reported.[15,21-23,25-33] Three clearly failed to reveal any prophylactic benefit of 3 g/day in outpatient-acquired colds[26] or against induced influenza B and rhinoviruses[15] and induced rhinovirus 44 infection[25] in adult human volunteers. Some authors[31,32] were unimpressed with the differences in the incidence and severity of the colds in earlier studies, but this interpretation has been debated.[7] On the other hand, a study by Ritzel[33] and more recent outpatient trials in school children in Dublin,[21-23] on a Navajo Indian reservation,[28] among university staff and students in Glasgow,[27] and among adults in Toronto[29] revealed that vitamin C significantly reduces the number of episodes of respiratory tract infections (and other ailments!) and that the catarrhal manifestations of a cold are more susceptible to amelioration than the toxic manifestations. Significantly less time was lost from school or employment among those receiving supplemental ascorbic acid. It is of interest that at the 200 mg per day dose, school girls were significantly benefited but not school boys. In fact, some manifestations were more severe among the latter,[23] suggesting sex-related differences in response to, and perhaps in requirements for, vitamin C.

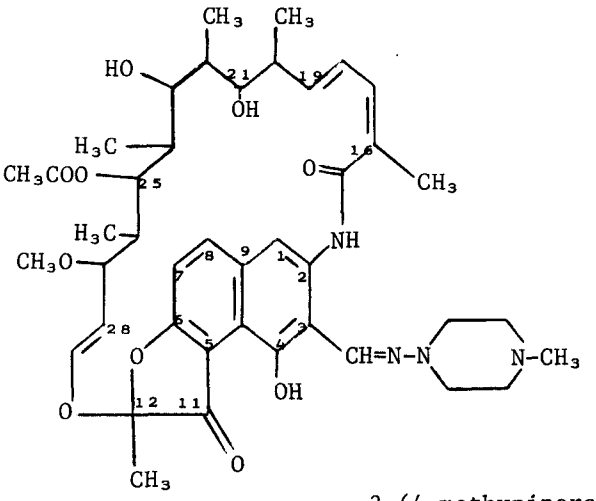
There has been no demonstrable effect of ascorbic acid (at 3 g/day) on the magnitude of viral multiplication or shedding from the nose, the rate of development or magnitude of local or circulating antibody, or the degree of infection-induced reduction in the flow of the nasal mucociliary blanket.[25,34] Interferon production appears to be neither enhanced nor suppressed by the presence of additional ascorbic acid or by the scorbutic state.

Overall, there does appear to be some benefit associated with supplemental ascorbic acid in preventing colds, but the mechanism and dosage requirements require further elucidation.

Rifamycin Antibiotics - Derived from the fermentation of Streptomyces mediterranei, this family of ansa-macrolide drugs possesses a very broad spectrum of antimicrobial activity against gram-positive and gram-negative bacteria, mycobacteria, chlamydia, and several viruses. The activity of rifampin, the only clinical candidate of this group, has recently been reviewed by Lester.[35] Its efficacy is presumably due to the inhibition of bacterial derived DNA-dependent RNA polymerase and the inhibition of viral RNA-dependent DNA polymerase (reverse transcriptase).

Viral-directed RNA synthesis by pox viruses is affected by high concentrations of rifampin (100 μg/ml); viral-directed DNA synthesis is partially affected, and late viral protein synthesis is blocked. The target seems to be the late synthesized particulate RNA polymerase that is to be incorporated into the intact virus. Riva and Silvestri[36] review the noteworthy inhibition of reverse transcriptase activity of rifampin in DNA vi-

ruses and RNA tumor viruses. Rifampin (the 3-iminomethyl derivative of rifamycin SV) is inactive against the reverse transcriptase of murine sarcoma virus (MSV), but if the 4-side chain on the piperazine moiety of rifampin (see accompanying structure) is lengthened (i.e., from methyl to octyl) it becomes an effective inhibitor. Lengthening of the 4-side chain by adding aromatic substituents may also yield derivatives which inhibit MSV polymerase activity.



3-(4-methypiperazinyliminomethyl)-rifamycin SV (rifampin)

The 3-hydrazonomethyl derivatives of rifamycin SV with bulky side chains (cycloheptyl and cyclooctyl groups) are excellent inhibitors of MSV polymerase. In 3-formyl rifamycin SV oxime derivatives, good correlation is seen between the length of the alkyl-o-substituent and RNA polymerase inhibitory activity. Rifamycin SV-3-substituted derivatives other than 3-iminomethyl, 3-formylhydrazo, or 3-formyl oxime, are either inactive or only minimally active against RNA polymerases. Very low concentrations of some of these derivatives may inhibit reverse transcriptase activity, probably by combining with the enzyme directly rather than by affecting the template. These compounds do not materially affect DNA-dependent DNA polymerases.

Yang, et al.[37] reported inhibitory activity of some rifamycin SV derivatives against normal and leukemic human lymphocyte-derived and murine (Rauscher) leukemia virus-derived DNA polymerases. The 3-piperazinoiminomethyl derivatives and 3-(2,4-dinitrophenyl hydrazonomethyl)rifamycin SV were more inhibitory against viral polymerase than against normal lymphocyte-derived DNA polymerase, and also preferentially inhibited the leukemic lymphocyte-derived DNA polymerase, but not to the same extent as the viral polymerase. This suggests a possible antiviral or antileukemic activity for these preparations.

Tischler, et al.[38] concluded that large and hydrophobic substituents at the 3-position are associated with high potency in studies of reverse

transcriptase (inhibition by rifamycin derivatives).

Gurgo, et al.[39] reviewed the structural relationships of 180 rifamycin SV and rifamycin B derivatives against MSV RNA-dependent DNA polymerase and also concluded that suitable modifications of rifamycin SV derivatives in the 3-position can inhibit virus-derived RNA-dependent DNA polymerase at low concentrations (5-20 ug/ml). The ansa ring structure is necessary for effective inhibition of both bacterial DNA-dependent RNA polymerase and MSV RNA-dependent DNA polymerase activity. The bacterial DNA-dependent RNA polymerase may be inhibited by rifamycin SV derivatives without a 3-side chain, but for successful inhibition of the MSV RNA-dependent DNA polymerase a large 3-substituent is essential. Compounds such as streptovaricin and topolymycin, which contain no 3-substituents but have the ansa ring, inhibit the bacterial RNA polymerase. Streptovaricins A, C, and D do not appreciably inhibit the MSV polymerase or do so only with weak activity. Geldanomycin, an ansomycin without a 3-substituent, does not inhibit MSV polymerase at 100 ug/ml. Further study of the antiviral and antineoplastic properties of these derivatives is desirable.[40]

Interferon and Interferon Inducers - Interferon and interferon inducers have been recently reviewed.[41-43] The effectiveness of local secretory interferon in induced infection appears to be limited and probably unrelated to its antiviral activity.[44] The local application of interferon to the nasal mucous membranes provided protection against subsequent challenge with a rhinovirus and parainfluenza I virus in monkeys[45] and against rhinovirus 4 challenge in man.[46] Large doses and a prolonged course of post-challenge applications were necessary to achieve this effect. Topical use of interferon inducers in HSV keratitis has been reviewed.[47] Non-interferon antigenic impurities in virus-stimulated cell-derived interferon preparations have been observed,[48] but their significance is uncertain. Induced interferon may not be the sole factor involved in the augmented host response to viral infection associated with polynucleotide administration.[49] The improved efficacy of interferon inducers plus specific gamma globulin therapy in HSV keratitis and vaccinia infections (even in the immune-suppressed host[50,51]), and of interferon inducers plus vaccines[52], has been reported.

The antiviral activity of polynucleotide interferon inducers is dependent upon many factors.[53] In Poly I:C the more stringent continuity and base pairing requirements of the poly I strand limit significant modifications to the poly C strand. The synthesis of guanidine or uridine "loops" in the poly C strand may be associated with more rapid hydrolysis, lessened toxicity, and preservation of the antiviral interferon-inducing activity.[54]

A series of bis-basic substituted aromatic compounds having direct antiviral and interferon-inducing effectiveness has been described.[6,42] The bisalkamine esters of fluorenone, fluorenol and fluorene had diminishing antiviral potency, respectively. Bis(β-dibutylaminopropyl)-9-oxofluorene-2,7-dicarboxylate dihydrochloride prolonged the survival of mice

against EMC, vaccinia, and Semliki Forest virus infections. Structure-activity relationships have been elucidated and the first member of this class of drugs--tilorone--has been extensively studied.

Another low molecular weight interferon inducer, BL-20803, has also been reported to be successful in inhibiting vaccinial infection.[55]

Pyrimidine Nucleosides - An improved animal model for the study of idoxuridine (IUDR) and the efficacy of topical antiviral therapy in the immune host has been described.[56] Topical application of Poly I:C enhances the efficacy of IUDR therapy for HSV keratitis and delays the emergence of resistant virus.[57]

Trifluorothymidine (F3T) has been found to be less toxic to corneal epithelium, in animals and man than are IUDR and cytosine arabinoside (Ara-C). The *in vitro* and *in vivo* spectrum of anti-DNA virus activity and the marked solubility in aqueous solution represent desirable properties. In a clinical trial comparing F3T and IUDR in HSV keratitis in man,[58] there was more rapid healing in the F3T group than in the IUDR group, with a 7.5% versus a 39% failure rate, respectively.

The potential for visceral abnormalities in the newborn animal, especially involving the retina, makes the use of systemic IUDR a serious undertaking for the therapy of congenital HSV or CMV infection.[59] The value of IUDR therapy in systemic HSV infection is still controversial, and a new approach to its use has been outlined[60] to minimize its toxic side effects. A controlled clinical trial is still required to establish efficacy in HSV encephalitis. Study in animal models[61,62] has emphasized the need for defined protocols of drug administration.

In an assessment of the *in vitro* susceptibility of HSV to antiviral drugs, utilizing a plaque reduction assay,[63] a rabbit kidney cell culture system produced lower MIC's than did a hamster kidney cell system. Of the two types of HSV tested (types I and II, a total of 21 strains), both exhibited an *in vitro* spectrum for susceptibility to IUDR, which may account for some of the differences previously reported. HSV-I was most consistently inhibited by IUDR and Ara-C, while Ara-C was most effective against HSV-II. Adenine arabinoside (Ara-A) and hypoxanthine-arabinoside were less effective against both HSV types.

2'-Deoxyuridine (UDR) enhances the activity of 5-fluorouracil (FU) and 5-fluoro-2'-deoxyuridine (FUDR) against L-1210 leukemia and adenocarcinoma-755 in mice. The resultant stabilization of the FUDR formed *in vivo* from FU in the presence of added UDR[64] might provide an interesting approach to augmenting the antiviral efficacy of IUDR as well.

Ara-C - Ara-C has been utilized in the therapy of MSV, CMV, and varicella-zoster (VZ) infections with inconclusive clinical results.[65-71] The only controlled clinical trial reported failed to reveal any benefit from the use of Ara-C in disseminated zoster and suggested that the disease may be prolonged by its use.[72] Ara-C may adversely affect the developing nervous

system,[73] and its use in congenital CMV and HSV infection should be controlled in view of the uncertain outcome of both the disease and the treatment.[74] HSV inhibition is apparent *in vivo* in rats, even with delayed therapy, but high doses appear less effective than lower ones,[75] perhaps because of increased immunosuppression, as in the case of disseminated zoster. The report of a remission associated with Ara-C therapy[76] in a case of progressive multifocal leukoencephalopathy (PML) is extremely exciting. PML may be caused by a papova virus, and thus further trials are indicated.

The frequently observed temporary inhibition of viral multiplication and shedding during Ara-C therapy, as well as kinetic studies *in vitro*,[77] suggest that Ara-C does not inhibit the development of already existing viral genomes into mature virions and that cellular damage may occur before the stage of viral DNA synthesis that Ara-C inhibits.

Structural modification of Ara-C may render it more resistant to deamination *in vivo*. The use of a poorly soluble prodrug, such as 1-β-arabinofuranosylcytosine-5'-adamantoate, or other 5'-acylates, results in a prolongation of active blood levels.[78] Cyclocytidine yields Ara-C by *in vivo* hydrolysis and is less toxic and more effective against the L-1210 mouse leukemia than is Ara-C. Substitution at C-6 of the pyrimidine ring improves resistance to deamination *in vitro*, but competing degradation reactions *in vivo* result in no increased stability.

Cyclic Ara-CMP synthesis has been described.[79] *In vitro* antiviral activity against HSV I and II, vaccinia and myxoviruses was comparable to that of Ara-C. *In vivo*, HSV I keratitis responded well to topical therapy, and intracerebral injection resulted in inhibition of HSV and vaccinia viruses. The increased cell penetration of this compound should make its further study of interest.

Adenine Arabinoside (Ara-A) - This compound has been reviewed.[80] The *in vivo* inhibitory effect against DNA viruses appears to be dose related, with equivalent sensitivity of HSV I and II.[81] The lack of noteworthy toxicity associated with Ara-A has led to controlled clinical trials in HSV infections in man.[82] Marked decrease in viral shedding and earlier healing of orofacial HSV I was noted. Less effect was noted on genital HSV II lesions. Encouraging results were reported in neonatal HSV infection, especially when treated early. The virustatic effect of Ara-A on CMV infections in man[83] and the equivalent efficacy to IUDR in the topical therapy of HSV keratitis[84] have been reported.

Modifications of Ara-A suggest that changes in the carbohydrate moiety at C-3 may eliminate the antiviral activity.[85] The water-soluble N^6-(dimethylamino)methylene derivative of Ara-A was found to protect against HSV and vaccinia, probably by conversion to Ara-A *in vivo*.[86] A series of 5'-esters of Ara-A showed *in vitro* efficacy of the 5'-palmitate; no activity against RNA viruses was noted.[87].

A number of 5-butylpyrimidine nucleosides revealed only limited ac-

tivity at high concentrations.[88] Carboxamide-ribonucleosides revealed in vitro activity against HSV I, rhinovirus 13 and parainfluenza 3 and in vivo activity against influenza A_2.[89,90]

Virazole, a β-D-ribofuranosyl derivative, has demonstrated a broad spectrum of in vitro inhibitory activity against adenovirus, HSV I and II, vaccinia, myxoma virus, parainfluenza, rhinovirus, Coxsackie virus and influenza A and B.[91] It is more potent than other anti-influenzal compounds in vitro[92] and is effective against HSV keratitis and localized vaccinia, Friend leukemia virus, influenza A and parainfluenza I in vivo. The antiviral effect may be due to the inhibition of guanosine-5'-phosphate synthesis in the infected cell.[93]

Benzimidazole Derivatives - The activity of hydroxy benzyl benzimidazole (HBB) against picornaviruses has been a stimulus to continued study of its derivatives. 2-(alpha-Amino benzyl)benzimidazole (ABB) and 1-phenyl ABB were significantly effective in reducing cytopathic effect of ECHO-12 virus at nontoxic concentrations.[94] Alkylation or acetylation of the amino function resulted in loss of antiviral activity. The presence of a 5- or 1-methyl group in the N-methylamino series increased the activity against ECHO-12. The addition of a phenyl substituent in the 1-position of the alpha-methoxy and alpha-amino derivatives of HBB also increased the activity against the ECHO viruses.

The activity of a series of 1-alkyl derivatives of HBB was dependent upon the length of the side chain, being maximal at C-3 or C-4.[95] Unsaturation in this side chain did not greatly influence activity.

Bisbenzimidazoles have demonstrable activity against rhinoviruses[96] and arenaviruses.[97] Heterocyclic analogues of HBB have not shown specific activity against wild strains of polio virus.[98]

Adamantine Derivatives - These compounds continue to be of interest. Adamantine spiro compounds have displayed a broad range of in vitro activity against myxoviruses, paramyxoviruses, and some picornaviruses. Of a series of N-substituted adamantine spiro compounds, N-methyladamantane-spiro-3'-pyrrolidine had the broadest spectrum of activity.[99] Six- and seven-membered ring analogues were found to be more cytotoxic.[100] Clinical trials[101] revealed prophylactic efficacy against influenza A/HK 68 but not against rhinoviruses 2 and 9.

Aziridines and annulene were compared to 1-amino adamantine and found to possess less in vitro anti-influenzal activity.[102] Further clinical trials with cyclo-octylamine[103] revealed prophylactic efficacy associated with topical use in volunteers challenged with A_2 influenza.

Isoquinolines - These compounds have shown in vitro activity against rhinoviruses, but have had little or no significant prophylactic efficacy in human trials.[104,105]

Isoprinosine - This drug has demonstrated a variable spectrum of activity

against several DNA and RNA viruses *in vitro* and *in vivo*.[106,107] A recent clinical trial suggests efficacy in the early treatment of primary herpes progenitalis infections in man.[108]

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Chapter 15. Antineoplastic Agents

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Introduction - A review of the progress made during 1973, with respect to the development of new antineoplastic agents, clearly reflects the profound difficulties encountered in the development of such drugs. Little concrete information is, generally, available concerning biochemical differences between tumor and normal tissues which could be exploited for selective therapy. Thus, with the exception of some of the hormones, the currently used antitumor agents are essentially inhibitors of cell growth, normal as well as tumor, and it is for this reason that some host toxicity is an almost invariable component of tumor chemotherapy. However, subtle biochemical differences between tumor and normal tissues are, in some instance, sufficient to allow for selective chemotherapeutic intervention, and remarkable progress has been made in the treatment of various neoplasms[1].

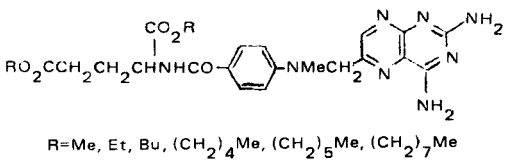
The question facing the medicinal chemist is not whether new antitumor agents should be developed-on that point the cancer chemotherapists are in nearly unanimous agreement - the question is what type of agents carry the greatest promise for success. Here the opinions diverge, depending on one's chemical interests, the type of tumor one wishes to treat, and the analogies one draws from past clinical or experimental experience gained with a given class of compounds. For, the development of antineoplastic agents falls, at best, under the heading of rational empiricism. Thus, the systematic introduction of small structural changes into metabolite molecules led to such useful antimetabolites as methotrexate, 6-mercaptopurine, 5-fluorouracil and 1-β-D-arabinofuranosyl cytosine. The routine screen for antibiotics provided such valuable agents as actinomycin D and daunomycin, and serendipity paired with perspicacity led to the use of alkylating agents such as the nitrogen mustards, mitotic inhibitors such as vincristine, and hormones including the corticosteroids and sex hormones. All further cancer drug development has relied, essentially, on these approaches. When one type of compound is found active, innumerable structural modifications are then carried out, aimed at decreasing toxicity and improving selectivity. New insights concerning novel structures with antitumor activity are encountered only infrequently. The antineoplastic agents reported during 1973 follow this pattern, and in reporting on some of the agents, an attempt is made to correlate structure with antitumor activity. From such correlations, rational advances in cancer chemotherapy may, eventually, emerge.

Vitamin Analogs - The folate analog methotrexate is of great value in the treat-

ment of acute leukemia, choriocarcinoma and some other solid tumors.

Its utility is limited by its pronounced toxicity. In some instances this toxicity can be ameliorated by leucovorin "rescue", [2,3] denoting the administration of folinic acid to a patient treated at some prior interval with high doses of methotrexate.

The observation that esterification of nucleoside analogs can improve their therapeutic properties, has now been applied to methotrexate as well [4,5]. Esterification of the carboxyl groups of the glutamoyl moiety of methotrexate with primary or secondary alcohols of various chain lengths (C_1 - C_8) provided derivatives (I) which were, however, only as active as the parent compound in increasing the life span of mice with both intracerebrally and i.p. implanted leukemia L-1210. As early as 15 minutes after i.p. administration, 80-90% of the inhibitory activity in the serum was due to free methotrexate. It is of interest, that these lipophilic esters did not apparently enhance the transport of methotrexate through the blood-brain barrier, as evidenced by the lack of increased activity against the intracerebrally implanted tumor. Of course, the observed rapid hydrolysis of the esters may have abolished the potential therapeutic advantage. The increased transport of methotrexate through the barrier is desired, to achieve concentrations high enough to be effective against leukemic cells sequestered in the brain.



I

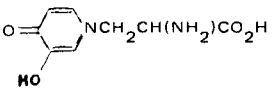
Among newly synthesized active inhibitors of dihydrofolate reductase is the water-soluble 1-[3-chloro-4-(m-dimethylcarbamoylbenzyloxy)phenyl]-4,6-diamino-1,2-dihydro-2,2-dimethyl-s-triazine ethanesulfonate[6], which is markedly effective against Walker ascites carcinosarcoma 256 in vivo. In a series of 2,4-diamino-6,7-bis(aralkyl)pteridines, the 3,4-dimethylbenzyl derivative was the most potent inhibitor of the growth of S. faecium in vitro, but was inactive against leukemia L-1210 or P 1534 in the mouse[7]. Proceeding on the assumption that a decrease in the electron density at position 10 (N^{10}) of folate may affect its one carbon transfer function, the synthesis of neohomofolic acid (N-[p - [[2-amino-3,4-dihydro-4-oxo-6-pteridinyl) methyl] amino]phenylacetyl] glutamic acid was carried out [8]. Although toxic to animals, the compound showed little inhibitory activity towards dihydrofolate reductase, and was inactive against leukemia L-1210 in mice. Correlations of the structure of various 2,4-diaminopyrimidine derivatives with their activity as dihydrofolate reductase or tumor inhibitors suggest that the extent of activity is related to the steric bulk and the hydrophobicity of the 5- substituent [9,10]. With an adamantyl group at position 5, the introduction of an alkyl chain (C_1 - C_3) at position 6 also increased activity[11].

Among a series of 6-halogen-substituted vitamin B_6 (pyridoxol, pyridoxal and

pyridoxamine) analogs, only the fluoro derivatives inhibited the in vitro growth of two tumor cell lines in the presence of low pyridoxal concentrations, but they were less active than 4-deoxypyridoxine[12]. In contrast, 4-deformyl-4-vinylpyridoxal was shown, in vivo, to be a more potent vitamin B_6 antagonist than is 4-deoxypyridoxine[13].

Amino acid analogs do not, currently, play a significant part in cancer chemotherapy. The therapeutic use of DON (diazo-oxo-norleucine) and azaserine has essentially been abandoned. However, because of the clinical utility of L-asparaginase[14], the preparation of analogs which interfere with the synthesis or utilization of asparagine appeared indicated. Among such agents, L-2-amino-5-bromo-4-oxopentanoic acid and its 5-chloro-analog inhibited the synthesis of L-asparagine by P 815 Y tumor cells, as well as the growth of asparagine dependent L-5178 Y lymphoblasts in culture [15].

A vivid example of the value of perspicacity in drug development is provided by the evaluation of mimosine (II) as an antitumor agent. Because sheep eating the browse tree, Leucaena glauca, shed their wool and experience weight loss and reversible infertility - a pattern of toxicity seen with several of the currently used antitumor drugs -the active principle, mimosine, was considered a potential antineoplastic agent [16]. When provided in the drinking water (0.4-0.8 g / 100 ml), a dose dependent reduction in the growth of Walker 256 carcinosarcoma and a concomitant prolongation of life-span was achieved. Similarly, by taking into account that prolactin is essential for the development of spontaneous and carcinogen-induced mammary tumors in rats [17], the lowering of prolactin levels by L-dopa [3-(3,4-dihydroxyphenyl-L-alanine] was envisioned to decrease tumor growth, which was indeed achieved in rats bearing carcinogen-induced mammary carcinomas[18]. Screening for new agents has furnished (αS, 5S)-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid (III) from Streptomyces sviceus, which has significant activity against leukemia L-1210 in vivo, with minimal toxicity [19]. Of interest to the researcher using tissue cultures is the observation that pyrrolidone carboxylic acid, formed spontaneously upon incubation of glutamine, at 37°, in phosphate or bicarbonate medium, inhibited the growth of a line of hamster fibrosarcoma requiring glutamine for growth[20].



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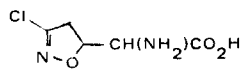
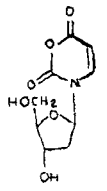


III

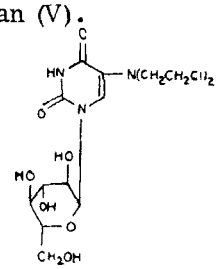
The utility of pyrimidine and purine analogs in cancer chemotherapy (e.g. 6-mercaptopurine, 5-fluorouracil, arabinosyl cytosine) serves as a continued stimulus for the preparation of new compounds of this type. Among these, 3-deazauridine [1-(β-D-ribofuranosyl)-4-hydroxy-2-pyridone] was markedly active against leukemia L-1210 in vivo [21]. On the other hand, the 5-fluoro derivative of this nucleoside was inactive [22]. Another pyrimidine nucleoside modified at the 3-position, 3-(2-deoxy-β-D-erythro-pentofuranosyl)-2,3-dihydro-1,3-6H-

oxazine-2,6-dione (IV) was found moderately active against leukemia L-1210 cells in vitro[23], whereas 1,2-dihydro-1-(2-deoxy-β-D- ribofuranosyl)-2-oxopyrazine-4-oxide was inactive against two in vitro tumor cell lines, but was a highly potent inhibitor (50% at 4 x 10^{-11} M) of bacterial cells[24]. This fact is mentioned because bacterial infections are a major cause of mortality of cancer patients[25,26], and the identification of a potentially useful antibacterial agent is acceptable compensation for the lack of antitumor activity. This finding also indicates that the evaluation of agents designed for potential antitumor action should not necessarily cease, once they are found to lack this potential.



IV

Among nucleosides isolated from natural sources or synthesized during previous years, pyrazomycin [3 (5)-ribofuranosyl-4-hydroxypyrazole-5 (3) - carboxamide] has demonstrated pronounced activity against various experimental tumors in vivo [27]. Also reported active[28] is α- (but not β) glucopyranosylnordopan (V).



V

New potential anticancer agents are also found among the purine analogs. Several 1-deazapurines (imidazo [4,5-b]pyridine derivatives) were found active against leukemia L-1210 in vitro[29]; the most effective of these, ethyl 7- {[bis (diphenyl) methyl] amino } -3H-imidazo [4,5-b]pyridine-5-carbamate provided a 59% increase in life span at a single dose of 40 mg/kg (toxic at 200 mg/kg). 8-Aza-inosine [30] prolonged the life span of the tumor bearing mice by 55%. Among a large series of 6-ureidopurines[31], phenylpropylureidopurine and phenylbutylureidopurine showed some inhibition of a cell line derived from human leukemia, and some unsaturated 6-methylthiopurine nucleosides (e.g. 9-(2,3-dideoxy-β-D- glycero-pent-2-enopyranosyl)-6-methylthiopurine were markedly cytotoxic to HeLa cells[32] Replacement of the furanose ring with cyclopentane furnished various carbocyclic analogs of purines. The 8-azaadenosine analog [(±)-trans -3-(7-amino-3H- ν - triazolo-[4,5-d]pyrimidin-3-yl)-trans -5-(hydroxymethyl)cis-1,2-cyclopentanediol) was effective against leukemia P 388 but not against L-1210[33]. The cyclopentyl analog of 8-azaguanosine and various carbocyclic analogs of 6-substituted purine ribonucleosides were similarly inactive against L-1210 , although some were cytotoxic to human epidermoid carcinoma # 2 cells[34,35]. Of interest is the observation that formaldehyde: RNA (5:1 and 10: 1 molar ratios) or formaldehyde: polyadenylic acid (20:1 molar ratio) significantly prolonged the survival of mice bearing leukemia P-388[36].

Cyclic Nucleotides . Reports on the inhibition of in vitro and in vivo tumor cell growth by cyclic AMP or its dibutyryl derivative, and loss of tumorigenicity of certain cell lines treated with these agents have accumulated extensively during recent years. To be added to this list are observations on the inhibition of the in vitro growth of human rhabdomyosarcoma[37],B16 melanoma[38],and murine

P 815 Y cells[39] by N^6, $O^{2'}$-dibutyryl cyclic AMP. The in vivo growth of two hormone-dependent mammary tumors was also arrested by this cyclic nucleotide ester[40], and when administered, in vivo, together with aminophylline, cyclic AMP decreased the growth of Ehrlich carcinoma significantly[41]. The apparent ability of cyclic AMP to suppress tumorigenicity was demonstrated with human cancer cells (KB) which, after growth in a medium containing this cyclic nucleotide, lost the ability to form solid tumors in hamster cheek pouches[42].

Carbohydrates. Relatively few pentose or hexose derivatives have been prepared as potential antitumor agents during the reporting period. In a series of deoxyfluoro-D-glucopyranoses, only 6-deoxy-6-fluoro-D-glucose inhibited (up to 90%) R-1 lymphoma growth in vivo[43]. Polysaccharides, on the other hand, are receiving wider attention. These polymers appear to stimulate the reticuloendothelial tissues, thereby activating the immune defenses of the host. For instance, mannan from Saccharomyces cerevisae injected into mice 1-2 weeks prior to sarcoma 180, prevented the growth of these tumors[44]. Similarly, zymosan, from yeast cell walls, enhanced the survival of rats bearing a benzpyrene induced tumor[45]. But stimulation of the RES may not be the only parameter of polysaccharide action, since the chitin derivative (1→ 4)-2-amino-2-deoxy-β-D-glucopyranuronan showed marked in vitro growth inhibition of leukemia L-1210 cells[46].

Lipids, too, have not fared prominently as potential antitumor agents, probably due, in part, to the difficulties involved in their isolation and characterization. But such materials do possess potential antitumor activity, as shown by the observation that certain lipid fractions from group A streptococci[47] and from trypanosomes (cruzi and lewisi)[48] exert an inhibitory effect on the growth of Ehrlich ascites and sarcoma 180 cells in mice. Indications are that it is the free fatty acids and monoglyceride components of these fractions which account for the observed activity. A glycopeptide fraction, isolated from bovine liver, was also reported markedly effective against the Ehrlich ascites tumor in vivo[49].

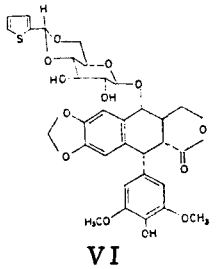
Hormone derivatives with reported antitumor activity include 2 α, 3 α-epithio-5 α-androstan-17 β - ol[50], which suppressed the growth of estrogen-dependent mammary tumors in rats, and enhanced the growth of androgen-dependent mammary carcinoma in mice. Similarly, in a large series of estrone and estradiol analogs prepared, some compounds such as dl-8-iso-D-homoestrone or dl-8-iso-D-homo-3 α, 17 β-estradiol displayed significant activity against mammary tumors of rat and mouse[51]. Androgen derivatives have also been prepared, and 9-α-fluoro-11 β-hydroxybenzo [d,e] testosterone 17-acetate was reported markedly inhibitory to 7, 12 dimethylbenzanthracene induced mammary tumors in rats[52].

Enzymes. The clinical utility of L-asparaginase has acted as a stimulus for the evaluation of other enzymes for possible antitumor activity. A phenylalanine ammonia-lyase from yeast rapidly decreased the plasma phenylalanine and tyrosine

levels in mice, and significantly increased the life span of mice with lymphoblastic leukemia[53]. Similarly, a L-methioninase from Clostridia effectively decreased the plasma methionine level of rats, and inhibited the growth of Walker carcinosarcoma 256[54]. A bacterial L-leucine dehydrogenase and a glutaminase increased the survival time of mice with Ehrlich ascites carcinoma by 200-300%[55], and a pumpkin ascorbate oxidase interfered with the growth of Sarcoma-180 in mice[56]. Modification of E. coli L-asparaginase with maleic anhydride, ethyl acetimidate or methyl β-dimethylaminopropionimidate gave enzyme derivatives with altered isoelectric points, but not significantly changed catalytic activity. The biological half-life increased with increasing isoelectric points, and this relationship was associated with increased therapeutic effectiveness in leukemic mice [57].

Diphtheria toxin , a protein secreted by lysogenic strains of Corynebacterium diphtheriae , has been shown to be an effective inhibitor of the growth of Ehrlich carcinoma, both ascitic and solid, in mice [58]. The effect is thought to be mediated primarily through inhibition of protein synthesis. Similarly, the parasporal crystal (δ -endotoxin) of B. thuringiansis has been reported to exert potent anti-Yoshida ascites carcinoma activity, with a high therapeutic index[59]. Shigella endotoxin, too, caused the regression of some established s.c. or intradermal tumors in mice, when administered systemically or directly into the tumor[60].

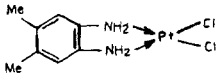
Materials from plant and animal tissues . Although empirical in approach, the isolation and evaluation of materials from natural sources has been extraordinarily successful in the development of antimicrobial agents, and has contributed significantly towards obtaining agents of great value in cancer chemotherapy (e.g. the antibiotics vincristine, actinomycin D, daunomycin and others). Thus, this source of antineoplastic agents deserves closest attention, since it also reveals information about chemical structures with antitumor activity. In this very restricted review it is obviously impossible to cite all the reports of natural products with antitumor action which appeared during the past year, and only a few examples are provided. Thus, extracts from Western Samoan plants (Barringtonia samoensis),[61] from lichens (Cetraria sp.)[62], from the evening primrose Oenothera caespitosa ,[63] from Steganotaenia araliacea (which yielded two lignan lactones[64], and from leaves of Pterocarpus indicus and vidalianus [65] had antitumor activity in vitro and/or in vivo . The glycosidic lignan derivative 4'-demethylepipodophyllotoxin 9-(4,6-O-2-thenylidine-β-D-glucopyranoside[66] (XI), and its ethylidine analog ,[67] proved highly effective against leukemia L-1210 in mice, and appear to be of value in clinical therapy[68]. Of various derivatives of daunomycin examined,[69] only 13-dihydrodaunomycin-HCl showed significant antitumor activity in vivo. Similarly, actinomycin D lactam,[1',1'-bis(L-threo-α, β-diaminobutyric acid] actinomycin D, had a lower therapeutic index against Ridgeway osteogenic sarcoma then had



VI

the parent compound[70]. Of interest is the observation that ergot alkaloids are effective in suppressing the growth of pituitary tumors in rats, relieving thereby the adrenal hypertrophy caused by tumor ACTH[71]. Tissue extracts with reported antitumor activity include an ethanolic fraction from the tentacles of Retetere-bella queenslandia[72], a tropical sea annelid used by early Hawaiians for cancer therapy[73], and thymic extracts from calf and horse embryos[74].

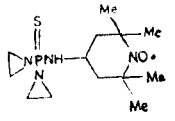
Platinum compounds and other metal derivatives: The promising antitumor activity of cis-dichloro-diammineplatinum (II)[75,76] has stimulated the search for related compounds with improved therapeutic properties. A comparison of a variety of Pt (II) complexes as active inhibitors of sarcoma 180 in mice showed[77,78] that, in general, only neutral complexes exhibited activity, whereas charged species were inactive. Cis - reactive ligands appear essential for antitumor activity, the trans -isomers being relatively inactive. Among newly prepared derivatives, dichloro (4,5-dimethyl-o-phenylenediamine-N,N') platinum (VII) increased the survival time of L-1210 bearing mice by 74%[79]. Of potential interest is the report[80] that sodium selenite, at 1 mg/kg i.p., retarded the growth of various experimental tumors in rats and mice. Also of interest to the medicinal chemist may be a discussion on the potential utility of boron[81] and silicon[82] compounds in cancer therapy.



VII

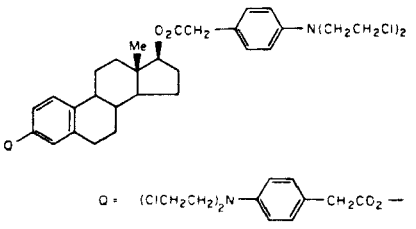
Thiosemicarbazones. Because of the pronounced antitumor activity of various α-(N)-heterocyclic carboxaldehyde thiosemicarbazones, some 4'-diethyleneoxy derivatives were prepared, in an effort to improve the therapeutic index. Among these, 4-hydroxyisoquinoline-1-carboxaldehyde 4'-diethyleneoxythiosemicarbazone and 5-hydroxypyridine-2-carboxaldehyde 4'-diethyleneoxythiosemicarbazone were less toxic but also less active than the corresponding unsubstituted thiosemicarbazones against sarcoma 180 in mice[83]. Similarly, the N^4-phenyl- and allyl thiosemicarbazone derivatives of 6-formylpurine were less active than the parent compound, or were inactive against 6-mercaptopurine resistant leukemia L-1210 in mice[84].

Alkylating Agents are continuing to receive attention as antineoplastic agents. In view of the favorable therapeutic indices of some aromatic carbamate mustards in the Walker 256 system, several new compounds were prepared, wherein the O-phenyl N- phenylcarbamate moiety was linked to bis (1-aziridinyl)- and bis (2,2-dimethyl-1-aziridinyl)phosphinate. No improvement of the chemotherapeutic index was achieved by this modification[85]. On the other hand, replacement of one ethyleneimine group in thio-TEPA with a methyl substituted oxypiperidine moiety (VIII), furnished a compound which reportedly[86] was less toxic, and exerted a greater antitumor effect than equivalent doses of thio-TEPA. Steroidal alkylating

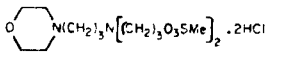


VIII

agents, such as "estradiol mustard"[87] (IX), and some homoaza-steroid mustards[88] showed significant inhibition of the growth of various experimental tumors. A comparison of the effects of "estradiol mustard" with those of its estrogen and alkylating moieties on a transplanted mammary carcinoma in the rat showed that the mustard produced a considerably smaller antitumor effect and toxicity than did an equimolar amount of its two constituents; however its uterotropic action was less profound[87]. A number of naphthoquinone derivatives with side chains potentially capable of alkylation after reduction, as for instance 2-chloromethyl-1,4-naphthoquinone[88], significantly increased the life span of mice bearing adenocarcinoma 755 and Sarcoma 180[89]. Similarly, some azobenzene derivatives containing an alkylating group chemically unreactive because of the deactivating influence of the azo linkage, were converted by liver azoreductase to toxic metabolites, suggesting their potentially selective use against liver tumors[90]. Polymers as carriers of alkylating agents have also been prepared, and poly (4-vinylpyridine) containing 10-40% sarcolysine was reported to have a similar or greater effect, as compared to free sarcolysine, against various experimental tumors[91]. Various compounds containing the methanesulfonate moiety have shown activity against a number of experimental tumor systems[92]. New members of this class of compounds include methanesulfonic acid esters of amino glycols, among which 3,3'-(morpholinopropylimino)di-1-propanol dimethanesulfonate (X) and the corresponding dibutylaminopropylimino ester were reported active against various experimental tumors[93]. Also effective were a p-toluenesulfonate derivative of N, N-bis (3-methylsulfonyloxypropyl)amine[94], and some bifunctional alkylating agents containing methanesulfonate as one component[95]. The methanesulfonic acid esters of aminoglycols have reportedly demonstrated encouraging results in the treatment of human malignant lymphoma[96], with minimal toxicity being encountered.

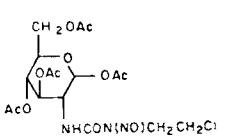


IX



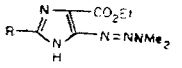
X

Nitrosoureas such as 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) have shown significant activity in a wide spectrum of animal and human neoplasms[97-99]. Of particular value is their ability to penetrate the blood-brain barrier. These compounds appear to act by alkylation of nucleic acids[100] and/or by cyclohexylcarbamoylation of the lysine residues of proteins[101]. 3-(Tetraacetyl glucopyranos-2-yl)-1-(2-chloroethyl)-1-nitrosourea (GCNU) (XI), which is structurally related to streptozotocin, is markedly active against leukemia L-1210, without the marked bone marrow toxicity exhibited by BCNU or CCNU[102]. Marked inhibition of L-1210 growth has also been reported for 1,1'-ethylenebis (1-nitrosourea) and for the hexamethylene derivative[103].



XI

Triazene derivatives also continue to receive attention as potential antineoplastic agents, based on the encouraging antitumor activity of 5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide[104, 105] in man. The compounds 4-carbethoxy-5 (3,3-dimethyl-1-triazino)-2-phenylimidazole, and the corresponding 2-methylimidazole derivative (XII) showed activity against various experimental rodents tumors comparable to that of 5-(3,3-dimethyl-1-triazino)imidazole-4-carboxamide[106, 107].



R = Ph, Me

In relating the structures of the newly synthesized compounds to their reported antitumor activity, one needs to keep in mind that the evaluation, even of members of the same class of compounds, was carried out in widely differing tumor systems, and that generalizations must, therefore, be derived with great caution. The pronounced inhibitory activity of a compound against a highly sensitive tumor, may be of only limited value in predicting its potential clinical utility. Nevertheless, the findings reported serve as indications that such a potential exists, and that a given structural modification may be useful in eliciting a tumor growth-inhibitory response.

What this brief review shows is, that the development of new antineoplastic agents is a slow and tedious process, but the achievements of past years clearly demonstrate, that each new contribution adds to the progress of cancer chemotherapy.

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Chapter 16. Immunotherapy of Cancer

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Many excellent review articles on the immunology and immune therapy of cancer have appeared in the last few years.[1,2,3,4,5,6,7,8,9,10,11] The major goal of this chapter is to provide the reader with information on immunotherapy of cancer which has been published during the past year.

The existence of an immunological response by a host to his tumor has been well documented.[12] Deficiencies in one or more components of this response may either predate and be a prerequisite for development of the tumor or be a consequence of progression of the tumor.[3,13,14,15] Immunotherapy aims at restoring immune competence or amplifying a competent but ineffective host response to the tumor.[1,4,5] Experience with immunotherapy has revealed that the host with the minimal residual tumor burden following surgery, radio-and/or chemotherapy receives the most benefit from immunotherapy.[16]

Active Specific Immunotherapy - The rejection of tumor cells following active specific immunotherapy is probably mediated through sensitized lymphocytes,[17] macrophages[18] and/or antibody.[19] Rejection of the tumor follows recognition of tumor specific transplantation antigens on neoplastic cells by an immune-competent host. Tumor antigens that are either weak or hidden may not be recognized by the host and thereby escape recognition;[20] some support for the existence of this phenomenon comes from Witz who has described the ubiquity of IgG_2 on tumor cells found only *in vivo* and not on tumor cells grown *in vitro*.[21] Thus the goal of active specific immunotherapy is the amplification of the afferent arc of the immune system by increasing the antigenic stimulus to the tumor bearing host.

Vaccines - Vaccines consisting of whole autogenous or allogeneic cells or membrane fractions from tumors have been used in animals to induce resistance to subsequent tumor challenge, and in man, to improve the immune response to an established tumor. All tumors induced by a specific virus have common antigens but chemically induced tumors do not share common antigens.

Gross demonstrated the immunization of 'strain 2' guinea pigs with L2C viable leukemic cell suspensions by superficial skin scarification.[22] The mortality of 30% due to the scarification alone was less that in previous trials with intradermal immunization. Prager and co-workers have shown that resistance to 6C3HED lymphoma in C3H mice could be produced by immunization with soluble 6C3HED membrane antigen(s).[23]

Human trials with osteogenic sarcoma[24] using frozen or fresh irradiated autologous tumor have been too small to offer conclusive results but the responses were as good as with conventional treatments. Other, larger studies have shown enough favorable response to warrant further trials.

The following vaccines have been among those used: aqueous pooled bronchogenic carcinoma extract in a series of patients with lung cancer;[25] irradiated, animal passaged and goat gamma globulin coupled vaccine in malignant melanoma;[26] and autogenous cell-free extracts in complete Freund's adjuvant in a series of patients with varied types of tumors.[27] Correlation of the clinical response to tumor vaccines with changes in skin reaction to recall and tumor antigens and in vitro measures of cell-mediated immunity and humoral immunity are in progress. In a randomized prospective trial in patients with glioblastoma multiforme no antitumor effect was produced following active immunotherapy.[28] Following surgery and radiotherapy, the survival of 27/62 patients receiving irradiated tumor cells was not improved over the control group receiving the conventional therapy alone.

Enzyme Treatment of Tumor Cells - Another mode of active specific immunotherapy has involved Vibrio cholera neuraminidase (VCN), an enzyme that can remove glycoproteins from the surface of tumor cells. Vaccines consisting of neuraminidase treated tumor cells have been shown to induce regression of preexisting tumors. To date, such vaccines have been limited to experimental systems but they offer the best model for clinical situations where therapy must be designed for a tumor-bearing host. Neuraminidase treatment increases the immunogenicity of tumor cells but the mechanism of tumor rejection has not been established. Rios and Simmons have shown that murine methycholanthrene-induced fibrosarcomas (MC-43), mammary carcinomas (M-2) and B16 melanoma up to 1 cm in size can be repressed with the respective specific neuraminidase-treated tumor vaccine and this rejection can be enhanced with BCG.[29] Such combined therapy is more effective when the tumor burden is reduced prior to the immunotherapy. VCN treatment is immunospecific, increasing the antigenicity of only the unshared antigens of mammary carcinomas and not the shared mammary tumor virus antigen. Other workers have extended this treatment modality to experimental leukemia with conflicting results.[30,31]

Active Non-Specific Immunotherapy - Active non-specific immunotherapy involves a general stimulation of the host's immuno system. Yashphe has recently written a very comprehensive review of this topic.[32]

BCG - Receiving much current attention is a strain of Mycobacterium bovis, bacillus Calmette-Guerin (BCG). Early studies revealed that BCG stimulated the reticuloendothelial system.[33] Administration of BCG to AKR mice decreased the incidence of spontaneous leukemia.[34] A retrospective study by Davignon revealed that BCG vaccination was associated with a decreased incidence of childhood leukemia;[35] however, other studies have refuted this conclusion.[36] Mathe reported that remissions produced in patients with acute lymphocytic leukemia following conventional therapy could be significantly prolonged in the absence of chemotherapy by treatment with Pasteur BCG plus irradiated leukemic cells.[37] An enlarged study by the British who used a different preparation of BCG (Glaxo) and no irradiated leukemic cells failed to support this finding.[38] Powles, on the other hand, has found that in acute myelogenous leukemic (AML) remissions on maintenance chemotherapy plus immunotherapy (BCG and irradiated

AML vaccine) were prolonged compared to chemotherapy alone.[39] The variation in clinical responses may stem from the use of different sources and preparations (fresh versus lyophilized) of BCG resulting in different numbers of live organisms at the time of use. Another problem in the failure to reproduce clinical trials has been the tendency to quantitate dosage by milligram of BCG protein rather than the number of viable organisms and to accept the number of viable organisms as stated by the supplier when using both the fresh or lyophilized preparations. The various methods of administration of BCG create variability in number of organisms actually penetrating into the subject. Mackaness has shown that cell mediated immunity is the dominant host response to viable organisms whereas non-viable organisms elicit a predominantly humoral response.[40] Experimental studies on the immune response to different strains have revealed the Pasteur strain to be the most immunogenic.[41] Finally it is possible that the antitumor effects of BCG may result from a direct antitumor effect rather than mediated through the immune system.

Gutterman used the scarification method of immunization with BCG in patients with Stage III and IV melanomas and produced a significantly lower rate of relapse with the Tice (lyophilized) strain than the live Pasteur strain.[42] In both groups, patients receiving the highest dose of organisms (6×10^8) developed the greatest skin reactivity on testing with a battery of antigens. Injections of BCG directly into intradermal malignant melanomas continues to produce strikingly positive results.[43,44] One study with BCG in malignant melanomas supports the hypothesis that BCG primarily augments the host's response to antigens to which the host has had previous exposure.[45]

BCG has also been studied in several experimental models. Maximum tumor inhibition and prevention of pulmonary metastases in animals with methylcholanthrene sarcoma followed BCG plus live tumor vaccine inoculated intralesionally.[46,47] Bansal and Sjogren have shown that BCG must be given before the tumor reaches a critical size; otherwise BCG enhances the blocking effects on the enlarging tumor.[48] Finally BCG decreases the tumor size in Moloney sarcoma virus infected mice which had been immunosuppressed by cytoxan.[49] Analysis of the guinea pig hepatoma model revealed that both the BCG cell wall suspension in oil and isolated BCG cell wall components were as effective as the live organisms.[50] Another BCG product, methanol-extraction residue (MER) has also been extensively studied and shown to stimulate a positive host response towards tumor rejection.[51]

Intralesional BCG therapy in strongly PPD-positive individuals has been complicated by malaise, influenza-like syndrome and hepatic dysfunction.[52,53] Noncaseating granulomatosis hepatitis developed in three patients who were PPD negative. Treatment with anti-tuberculous agents have reversed the more severe complications of BCG.

Corynebacterium parvum - The anaerobe Corynebacterium parvum (C. parvum) has been studied intensively because of its ability to stimulate an immune response resulting in tumor regression. Contrary to BCG whose activity depends upon viability, C. parvum is active when heat-killed or

as a phenol extract. In animal systems, it is most active when inoculated directly into an established tumor or administered with the tumor inoculum. Inoculation into the contralateral limbs or as intravenous doses was not effective. Maximum tumor regression occurred about 14 days after treatment, and the tumor-specific resistance that followed inoculation of 5 x 10^7 organisms was permanent.[54,55] The mechanism of regression appeared to involve cellular immunity.

Several large clinical series with C. parvum have been reported by L. Israel.[56] In one, composed of patients with metastatic cancer, the treated group survived longer than the controls. A subsequent study involving squamous cell cancer of the lung compared chemotherapy alone with chemotherapy plus C. parvum. The mean survival (MS) was 6.1 months and 10.5 months, respectively. Although the response rate was the same in each group, the duration of response was longer in the group with the combined treatment. Of further interest is a breakdown of the combined groups into PPD status: those which were PPD positive had a MS of 13 months whereas those which were negative had a MS of 3.6 months.

Listeria monocytogenes - Listeria monocytogenes (LM) has also been effective in experimental tumor rejection.[57] Inbred mice previously immunized with LM were able to impede or prevent growth of a syngeneic sarcoma when the tumor cells were inoculated together with viable LM. The mechanism of action was consistent with a delayed-type of hypersensitivity at the site of tumor implantation.

Macrophage Activation - The role of macrophages in tumor rejection is being actively investigated.[58] Certain compounds which exhibit antitumor activity has been shown to act via the macrophage, probably through macrophage-mediated cytotoxicity.[59] These include endotoxin and double stranded Penicillium stoloniferum RNA,[60] Triton WR 1339,[61] and pyran.[62,63] One such compound, tilorone, is in a Phase II human trial and has produced antitumor responses in 5/41 cases.[64]

Interferon - The role of interferon in tumor rejection is unknown. Interferon enhances phagocytosis,[65] antibody production,[66] sensitized lymphocyte mediated cytotoxicity and tumor cell antigenicity[67] and thus may induce host-mediated anti-tumor effects in addition to a direct tumoricidal effect. Interferon prolonged survival in DBA/2 mice inoculated with interferon resistant strains of L1210. In these mice, lymphocyte and macrophage functions were compromised with anti-lymphocyte serum and silica, respectively, leaving the mode of action of interferon unexplained.[68] Statolon, a double stranded RNA mycophage from Penicillium stoloniferum, induces interferon in DBA/2 mice and suppresses established Friend virus leukemia (FV).[69] Suppression of FV erythroleukemia is dependent upon the immunostimulatory effects of statolon, a property not shared by other interferon inducers such as Newcastle disease virus and poly I:poly C, that have no leukemosuppressive effects.[70]

Macrophage Recognition Factor - Macrophage recognition factor (MRF) may be important as a mediator of immunotherapy.[71] Found in the α_2-globu-

lin fraction of normal plasma, MRF enhances phagocytosis. Depleted levels of MRF in tumor patients return to normal after reduction of tumor load. Inoculation of MRF into a subcutaneous Shay chloroleukemic tumor reduced the tumor size by 86% compared to controls. Direct cytotoxicity by MRF for tumor cells was not demonstrated.

Allogeneic Cell Transfers - Another approach to immunotherapy has been the initiation of a graft versus host reaction in an immunocompetent tumor-bearing host. As the only form of therapy transfer of non-sensitized allogeneic immunocompetent lymphocytes significantly altered the course of guinea pig leukemia L_2C, and produced long-term protection in 21% of cases.[72,73] Optimum protection resulted when the allogeneic lymphocytes were transferred one week prior to leukemic cell challenge; prolonged survival still occurred when a 30-fold increase in the lethal dose of L_2C leukemia was used. Significantly prolonged survival also occurred when the allogeneic cells were transferred after the leukemic challenge. The use of human allogeneic cell transfers in early malignancies in immunocompetent host was suggested.

Skin Sensitizers - A final mode of active specific immunotherapy utilizes primary skin sensitizing agents such as 2,4-dinitrochlorobenzene (DNCB), 2,3,5-trisethyleneiminobenzoquinon and 5-mercapto-2-deoxyuridine. Reports show 54-95% favorable response in premalignant and primary cutaneous neoplasia and 33% in neoplasias metastasizing to skin.[74] Anti-tumor effects in neoplastic lesions distant to the site of sensitization and challenge were also observed. A response was defined as the complete disappearance of a lesion for the period of survival (12 months) or period of observation (20 months). Anergic patients who could not respond to primary antigens were challenged with antigens which could test for retained immunological memory. A much smaller study limited to the response rate of basal cell carcinoma to sensitization and challenge by DNCB revealed that only 32% of lesions showed complete, and 29% partial regression.[75] Large lesions had the poorest anti-tumor response and no distant effects were noted. Croton oil, which is an irritant without being a sensitizer, produced complete regression in 23% of treated basal cell carcinomas. This sheds doubt on the mechanism of response to DNCB as being due to cell-mediated immunity. The authors noted, however, that the patient most sensitive to DNCB had the best response and the patient who was most tolerant to DNCB had the poorest response. This supported a host-mediated immunological reaction to DNCB.

Miscellaneous - The anti-tumor effects of nonspecific stimulants of the immune system were first described in 1906 by Coley who reported a series of 31 patients with sarcoma who improved after receiving extracts of hemolytic streptococci and Serratia marcesans.[76] In a retrospective study Ruckdeschel found that 50% of the patients with post-operative empyema were alive following surgery for lung cancer compared to a non-empyema control series where the survival was 18%.[77] By excluding patients with metastatic symptoms, the survival rates increased to 73% in the study group compared to 23% in the control. He concluded that the host responses to infection may influence survival in the cancer patient.

Passive Immunotherapy - In passive immunotherapy, immune modalities are given to a tumor host which will enable it to more effectively reject its tumor. These modalities may consist of activated mononuclear cells, extracts of sensitized lymphocytes or serological factors such as specific antibodies or complement. Lymphocytes, macrophages or thymocytes may be transferred in passive immunotherapy.

Lymphocytes Activated In Vivo - Lymphocytes may be presensitized either in a syngeneic or an allogeneic host. An example of syngeneic cell transfer comes from guinea pig primary lymphatic leukemia, L_2C.[78] All animals were protected from a lethal dose of leukemic cells if 580-750 x 10^6 sensitized spleen and lymph node cells were transferred 2 days after the leukemic challenge; cell transfer 4 and 7 days after leukemic challenge was less successful. Gaugas et.al.[79] similarly showed that transfer of sensitized syngeneic spleen cells to thymectomized and/or antilymphocyte globulin-treated (ALG) mice inhibited polyoma virus and mammary tumors.

Passive immunotherapy with sensitized allogeneic lymphocytes has been employed in patients with metastatic carcinoma. Pairs or groups of 3 patients, matched for ABO-Rh and similar tumors, were serially immunized with another patient's tumor. Then, sensitized lymphocytes were serially transfused into the host with the target tumor. Krementz et.al.[80] reported a series of 64 patients and observed an overall anti-tumor response of about 20%. This type of immunotherapy was most effective when the patients were immunocompetent and carried a minimal amount of residual tumor.

Lymphocytes Activated In Vitro - Lymphocytes activated in vitro may also be effective in tumor inhibition. Balb/C spleen cells sensitized in vitro for seven days to a syngeneic plasma cell tumor, HPC 108, exhibited in vitro cytotoxicity for this target cell.[81] In this system sensitized lymphocytes admixed with tumor cells at a 20:1 ratio inhibited subcutaneous growth of tumor.

Autochthonous lymphocytes activated in vitro with phytohemaglutinin (PHA) and applied to subcutaneous metastatic nodules resulted in a greater percent regression than non-activated lymphocytes but the degree of regression in those nodules which were affected was not significantly different from the control group.[82] This study reemphasized the need for effector lymphocytes at the site of the tumor.

Non-Activated Lymphocytes - Non-activated lymphocytes from healthy HLA-identical siblings have been harvested and transfused into the other sibling with advanced cancer with the rationale of increasing the number of immunocompetent cells for the tumor host.[83] It is conceivable that these lymphocytes may have been sensitized in the healthy donor to the same carcinogenic stimulus as was present overtly in the recipient. In any case transfer of lymphocytes had no effect on the tumor. Non-specific or specific in vitro lymphocyte activation plus active immunotherapy were suggested for the future.

Macrophages - Adoptive transfer of BCG-activated macrophages has also been shown to play a role in tumor rejection.[84] Transfer of these cells together with tumor-immunized lymph node cells and tumor cells into normal syngeneic hamsters resulted in significant reduction of tumor growth. Substitutions of non-activated macrophages or of normal lymph node cells was not effective. This study was unique in that extracellular BCG itself was not present in the inoculum. Furthermore, the requirement of both lymphocytes and macrophages in the inoculum for antitumor effects indicated that cell-cell cooperation was needed for inhibition of tumor growth.

Thymic Cells - There are scattered reports of the anti-tumor effects of thymic transplants in immunodeficient tumor-bearing hosts.[85,86] In contrast, ablation of the thymus has been cited as cause for decreased incidence of experimental neoplasias.[87] The role of the thymus in neoplasia is implicated by a retrospective study which described an increased incidence of neoplasia in patients with myasthenia gravis prior to thymectomy. After the second post-thymectomy year the incidence of neoplasia in the thymectomized patient equaled the general population.[88]

Cellular extracts - Sensitized lymphocytes have served as a source of extracts such as immune RNA[89,90] or transfer factor.[91] Immune RNA from presensitized lymphoid tissue has been given either directly to the tumor bearing host or to lymphoid cells *in vitro* which have then been adoptively transferred to such hosts. Such treatment has conferred immunity to tumor specific transplantation antigens; its use in conferring tumor immunity has been largely limited to experimental systems. However, immune-RNA has been shown to transfer cell-mediated skin reactivity in Hodgkin's disease and other neoplastic states.[92]

Since transfer factor has been strikingly effective in the restoration of cell-mediated immunity in some immunodeficiency diseases,[93] its use in a variety of human neoplasias is being tested.[80] Its specificity, non-antigenicity, ability to be stored and quantitated *in vitro* and its long lasting effects would make it a desirable form of immunotherapy.

Serotherapy - The administration of tumor-specific antisera to a tumor-bearing host is an old approach to tumor therapy and early reports were disappointing.[94] In contrast, the intraperitoneal administration of heterologous immune antisera to animals with experimental peritoneal ovarian carcinomas[95] produced 100% survivors when administered concurrent with a lethal dose of tumor cells. Survivors were reduced to 30% when administration of the antisera was delayed until the fourth to the eighth day. The intraperitoneal administration of antisera may have avoided the dilution or non-specific organ binding of heterologous antibody given intravenously.

With the recognition that certain antibodies can protect tumor cells against the host's immune response thereby resulting in tumor enhancement, therapy with unblocking antibodies has been attempted. Bansal and Sjogren[96] studied a rat polyoma tumor system and found unblocking activity

in sera from rats in which tumors were excised and bled 5-7 days later or from animals non-specifically immunized with BCG 14 days prior to grafting with the polyoma tumor and again bled 5-7 days after grafting. Blocking effects were abrogated by such sera and growth of tumors in vivo was either arrested or regressions observed. DiLorenze et.al.[97] has vitiated the 'blocking effects' in a mouse mammary carcinoma with low dose chemotherapy, i.e. cytosine arabinoside.

The use of antibodies as carriers of cytotoxic drug, radioisotopes, toxins, and enzymes offers hope of achieving greater specificity by chemotherapeutic means.[98,99]

Drake et.al.[100,101] found that endogenous complement may be a limiting factor in serotherapy and showed that exogenous complement plus heterologous antisera was needed to achieve in vivo tumor cytolysis.

A $\overline{C1}$ inhibitor produced by tumor cells in culture and found on human leukemia and carcinoma cells has been described and may serve to block host effector mechanisms; the overall scope of this phenomena is being studied.[102]

Immunotherapy today is in its infancy, but recent results are encouraging and prospects for the future are bright. The wide range of approaches to immunotherapy and the multiple agents which can be employed in each approach will form the basis for much research in the coming years.

Several considerations should serve as important guidelines for effective immunotherapy: 1) early diagnosis is essential since maximum beneficial effects are produced in patients with the smallest tumor load; 2) immunotherapeutic agents must be selected with great caution since they may enhance production of factors that block the host's normal immune response to tumor cells; 3) immunotherapy demonstrated to be effective against one tumor may work to the detriment of the host bearing another tumor. Ideally immunotherapy should be directed at amplification of the specific host function that is crucial to the defense against a specific tumor.

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Section IV - Metabolic Diseases and Endocrine Function

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Chapter 17. Prostaglandins and Related Compounds

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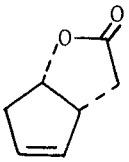
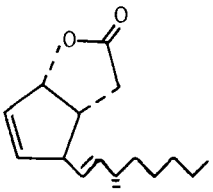
This review will include advances in chemical synthesis that have occurred since the last article in this series[1] and selected aspects of prostaglandin biosynthesis. Specialized up-to-date reviews of most areas in prostaglandin biological research have appeared; cAMP interactions,[3,4,5] prostaglandin antagonists,[6] effects on the respiratory system,[7,8,9] in blood,[10,11] in cardiovascular-renal,[15,16,17,18] occular,[19,20,21] fertility and reproduction,[22,23,24] gastro-intestinal,[25,26,27] nervous system,[28,29] radio-immunoassay,[30] and veterinary applications.[31] Chemical reviews[12,13,14] and general books and monographs have been published.[32,33,34]

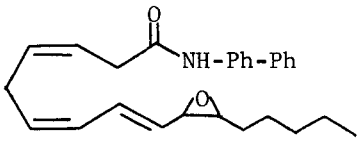
Synthesis of natural prostaglandins and their analogs. Corey has disclosed three direct syntheses of PGA_2. The first involves conversion of the key intermediate 1 to 2 [R = $Si(CH_3)_2C(CH_3)_3$] and subsequent treatment by previously reported procedures to yield PGA_2 as the primary product.[35]



1

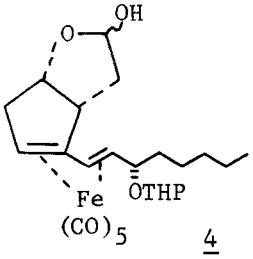


2



3

The second sequence involves the preparation of 2 from a bicyclo[2,2,1]-heptene intermediate.[36] Addition of the carboxyl sidechain in the usual fashion followed by Collins oxidation of the resulting 9-hydroxy-PGA_2



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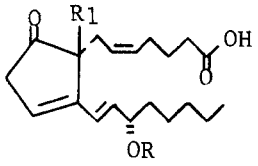

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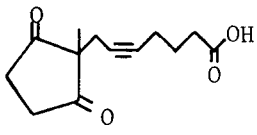

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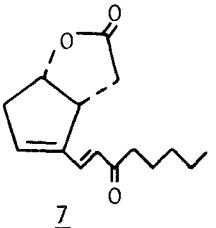
formed gave PGA_2. The third is a biosynthetically derived process which involves Lewis acid cyclization of 3 to give dl-2 (R = H) and its 15-epimer.[37] Corey has also disclosed[38] a total synthesis of PGC_2, 5 (R = R' = H) involving reduction of 2 (R = THP) to the lactol which gave 4 when treated with $Fe_3(CO)_{12}$. Addition of the carboxyl sidechain and Collins oxidation, which also disrupted the iron carbonyl complex, gave the desired product. 8-Methyl-PGC_2, 5 (R = H, R' = CH_3), synthesized from 6, had about 3% of the activity of PGE_2 in "the smooth muscle test". The epimeric methyl esters had about 60% of the potency of PGA_2 as inhibitors of gastric secretion in the rat.[39] Crabbé has also reported[40] the synthesis of 5 (R = THP, R' = H) via 7 and 8. A synthesis of optically pure 1 was published, which



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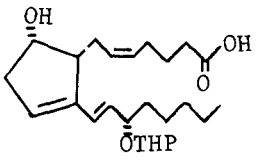

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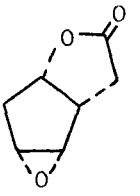

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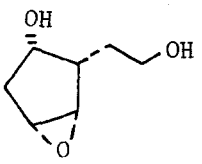

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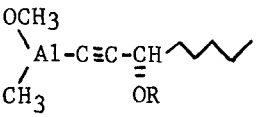

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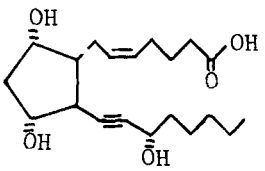
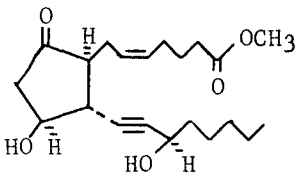


12

is based on a highly stereoselective hydroboration with (+)-di-3-pianylborane.[41] Epoxidation of 1 with peracetic acid gave a 36% yield of the epoxylactone 9. This represents an efficient route to the optically pure intermediates 9 and 10, which have been converted to the natural prostaglandins by Corey and Fried respectively. Fried and his group disclosed[42] the details of their method for obtaining 100% regioselectivity in the ring opening of epoxide 10 by displacement with the organoaluminum reagent 11 [R = $OC(CH_3)_3$]. This process, which has been shown to lead to the natural prostaglandins,[1,2] was used to prepare 13,14-dehydro analogs of PGE_2, $PGF_{2\alpha}$ (12), and their enantiomorphs.[43] Compound 12 is 2 times more potent subcutaneously and 5 times more active orally than the natural product in a hamster anti-fertility test, while having about 1/3 of its activity in vitro on the gerbil colon, thus showing some separation of pharmacological activity. The enantiomer of 12, although less potent than the natural isomer, shows an even broader separation of smooth muscle and anti-fertility activities and effectively inhibits prostaglandin 15-dehydrogenase, the enzyme primarily responsible for inactivation of the natural compounds. The most active dehydrogenase inhibitor in the series, 13, has a Ki of 7 μm.

The lactone 14 (R_1,R_2 = O) or closely related intermediates have been the focal point of work from a number of laboratories. A Woodward synthesis of $PGF_{2\alpha}$ was based on the conversion of 15, which was made starting

with cis-cyclohexanetriol, via the amine 16 to give 14 (R_1 = H, R_2 = OCH_3).[44] The key reactions were diazotization followed by rearrangement of the resulting carbonium ion. Corey has disclosed[45] a similar sequence starting with the cyclohexene analog of 2, whose resolution was reported this year.[46] An additional variant started with norbornadiene and proceeds through



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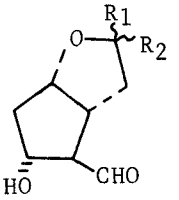

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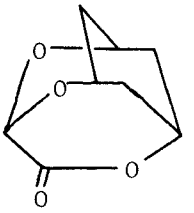

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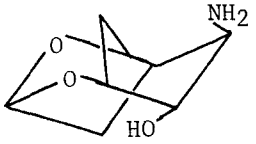

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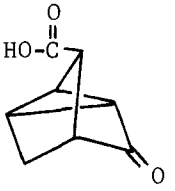

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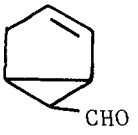

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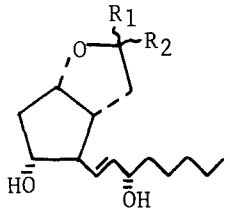
intermediate 17.[47] Kelly has reported[48] on an improved synthesis of $PGF_{2\alpha}$ which starts with the endo-aldehyde 18 and utilizes intermediate 19 (R_1, R_2 = 0). The resolution of a key cyclobutanone intermediate was discussed in a separate paper.[49] An alternate synthesis of 19 was reported by another group.[50] Turner has disclosed the synthesis of dl-$PGF_{2\alpha}$ via 19 (R_1 = H, R_2 = OH), starting with dicyclopentadiene.[51]



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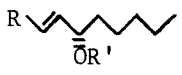

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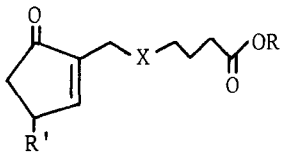
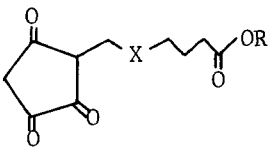


21 a (X=CH=CH)
b ($X=CH_2CH_2$)

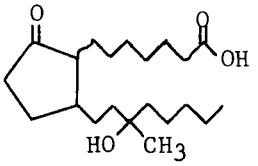
A synthesis of dl-PGD_2 by oxidation of the 15-THP derivative of $PGF_{2\alpha}$ was described;[52] PGE_2 and 11-keto PGE_2 were also obtained. In a second communication,[53] 16(R) and 16(S)-methyl PGE's and PGF's of both the 1 and 2 series were disclosed. Another laboratory has also published the synthesis[94] and biological activity[123] of alkyl prostaglandins. 16(R)-Methyl-PGE_2 is reported to be 100 to 200 times more active than PGE_2 as an inhibitor of gastric secretion in the rat. The 15-epi compound is also reported to have "strong activity". Two additional papers[54,55] disclose the synthesis, but not the biological activity, of 2-carboxy- and 15,16-dimethyl prostaglandins in the E, F, and A series. Gandolfi has reported[56] a synthesis of 14-chloro-PGE_2 and 14-chloro $PGF_{2\alpha}$. Base catalyzed elimination of chloride yielded

the corresponding 13,14-dehydro derivatives whose biology is reported above.

The 1,4-addition of organometallic reagents to cyclopentenone derivatives continued to be pursued. dl-11-Deoxy-PGE_2 and its 15 epimer were made[57] by the addition of 20 (R = Li, R' = $CH(CH_3)OC_2H_5$) to 21a and the 1,4-alanate addition, reported last year,[1] was further developed to yield PGE_1 using 21b (R = THP, R' = OTHP) as substrate.[58] A second paper reports[59] the tri-n-butylphosphine copper (I) iodide complex catalyzed addition of the Grignard reagent 20 (R = MgBr, R_1 = trityl) to the same substrate. The synthesis[60] of 20 (R = I, R_1 = H) by microbial reduction of the corresponding ketone in 10% yield with an optical purity of 80% was reported. The reduc-



22 a (X=CH_2CH_2)
b (X=CH=CH)



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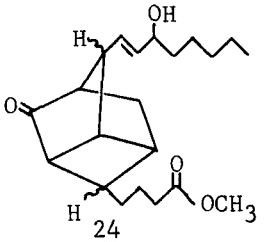


24

tion of the 11-ketone in 22b was also accomplished by fermentation, thus expanding the synthesis reported last year.[1] Compound 21b (R = OH, R_1 = H) has been microbiologically hydroxylated to 21b (R = OH, R_1 = OH).[61] Details of the bacterial and chemical resolution of 7-(2-trans-styryl-3-hydroxy-5-oxocyclopentenyl)-n-heptanoic acid have been published.[62,63] Another group used 22a as starting material for the synthesis of 8-epi-$PGF_{1\alpha}$ by a multistep pathway.[64] Alvarez and Wren reported[65] the Michael addition of nitromethane to 21a (R = R_1 = H) as the basis of a synthesis of 11-deoxy PGE_1, $PGF_{1\alpha}$, and $PGF_{1\beta}$. 15-Keto-tetrahydro-PGA_1 has been resolved[67] using fermentation methods and both enantiomers have served as substrates for the synthesis of 23. Minimal anti-fertility action was observed, whereas both enantiomers had oral and parenteral anti-secretory/anti-ulcer effects, with (+)-ent-23 being the more potent.[68,69] 9-Deoxy-PGE_1 has been synthesized by another group.[66]

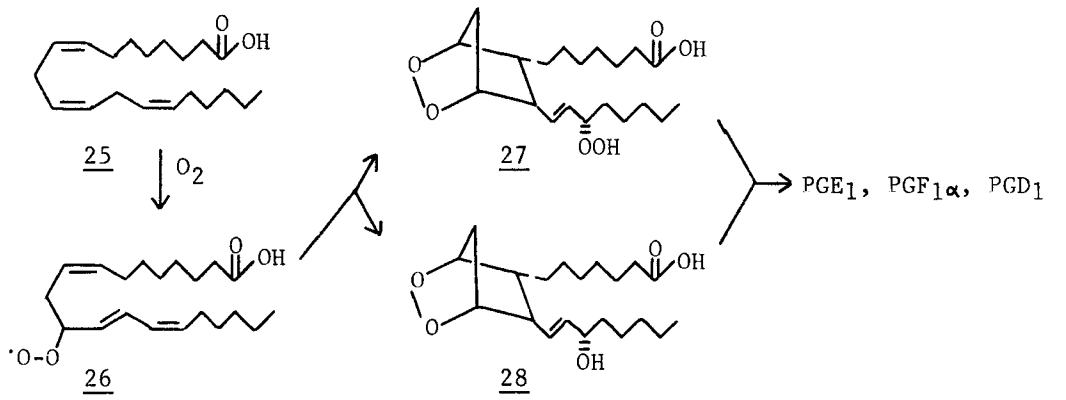
PGA_2 from the coral, P. homomalla, continued to provide starting material for the synthesis of the other natural prostaglandins and their analogs. Crabbé reported[70] on the intramolecular photochemical addition of the 5,6 and 10,11 double bonds of PGA_2 in non-protic solvents which afforded three isomers of 24. A full paper has appeared[71] on the photochemical addition of enes and dienes to PGA_2 disclosed[1] earlier. Also reported[72,73] were the synthesis of 13,14-dihydro-14-methyl-PGB_2 methyl ester, 11-methyl-PGE_2, 11-deoxy-PGE_2, $PGF_{2\alpha}$, their 15-methyl analogs, and 9,11-dimethyl-11-deoxy-$PGF_{2\alpha}$ by the reaction of PGA_2 with lithium dimethyl copper and of the difluoromethylene adduct of 7 and the resulting PGE_2 and PGF_2 derivatives. The reaction of PGA_2 with diazomethane was investigated in a separate study.[74] Photochemical decomposition of the pyrazoline derived addition to the 10,11-double bond gave both 10,11β-cyclopropyl-PGA_2 and 11-methyl PGA_2. PGA_2 was

converted into PGE_2 via epoxidation of the 10,11-double bond and aluminum amalgam reduction by researchers at Upjohn.[75] The total yield was 47%, 40% without chromatography. In a second full paper, catalytic reduction of various prostaglandins was discussed and complete experimental details were given.[76] The hydrolysis of PGA_2 esters using enzymes endogenous to the coral was described[75] and a group at Syntex reported[77] details applicable to large (10 kg) batches. Corey has also disclosed[78] the conversion of PGA_2 into PGE_2 by epoxidation of the 10,11-double bond and subsequent reduction. The key step is the use of the bulky tri-p-xylylsilyl derivative of the 15-hydroxyl to give an α/β epoxide ratio of 94/6. He has also published[79] details for converting $PGF_{2\alpha}$ into PGE_2 using n-chlorosuccinimide/dimethyl sulfide in 90% yield and the preparation of aldehyde 14 (R_1,R_2 = 0) in 93% yield from the corresponding alcohol using chlorine/methyl phenyl sulfide.

In other publications, Finch,[90] Miyano,[91] Sih,[92] and Taube[93] have disclosed details or improvements of their previously reported[1,2] syntheses of prostaglandins. PGA_1 was tentatively identified as a constituent of yellow onion[80] and a metal ion assisted (especially $FeCl_3$) thin layer chromatographic method for separating and identifying PGA_2 and PGB_2 was reported.[81] Reports on the PMR spectrum of prostaglandin derivatives using shift reagents[82] and ^{13}C nmr spectra[83] have appeared. The X-ray structure of PGA_1 has been completed.[84] Five papers[85-89] were published on the stability and/or kinetics of decomposition of the natural prostaglandins or their salts.

Prostaglandin synthetase mechanism studies and inhibition by non-steroidal anti-inflammatory compounds. Although many details of prostaglandin biosynthesis have yet to be worked out, the early work of Bergstrom, Samuelsson and their co-workers from Sweden and that of Van Dorp and his group in the Netherlands forms much of the basis for a possible reaction pathway shown in Scheme I.[1,2,32,34] The synthesis of the so-called natural prostaglandins proceeds in several steps and the enzyme catalyzing these reactions are referred to collectively as prostaglandin synthetase (PGS'ase). Many mammalian tissues as well as some cell cultures, invertebrates and fish gills are capable of converting substrate to prostaglandins.[95,96]

It is implied that PGS'ase is not one enzyme, but rather an enzyme complex. The specific enzymes catalyzing the formation of the PGE's, PGF's, PGD's and PGA's have not been identified to date. Isomerase activity is postulated in the formation of PGE's and PGD's and a reductase activity for formation of PGF's.[103] The enzymatic formation of PGA_2 in kidney has been claimed[97] but it is still uncertain whether the material found was formed enzymatically or by non-enzymatic dehydration of PGE_2. Corey et al.[100] have demonstrated that P. homomalla can synthesize PGA_1 and PGA_2; little or no PGE synthesis was detected.



Scheme I

Both the Swedish and Dutch workers have now confirmed the existence of the endoperoxides 27 and 28. Hamberg and Samuelsson[102] and Nugteren and Hazelhof[103] have isolated 28 from ram seminal vesicle PGS'ase incubations with eicosatetraenoic acid and shown enzymatic as well as non-enzymatic conversion of 28 to PGE_2, $PGF_{2\alpha}$ and PGD_2. Nugteren and Hazelhof were also able to isolate 27. Compounds known to act as cofactors in the biosynthesis of prostaglandins in vitro were shown by the Dutch workers to affect non-enzymatic breakdown of both 27 and 28. Thus, glutathione (GSH), a "stimulator" of PGE_2 biosynthesis in vitro reduced the 15-hydroperoxy group (measured by reduction of 15-hydroperoxy PGE_1 to PGE_1).[95,103] In combination with ferrihaem, GSH addition catalyzed the reduction of 28 to $PGF_{2\alpha}$. The ratio of prostaglandins formed from 28 differs quite markedly in different tissues; for example, rat lung homogenate produced predominantly PGD_2 while ram seminal vesicle tissue gave predominantly PGE_2.

That the endoperoxide does exist in detectable concentrations during in vitro biosynthesis for a fairly long time period - $T_{\frac{1}{2}}$ = 2-5 min - has raised the question of its role as an active prostaglandin. Samuelsson has shown 28 to be more active than some of the other natural prostaglandins in various biological tests.[104] These data and that of others has led Samuelsson to propose that 27 and/or 28 may be the physiologically active agent in some tissues or cell types while in others, the PGE's or PGF's may be the physiologically active prostaglandins.

Whatever the "natural" prostaglandin may be, it is clear that inhibition of the initial dioxygenase step will stop production of prostaglandin by PGS'ase. It was shown in 1970 by Downing[105] that 5, 8, 11,14-eicosatetrynoic acid inhibited the formation of PGE_1 from 8, 11,14-eicosatrienoic acid and the following year Vane and Willis published their data showing that aspirin inhibited prostaglandin biosynthesis in human platelets, guinea pig lung and dog spleen homogenates.[106,107,108] What has followed these initial papers can be described as a flurry of activity directed toward the following objectives:

1. to explain the anti-inflammatory, antipyretic and analgesic activity of aspirin on the basis of its observed inhibition of PGS'ase.
2. to establish that most, if not all, non-steroidal anti-inflammatory and antipyretic drugs are capable of inhibiting PGS'ase *in vivo* and thus, like aspirin, owe their efficacy to this activity.
3. to utilize inhibitors of PGS'ase to explore the role of prostaglandins in normal physiological processes (as opposed to their pharmacological effects).
4. to develop structure-activity relationships for inhibition of PGS'ase.

Vane proposed that antipyretic, analgesic and anti-inflammatory drugs owe their activity to their ability to inhibit PGS'ase.[106] Flower showed a remarkable correlation between potency of PGS'ase inhibitors and their anti-inflammatory activity (carrageenin adema test in rat hind paw).[111] Evidence for the specificity of the enzyme system is that analogs of aspirin lacking anti-inflammatory activity and an enantiomer of naproxen with lowered antipyretic and anti-inflammatory potency also showed much less inhibitory action against PGS'ase. Clinical studies involving the measurement of prostaglandins before and after drug therapy are just beginning to appear. Hamberg has shown[112] that the appearance of urinary metabolites of PGE_1 and PGE_2 in man is strongly suppressed after therapeutic doses of indomethacin or aspirin. This offered early clinical support for the Vane hypothesis.

Table I

Inhibition of PGS'ase by Non-Steroidal Anti-Inflammatory Agents

Compound	Tissue Source of PGS'ase[a]	ID_{50}* (μg/ml)	Ref.
Meclofenamic Acid	Dog Spleen-M	0.03	111
Niflumic Acid	Dog Spleen-M	0.03	111
Indomethacin	Dog Spleen-M	0.06	111
Mefenamic Acid	Dog Spleen-M	0.17	111
Phenylbutazone	Dog Spleen-M	2.23	111
Aspirin	Dog Spleen-M	6.61	111
Paracetamol	Dog Spleen-M	100	111
Paracetamol	Rabbit Brain-H	14	120
Indomethacin	Rabbit Brain-H	1.3	120
Naproxen	Bovine S. V.-M	0.37	121
Ibuprofen	Bovine S. V.-M	2.0	121
2,7-dihydroxynaphthalene	Bovine S. V.-M	2.0	122
Dexamethasone	Dog Spleen-M	≤10% Inhibition†	111
Hydrocortisone	Dog Spleen-M	≤10% Inhibition†	111

[a]S.V. - seminal vesicle; M = Microsomes; H = homogenate.
*Dose at which compound produced 50% inhibition of PGE_2 synthesis
†% inhibition at 100 μg/ml.

Research efforts on the inhibitory mechanisms of these drugs against PGS'ase is at an early stage; however, Smith and Lands have demonstrated that indomethacin and aspirin irreversibly inhibited the dioxygenase activity.[113] This allows one to interpret the actions of these drugs on prostaglandins physiologically, irrespective of whether the active prostaglandin *in vivo* is the endoperoxide or one or more of the end products of the biosynthesis.

Table I contains a list of drugs with confirmed activity of PGS'ase inhibitors. Shen[114] and Gryglewski[115] have independently proposed structure activity relationships to account for the inhibitory action of most, if not all, of the compounds mentioned in Table I. Other structures reported as PGS'ase inhibitors are corticosteroids,[116] some psychotropic drugs,[117] antiestrogens,[118] fatty acid hydroperoxides,[103] and various acetylenic fatty acids.[119]

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Chapter 18. Disorders of Lipid Metabolism

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Introduction - Previous reviews in this series have been limited to lipid abnormalities predisposing to cardiovascular disease which accounts for more than fifty percent of all deaths in the United States. Although progress in atherosclerosis research continues to be the main focus of this review, important advances in other aspects of lipid metabolism have prompted us to expand our scope and consider several potential new approaches in other therapeutic areas. Prominent among these are enhancement of cholesterol gallstone dissolution by increasing the relative concentration of biliary bile acids and correction of the inappropriate mobilization of fatty acids during the period immediately following a myocardial infarction.

Pathogenesis of Atherosclerosis - Two new hypotheses on plaque development have been proposed. Ross and Glomset suggest that the lesion is initiated by the accumulation of smooth muscle cells at the damaged intimal site between the endothelium and the internal elastic lamina at arterial branch points where the endothelial structure is thought to be under greatest hemodynamic stress.[1] With increasing age, numbers of cells increase, the intima thickens, and extra- and intra-cellular lipids and connective tissue are deposited. The multiplication of internal smooth muscle cells is thought to be under the influence of plasma lipoproteins (LP). Once plaque formation has been initiated, the lesion may remain fixed, regress or become a nidus for deposited cholesterol. The second proposal, like the first, suggests that the lesion is a proliferation of smooth muscle cells in the arterial wall.[2] However, it maintains that the smooth muscle cells in plaques originate from a single mutant cell produced either by chemical mutagens or viruses. Additional studies are needed to determine whether such transforming factors play a causal role in atherosclerosis.

Attempts to elucidate the mechanism of lipid accumulation in plaques continue. Cholesterol in atheromata is known to be derived from plasma cholesterol and not from synthesis in situ, but the processes of accumulation and combination with other substances remain poorly understood. Smith and Slater[3,4] examined postmortem aortic LP-bound and unbound cholesterol in patients whose blood cholesterol levels were measured within a week of death and found a correlation between the amount of low density lipoproteins (LDL) in normal intima and plasma cholesterol levels. An increase of 100 mg percent in serum cholesterol gave a 50% increase in intimal LP-bound cholesterol, but did not appear to increase the unbound cholesterol. The finding that in normal aortic intima there is intact LP which appears to be in equilibrium with plasma LP has prompted Adams[5] to suggest that the arterial (intimal) LP cholesterol pool should be more accessible to metabolic and exchange processes than the unbound cholesterol pool. Smith and Slater[6] conclude that the perifibrous lipid in normal intima, the pool of amorphous atheroma lipid under raised plaques and the lipid within fat-filled cells in fatty streaks are no longer in the form of intact LP in equilibrium with plasma LP, but represent unbound cholesterol. If this

is true, reducing intimal LP cholesterol levels should reduce the rate of deposition of unbound cholesterol but is not likely to remove unbound cholesterol from this pool. A significant correlation between aortic and serum cholesterol levels in atherosclerotic swine demonstrates that serum cholesterol levels are indeed good measures of atherogenesis.[7] The work of Smith and Slater suggests that the epidemiological results establishing high serum cholesterol levels and elevated blood pressure as additive risk factors predisposing to coronary heart disease may be explainable by the simple fact that hypertension increases the total volume of serum entering the intima, and hence increases the deposition of LP cholesterol. The factors which concentrate and keep intact LP within the intima are not known. However, these findings provide evidence of an equilibrium between plasma LP cholesterol and aortic LP cholesterol which underscores the value of reducing plasma cholesterol levels. Fibrinogen, another plasma component, has also been shown to enter the intima.[8] Additional studies will be required to determine whether fibrinogen is converted to fibrin which could contribute to the formation of the atherosclerotic lesion.

There has been an increased interest in the role that plasma and cellular enzymes play in cholesterol esterification and hydrolysis, and the pathological consequences thereof. For example, it appears that the accumulation of cholesterol esters in the arterial wall, either by filtration or synthesis, is a characteristic feature of atherogenesis. Either after or during the passage of free cholesterol into the arterial wall, metabolic changes occur there, which promote the synthesis of fatty acids.[9] The fatty acids combine with cholesterol to form cholesterol esters which are unable to diffuse out, and remain fixed. The enzyme that catalyzes the formation of cholesterol esters in plasma is lecithin:cholesterol acyl transferase (LCAT).[10] Originally thought to be a cholesterol esterase of little importance in plasma LP metabolism, it has now been shown to catalyze the transfer of fatty acids from lecithin to the cholesterol of plasma LP. LCAT is mainly extracellular and is secreted into plasma by the liver. Evidence in man suggests that cholesterol enters the plasma in the free form and is esterified within the plasma by the action of LCAT for which the preferred substrates are high density lipoprotein (HDL) cholesterol and lecithin. LCAT indirectly reduces the unesterified cholesterol and lecithin of plasma LP other than HDL, by virtue of the non-enzymatic equilibration of these lipids among other LP. The physiological role of the plasma LCAT reaction is not fully understood, but it is thought to be of major importance for cholesterol homeostasis. It has been suggested that LCAT, by increasing the level of plasma HDL cholesterol esters at the expense of HDL free cholesterol, promotes the uptake and transport of free cholesterol from peripheral tissues, such as the arterial wall, to the liver.[11] The fact that cholesterol esterification in blood is impaired in patients with acute myocardial infarction and coronary artery disease tends to support this suggestion.[12] Additional support for this hypothesis is found in the report that the turnover of HDL and LDL cholesterol esters is reduced in type II subjects.[13] If one assumes that decreased cholesterol ester turnover is mainly due to decreased cholesterol esterification, then the increased risk of atherosclerosis observed in such subjects may be due to reduced LCAT activity. On the other hand, the speculation of Glomset

and Norum that the function of LCAT might be to insure that cell membranes receive an adequate supply of free cholesterol from plasma appears to be less tenable.[10] The possibility that the primary function of LCAT is to maintain LP structure and surface area has also been suggested.[14]

Cholesterol esterification (and hydrolysis) takes place at a number of other sites, e.g. liver, pancreas, small intestine, skin, aorta,[15,16] ovaries (corpora lutea and interstitial tissue[17]) and adipose tissue.[18] Liver and arterial tissue are of significance in atherogenesis since both are involved in the uptake and degradation of plasma cholesterol esters. In man, a reversible hepatic acid cholesterol esterase whose main function is probably ester hydrolysis has been described.[19] Since cholesterol esters in plasma LP are good substrates for this enzyme, it has been proposed that it facilitates the hydrolysis of LP cholesterol esters prior to biliary secretion. In normal and atherosclerotic arterial tissue, both the synthesis and hydrolysis of cholesterol esters have been shown to occur. Two pathways for arterial cholesterol esterification have been claimed, direct esterification with acyl-CoA and transesterification via a tissue LCAT. The remarkable increase in the acyl CoA-dependent esterification during atherogenesis suggests that the former reaction is of substantially greater significance.[20] Both the enhanced rate (28-40 fold) of cholesterol esterification in the atherosclerotic intima and the greater permeability of LP cholesterol ester into atherosclerotic lesions suggest that these processes may cause the observed accumulation of cholesterol esters.[20,21] In addition, an impaired rate of cholesterol ester hydrolysis may contribute to cholesterol ester accumulation.[22] There appears to be little doubt that cholesterol esterification and the hydrolysis of cholesterol esters are ubiquitous and important processes in cholesterol economy. However, until there is further clarification of the significance of these reactions in tissue and plasma, they appear to offer a limited basis for a therapeutic approach to atherosclerosis.

Early Detection and Treatment of Hyperlipidemia - The recognition that certain forms of hyperlipidemia are familial in nature has stimulated investigations into the underlying patterns of inheritance. Only in the case of Fredrickson type I disorder, which arises from an autosomal recessive trait of lipoprotein lipase deficiency, has the pattern been established.[23] Three familial lipid disorders, designated familial hypercholesterolemia, familial hypertriglyceridemia and familial combined hyperlipidemia were identified.[24-26] In familial hypertriglyceridemia and familial combined hyperlipidemia reduced expression of the genetic defect (incomplete penetrance) was observed in relatives below age 25. The authors conclude that the three major familial lipid disorders represent dominant expression of three different autosomal genes.

Several studies[27-32] have been undertaken to determine whether lipid analyses of umbilical cord blood are predictive of hyperlipoproteinemia in later life. These studies suggest that diagnosis of the type II disorder can be made on the basis of high cord blood serum cholesterol concentrations. On the other hand, Darmady et al.[33] concluded that cholesterol values in cord blood are not a reliable basis for early detection of type

IIa hypercholesterolemia. The weight of the several studies indicates that diagnosis at birth on the basis of plasma cholesterol concentrations is, at best, difficult and can be improved markedly by a knowledge of LDL levels and the family history with regard to essential hypercholesterolemia. The ability to detect hyperlipidemia in childhood has led to National Heart and Lung Institute-supported detection programs which are presently being carried out at three designated research centers and will be expanded to include twelve centers at a later date.

Detectability of type II hyperlipoproteinemia in early childhood suggests the possibility of early therapeutic intervention. Toward this end, several studies have examined the effects of dietary manipulation on infant plasma cholesterol levels. In type II hyperlipoproteinemic infants,[34] a progressive hypercholesterolemia develops on cow's milk or breast milk diets. On the other hand, formulas rich in corn oil and low in cholesterol depress plasma cholesterol to levels seen in normal infants. This responsiveness to dietary management persists through the first year of life but in older children with the type II defect, a much reduced responsiveness is observed.[34-36] In addition to dietary manipulation, West and Lloyd[37] have administered cholestyramine to heterozygous type II children and produced a 36 percent reduction in cholesterol levels. Dietary modification had no additional effect on serum cholesterol values.[38] In a limited number of homozygous type II subjects, nicotinic acid and cholestyramine, alone or in combination , produced limited effects on plasma cholesterol. However, the fact that the combination reduced the size of atheromatous lesions suggests that the mass of total body exchangeable cholesterol was decreased. In a similar but larger study,[39] more prominent cholesterol decreases and lesion regressions were reported. A combination of clofibrate and a low saturated fat diet supplemented with corn oil produced a serum cholesterol decrease of 33 percent in heterozygous type II children.[36] These preliminary findings provide a basis for optimism in the treatment of heterozygous type II children. However, the results of studies with homozygous children are less promising and additional studies will be required to provide a satisfactory treatment for children afflicted by this malevolent form of hyperlipoproteinemia.

Although the liability represented by elevated serum cholesterol levels has been amply studied, the risk of cardiovascular disease related to high serum triglyceride levels has only recently been characterized. In view of the increased risk of ischemic heart disease in hypertriglyceridemia, attempts have been made to detect this abnormality at birth. Tsang and Glueck[40] failed to detect hypertriglyceridemia in cord blood of neonates from families with elevated serum triglyceride levels. Other studies[41,42] have detected significantly elevated cord blood triglycerides. These findings suggesting that hypertriglyceridemia may be detected at birth must be weighed against the observation of limited penetrance of familial hypertriglyceridemia and combined hyperlipidemia in subjects below the age of 25.[25] In view of delayed triglyceride elevation, cord blood triglyceride analyses would be expected to detect only a limited portion of that population destined to become hypertriglyceridemic.

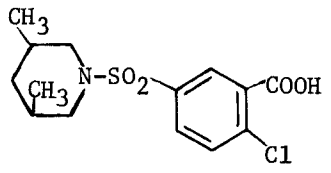
Hypolipidemic Drugs - The clear designation of elevated serum lipid levels as risk factors in the development of atherosclerotic heart disease has

stimulated efforts to discover agents which reduce circulating lipid levels. Clofibrate, the most widely used hypolipidemic agent, is the subject of two new studies evaluating long-term effects on morbidity and mortality,[43,44] but no results have been described.

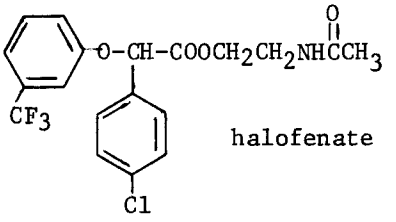
Based on the initial observations of an inverse relationship between plasma triglyceride levels and glucose tolerance[45] and improved glucose tolerance with clofibrate therapy,[46,47] a number of studies have been undertaken to delineate the effects of clofibrate on plasma glucose levels, glucose tolerance, and glucose- and arginine-stimulated insulin secretion. In rats, clofibrate administration reduced circulating levels of plasma insulin during fasting and after stimulation of secretion by glucose or glucagon. Increased sensitivity to insulin *in vivo* and *in vitro* was observed in clofibrate-treated animals.[48] Eaton and Nye[49] found that clofibrate administration to hyperlipemic patients decreased glucose-stimulated insulin secretion and improved glucose tolerance. In normal humans, clofibrate decreased the rate of arginine-stimulated insulin secretion and glucose tolerance was either improved or unchanged.[50] Fenderson *et al.*[51] reported that clofibrate treatment decreased blood glucose and insulin elevations during glucose tolerance tests in normal and hyperlipoproteinemic subjects as well as fasting blood glucose and/or plasma insulin levels in the latter. The evidence provided by these investigations indicates that clofibrate treatment reduces circulating insulin levels while either decreasing or having no effect on blood glucose values, strongly suggesting increased insulin sensitivity as the basis for improved glucose tolerance. Additional studies are required to elucidate whether clofibrate increases insulin sensitivity directly or as a result of its hypotriglyceridemic effect.

Estrogen, initially included in the Coronary Drug Project at doses of 2.5 and 5.0 mg/kg/day, has now been removed from the study because of lack of therapeutic effect and a suggestion of adverse trends with regard to morbidity and mortality.[52] The deletion of estrogen leaves clofibrate and nicotinic acid as the only drugs remaining in the study.

Tibric acid, a new, structurally distinct, hypolipidemic agent, depressed plasma triglyceride levels of type IV subjects for up to twelve months. The drug was well tolerated with minimal side effects.[53]



tibric acid



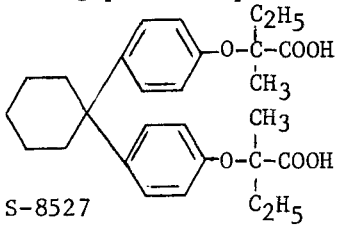
halofenate

Studies with halofenate continue to establish the uricosuric effect of this hypolipidemic agent.[54-56] The earlier pattern of plasma triglyceride lowering with limited effects on plasma cholesterol levels continues to be observed and the drug appears to be of value in treating patients with hypertriglyceridemia and hyperuricemia.

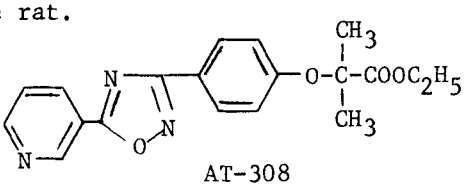
Probucol, a hypocholesterolemic agent which has no consistent effect on plasma triglyceride values, caused sustained decreases in plasma cholesterol levels of approximately 15 percent.[57] The drug appears to be well tolerated in man and the unexpected toxicity of probucol in dogs appears to be due to species-specific sensitization to epinephrine-induced ventricular fibrillation.[58,59]

Colestipol was well tolerated and reduced plasma cholesterol levels during a 2 year study in types II, III and IV hyperlipoproteinemic subjects.[60] In another study, colestipol, at a daily dose of 15 g, decreased plasma cholesterol levels in type II and IV subjects. The addition of clofibrate further reduced cholesterol values. Serum triglyceride levels were elevated by colestipol but the effect could be reversed by clofibrate.[61] Similar triglyceride elevations have been observed with cholestyramine[62] which, during the past year received FDA approval for a lipid-lowering indication.

A number of phenoxyacetic acids related to clofibrate were described; two of these, S-8527[63] and AT-308,[64] are more potent than clofibrate in reducing plasma lipid levels in the rat.

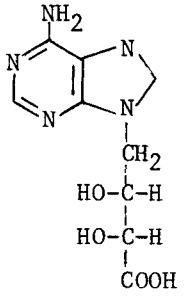


S-8527



AT-308

Eritadenine is approximately ten times as potent as clofibrate in rats. Dietary hypercholesterolemia was suppressed by eritadenine but limited effects on Triton-induced hyperlipidemia were observed. The oral route of administration appears to produce superior lipid reductions and the drug causes a limited but significant enhancement in the rate of removal of cholesterol from the plasma compartment.[65]



eritadenine

Treatment During the Post-Myocardial Infarction Period - Although clinical emphasis has been directed toward influencing the major risk factors predisposing to atherosclerotic heart disease, significant efforts are being made to alter the course of events immediately following an infarction. The survival of patients hospitalized with acute myocardial infarction has been improved by preventing fatal arrhythmias through the administration of antiarrhythmic drugs.[66] The cause of these arrhythmias has come under investigation and the Edinburgh group[67] has demonstrated that plasma free fatty acid (FFA) levels are markedly elevated during the first few hours following an acute myocardial infarction. These findings have been confirmed[68] and extended to show that, in the crucial, immediate post-infarction period, total plasma catecholamine, cortisol and glucose levels are significantly increased.[69] Plasma FFA concentrations have been

shown to be particularly elevated in those patients who develop fatal arrhythmias.[70] Elevated FFA levels may, therefore, have predictive value in identifying infarction patients who are at special risk. In infarction patients, heparin, which increases plasma FFA levels, does not clearly increase the incidence of arrhythmias[71] although a recent study[72] suggests a potential risk which must be weighed against the questionable positive value of heparin.

The mechanism by which elevated FFA levels lead to arrhythmias has been examined and it has been proposed that, in the presence of myocardial ischemia, increased FFA levels lead to increased uptake and oxygen consumption.[73] It has been claimed that the oxidation of fatty acids requires more oxygen than does glucose oxidation. Since oxygen supply is limited in myocardial ischemia, FFA has been viewed as an inappropriate substrate during the post infarction period. If, however, one considers the efficiency of oxygen utilization, i.e., caloric yield per unit of oxygen consumed, it becomes clear that FFA is oxidized almost as efficiently as is glucose. On this basis, it would appear that excessive mobilization of oxidizable substrate of any sort should be minimized during myocardial ischemia. With this objective, the effects of a variety of lipolysis inhibitors have been examined. 5-Fluoro-3-pyridinemethanol has been shown to suppress the appearance of arrhythmias produced by heparin administration to dogs with ligated coronary arteries.[74] In addition, this agent depresses the elevated plasma FFA levels observed in man immediately following acute myocardial infarction.[75] The recent findings of Kjekshus and Mjøs indicate that 3-pyridinemethanol, a nicotinic acid precursor, reduced the extent and severity of myocardial infarction in dogs.[76] These findings offer encouragement that lipolysis inhibitors may reduce the incidence of post-infarction arrhythmias in man.

Cholesterol Gallstones - Another aspect of cholesterol metabolism that has received a great deal of attention is the formation and dissolution of cholesterol gallstones. More than 70% of the crystalline matter in most gallstones found in patients in the United States and western countries is cholesterol. Their formation in man is associated with abnormalities in the relative concentrations of the major biliary lipids, cholesterol, bile acids and lecithin. Under normal conditions sufficient concentrations of bile acids and lecithin are present in the bile so that the cholesterol secreted from the liver can be solubilized by formation of mixed micelles. Gallstones are formed when the liver secretes bile that is deficient in bile acids and phospholipids and supersaturated with respect to cholesterol causing excess cholesterol to precipitate during concentration and storage in the gall bladder. These cholesterol crystals, if not emptied from the gall bladder or redissolved, will aggregate, be cemented together with biliary mucous substances and grow into macroscopic cholesterol gallstones.[77] It is not yet clear whether the primary abnormality is in the hepatic cells that produce bile, or in the gallbladder itself. Some studies of the effect of cholecystectomy on bile acid pools, kinetics and biliary lipid composition indicate that the formation of lithogenic bile does not depend on the presence of a gallbladder, suggesting that the gallbladder is merely a storage site contributing to stone formation, possibly by stratification of bile or by its inability to empty completely.[78] Others have re-

ported however, that biliary lipid composition tends to return to normal after cholecystectomy.[79] If this be so, the gallbladder must have an active role in causing hepatic secretion of lithogenic bile.[80,81] In either case, hepatic bile supersaturated with cholesterol is required for gallstone formation; there is ample evidence suggesting that the abnormality, at least in part, results from increased hepatic secretion of cholesterol and a decreased bile acid pool size. A major breakthrough in understanding the conditions under which gallstones form was the definition of cholesterol solubility in bile in terms of the triangular phase diagram. Since the original report by Admirand and Small,[82] a number of others have shown overlap between the micellar zones of normal and abnormal bile. By using more rigorous solubility techniques to insure equilibration, it has now been demonstrated that the micellar solubility zone is smaller than first postulated.[83] It has been reported that some normal biles are supersaturated with respect to cholesterol.[84] The index of saturation utilizing rectangular coordinates has been proposed as a method which better expresses phase diagram data than do triangular coordinates. Studies show that human gallstones dissolve extremely slowly either in vitro or in vivo. Higuchi has presented evidence of an interfacial barrier to account for this, and has found that a number of quaternary ammonium salts interfere with the barrier and greatly increase cholesterol dissolution in vitro.[85]

Cholecystectomy is presently the only accepted treatment for gallstones. Progress toward a chemotherapeutic approach has been encouraging. Chenodeoxycholic acid (CDC) has been shown in a number of studies to be effective in dissolving cholesterol gallstones.[86] Administration of CDC caused the disappearance of stones or reduction in size in a number of patients after six months of treatment. In one study, CDC depressed plasma triglyceride, but had no effect on plasma cholesterol levels.[87] Controversy exists concerning the mechanism whereby CDC dissolves gallstones. Expansion of the bile acid pool as the basis of gallstone dissolution has been challenged.[86,88,89] CDC has been reported to decrease the relative secretion of cholesterol into bile.[88,90] It has also been shown to inhibit hepatic β-hydroxy-β-methylglutaryl CoA reductase, the rate-limiting enzyme of cholesterol synthesis.[91,92] It is tempting to suggest that the reduced cholesterol secretion observed during CDC therapy is related to reduced hepatic synthesis. Efficacy studies with cholic acid produced no change in gallstone size, although sodium cholate has been reported to dissolve retained stones in the common bile duct.[93] Although CDC is effective and well tolerated in man,[94] studies in Rhesus monkeys have demonstrated hepatic changes.[95,96] No comparable lesions have been observed in man.[97]

Another experimental approach to gallstone therapy is the use of phenobarbital. In Rhesus monkeys it increases bile salt and phospholipid secretion into bile without significantly changing cholesterol secretion.[98] Furthermore, it has been reported to decrease cholesterol saturation in hepatic bile in man.[99] Phenobarbital's ability to decrease the relative cholesterol content in bile may allow dissolution of cholesterol gallstones after long-term therapy. Neither lecithin nor cholestyramine are effective in human gallstone disease.[100]

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Chapter 19. Diabetes Mellitus

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This review attempts to survey key developments in the field of diabetes mellitus since 1971, when the last review on the subject appeared in this series. The concept that diabetes mellitus is a disease of diverse nature has been affirmed by further understanding of its etiology and associated hormonal and metabolic defects[1,2]. Although heredity is known to play the major role in the pathogenesis of diabetes[3], other possible causative agents such as viruses[4] have also been implicated. While insulin deficiency remains as the primary hormonal imbalance in diabetic patients, several other endocrine factors also contribute to diabetic abnormalities[5-8]. An increasing number of metabolic derangements, as in the syntheses of sorbitol[9], glycoproteins[10], cholesterol[11], proteins[12]and ribonucleic acids[13], have been reported in the state of diabetes.

Insulin

Proinsulin biosynthesis. Since the discovery of proinsulin by Steiner and Oyer in human islet adenoma[14], the existence of a precursor in insulin biosynthesis has been demonstrated in isolated islets from many species, including cod[15], Chinese hamster[16], rat[17], angler fish[18], bullhead[19], and in bovine fetal pancreatic slices[20]. Cell-free synthesis of insulin was shown for the first time in membrane-bound polysomes from rat islets[21]. Polypeptides produced in this system were identified as insulin by immunological assay[22] and gel filtration chromatography[21]. Using this cell-free preparation and a rapid and specific immunoprecipitation assay to monitor amino acid incorporation into proinsulin and insulin, Permutt and Kipnis concluded that glucose exerts both transcriptional and post-transcriptional effects in stimulating insulin biosynthesis[23]. Specifically, glucose promotes the overall rate of islet RNA synthesis and alters the nature of the nascent RNA species toward increased guanosine + cytosine content, greater ease of hybridization with DNA, and higher proportions of large molecular weight RNA[24]. Glucose supplies not only energy and substrates for this process, but also some "triggering" factor(s), presumably produced during its metabolism[25].

Conversion of proinsulin to insulin. After proinsulin is formed in islet endoplasmic reticulum, it is quickly stored in the secretory granules, where the cleavage of proinsulin to insulin occurs[26]. The conversion process seems to be the rate-limiting step of insulin biosynthesis[27]. The converting enzyme appears to be membrane-bound[26] and a protein possessing such enzymatic activity has been purified from a bovine pancreatic extract[28]. However, it remains to be established whether this protein indeed plays such a role *in vivo* in the islet β-cells. Using a different approach, Steiner and his co-workers demonstrated that the conversion can be achieved *in vitro* with a combination of carboxypeptidase B and trypsin under conditions where the tryptic activity is rate-limiting[29]. Furthermore, they have partially characterized the converting protease(s) in a subcellular fraction of rat islet secretion granule and their results were in accord

with such a scheme of conversion[30]. In angler fish islets, the emergence of insulin also appears to proceed via two sequential steps[18]. However, the precise nature of the conversion process remains to be clarified.

Intermediates and C-peptides. Proinsulin is converted via intermediates a (residues Lys_{59}-Arg_{60} missing) and b (Arg_{31}-Arg_{32} missing) to form insulin, lys, 3 arg and a connecting-peptide (C-peptide)[31]. The biological activities of the intermediates, measured in terms of glucose incorporation into CO_2 and lipids and the antilipolytic activity in the adipose tissue, were higher than that of proinsulin but lower than that of insulin[32]. C-Peptide showed neither insulin-like activity nor antagonistic or potentiating effects on insulin action in fat tissues[32]. C-Peptides have been isolated from the pancreatic extracts and found to be present with insulin in a one to one ratio. The primary structures of C-peptides have been determined in a number of species[31,33]. There is considerable interspecies variability, yet several features of their primary structures appear to have been conserved[31]. Since C-peptide does not cross-react with its corresponding insulin antibodies, it serves as a convenient handle to measure β-cell secretory function, especially in diabetic patients with circulating antibodies in response to insulin therapy[34]. This technique has been applied clinically; serum C-peptide immunoreactivity (CPI) was unmeasurable in juvenile-onset diabetic patients and it correlated well with immunoreactive insulin levels[35]. In all patients, serum CPI increased during remission and decreased during clinical relapse[34].

Secretion. The process of insulin secretion involves the movement of the secretory granules, in which the conversion of proinsulin to insulin occurs[26], to the periphery of the cell through a microtubular-microfilamentous system[36]. The participation of such a system has been experimentally confirmed by studying the influence of a series of drugs, known to interfere with the function and structure of microtubules and microfilaments, upon the β-cells[37-39]. An abnormality of the microtubular system has also been suggested as a cause of the diabetic syndrome found in the spiny mouse[40].

A large variety of compounds serve as secretagogues for insulin and these include sugars, amino acids, hormones, cations, metabolic modifiers and hypoglycemic agents[41-48]. The regulation of this complex process is multifactorial and it plays an immensely important role in maintaining normoglycemia in animals[40]. Although the precise nature of the control mechanism is still far from being understood, two aspects of insulin release have been extensively studied. The first of these is how glucose, the primary secretagogue, stimulates insulin release. A number of investigators championed the theory that metabolism of glucose via pentose phosphate shunt[45,49] or glycolysis[50], may be coupled to excitation of the secretory process. Matschinsky and his co-workers, on the other hand, favored the hypothesis that glucose reacts directly with a glucoreceptor molecule located in the β-cell membrane that excites the secretory process[51]. They have also showed that the fuel and insulin-releasing functions of glucose in the islets can be dissociated by means of specific blockers[52]. The presence of a possible glucoreceptor has been demonstrated in the extracts of dog pancreatic islet membranes[53]. The second well-studied aspect of in-

sulin secretion is the requirement for Ca^{++} and cAMP. Malaisse and his co-workers have demonstrated an invariable relationship between the net uptake of Ca^{++} and the subsequent release of insulin in the isolated islets[54]. There is also a correlation between the rate of insulin secretion and the level of cAMP in the islets [55,56], and cAMP may exert its effect through the action of cAMP-dependent protein kinase[57].

Receptors. The initial observation by Cuatrecasas[58] that an insulin-agarose complex incapable of penetrating the cells showed biological activity has led to an outburst of reports on the isolation of insulin receptors from cell membranes of liver[59,60], fat[60,61], lymphocytes[62,63] and muscle[64]. The receptors appear to be glycoproteins containing sialic acid, which is intimately involved in processes transmitting the effects of insulin on glucose transport and lipolysis in the adipocytes[65]. The glycoprotein nature of the soluble receptor was further substantiated by the ease with which it binds lectins such as concanavalin A and wheat germ agglutinin[66].

The studies on the insulin receptor of human lymphocytes[63] led to the development of a sensitive radioreceptor assay[67] which complements the radioimmunoassay in measuring minute quantities of substances in plasma with insulin bioactivity. Using this technique clinically, a patient with a primary defect in insulin receptor deficiency was reported[68]. In the same study, acquired and reversible receptor deficiency was also observed in obese patients[68]. In the obese-hyperglycemic mutant mouse, insulin-resistance appears to correlate directly with an irreversible deficiency in insulin receptor activity on the adipocyte[69] and hepatocyte[70] plasma membranes. Cuatrecasas and his co-workers, on the other hand, reported the same number of insulin-binding sites in adipocytes from obese and insulin-resistant rats as were present in those from normal animals[71]. They proposed that the metabolic defect in these animals resides in the coupling of the signals of the insulin-receptor complex to glucose transport.

Mechanism of action. In addition to the well documented effects of insulin on the transport of glucose, amino acids and ions, and the metabolism of carbohydrates and lipids[72], it has also been reported that insulin regulates the synthesis[73] and activity[11,74,75] of a number of enzymes and stimulates protein[12,76] and nucleolar and extranucleolar RNA syntheses[14] in a variety of tissues. It is unlikely that insulin exerts its effects on such diverse activities by a common mechanism. There is, however, some experimental evidence suggesting that some of the effects of insulin can be attributed to changes in cellular concentrations of cyclic nucleotide messengers such as decreases in cAMP[77,78] and increases in cGMP[79].

Degradation. In a series of studies on insulin degradation[80-86], Varandani et al. proposed that insulin is degraded in a sequential manner: a preliminary splitting into A and B chains by glutathione-insulin transhydrogenase, which is followed by proteolysis of separated chains[82,83]. The transhydrogenase has been detected in a number of tissues from various species[80,81] and it is located in the microsomal membrane[84,87]. This enzyme has been purified from rat liver[86,89]. It degrades insulin via sulfhydryl-disulfide interchange between the disulfide bonds of insulin and a thiol donor; a variety of thiol proteins, in addition to glutathione, serve as effective

co-substrates for the purified enzyme[86]. This enzyme appears to be identical to the sulfhydryl-disulfide interchange enzyme[88]. A protease which specifically degrades insulin into small molecules has been purified and characterized in liver[89] and muscle[90,91]. This enzyme is sulfhydryl-dependent[90] and it shows relatively low activity toward proinsulin and various proinsulin intermediates[91]. Degradation of ^{125}I-insulin in the medium by incubated fat cells was also reported[92], but the nature of this degradation is not clear.

Other Endocrine Factors

Glucagon. An implication of glucagon in diabetes mellitus has long been suggested by its opposing biological effects to those of insulin. Recently, it has been reported that patients in diabetic coma frequently have very high concentrations of plasma glucagon[5]. Studies in the isolated perfused pancreas of genetically diabetic mice (db/db) also showed a pattern of non-suppressible excessive glucagon release, in spite of the high levels of endogenous insulin[93]. Buchanan and McCarroll observed in their investigation with newly diagnosed and untreated human diabetic patients that these patients responded to arginine infusion in glucagon secretion and concluded that insulin deficiency is the major cause of abnormal glucagon secretion in diabetes mellitus[94]. Gerich et al. confirmed these results but further demonstrated that, during insulin-induced hypoglycemia, plasma glucagon did not rise in juvenile-onset diabetic patients whereas glucagon in the controls rose significantly[95]. Thus an intrinsic alpha cell dysfunction may be operative in juvenile-onset diabetes mellitus; a common defect in the secretory apparatus in the alpha cells can be excluded since the diabetics still retain glucagon response to arginine infusion.

Potentially useful antidiabetic therapy with glucagon antibodies resulted from two lines of experimental evidence. First, Epand and Douglas observed that rabbits immunized with glucagon show hypoinsulinemia and yet they are normoglycemic and able to rapidly utilize intravenously injected glucose[96]. These results demonstrated that normoglycemia can be maintained with glucagon antibodies despite hypoinsulinism. Secondly, Stahl et al. reported the production of glucagon antibodies in human subjects[97].

Pituitary hormones. Increased serum levels of growth hormone have been reported in juvenile diabetic patients[98] and may be a causative factor in diabetic angiopathy[99]. The diabetogenic effects of growth hormone have been postulated to be mediated by an insulin-antagonistic peptide, somantin, which is released at the cell membranes of growth-hormone sensitive tissues[7]. Diabetogenic peptide(s) isolated from urine[100] and pituitaries[101,102] has also been reported to possess insulin-antagonistic effects. However, the significance of these findings to the etiology of diabetes remains to be demonstrated.

Hypothalamic factors. A growth hormone-release inhibitor (somatostatin) has been isolated from hypothalamic extracts of sheep and its primary structure determined[103]. This tetradecapeptide can suppress exercise-induced plasma growth hormone rise in normal and diabetic subjects[99]. However, the therapeutic potential of somatostatin in treating diabetic

angiopathy is complicated by the observation that it also inhibits insulin secretion by acting directly on the β-cells[104].

Diabetic Complications

Glomerular basement membrane. Diabetic renal lesions include the thickening of the peripheral glomerular-capillary basement membrane and an increase in the mesangium, the interstitial tissue between glomerular capillaries. Spiro et al. reported a marked increase in hydroxylysine content and in the number of glucosylgalactose disaccharide units in basement membrane isolated from diabetic human glomeruli[105]. They also attributed these changes to high levels of glucosyltransferase, the enzyme responsible for attaching glucose onto basement membrane glycoproteins[106]. Kefalides, however, reported no significant increase in the amount of hydroxylysine or in the hydroxylysine-linked disaccharide in diabetic human kidneys[107]. The difference in the results obtained by these two groups of investigators may be due to the degree of contamination in the basement membrane preparations by the surrounding epithelial or endothelial cells; the cause for the thickening of glomerular basement membrane remains to be determined.

Sorbitol metabolism. Excessive sorbitol formation from glucose has been suggested as a cause for diabetic complications, especially neuropathies[108] and cataracts[9]. One approach to the control of these diabetic complications would be to limit sorbitol production using inhibitors of aldose reductase, the enzyme which converts glucose to sorbitol. A study in streptozotocin-induced diabetic rats with AY-22,284 (I), an orally active aldose reductase inhibitor, has been reported to be successful[109].

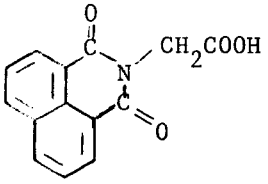
Oral Hypoglycemic Agent

Sulfonylureas. Extensive clinical studies have been carried out with several highly potent sulfonylureas, i.e. Glyburide (HB-419, U-26452, II)[110,111] glipizide (K-4024, III)[112-114], and glibornuride (Ro 6-4563, IV)[115,116]. A new highly effective sulfonylurea, gliclazide (S-1702, V), showed some unique pharmacological properties in animal studies[117,118]. It inhibits platelet aggregation and induces disaggregation. It also shows fibrinolytic properties and diminishes the permeability of capillaries. The antidiabetic action of sulfonylureas is still attributed mainly to the stimulating effect on insulin release. However, several extrapancreatic activities have been demonstrated with tolbutamide. It was found to alter intracellular pattern of carbon flow[119], and to inhibit cAMP phosphodiesterase[120]. The significance of these findings to its therapeutic action in the treatment of mild adult-onset diabetes remains obscure.

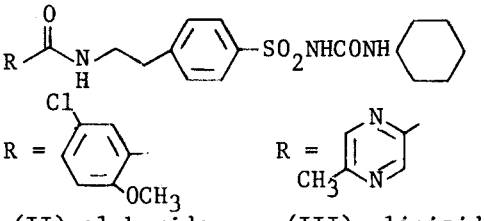
Biguanides. Mechanism of antidiabetic action of biguanides has been attributed to the inhibition of glucose absorption[121], of endogenous triglyceride production[122] and of digestive absorption of lipids[123]. A differential inhibition of phenformin on gluconeogenesis in normal and diabetic rat livers was also reported and it is paralleled by the selective hypoglycemic action of phenformin in diabetic subjects[124]. In a series of studies, Losert et al. conclusively demonstrated the two distinct hypoglycemic

effects of biguanides[125]. At high doses, biguanides decrease cellular ATP/AMP ratio by inhibiting respiration; as a result, anaerobic glycolysis increases and gluconeogenesis decreases. This explains the hypoglycemic action of biguanides in alloxan-diabetic animals. On the other hand, the therapeutic effect of chronic administration of low doses of biguanides in maturity-onset diabetes is mediated mainly by potentiating insulin action on glucose uptake and oxidation in peripheral tissues.

Others. An iminopyridine, MK-270 (VI), has been reported to be orally active in diabetic subjects not well controlled with sulfonylureas[126]. 3-Methylisoxazole carboxylic acid (VII) showed a short-term hypoglycemic effect in maturity-onset diabetic patients by inhibiting lipolysis and reducing plasma free fatty acid levels[127]. Diisopropyl ammonium dichloracetate is effective in lowering blood sugar and clearing ketosis in insulin-deficient diabetic rats[128]. Several new classes of compounds were also reported to possess oral hypoglycemic activity in animals and these include camphidines (VIII)[129], quarternary 4-(5-)azolylpyridazinium salts (IX)[130], cycloalkyl lactaminides (RMI 11,894, X)[131], monoguanidines[132], 2-piperazino-4-(3H)-quinazolinone monoacetate (XI)[133] and diphenyleneiodonium (XII)[134]. (+)-Decanoylcarnitine inhibits ketone body formation and the combined therapy of insulin and (+)-decanoylcarnitine may yield great benefits to patients with diabetic ketoacidosis[135].

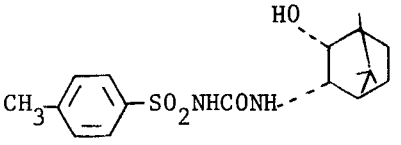


(I) AY-22,284

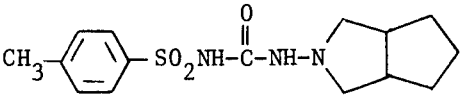


(II) glyburide

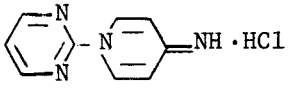
(III) glipizide



(IV) glibornuride



(V) gliclazide



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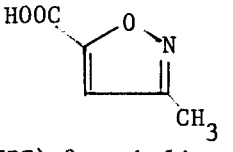
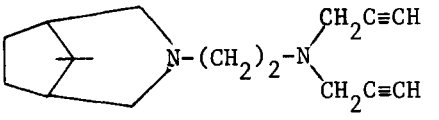
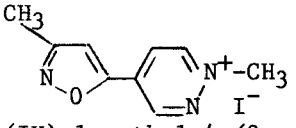


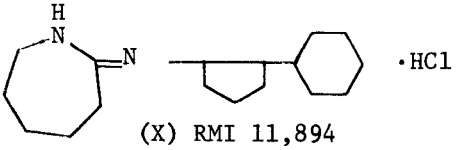
(VII) 3-methylisoxazole carboxylic acid



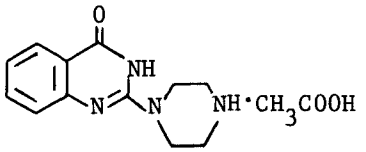
(VIII) 3-(2-dipropargylaminoethyl)camphidine



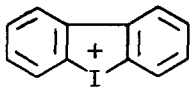
(IX) 1-methyl-4-(3-methyl-5-isoxazolyl)pyridazinium iodide



(X) RMI 11,894



(XI) 2-piperazino-4(3H)-quinazolinone monoacetate



(XII) diphenyleneiodonium

UGDP studies. The controversial reports by the University Group Diabetes Program relating the treatment of diabetes with hypoglycemic agents to the incidence of vascular complications[136] continue to draw criticisms[137,138] and defenses[139,140]. A more cautious assessment of the cause-effect relationship has also been offered[141,142].

Insulin and oral agents. A comprehensive review on the physiological, pharmacological and clinical aspects of insulin and oral hypoglycemic agents has been published in two parts[143].

Spontaneous Diabetes in Laboratory Animals

Study of human diseases in small laboratory animals with similar pathological features offers many advantages, especially in those diseases which involve both genetic and environmental determinants as in diabetes mellitus. These animals have relatively short generation time which facilitates the study of the mode of genetic transmission of the disease. The interplay of genetic and environmental factors in pathogenesis may also be unraveled by manipulating both the genetic and physical backgrounds of the animal population. The intensive search for an ideal animal model for human diabetes in the past few years has resulted in the discovery of spontaneous diabetes in many species of animals. The striking resemblance of the diabetic features in some of these animals to those in humans greatly enhances the prospects for understanding the pathogenesis of diabetes and for discovering novel approaches to treat the disease.

Mus musculus. Several strains of mice exhibit symptoms similar to diabetic humans. Severe diabetes, characterized by marked hyperglycemia, hyperphagia, and islet degeneration, was produced by mutant genes db and db^{2J} on the C57BL/KsJ background[144,145]. In contrast, mild diabetes with transitory hyperglycemia and islet hypertrophy was found in animals produced by mutant genes db^{2J} and ob on the C57BL/6J background[145,146]. The markedly different syndromes resulting from the same gene, db^{2J}, in two closely related inbred strains point to the importance of genetic background on the expression of the mutant gene. Other mutant strains showing diabetic symptoms include the New Zealand Obese (NZO)[147,149], the yellow KK[150] and the KK[151].

Cricetulus griseus. Considerable data have been accumulated in the epidemiology, genetics, physiology, morphology and biochemistry of the diabetic syndromes in the Chinese hamsters[152]. Both juvenile- and maturity-onset types have been observed. The complexity in the mode of genetic transmission of the disease and in the diabetic syndromes in this animal closely

resembles that in humans.

Others. A colony of Acomys cahirinus (spiny mouse) showed glucose intolerance, obesity and defective insulin secretion[153]. Latent diabetes has been reported in Psammomys obesus (sand rat) which developed hyperglycemia and obesity on caloric loading[154]. Spontaneous diabetes also arises in other animals such as Macaca nigra (black Celebes apes)[155], Mystromys albicaudatus (South African Hamster)[156], Meriones unguiculatus (Mongolian gerbil)[157], and Cavia porcellus (guinea pig)[158].

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Chapter 20. Non-steroidal Antiinflammatory Agents

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Introduction - Many new agents continue to appear, some in advanced clinical trials, but the most exciting developments are in the advancing understanding of the interrelationships and roles of immunological factors, prostaglandins and control of degradative enzyme release. Several excellent texts[1-3] reviews and symposia[4-8] have appeared which summarize these trends in inflammation and non-steroidal antiinflammatory agents (NAA).

Pathogenesis - The continued inquiry into the nature of infections of bacterial and viral origin and their involvement in initiating degenerative diseases[9] has been useful in identifying a number of potentially answerable questions which encourage speculation and provide direction for future research. Speculation continues that slow viruses (myxovirus) may cause a variety of degenerative diseases including rheumatoid arthritis by inducing persistent new antigens.[10,11] Additionally, there are studies which show that mice systemically infected with Mycoplasma pulmonis develop cellular hypersensitivity to synovial tissue[12] and that rabbits, given a single intraarticular injection of herpes simplex virus, develop a chronic explosive arthritis.[13] Yet, detailed studies have failed to demonstrate the presence of mycoplasmas, viruses or a transmissible agent in human rhematoid synovial tissue[14] or in cell cultures derived from nodular tissue, cartilage or synovia of patients with arthritic diseases.[15,16]

Perpetuation of the inflammatory response by a variety of immunologic mediators such as rheumatoid factor,[17] lymphokines[18] and complement[19] remains of great potential importance and its relationship to other facets of inflammation continue to be elucidated. More recent findings have shown that cellular immune mechanisms, while active in the synovial lesion of rabbits with antigen-induced synovitis,[20] are not necessarily active in humans with rheumatoid arthritis as has been demonstrated by an anergy to delayed hypersensitivity skin tests in almost half of 84 rheumatoid arthritic patients tested with 5 different antigens.[21] Other studies have shown that a demonstrable inverse relationship exists between the percentage of blood T-cells and clinical disease in rheumatoid patients,[22] that the majority of small lymphocytes which infiltrate the rheumatoid synovium are B-cells,[23] and that microtubular subunits are essential for lymphocyte-mediated cytotoxicity thus adding another example of modulation of this phenomena by cyclic AMP.[24] Finally, an interesting in-vitro model designed to mimic cartilage destruction in the rheumatoid joint has demonstrated that human leukocytes release neutral proteases which are capable of degrading the non-collagenous protein mucopolysaccharide matrix of cartilage.[25]

Current knowledge and newer concepts of immunology[26-28] as well as supportive evidence of a pathogenetic concept of rheumatoid arthritis[29] lend direction and promise to future research efforts into areas

concerned with antibody, delayed hypersensitivity, immune complex formation, complement activation, chemotaxis, leucocytic infiltration and release of tissue-destroying enzymes.

Mechanism of Action - Studies on the effects of existing drugs on deleterious intracellular enzymes indicate that prostaglandins, cyclic AMP, and catecholamines inhibit, but cyclic GMP and cholinergic agents enhance lysosomal enzyme release from rat tissues,[30] or guinea pig leucocytes[31] while colchicine and gold can inhibit degradative neutral proteases released from human leucocytes.[25] Other biochemical effects of the NAA include: a lack of inducing effects on rat hepatic tryptophan pyrrolase, an enzyme thought to play a role in the antiinflammatory action of steroids;[32] a profound effect on glycolysis in tissue culture;[33] and an ability to restore the impaired serum SH-SS interchange reaction of adjuvant arthritic rats.[34]

Involvement of the prostaglandins (PG) in the inflammatory process revolves around 3 major areas of activity. (a) An inflammatory effect: when PGE_1 is injected intradermally in volunteers, a cutaneous inflammatory reaction characterized by a moderate mononuclear cell infiltrate is apparent.[35] PGE_1 also lowers the threshold of human skin to histamine-evoked itching,[36] and potentiates the effects of bradykinin on vascular permeability in the rat skin.[37] Similar effects on bradykinin or histamine are obtainable with PGE_1, E_2, A_2 and $F_{2\alpha}$ in guinea pig skin.[38] The involvement of PGs in periodontal and eye inflammations and their general role as mediators of inflammation was reviewed.[39] (b) Anti-inflammatory effects of the PGs: histopathologic studies of joints from rats with adjuvant arthritis show complete suppression of the disease when rats are treated prophylactically with PGE_1. The established disease is also favorably affected.[40] Further investigation of this activity shows that at least part of this effect is not mediated by the adrenal-pituitary axis, but may be due to inhibition of chemotaxis and/or inhibition of lysosomal enzyme and inflammatory mediator release.[41] (c) Inhibition of PG biosynthesis as a mechanism of the analgesic activity of NAA : intrasplenic injection of bradykinin in the dog increases the concentration of endogenously released PGs resulting in facilitation of hyperalgesia. Inhibition of this response by indomethacin is proposed as a mechanism of the analgesic activity of NAA.[42]

A review of the role of the PGs in inflammation and a discussion of the various sensitivities of different tissue synthetases to NAA, underscores the fact that only the most preliminary aspects of this fascinating subject have been uncovered.[43] It has been speculated that the inhibition of PG synthetase of brain, but not of several peripheral tissues, by acetominophen is responsible for its analgesic effect and lack of antiinflammatory activity, and serves to suggest new approaches in the search for more specific agents.[44] (See Chap. 17 for additional discussion).

Test Systems - The development of more valid systemically induced immunopathologic model systems of human disease suitable for detection of new therapeutic agents, remains of primary importance. Some inroads

were accomplished in investigating established models but no significant new system was evident.

The essential role of certain complement components in the adjuvant-induced arthritic (AIA) rat was studied in cobra venom factor - treated animals,[45] as was impaired liver microsomal drug metabolism in the AIA rat and the pitfalls of new drug testing in animals with impaired liver function.[46] Observations also indicate AIA rats to be more sensitive to the ulcerogenic potential of indomethacin during the later stages of the disease,[47] that polyarthritis can be induced in rats by the intra-lymph node injection of a variety of cell wall preparations from gram-positive bacteria,[48] that a paradoxical increase in polyarthritis induced in rats by Mycoplasma arthritides occurs with salicylate treatment,[49] and that arthritis due to mycoplasma in rats and swine is not to be considered a specific model of the human condition.[50] Oral feeding of 6-sulfanilamidoindazole and intravenous endotoxin induces both local and generalized Shwartzman reactions in rats,[51] and may serve as a unique model for further exploration with drugs. Using experimental allergic encephalomyelitis (EAE) in guinea pigs as a model, it was shown that human serum contains a component capable of neutralizing the ability of the highly antigenic encephalitogenic protein which hypothetically might be released after brain injury.[52] A localized form of hyperacute EAE which was produced in rats within 1 day of passive transfer,[53] might conceivably be adopted as a rapid screen.

Locally induced models of rheumatoid joint disease received considerable attention this year. Chronic arthritic knee joints were produced[54] and treated with antibiotics[55] in rabbits and monkeys who received intra-articular injections of bedsonia isolated from a patient with Reiter's syndrome, in rabbits receiving cell free extracts of group A streptococci[56] or the membrane disrupting antibiotic filipin,[57] in guinea pigs or rabbits administered immune complexes,[58,59] and in dogs given M. butyricum in Freund's adjuvant.[60] Acute arthritis was induced in sensitized rabbits by the intra-articular injection of horseradish peroxidase,[61] and in chickens with sodium urate or calcium pyrophosphate which were then treated with colchicine.[62] Clearance of radio-labeled technetium was used as a non-invasive measure of joint inflammation in rats, rabbits and humans.[63]

The effects of several agents on an experimental model of hyperuricosuria and gouty nephropathy in the rat were described.[64] The relationship between the ability of an agent to displace urate from protein in vitro, and uricosuric properties in vivo was proven by clinical testing.[65] This represents a rare and extremely valuable validation of an experimental hypothesis and it is hoped that further such studies will appear in the future.

Miscellaneous test systems of more than routine interest included a test for topical agents on external ocular inflammation in rats,[66] a test for immunosuppressants in localized graft-versus host in rats,[67] two in vitro systems for assaying granulocytic chemotaxis[68] or

macrophage phagocytosis and cytotoxicity[69] using radiolabeling techniques, a short-term clinical screen utilizing patient preference,[70] and two clinical assays using suppression of tetrahydrofurfuryl nicotinate-induced skin erythema,[71] or analgesia[72] as the criterion for antiinflammatory activity.

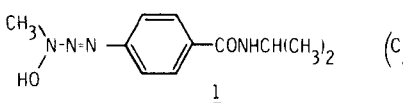
Compounds Under Investigation

Immunosuppressants - Encouragement from recent disclosures concerning selective effects of both antigens and drugs on cell populations responsible for the production of circulating antibodies (B cells) and those involved in cellular immunity (T cells)[73, 74] have brightened the prospects for uncovering useful, specific immunoregulants. A number of immunosuppressants were tested in guinea pigs and mice for their differential effects on B and T lymphocyte functions[75,76] and the selective effect of cyclophosphamide was assessed in patients having a variety of immunopathic diseases,[77] rheumatoid arthritis,[78] or multiple sclerosis.[79] Contradictory results in both species indicate that more studies, using similar procedures are required before definitive conclusions can be reached. It appears, however, we are succeeding in more accurately defining the different effects of agents on basic immune mechanisms and further efforts to apply these findings clinically may provide the key to development of superior therapeutic agents.[80]

The clinical use of immunosuppressant drugs in nonmalignant disease[81-83] and their inherent dangers,[84-86] underscore the need for more specific agents. The therapeutic usefulness of cyclophosphamide, azathioprine and 6-MP in systemic lupus, polymyositis,[87] rheumatoid[88-90] and psoriatic arthritis,[91] is apparent but is severely limited by side effects.

Chemical, pharmacological and pharmacokinetic properties of immunosuppressants to a great extent determine their relevance for future use in man.[80,92,93] Oxisuran,[94] the selective suppressant of cell mediated immunity,[95] was found to be rapidly absorbed, negligibly bound to plasma proteins and to have a longer half-life in man than the rat or dog. Interestingly, the differential immunosuppressive properties of oxisuran reside in the reduced metabolites, the alcohols, which are found in higher concentrations in man than the dog or rat.[96]

One of a series of arylhydroxytriazenes, PR-H-286 BS (1) was effective in both humoral and cell-mediated immune responses and in rat adjuvant-induced arthritis.[97] The orally active antiviral agent tilerone (2), effectively inhibited symptoms of adjuvant arthritis and allergic encephalomyelitis in rats but paradoxically, it potentiated the humoral immune response to the thymus dependent and independent antigens as well as to homocytotropic antibody.[98]



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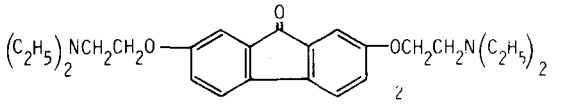

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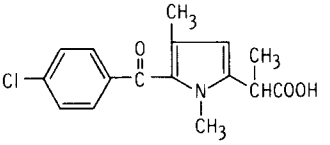
Arylalkanoic Acids and Related Compounds - In a number of clinical studies the therapeutic ratios for newer agents were compared to the standard drugs. In a 16 week double-blind crossover study in 30 patients with rheumatoid arthritis (RA) aspirin was slightly more effective than fenoprofen but substantially more toxic.[99] Using a new trial designed to minimize the unpredictable course of RA, naproxen (400-500 g daily) was found effective without significant gastrointestinal side effects in 117 patients.[100] Ketoprofen (Orudis, RP-19583, 100 mg daily) had significant activity with few side effects in 14 patients with RA and had no effect on the microsomal drug-metabolizing enzymes of the liver as shown by lack of effect on antipyrine clearance.[101] In a double blind crossover study in 35 patients with RA, the analgesic and antiinflammatory activity of 150 mg daily of ketoprofen was superior to 1,200 mg of ibuprofen.[102] Aspirin antagonized the effects of various NAA in the arthritic rat,[103] and lowered the plasma concentrations of fenoprofen and indomethacin in humans,[104] and naproxen in the rat,[105] again emphasizing the need to assess the presence of salicylates in the clinical evaluation of new agents, particularly aryl acids.



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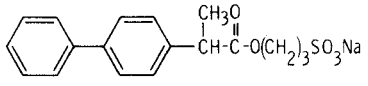

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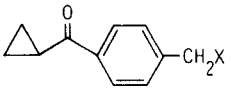
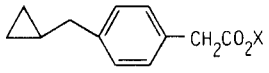
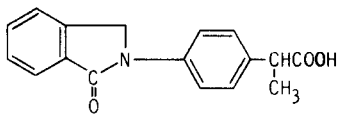


X = CO_2H, CO_2Na 5



X = H, Na

An analog of tolmetin,[106] McN-2981 (3) was found to have similar antiinflammatory properties to the parent drug.[107] The antiinflammatory[108] and analgesic[109] properties of clonixin,[106] were demonstrated in rats and monkeys. Despite initial clinical failure, 6/137 (4) had sufficient activity and safety in animals to warrant further testing.[110] Small ring compounds synthesized in the hope of altering metabolism and thus biological profiles, resulted in development of several active agents (5) in the potency range of phenylbutazone.[111]



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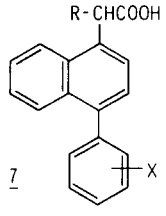

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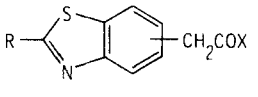

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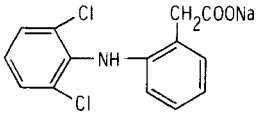
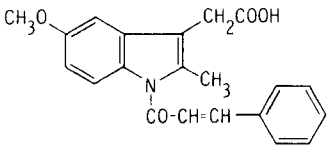


9

K 4277 (6)[112] and the naphthaleneacetic acid derivatives (7),[113] were more potent than phenylbutazone when compared in several pharmacologic assays. The benzothiazolacetic acids (8),[114] were less potent, while GP 45840 (Voltaren, 9),[115] was as potent as indomethacin but much less acutely toxic in mice.

Heterocyclic Alkanoic Acid Derivatives - Less toxicity and potency than the parent indomethacin was reported for the analogs cinmetacin (10),[116] and 11,[117] in rodents.



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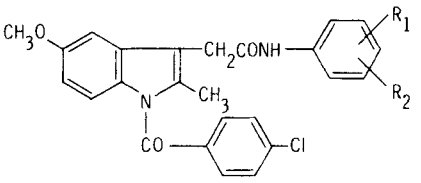

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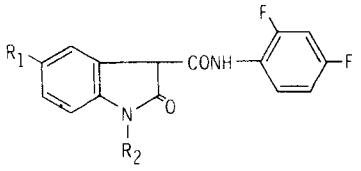
Heterocyclic Carboxamide Derivatives - In the carrageenin rat paw assay carboxanilides of the oxindole ring (12) demonstrated antiinflammatory activity somewhat less potent than indomethacin, having long dog plasma half-lives.[118]



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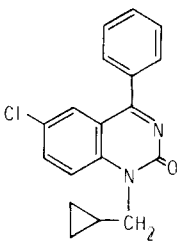

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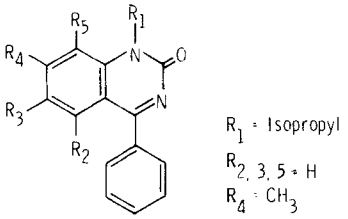


14

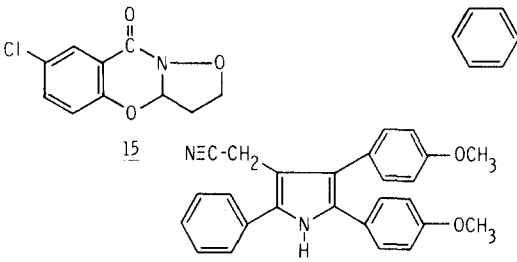
R_1 = Isopropyl
$R_{2,3,5}$ = H
R_4 = CH_3

Quinazolines - SL-512 (13) caused minimal gastrointestinal toxicity, was equipotent to phenylbutazone in acute models of inflammation, and was less effective in chronic models such as the adjuvant rat.[119] Comparisons of 14 with indomethacin and phenylbutazone (most potent analog is shown) were sufficiently favorable to warrent further testing of members of this series.[120]

Salicylates - Gastrointestinal Side Effects - Benorylate (6 g daily) was superior to aspirin (4 g daily) as an antiinflammatory agent with less gastrointestinal side effects in 33 outpatients with RA.[121] Previously

described tolerance in dogs to the gastrointestinal side effects of aspirin was reinvestigated in 9 volunteers who showed no evidence of such tolerance during a 4 week test period. However, a daily dose of 4 or 5 g aspirin induced a uniform rate of gastrointestinal blood loss.[122] Using a labeled erythrocyte assay, fenoprofen and aspirin at equipotent doses caused measurable gastrointestinal bleeding; bleeding was significantly less with fenoprofen.[123] When the labeled erythrocyte method was modified for the rat, aspirin (321 mg/kg) and phenylbutazone (155 mg/kg) but not seclazone (W-2354, 15, 321 mg/kg) were shown to increase gastrointestinal bleeding.[124] There was less gastrointestinal blood loss from sodium salicylate tablets than from comparable doses of aspirin over a 7 day period in 13 volunteers in a crossover study.[125] The continued interest in the gastrointestinal side effects of NAA emphasizes the difficulty which exists in attempting to dissociate activity from toxicity. The continuing effort to quantitate this side-effect in man is a welcome addition to laboratory studies which thus far have not been as predictive as desired.

Miscellaneous Agents - The potent antiinflammatory activity of U-24568 (16) correlated closely with plasma concentrations in arthritic rats.[126] In a double-blind multicenter trial Abbott 29590,[94] (1200 mg/day was equivalent to aspirin (3900 mg/day) in the control of RA in 107 patients.[127] The pharmacologic properties of seclazone (15) are marked by lack of ulcerogenic activity in rats and diuretic activity in rats and beagles.[128] Seclazone is almost completely metabolized to 5-chlorosalicylic acid in the rat, dog and rhesus monkey.[129] The oxazole, ditazol (17), possesses potency similar to that of aspirin, has a low level of toxicity, an absence of gastrointestinal irritation in rats and dogs, and a long half-life in man.[130]



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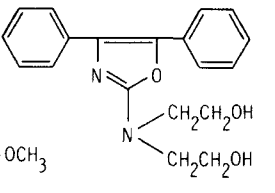

17

Disease activity was suppressed in an uncontrolled study in seven patients with RA treated with penicillamine; initial doses of 300 mg/day were increased to 1.8 g/day. Side-effects eventually demanded cessation of treatment.[131] At doses up to 1.5 g/day, penicillamine resulted in significantly lowered serum IgG and IgM but not IgA levels at 3 and 6 months in patients with RA, suggesting that at least part of its immunosuppressive effect is at the cellular level, and may be specific for cells which produce certain classes of immunoglobulins.[132] Radiation synovectomy with intra-articular injections of yttrium-90 in 44 rheumatoid knee joints benefited 50% of the patients, however the long-term danger

of this therapy is unknown.[133]

Natural Products and Related Substances - A basic peptide fraction of bee venom exhibits powerful antiinflammatory effects in the carrageenin paw and adjuvant rat tests;[134] whole bee venom is ineffective in adjuvant arthritis in adrenalectomized rats;[135] lymphocyte chalone concentrate from spleen exhibits immunosuppressive properties;[136] vitamin A stimulates humoral and cell-mediated response[137] and vitamin E exerts antiinflammatory effects in rats and on human skin.[138] Improved methods of purification, preparation and characterization of immunosuppressive material derived from bovine serum,[139] antiinflammatory material from human plasma,[140] or gram-negative bacteria (Pseudomona aeruginosa),[141] were described.

Gold - In a multiclinic trial by the Cooperating Clinics Committee of the American Rheumatism Association, suggestive evidence indicated beneficial effects for RA patients treated with gold thiomalate but the overall therapeutic effects were disappointing, perhaps because of the small number of patients who completed the study.[142]

Comment - Some initial advances in 1973 towards a more scientific approach in the search for new antiinflammatory agents suggest an end of the "me too" era. The recognition of, and specific testing for, compounds capable of individually affecting T and B cells, heralds the development of a long-sought-for class of selective, and thus safer "immunoregulants" which are effective against the basic aspects of inflammatory disease, i.e. the immunologic component. Similarly, the recently disclosed description of substances affecting specific inhibition of various prostaglandin synthetases should speed up the development of superior NAA at a rate more responsive to the needs of research and medicine. Initial clinical reports on several of the agents described in this and past reviews are encouraging and will continue to influence the direction of future endeavors.

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Chapter 21. Cyclic Nucleotides and Drug Discovery

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The explosive growth of cyclic nucleotide research is significantly advancing our understanding of a variety of diseases and of the mechanisms of action of both new and old drugs. In drug discovery, it is opening up important new opportunities for the design of new agents expected to act *via* regulation of cyclic nucleotides, particularly as evidence of their apparent involvement in an ever-expanding number of disease states accumulates and greater understanding of the factors responsible for controlling their intracellular concentrations is achieved. Primarily because of space limitations only representative examples of research in this area directly concerned with drug discovery will be discussed in this article. References to other pertinent articles can be found in the three already published volumes of *Advances in Cyclic Nucleotide Research*[1] and the proceedings of two symposia on cyclic nucleotides in disease[2,3].

The role that cyclic nucleotides may play in the etiology and/or maintenance of a number of disease states is becoming clearer. In asthma, the ability of patients with this disease to excrete increased amounts of cyclic AMP in urine in response to epinephrine is greatly diminished, possibly due to a disease-related decrease in the sensitivity of adenylyl cyclase (AC) to the catecholamine[4]. This lack of AC sensitivity however could be reversed by corticoid therapy[5,6] in accord with the long established permissive effects of these hormones on cyclic AMP synthesis. In nephrogenic diabetes insipidus, diminished urinary production of cyclic AMP in response to ADH was also reported[7]. Other conditions that may involve deficient cyclic AMP production include hypertension[8], psoriasis[9] and stroke[10]. On the other hand, cyclic AMP production appears to be excessive in mania[11], cardiomyopathy[12], Down's Syndrome[13], epilepsy[14,15], thyrotoxicosis[16], magnesium deficiency[17] and diabetes[18,20]. Cyclic GMP, which appears to share with cyclic AMP the secondary messenger function in many systems[2,3,21], also appears to be elevated in psoriasis[9] and hypertension[22-24]. Clinically the determination of cyclic nucleotide levels in biological fluids is beginning to yield promise as a diagnostic tool for a variety of diseases[25] and as a reliable indicator of fetal health[26].

Cyclic nucleotides now appear to be involved in the control of sperm activity and motility[27] and sperm capacitation[28] and as mediators of viral infection[29,30], cellular growth[31] (including that of bacteria[32]), the immune response[33-36], inflammation[37], anaphylaxis[38,39], histamine release[40,41], hypersensitivity[42,43] and neurotransmitter release[44,46], among the many other important cellular processes previously known. The potential of the cyclic nucleotides or their derivatives as drugs is already apparent in many areas including contraception[47], radioprotection[48,49], inhibition of drug metabolism[50] and antagonism of anesthetics[51] to mention a few.

Although cyclic AMP and cyclic GMP appear to mediate antagonistic events in many systems support for the simplistic view that they always have antagonistic functions is dwindling. Newer studies appear to stress the independent role played by each cyclic nucleotide in the mediation of specific cellular responses. Thus, the demonstration of the existence and reciprocal responsiveness of cyclic GMP in a particular system is no longer sufficient to assign to it merely a role as an antagonist of cyclic AMP in that system.

The sites at which appropriately designed drugs could act to alter cyclic nucleotide-mediated events have been previously outlined[52] and are shown in the accompanying figure 1. The sites of possible drug action are enumerated in the figure and will be discussed in the same order as they appear therein.

I. CONTROL OF THE INTRACELLULAR LEVELS OF CYCLIC NUCLEOTIDES

A. Synthesis: The synthesis of each of the two naturally-occurring cyclic nucleotides is catalyzed by a specific cyclase that uses the corresponding nucleoside triphosphate (ATP or GTP) as substrate. The activities of these cyclases determine to a great extent the intracellular levels of these messengers. Consequently, stimulation of the cyclases represents an important site for possible drug effects (Site 1) on this system. Stimulation of the cyclases has long been limited to only hormones and their immediate derivatives since these cyclases appear to represent the membrane sites for hormonal regulation and have consistently demonstrated marked specificity to certain hormones in target tissues. Although some drugs, *e.g.*, isoproterenol, are known to stimulate the cyclase enzymes, these for the most part have represented only minor modifications in the structures of the parent hormones. However, compounds less closely related to the natural hormones now appear to be capable of cyclase stimulation in a variety of tissues, enlarging the possibilities for drug discovery. Among these are a group of insecticides including α-chlordane and heptchlor[53] on liver AC, tolbutamide and ethanol on pancreatic islet AC[3,54] and the long described effects of fluoride ions. Similarly direct inhibition of the activity of the cyclases is an important site for the control of the intracellular cyclic nucleotide levels (Site 2). Lithium ions were reported to directly inhibit AC in brain[55], platelets[56] and thyroid[57]. Valinomycin[58] and possibly other antibiotics as well[59,60] also appear to inhibit AC. Adenosine acts at least partially *via* a similar mechanism[61,62]. The β-adrenergic blocker propranolol also inhibited AC in cat myocardium[63] and turkey erythrocytes[64], suggesting that AC inhibition may underlie its membrane effects unrelated to β-adrenergic receptor blocking activity. Sotalol, a β-adrenergic receptor blocker devoid of these membrane effects, did not inhibit AC under similar conditions. Alloxan[65] and ethacrynic acid[66] apparently act *via* AC inhibition in the pancreas and kidney, respectively. There are indications from studies with brain tissue *in vitro* that cobalt ions may inhibit guanylyl cyclase (GC) activity[3]. In addition to either stimulating or inhibiting cyclase activity, blockade of enzyme stimulation by natural stimulants represents a major site for the action of potential drugs (Site 3). Hormone antagonists[67-69] and β-adrenergic[70],

as well as α-adrenergic, blockers[71] probably act at this site. In general, compounds that are capable of antagonizing hormone-induced effects in intact systems are also capable of antagonzing hormonal effects on the cyclic nucleotide system. This applies equally well to those derivatives of peptide, steroid and other hormones that antagonize hormonal effects. Frequently, hormonal derivatives devoid of hormone-like actions fail to induce responses similar to those induced by the active hormones on the cyclic nucleotide system.

Studies continue to advance the understanding of the mechanisms involved in cyclase activation and/or inhibition. It now appears that net AC activity represents an equilibrium between two forms of the enzyme, a phosphorylated inactive and a dephosphorylated active form[72]. Phosphorylation of AC is catalyzed by a cyclase kinase, the properties of which are under investigation and may hold the answer to many questions related to the actions of drugs on AC activity. It appears, for example, that fluoride and prostaglandins (PDE's) activate AC by shifting the cyclase kinase reaction toward the active, dephospho-form. Other drugs stimulating or inhibiting cyclase activity may well be expected to act on the cyclase kinase shifting the reaction toward the phospho- or the dephospho-forms, respectively. It is also possible that GC may be similarly regulated.

Studies on AC sensitivity *in vivo* are more difficult and less conclusive because of the complex patterns of interacting factors affecting the AC response. Ethanol is thought to induce lower AC activity in certain areas of the mouse brain mainly by inhibiting adenosine release. Although ethanol does not seem to affect AC activity directly, its *in vivo* effects could be easily mistaken for a direct effect on the enzyme.

A hypothetical, three component model for AC was proposed and appears to fit well with the known aspects of the enzyme action[73]. According to this model the enzyme is composed of a discriminator, a transducer and an amplifier. The discriminator unit is that part exposed on the exterior surface of the cell and may be indistinguishable from the long sought hormonal receptor. It embodies the specific structural features necessary for the recognition of and binding with the natural hormone. The amplifier unit is that part facing the cell interior which catalyzes the conversion of ATP to cyclic AMP. It is also called the catalytic subunit. The discriminator or receptor unit is coupled to the amplifier or catalytic unit *via* the transducer or coupling unit. This latter subunit transmits the information from the receptor after hormonal interaction to the amplifier unit leading to increased or decreased catalytic activity. Although the structural requirements for interaction with the receptor subunit are well-defined in many systems and the cofactor and other requirements for optimal activity of the catalytic subunit are also well-identified, little, if any, information is available about the structure and/or function of the transducer or coupling subunit. It appears to be the locus where cation interactions with the AC system occur. Furthermore, most disease-related aberrations in the cyclic AMP system seem to be closely-related to abnormal function of this subunit, since it has the main role of regulating the overall responsiveness or sensitivity of the entire system.

Drugs could act on any of the three AC components. As examples, most hormones appear to act at the discriminator, Ca++ at the transducer and F^- at the amplifier level. The situation with GC may be more complex since its activity is not limited to cellular membranes and soluble GC activity appears to exist in a number of tissues[1].

The sensitivity of AC and possibly also GC to stimulation by natural hormones appears to be an important component of a number of disease states, including hypertension[8], leukemia[74], hepatoma[75,76], psoriasis[78], asthma[79] and possibly aging[80-83]. In addition, decreased AC responsiveness may underlie the process of carcinogenesis[84], bacterial infection[85], and possibly morphine[86] and alcohol dependence[87]. In all these conditions, the factor (or factors) essential for the optimal function of the coupler unit apparently involved in this loss of cyclase sensitivity is (are) unknown. The importance of the permissive or maintenance hormones, in particular growth, thyroid and steroid hormones in maintaining AC and/or GC sensitivities should be explored. GTP also appears to be an obligatory requirement in the stimulation and inhibition of AC activity in platelets[88], rat anterior pituitary gland[89], adipose tissue[90,91], and liver[92], apparently by enhancing the dissociation of the active ligands after action on the enzyme[93]. Many other factors appear to influence the sensitivity of AC to stimulation but their exact mechanisms are less understood. These factors include a reversible macromolecular inhibitor[94], phospholipids[95,96], adenosine[97], corticoids[98], fatty acids[99] and the prostaglandins. The possible involvement of these and other factors in disease-associated loss of cyclase sensitivity represents an important area for future research toward understanding of the disease process as a whole and may also provide additional insights into new mechanisms for drug action.

Another important factor that should be stressed is that AC sensitivity also appears to reflect to a certain extent the recent history of the enzyme. Perkins[2] showed that brief exposure of human astrocytoma cells in culture to concentrations of norepinephrine resulted in decreased responsiveness of the same cells to the addition of the same or higher concentrations of the hormone. This loss of AC sensitivity is ligand-specific since the response to PGE_1 is unaltered after exposure to norepinephrine. The responsiveness of these cells to PGE_1 is, however, reduced after brief exposure to the prostaglandin while the response to norepinephrine remains intact. The exact mechanisms involved here are unknown.

B. Loss to Extracellular Fluid: The loss of the cyclic nucleotides from cells is an important factor in the control of their intracellular levels. Released cyclic nucleotides can be a valuable indicator of hormonal activity[100] since they reflect the "free", metabolically-active pool(s)[101]. The system regulating cyclic nucleotide leakage represents an important site for drugs that can act by stimulating or inhibiting the release of cyclic nucleotides to the extracellular fluids and thus appreciably affecting the size of their free active pools (Site 4). Whether the same factors are involved in the transport of the cyclic nucleotide into the cells[102] is not at all clear. Present evidence seems to indicate that loss of cyclic

nucleotides to extracellular fluid accounts for the readily-assayable levels of these nucleotides in plasma and to the assignment of a possible primary messenger function to them. However, this view is complicated by the notorious inability of these nucleotides to cross cellular membranes necessitating extremely high extracellular levels, far above those encountered *in vivo*, to accomplish such a role. Loss of cyclic nucleotides to the outside of cells may at least partly explain the rapid decay of the hormonally-induced rises in intracellular cyclic nucleotides in some systems even in the continued presence of the stimulating hormone and complicates correlation studies between cyclic nucleotide levels and hormonal responses[1]. This leakage of cyclic nucleotides to the extracellular fluid appears to be regulated to a certain extent and to be affected by drugs. In man, probenecid caused a substantial increase in the cyclic AMP level in lumbar spinal fluid[103]. Adenosine enhanced the release of cyclic AMP from cortical tissue and that effect was inhibited by Ca^{++}[104].

C. Degradation: Stimulation and inhibition of cyclic nucleotide degradation (Sites 5 and 6) are very important mechanisms for controlling their intracellular levels, and to date represent the most fertile sources of prototype drugs. The variety of structures that can influence the activity of the phosphodiesterase enzymes (PDE's) and the importance of these enzymes in drug development have been recently reviewed[105]. Since PDE's are influenced by a number of hormones, *e.g.*, insulin and cholecystokinin, it is at least theoretically possible that some drugs could block the action of these hormones on PDE's (Site 7). However, no such drug has yet been found.

Newer advances in this area strongly suggest the possible presence of specific cyclic AMP-PDE's and cyclic GMP-PDE's in contrast to what was previously believed[105]. Each cyclic nucleotide appears to significantly influence the rate of hydrolysis of the other. Thus a drug acting on the particular PDE of either cyclic nucleotide would be expected to change its intracellular concentration, which could in turn influence the rate of hydrolysis and the intracellular concentration of the other cyclic nucleotide. This may explain at least in part the opposite responses of the two naturally-occurring cyclic nucleotides in a variety of circumstances, *e.g.*, during cardiac contraction[106] and under the influence of β-adrenergic stimulants[107]. Therefore, the effects of PDE inhibitors and/or activators could be more complex than appears at first sight. Selectivity of drugs for either cyclic AMP-PDE's or cyclic GMP-PDE's is beginning to be recognized[108] and may explain the previously nonconforming pharmacologic effects of some well-known PDE-inhibitors[109]. This is underscored by the apparently opposite events mediated by the two cyclic nucleotides in many systems [1,71]. An example is the divergent effects of three PDE-inhibitors on gastric acid secretion by the mammalian stomach. While theophylline potentiates, papaverine has no effect on, and glycyrrhetinic acid[110] inhibits acid secretion. Theophylline was found to be more selective in inhibiting cyclic GMP-PDE's than cyclic AMP-PDE's. Glycyrrhetinic acid showed the reverse selectivity, being more active on cyclic AMP-PDE. Although a potent inhibitor for both enzymes, papaverine showed no selectivity for

either. Since cyclic GMP appears to mediate the stimulation of acid secretion[109] and cyclic AMP its inhibition[21], the selectivity of PDE inhibitors for the enzymes specific for the two nucleotides determines to a large extent the relative *in vivo* rise in the levels of these mediators and consequently the effect on mammalian acid secretion. This is very important, since PDE-inhibitors, particularly the methylxanthines, are used extensively as tools in the elucidation of mechanisms involving the cyclic nucleotides. In most systems, the methylxanthines exhibit greater ability to inhibit the cyclic GMP-PDE's, resulting in a greater rise in cyclic GMP than cyclic AMP levels and should be expected to mimic those effects mediated by cyclic GMP rather than by cyclic AMP in most systems studied.

II. CONTROL OF THE INTRACELLULAR ACTIVITY OF THE CYCLIC NUCLEOTIDES

A. Alteration in Free and Bound Pools: Several lines of evidence strongly indicate the presence of at least two pools of cyclic AMP: one free and one bound[52,111-113]. A similar situation may also prevail for cyclic GMP[114]. Bound cyclic nucleotides are apparently unavailable for PDE hydrolysis[115] and there is evidence for the existence of at least one membrane-bound pool[116]. Methods of measuring both bound and free pools are becoming available[112,113] and cyclic nucleotide effects appear to be related to the size of the free pools. Although no drugs are yet known that can change the sizes of these pools (Site 8), this remains an important potential mechanism for drug action.

B. Alteration of Cyclic Nucleotide-dependent Protein Kinase Activities: The relative inability of the natural cyclic nucleotides to cross cellular membranes limits to a great extent their utility as drugs in cyclic nucleotide-deficiency diseases. This has stimulated a search for cyclic nucleotide derivatives that can penetrate cellular membranes with greater ease, while retaining the ability to activate the cyclic nucleotide-dependent protein kinases (Site 9). The importance of this potential drug mechanism is underscored by the possible involvement of protein kinase in disease[2,117,118] and in the mechanism of action of at least some hormones[119]. Several cyclic nucleotide derivatives have been prepared and tested for activity against protein kinases from a variety of sources with excellent drug possibilities[120-124]. Some well-known drugs, including tolbutamide[125], furosemide[126], and mefenamic acid and phenylbutazone[127], were shown to directly affect protein kinase activity. Thus, the possibilities of this site for drug development appear quite promising.

C. Antagonism of Cyclic Nucleotide Effects: Agents capable of antagonizing the intracellular effects of the cyclic nucleotide (Site 10) represent a potential approach that would be particularly useful in situations where excessive cyclic nucleotide synthesis may represent a disease-related defect, *e.g.*, cyclic AMP in diabetes[17-19], alcoholism[128], cholera[129], and mania[10], and cyclic GMP in psoriasis[8] and hypertension[22-24]. This area has been recently reviewed elsewhere[52].

III. INDIRECT CONTROL OF CYCLIC NUCLEOTIDE METABOLISM

A. Prostaglandins: Cyclic nucleotides appear to be obligatory intermediates in the action of the prostaglandins[130 132] and the latter thus represent an important target for indirect cyclic nucleotide modulation (Site 11) as do prostaglandin antagonists (Site 12) and prostaglandin synthesis inhibitors (Site 13). Prostaglandins also appear to be obligatory intermediates in the actions of at least some hormones on the cyclic nucleotide system[133-135]. It is also of interest to note that cyclic nucleotides can modulate the synthesis of prostaglandins[136-138], suggesting a complex system involving both prostaglandins and cyclic nucleotides in the regulation of tissue responses to hormones. When applied exogenously, prostaglandins of the E type appear to stimulate the synthesis of cyclic AMP, whereas those of the F type appear to act *via* increased cyclic GMP. Thus, there are reasonable grounds for the assumption of strict coupling between the prostaglandin system on the one hand and the cyclic nucleotide system on the other.

B. Co-factor Control: Since several enzymes with varying co-factor requirements are involved in the cyclic nucleotide system, it is at least theoretically possible to modulate the system significantly by controlling the availablilty of the essential co-factors. Of these factors, Ca++ occupies a commanding position. Ca++ generally inhibits AC activity, is essential for GC activity, modulates PDE activity and its efflux appears to be controlled by the prostaglandins[139]. Furthermore, activation of protein kinase by cyclic AMP seems to enhance Ca++ uptake[140]. The importance of Ca++ to the functioning of cyclic nucleotide system is indicated by the lack of hormonal response in Ca++-free media in many systems in spite of an observed accumulation of the cyclic nucleotides.

In summary and on the basis of the above considerations, it seems evident that the cyclic nucleotide system presents significant challenge for the design of more selective and specific drugs. To exploit that challenge will require the intimate cooperation of medicinal chemists and molecular biologists and pharmacologists. An even further expansion of our knowledge of first the basic lesions in cyclic nucleotide metabolism in the various disease states and second the most logical and pertinent approaches to correct those lesions is fundamental to the successful discovery of new and better drugs for the medical armamentarium.

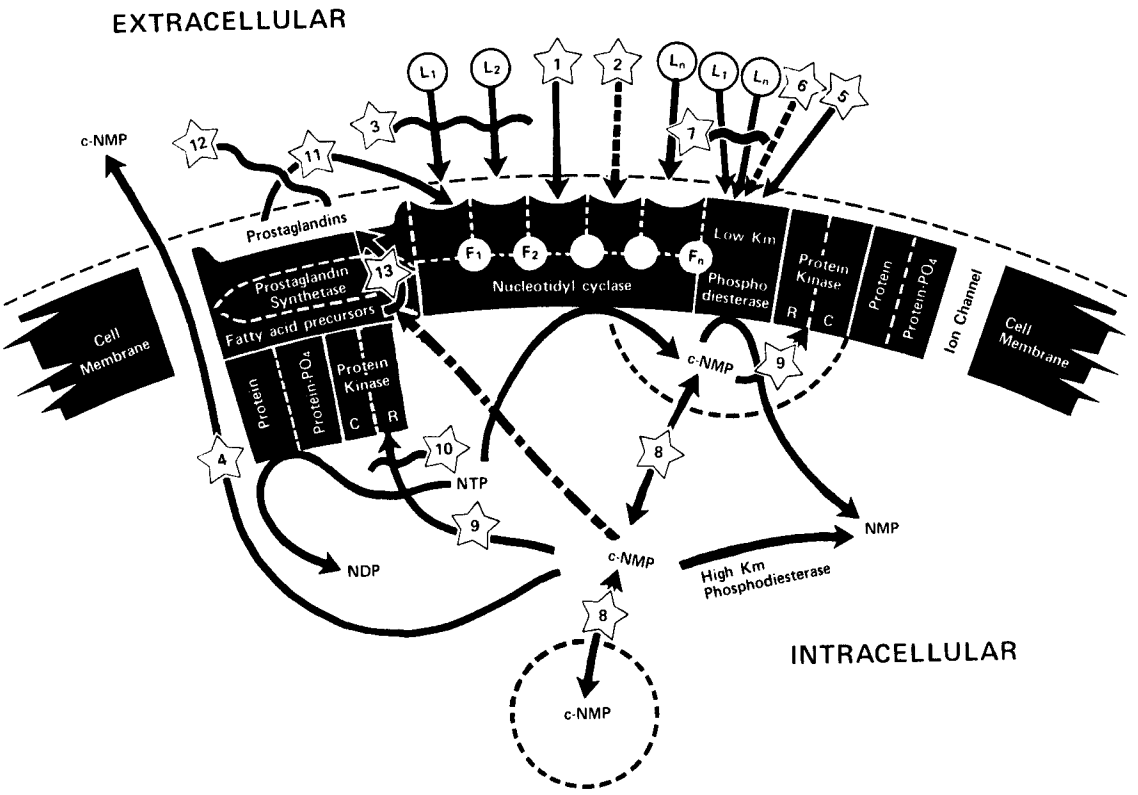

Figure 1: Possible sites of drug activity on the cyclic nucleotide systems

c-NMP = cyclic nucleotide-3',5'-monophosphate
NTP = nucleotide triphosphate
NDP = nucleotide diphosphate
NMP = nucleotide monophosphate
Ⓕ = cofactor, *e.g.*, Mg++ and Mn++
Ⓛ = ligand, *e.g.*, norepinephrine or acetylcholine
= possible drug action site
= stimulation
= inhibition
= modulation
= blockade
= bound c-NMP
R = regulatory unit
C = catalytic unit
Km = Michaelis Menten Constant

Note the multiple receptor sites on nucleotidyl cyclase and the membranous location of the low Km phosphodiesterase. Two bound (one membrane-associated) and one free pools of the cyclic nucleotides are also shown.

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Section V - Topics in Biology

Editor: William J. Wechter, The Upjohn Co., Kalamazoo, Michigan

Section Editorial

During my tenure as editor of the section Topics in Biology, I have tried to emphasize new developments in Biochemistry and Biology, which should impact on the practice of medicinal chemistry. This was particularly exemplified by the chapters on transition state analogs, gene therapy, reverse transcription, and the recent chapter on Enzyme Suicide. In more biological terms I have tried to raise the level of understanding by the medicinal chemist of the general subject of immunology. This, I hope, was accomplished through the two chapters on immediate type hypersensitivity, a chapter on the complement system, a chapter on the delayed mediators, and finally, the recent chapter on immune adjuvants. The only subject that is noticeably lacking here is that of the biology of immunosuppressants. This subject has been adequately covered in a number of recent articles (for example, see Progress in Drug Research, 16:67(1972)). I cannot overemphasize the importance of immunology, a field now approaching its golden age. It is not, however, the continuous advances in immunology that should excite the imagination of the medicinal chemist, but rather the implications of the role of immunology in the majority of disease states with which we deal. Immunologic mechanisms are implicated in asthma and other atopic diseases, lupus, rheumatoid arthritis, glomerulonephritis, myasthenia gravis, pernicious anemia, immune infertility, diabetes mellitus, pemphigus, cirrhosis, ulcerative colitis, myocardial disease and a host of less common syndromes. Unquestionably, the advances in immunology will provide a means with which to deal with many of these diseases. Consequently, I look to the subjects of the chapters which have appeared in the last three years in Topics in Biology as a proper focus of activity in academic Medicinal Chemistry. These are the areas from which we, in the applied sector of health science should take our lead.

By no means do I see the problem of communicating these advances to our community as complete. I leave immunology and other subjects to the catholic tastes of my successors. Thus, Annual Reports can serve as a means of providing a biological direction for medicinal chemistry. Doubtless, it is a working understanding of what is new in biology, which provides not only impetus for innovation, but also a wealth of purely chemical problems that challenge biology. These are exemplified by questions such as what are the chemical structures of SRS-A and the mediators of delayed type hypersensitivity (Volume 8, Chapter 9).

I make a particular point of the importance of this type of work as "true" academic medicinal chemistry, because it clearly has potential for relevance in human medicine. Thus, I leave the charge to my successors to show us the way.

Section V - Topics in Biology

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Chapter 22. Anti-aging Drugs

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Introduction — Research on aging has long been regarded as an impractical pseudoscience, and proven prolongation of life by pharmacological means apparently still remains in the realm of science fiction. Only in recent years, with the application of scientific method and control, has experimental gerontology begun to be taken more seriously.[1] A major stimulus to this increasing interest has come from social and economic[2,3] considerations. One welcome consequence of this interest has been an improvement in the quality of research on senesence phenomena and a discernible shift from purely descriptive studies to studies on mechanism. The present discussion focuses on some recent studies and theories in gerontology which have yielded significant results, and the one aspect of gerontological research which perhaps most immediately concerns the medicinal chemist, viz., the current status of agents purported to affect the aging process.

Theories of Aging — Theories of aging are legion. While several books highlighting various aspects and individual theories have appeared in recent years, the host of theories proposed to explain why aging occurs have been dealt with in comprehensive reviews.[4-7] Several of the theories simply attempt to explain obvious deleterious changes associated with senesence and therefore must be approached with caution.

Aging processes are increasingly being thought of as originating at the cellular or subcellular level, even though there is no general agreement as to the nature of these processes. However, there is no reason to believe that there is only one fundamental type of cellular or subcellular aging process. Currently attractive hypotheses encompassing similar or identical cellular or molecular processes have been consolidated together by La Bella[8] in the coalescence-accretion-stabilization hypothesis of aging. There have been other attempts at developing integrated theories of aging.[9,10]

Aging as Impaired Cellular Information Phenomenon — The most attractive general hypothesis of aging is that senesence is primarily a cellular information loss phenomenon originating at the molecular level, and that dyshomeostasis of other kinds through loss of neuronal, endocrine, and immunological information is of a secondary nature.[2] Taken in the broadest sense this hypothesis may be related to any theory of aging. Interference with the flow of cellular information could occur anywhere in the sequence from DNA to RNA to protein synthesis, building up to Orgel's cytoplasmic "error catastrophe" due to impaired protein synthesis.[11-13] Alternatively, impaired cellular function due to breakdown of essential cellular components, rampant lysosomal enzyme activity, autoimmune reactions, increased cross-linking,

accumulation of lipofuscin pigments and waste products could also result in cellular information loss.

Programmed vs Unprogrammed Cellular Death – Goldstein[14] has reviewed the two opposing, though not necessarily mutually exclusive, views on whether loss of cellular mitotic potential and information flow is caused by a random accumulation of faults in informational macromolecules or an inbuilt genetic control mechanism related to differentiation. Although experiments have been designed to distinguish between these two possibilities, the results can be interpreted in favor of either theory.[15]

Aging of Tissue Culture Cells[16] – Diploid fibroblasts of human[17-20] and chick embryo[21-24] origin have a finite capacity for replication in vitro and are therefore being used increasingly as a model for studying aging. Hayflick[25] attributed this limited life-span (50 ± 10 doublings) to an intrinsic programmed expression of aging at the cellular level (clonal aging theory), and has suggested that this finite limit is rarely, if ever, reached by cells in vitro, and that viral transformations may be a prerequisite for conversion of such cells into permanent lines.[26] Evidence in support of clonal aging is based on the inverse correlation existing between age of donor in vivo and life-span obtained in vitro.[26,27] Moreover, physiologic rather than chronologic age appears to be important since fibroblasts from the premature-aging syndrome of progeria[28] and Werner's syndrome[26] have decreased growth potential in comparison with age-matched controls. Support for the view that genetic material of a given tissue is programmed to undergo a given number of cell divisions has been obtained by Daniel et al.[29-31] who studied the growth rate of mouse mammary epithelium and found it declines during serial transplantation in vivo. These authors compared transplant lines in which cells proliferated continuously, with lines in which growth was restricted and showed that the decline in growth rate was related primarily to the number of cell divisions undergone rather than to the passage of metabolic time. There have been reports confirming the fact that in vitro finite life span of human diploid cells is related to the number of cumulative population doublings and occurs independently of metabolic time.[20]

Maynard-Smith[32] has summarized some results from serial transplantation technique which seem to argue against the view that clonal aging of mitotic cells is an important cause of aging. Nevertheless, limited life-span of fibroblasts has now been observed sufficiently often in different laboratories for it to be regarded as a phenomenon that calls for an explanation, whether or not aging of individuals can be attributed directly to the limited life-span of stem lines.

Aging Mechanisms

Theoretical Models – Strehler et al[33] have dealt with programmed senesence rather extensively at the theoretical level in their "codon-restriction theory" of aging. They propose that DNA is read differentially because of changes in transfer RNA and aminoacyl-tRNA synthetase in various tissues during differentiation and aging. Other cellular factors could also

affect these changes in codon usage, i.e., feed-back loops alter the cell's language, and negative feed-back effects (repressors) may be the ultimate cause of aging.

In all tissues examined thus far, the rate of production of new cells is controlled by a tissue-specific antimitotic messenger molecule called a chalone (negative feedback system). Bullough[34] suggests that the onset of aging and the rate at which it proceeds is probably controlled through a chalone mechanism by a non-mitotic tissue which acts as a pacemaker. There is reason to believe that deterioration of post-mitotic cells may well be a major cause of aging in mammals.[32] Evidence has been presented that changes in neurons and in cells in the microvascular system are the main cause of aging in mammals.[35] Since the antimitotic power of chalones is known to be strengthened by stress hormones, such as adrenaline and glucocorticoid hormones, one test of the chalone theory would be to discover whether it is possible to affect the life-span by strengthening or weakening the chalone action. The marked increase in life-span of rats on restricted caloric intake[14] has been explained on basis of continued high level of stress hormone secretion due to strengthened chalone action.[34]

Gelfant and Smith[36] have recently presented a model in which cellular and tissue aging is regarded as the result of progressive conversion of cycling to noncycling cells in tissues capable of proliferation. Cycling cells are regarded as cells that are actively moving through the cell cycle of periods of nuclear DNA synthesis and mitosis. A cell may be blocked at any of the intervening gaps.[37] These authors suggest that immune mechanisms may be involved in cellular aging by keeping noncycling cells in restraint. Reversal of cellular aging by hydrocortisone, an immunosuppressive agent, has been rationalized as a release of noncycling cells.[36,38]

Hormonal Control of Aging – The diversity of regulatory functions performed by the hypothalamus has led to the suggestion that age-dependent changes in the hypothalamus are responsible for the functional and metabolic changes associated with aging. Frolkis[39,40] has presented evidence that the hypothalamus undergoes structural changes and exhibits a decrease in functional activity with age. There is a reduction in the activity of neurosecretory processes and the hypothalamic structures show an increased sensitivity to such physiologically active agents as catecholamines, acetylcholine and insulin.[39] Dilman[41] suggests that the key process in the genetic program of development and aging is a gradual reduction in sensitivity of the hypothalamus to feed-back control. This leads to increased secretion of "aging hormones", and disruption of the two basic homeostatic systems regulating reproduction and energy metabolism. Everitt[42] has further examined the relationship between hormones and aging, stressing the role of hypothalamic-pituitary regulation of the rate of aging and onset of age-related pathology.

Impaired Protein Synthesis – Aging may affect protein synthesis either at the DNA level by mutation, single strand breaks or other structural changes, or at subsequent stages.[5,43,44] However, questions of age-associated changes in DNA have been particularly difficult to pursue because of the problem of detecting subtle molecular changes at that level. Animals receiving sublethal doses of X-irradiation have been shown to become prematurely senile and susceptible to fatal diseases. X-irradiation has been used to induce single strand breaks and to apparently

duplicate age-associated changes that occur naturally in DNA of senescent brain cells.[45] However, in view of the rather remarkable ability of cells to rejoin broken strands and to replenish altered base units in DNA[46,47] the relevancy of radiation to aging is debatable. This does not exclude the possibility, of course, that damage could build up if DNA-repair enzymes are progressively lost[44] or if an age-associated rampant increase in the release of hydrolytic enzymes occurs. Cellular aging has also been attributed to an irreversible nuclear protein-DNA interaction[48,49] or RNA polymerase inactivation by arginine-rich histones.[50] Orgel[13] has recently reviewed how errors in the synthesis of RNA and information-handling enzymes can accumulate progressively, decrease the fidelity of protein synthesis and eventually jeopardize the entire protein-synthesizing machinery ("error catastrophe").[11,12] Frolkis has discussed the interrelationship between cell function and protein biosynthesis in the process of aging.[51]

Agents Affecting Protein Synthesis – Agents that enhance learning and memory[52] have been examined in geriatrics since they supposedly act on brain RNA and protein synthesis. 5,5-Diphenyl hydantoin reportedly prevents polyribosomal disaggregation in the aging brain tissue and renders RNA more resistant to hydrolysis by RNAse. The drug has been shown to improve the avoidance response and learning retention in aged rats.[53-55]

Procainamide has been tested for learning enhancement activity in experimental animals of different ages and found to improve behaviour and learning deficits in aging rats.[53] Procainamide was shown to increase brain RNA content and to redistribute intracellular amino acids from the free pool to an RNA-bound form.[54]

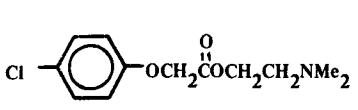
Impaired Cellular Function

Accumulation of Cell Debris – Lipofuscin granules are fluorescent lipoprotein masses, appropriately called age-pigments, which tend to accumulate with advancing age, especially in postmitotic cells such as those of the brain, myocardium, skeletal muscle and adrenals. Lipofuscin pigments may be debris left over from free-radical attacks on cell components[56], and might be an important indicator of the degree of cellular insufficiency in aging.[57,58] However, there is as yet no direct evidence that they interfere with normal cell processes.

Meclofenoxate (centrophenoxine) (I) is a geriatric therapeutic agent useful for treatment of patients suffering from confusional states, Parkinsonism and other senile mental disorders. Subsequently, Nandy and Bourne[59,60] showed that meclofenoxate treatment could reverse the accumulation of lipofuscin pigments in neurons of senile guinea pigs, and suggested that reduction of lipofuscin may be one of the ways by which the drug exerts its beneficial effects on the CNS. There was also a marked reduction in the activity of a number of oxidative enzymes, as determined histochemically.[60] The drug is said to promote an increase in glucose-6-phosphate dehydrogenase activity by diversion of glucose metabolism to the pentose cycle, the common pathway of glucose metabolism in cells undergoing rapid growth or multiplication, which results in an unexplained elimination of the accumulated pigments.

Free-Radical Damage – Free-radicals can arise from a number of sources in the living system.

Harman has suggested that free-radical mediated damage of macromolecules and other cellular components can result in cumulative degeneration of cellular function which expresses itself as aging.[61-63] Several antioxidants and free-radical scavengers, such as cysteine, 2-mercaptoethylamine (2-MEA), 2,2'-diaminodiethyldisulfide, butylated hydroxytoluene (BHT) (II) and ethoxyquin (III) have been tested for antiaging effects. BHT and 2-MEA were amongst the most effective agents in increasing the mean life-span in animal colonies, but had no significant effect on maximum life-span.[63,64] Antioxidants which are ineffective in increasing survival may have achieved inadequate tissue distribution or concentration. The role of unsaturated fatty acids as a source of free-radicals has been stressed.[56] Vitamins C and E, cysteine, glutathione and selenium compounds which have been shown to inhibit peroxidation and can act as free-radical scavengers[56] are being investigated as antioxidant therapy in humans.[65]



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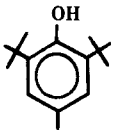

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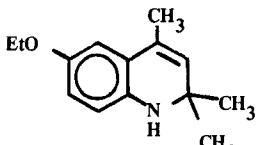


III

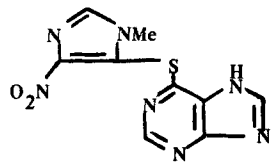
Lysosomes and Membrane Damage – Labilization of the lysosomal membrane and rampant lysosomal action have been suggested as a cause of cellular death during aging.[66] A detailed discussion of the possible involvement of membrane breakdown in aging has been presented.[67] Hochschild[67] notes that leakage of lysosomal enzymes into the cytoplasm could, in principle, be responsible for several processes suggested as primary aging mechanisms, such as reduced fidelity of protein synthesis. In this context membrane damage in inositol-starved mutants of Neurospora has been shown to result in synthesis of altered proteins.[68] Several agents are known to stabilize lysosomal membranes (e.g., corticosteroids).[69] Cortisone[70] and hydrocortisone[71] have been shown to prolong the mitotic capacity of human embryonic fibroblasts. However, it is as yet unclear whether corticosteroids act on aging systems by stimulating protein synthesis or by stabilizing lysosomal membranes. Dimethylaminoethanol (DMAE), another agent believed to stabilize membranes, when administered to senile mice in drinking water resulted in a significant increase in their life span.[72,73] FDA-approved clinical studies with DMAE are underway.[74]

Cross-linking of Connective Tissue – Agents which interfere with crosslinking of peptide chains in fibrous connective tissue macromolecules and collagen are expected to retard biological aging.[75-79] Lathyrogens inhibit the oxidative deamination of free ε-amino groups of lysyl residues to aldehydes which is the first step in the cross-linking of lysine residues on different peptide chains. Therefore, laythrogenic compounds, such as β-aminopropionitrile, penicillamine[77] and semicarbazide, which prevent maturation of collagen, have been suggested as anti-aging drugs. La Bella[8,75] has obtained some evidence that these drugs will extend the average life-span (but not the maximum life-span) of rats if given in doses sufficient to slow down crosslinking but not enough to cause undesirable side-effects. The use of penicillamine was accompanied by an inhibition of wound healing and by increased skin fragility.[80,81]

Autoimmunity – Walford[82,83] and Burnet[84,85] have elaborated on the idea that aging is an

autoimmune phenomena. They propose that aging is a consequence of increasing immunogenetic diversification of dividing cell populations of the body. The age-dependent-decline of immunocompetence is manifested in a progressive failure of the organism's own cells to distinguish self from foreign cells with damaging immunological reaction against them ("unleashing of self-destroying process"[83]), or the development of damaging autoimmune cell clones (forbidden clones) due to somatic mutation of immunocytes and a decreased responsiveness to extrinsic antigens. Burnet[85] has emphasized that decreased immunological surveillance may originate in the thymus-dependent segment of the immune system, and the latter, in turn, may be dependent on exhaustion of the Hayflick limit (inbuilt-metabolic clock) in thymus cells and dependent tissues. Based on experiments with "nude" mice (a C3H derived strain of mice born without a thymus which suffer from immunodeficiencies similar to, but more acute than those produced experimentally by thymectomy), Pantelouris[86] suggests that senescent normal animals have an excess of IgM antibodies over IgG antibodies. The IgM antibodies mainly produced in response to bacterial polysaccharides often cross-react with the mammal's own tissue (cross-reaction autoimmunity). This gradually increasing dependence on IgM antibodies has been correlated with involution of the thymus[87] and may be an important process in "immunological aging" independent, though not exclusive of forbidden clones and somatic mutations. In general, immune function does show a decline in intensity with advancing age. There is a progressive incidence of autoantibodies such as antinuclear, rheumatoid, antithyroid and antiparietal factors in the aged.[82,88-90] This occurs despite a decrease in the numbers of antibody-forming cell precursors in both the spleen and bone marrow with increasing age.[91]

On the basis of the autoimmune theory, Walford tested the effect of an immunosuppressant, azathioprine (Imuran) (IV), on the life-span of aging mice.[92] Animals receiving a daily dose of 100 mg/kg showed a mean survival time 10 weeks longer than controls, but the maximum life-span (135-140 weeks) was not affected. Similar results were obtained with another immunosuppressant, cyclophosphamide (V).[92] It seems probable that anti-aging therapy at this level may be too late in the chain of events. Moreover, suppression of the immune system may accelerate death from other causes.



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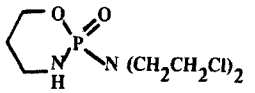

V

Conclusion – The enigma of human aging continues to persist, although it is becoming increasingly evident that aging may be caused by a number of different mechanisms operating simultaneously in the individual. There is growing research emphasis on some theories of aging that are especially promising. Most impressive has been the accumulation of evidence that aging is caused, at least in part, by a progressive breakdown of the body's immunological defenses – whether programmed or random. While on the basis of present experimental evidence it is difficult to recommend any treatment to inhibit aging, a clearer understanding of

the fundamental aging process will surely lead to a rational approach to retarding human aging during the next two decades.

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Chapter 23. Affinity Labeling of Hormone Binding Sites

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Affinity labeling (AL) is a technique for covalently labeling protein binding sites. According to this method, a reactive functional group (attaching function) is incorporated within the structure of a ligand molecule in such a manner that the modified ligand is bound by the protein and reacts with amino acid residues in or near the binding site, forming a covalent bond through the attaching function.

Most AL studies have utilized as attaching functions the alkylating and acylating groups typically used in protein modification studies ("conventional" affinity labeling; CAL) and have been directed at binding sites in homogeneous protein preparations, particularly the active sites in enzymes. Several comprehensive reviews of this work are available.[1-7] (Metabolically-activated irreversible enzyme inhibitors are reviewed in Chapter 24 of this volume.) More recently, the scope of AL studies has expanded to include antibody binding sites and binding sites associated with the allosteric control of enzymes and membrane transport (regulatory sites) and with the action of hormones, drugs, and neurotransmitters (receptor sites). A new dimension in labeling selectivity has been demanded in many of these latter applications, as the binding proteins generally constitute only a very small fraction of the total aggregate of macromolecules. The development of many new attaching functions, most notable of which[8] are those which require photoactivation ("photoaffinity" labeling; PAL), has improved the versatility of this technique. This review will focus on the developments in the area of AL relating principally to hormone binding sites. Fundamental methodological considerations of labeling selectivity in heterogeneous systems will also be covered.

Fundamental Considerations - The salient feature of an AL process is that covalent bond formation takes place within a complex of protein and labeling reagent; the high, localized concentration of reagent in the complex results in an enhanced reaction between the reagent and nearby amino acid residues (proximity effect), which is the basis for the labeling selectivity. Baker[4] and others[9] have outlined certain kinetic features that a binding-preceeded labeling process should display: (1) There should be evidence for a reversible equilibrium between reagent and binding site; (2) the labeling reaction should display a rate saturating effect; (3) non-reactive ligands which compete for the same binding site should exert a protective effect, reducing the rate, but not extent of labeling, and (4) there should be a stoichiometric relationship between the extent of labeling and the extent of inactivation. These properties are generally used as the criteria for establishing that a labeling process is indeed occurring by an AL mechanism, as opposed, in particular, to a simple bimolecular mechanism.

Labeling Selectivity in Heterogeneous Binding Systems: Conventional versus Photoaffinity Labeling - Metzger, Wofsy, and Singer[10-12] have proposed a kinetic model for the selectivity of an AL process in a homogeneous binding system. This model can be expanded to cover heterogeneous systems.

$$P^s + L\text{-}x \underset{}{\overset{K_a^s}{\rightleftharpoons}} P^s \cdot L\text{-}x \xrightarrow{k^s} \overset{\frown}{P^s \cdot L\text{-}x} \quad \ldots\ (1)$$

$$P^{ns} + L\text{-}x \underset{}{\overset{K_a^{ns}}{\rightleftharpoons}} P^{ns} \cdot L\text{-}x \xrightarrow{k^{ns}} \overset{\frown}{P^{ns} \cdot L\text{-}x} \quad \ldots\ (2)$$

$$P + x\text{-}L \xrightarrow{k^b} \overset{\frown}{P\ x\text{-}L} \quad \ldots\ (3)$$

The AL reagent L-x consists of a ligand portion L and an attaching function x. It is in reversible equilibrium (K_a^s) with a specific binding protein (P^s) whose labeling is desired, and covalent attachment proceeds within this complex by a first order process (k^s) (eqn 1). Undesired labeling can take place by two mechanisms: The labeling reagent, particularly if non-polar, may associate in a low-affinity, non-specific manner with hydrophobic regions of other proteins, and labeling can take place via this complex (eqn 2). In addition, the labeling reagent may react with exposed residues on any of the proteins in a bimolecular fashion, i.e., without a prior binding equilibrium (eqn 3). Labeling selectivity, thus, constitutes a predominance of process 1 over processes 2 and 3.

An expression for the ratio of the rates of labeling can be derived in a straightforward manner,[12] and the value of its integral over the time of labeling gives the labeling selectivity. However, it is equally instructive to consider a simpler expression which represents the maximum selectivity (selectivity limit, SL) expected under optimum conditions (low concentration of L-x and low extent of labeling).

$$SL = \frac{(P_o^s \cdot K_a^s) \cdot k^s}{(P_o^{ns} \cdot K_a^{ns}) \cdot k^{ns} + k^b \cdot P_o}$$

Three terms can be recognized in this expression: (a) A binding term ($P_o^s \cdot K_a^s / P_o^{ns} \cdot K_a^{ns}$), which describes the distribution of bound reagent between the specific and non-specific sites; (b) an attachment rate or efficiency term (k^s/k^{ns}), which relates how rapidly or efficiently covalent attachment takes place within the two types of complexes, and (c) a bimolecular labeling term ($k^b \cdot P_o$).

A two-point distinction can be made between conventional and photoaffinity labeling processes: (1) Dark vs. irradiation conditions gives one external control over the reactivity of the PAL reagents, and (2) the covalent bond-forming reactions with CAL reagents (alkylation and acylation) have sufficiently high activation energies to make them slow relative to the rates of complex formation and dissociation, while the reactions of the highly energetic species (ketone triplets, carbenes, nitrenes, etc.) produced by photolysis of the PAL reagents is rapid relative to the rates of complex association and dissociation.

The external control can be put to two advantages to increase labeling selectivity. Once the system has reached binding equilibrium (in the dark) the system can be perturbed by rapidly removing the excess free reagent by molecular sieving or adsorption. As the system strives to reequilibrate, bound reagent will dissociate more rapidly from the low affinity, non-specific sites, causing a temporary increase in the value of the binding term (a), at which time the system is irradiated. Removal of excess free reagent also reduces the value of term (c). Such an equilibrium-perturbation approach cannot be applied to a CAL process.

The rapid, indiscriminant reaction of the PAL reagents also has two potential advantages. The relatively slow and selective attachment reactions associated with the CAL reagents may well result in a wide variation in the rate of covalent attachment within the different types of complexes that will form in a heterogeneous binding preparation (value of term b uncertain), while the PAL reagents will presumably react with reasonable efficiency with residues in any binding site, regardless of its precise composition or steric layout (term b $\sim$ 1). Furthermore, in PAL, but not in CAL, a scavenger capable of intercepting those reagent molecules which are photoactivated in solution before they react with protein, can be added to reduced bimolecular reaction.

Steroid Binding Sites: - (1) Enzymes Involved in Steroid Biosynthesis and Metabolism - Although the steroid is playing the role of a substrate rather than a hormone, the study of these systems is the most advanced.

Δ^5-3-Ketosteroid isomerase has been the subject of three recent studies. 6β-Bromotestosterone acetate (BTA), a potent competitive inhibitor, causes irreversible inactivation of the enzyme upon long-term incubation.[13] Protection against inactivation is afforded by 19-nortestosterone, and the pH-rate profile of inactivation suggests the involvement of histidine. Labeling studies with ^{3}H-BTA have shown that each mole of enzyme can react with ca. three moles of ^{3}H-BTA (cf. trimer structure). In striking contrast to the reactivity of the 6β-bromo derivative, five different 2α-, 4α-, and 6α-bromoketoandrostane substrate analogs were shown to have no capacity for irreversible inhibition,[14] even though they all are even more potent competitive inhibitors than BTA.

In a particularly intriguing approach to labeling the steroid isomerase, "natural" ligands, having the Δ^4-3-keto chromophore (testosterone and 19-nortestosterone acetate, NTA) were used as photoaffinity labels.[15] Enzymatic activity was lost when the enzyme was irradiated in the presence of these derivatives, and the rate of inactivation paralleled the fractional saturation of the active site. Competitive inhibitors lacking the Δ^4-3-keto structure exhibited specific protection against irradiation-induced inactivation by NTA, suggesting that reaction was occurring at the active site. Irradiation in the presence of ^{14}C-NTA resulted in covalent labeling of the enzyme.

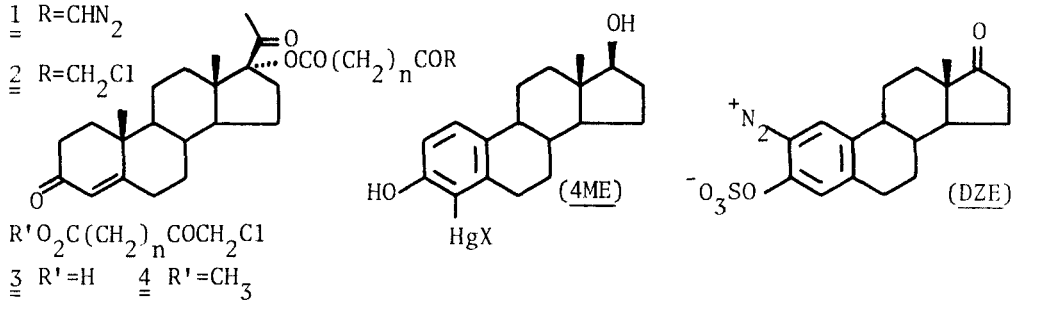
The 17β-hydroxysteroid dehydrogenase is irreversibly inhibited

by 16α-iodo-3-acetoxyestrone.[10,17] Both estradiol and NADP act as protectors against inactivation, and the alkylation process exhibits a rate saturation effect, consistent with an AL mechanism. The 3-O iodoacetyl derivatives of estrone (IAE_1) and estradiol (IAE_2) also rapidly inactivate the dehydrogenase.[18] The rate is slowed by estradiol and is also affected by NADP and NADPH. Reaction with ^{14}C-IAE_1 plus NADPH is rapid, giving a labeling stoichiometry of 0.9, and chymotryptic digestion produces a single labeled peptide, which contains a carboxymethyl histidine. The alkylated enzyme undergoes a slow reactivation upon prolonged incubation at 37°, with concomitant release of the steroid by hydrolysis of the 3-O ester bond.[19]

Warren has utilized a number of halogenated progesterone (P) and cortisone (C) derivatives in a comprehensive study of the 20β-hydroxysteroid dehydrogenase.[20-25] All of the derivatives act as substrates for the dehydrogenase, but prolonged incubation in most cases, results in irreversible inactivation, which is slowed by the addition of progesterone or cortisone and in some cases also by coenzyme. Reaction using tritium-labeled derivatives results in each case in the labeling of a single amino acid: 6β-bromo- and 6β-bromoacetoxy-P → cysteine, 11α-bromoacetoxy-P → methionine, and 16α-bromoacetoxy-P and 21-iodoacetyl-C → histidine. Thus, one can establish with considerable precision the relative spatial arrangement of the three nucleophilic residues in the active site.

16α- and 11α-Bromoacetoxyprogesterone and 20-acryloxyprogesterone irreversibly inactivate the 11β- and 21-hydroxylase activities in the rat adrenal;[26] progesterone can protect against the effect of the 16α- and 20-derivatives.

The diazoketone derivative 1 aroused interest in connection with its apparent, rapid, irreversible inactivation of corticosteroid acetyltransferase.[27] The inhibitory activity, however, was traced to the corresponding α-chloroketone 2 which contaminated the preparation. Two straight-chain (non-steroidal) chloroketone derivatives (3 and 4) also show considerable irreversible inhibition.



(2) Enzymes Affected Allosterically by Steroids - The activity of several metabolic enzymes is affected by steroids: glucose 6-phosphate dehydrogenase,[28] glutamate dehydrogenase,[28] aldehyde dehydrogenase,[28] and pyruvate kinase.[29] As the steroid is acting as an allosteric effector, these regulatory binding sites may be more appropriate models for steroid

hormone receptor sites than are the catalytic sites discussed above.

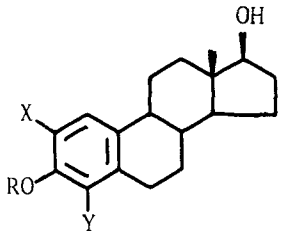
The two estrogen derivatives, 4-mercuriestradiol (4ME)[30] and 2-diazoestrone sulfate (DZE)[31] react rapidly with cysteine residues in glutamate dehydrogenase; DZE also reacts with pyruvate kinase. Estradiol slows the rate of reaction of these derivatives, which is consistent with an affinity labeling mechanism. The reactivity and instability of these derivatives, however, limit their utility.

Kallos and Shaw[32] found that 1 mole of ^{3}H-bromoacetyl diethylstilbestrol would react covalently with glutamate dehydrogenase. Both DES and ADP afforded protection against this alkylation reaction. The modified enzyme was insensitive to the allosteric effectors ADP and GTP, and cleavage of the DES group with hydroxylamine failed to restore normal enzymatic activity.

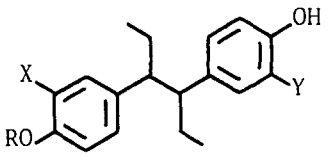
(3) Target Tissue-Specific, High Affinity Binding Proteins (Steroid Hormone Receptors) - (a) Estrogens - Liarakos and May[33] have described the preparation of some 3-estradiol ω-bromoalkyl ethers as potential alkylating agents for the estrogen receptor in rat uterus. Although no labeling studies were done, the low binding affinity and biological potency of these derivatives suggests that they have little potential as labeling agents. The 16α, 17α-, and 16β, 17β-epoxides of estradiol were also prepared.[34] Both derivatives have high uterotrophic activity, but show no indication of prolonged activity or inhibition.

The reported sensitivity of the uterine estrogen receptor to organomercurials prompted Warren and coworkers[30] to investigate 4-mercuriestradiol (4ME) as a potential AL reagent. This derivative demonstrates more persistent estrogen activity than estradiol[35-37] which is consistent either with prolonged occupancy of the estrogen receptor site or a slower metabolic clearance rate,[25] and its binding affinity is roughly 30% that of estradiol.[38] Direct incorporation studies using ^{3}H-4ME have shown that radioactivity becomes associated in a solvent-inextractable manner with proteins in rat uterine cytosol.[39] Estradiol can partially block the labeling, and the principal labeled protein sediments as an 8S species on sucrose density gradient centrifugation.[36,39] Detailed studies using this derivative are hampered by the relative instability of the mercury sulfur bond.[25]

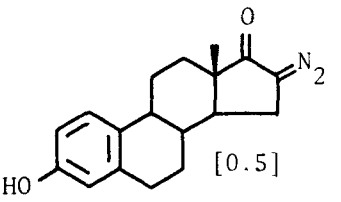
The preparation of a number of photosensitive azide and diazo derivatives of estradiol, estrone, and hexestrol has been described.[40] Their promise as selective photoaffinity labels for the rat uterine estrogen receptor has been evaluated systematically by measuring their receptor binding affinity in uterine cytosol (see values in square brackets, % of estradiol affinity)[41] and by determining their ability to consume estrogen binding activity in uterine cytosol upon irradiation. Reaction efficiencies, i.e., percent inactivation, range from 0-21%.[42,43] Four antiestrogens of the triarylethylene class (Upjohn U 11,100A, Parke-Davis CI-628, -680, and 9411X27) and 6-oxoestradiol were also shown to be highly photoreactive.[43]



$R=CH_2COCHN_2$; X,Y=H [1.4]
$X=N_3$; R,Y=H [3.0]
$Y=N_3$; R,X=H [0.9]



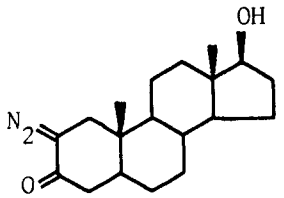
$R=CH_2COCHN_2$; X,Y=H [1.8]
$X=N_3$; R,Y=H [69.0]
$X,Y=N_3$; R=H [12.0]

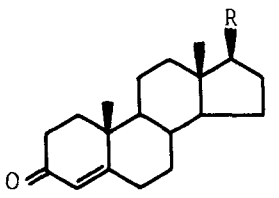


The preparation of several steroid derivatives (estrogens, androgens, progestins, corticosteroids) bearing nitrogen mustard or ethylene imine alkylating functions has been described.[44,45] The p-(N,N-bis(2-chloroethyl)aminophenylacetic esters, in particular, showed activity against DMBA-induced, transplantable mammary adenocarcinoma and a variety of rat leukemias.[44] It is not clear, however, whether the steroid carrier conveys any particular selectivity to these derivatives.[45]

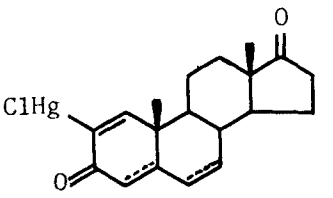
(b) Progesterone - Solo and Gardner[46-48] have prepared a series of progesterone derivatives bearing reactive alkylating functions at positions 16α or 17α (cf. 1 and 2). The relatively low level of progestational activity and the absence of persistent hormonal or antihormonal activity of these derivatives were taken to mean that no covalent interaction with the receptor site was taking place.

A number of reactive halo, thio, diazo, and mercuri-substituted progesterone and testosterone derivatives have been synthesized as potential affinity labeling reagents.[49,50] The binding affinities of 6α- and 6β-bromoprogesterone and 17β-thiol- and 17β-methylthio-4-androsten-3-one in human uterus and chick oviduct are sufficiently high to merit further study in these systems.[51]





$R=COCHN_2$; R=SH



(c) Androgens - A preliminary report describes the synthesis of several 7α-substituted testosterones, including the 3'-chloropropyl derivatives.[52]

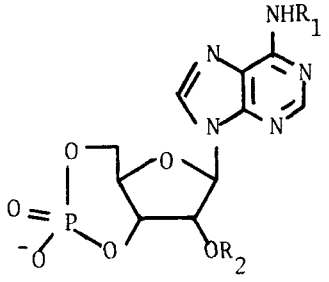
Protein Hormone Binding Sites - Bromoacetyloxytocin (BrOXY) has been used to study the neurohypophyseal hormone receptor associated with the adenyl cyclase system of toad bladder and rabbit kidney medulla.[53] BrOXY itself does not stimulate cyclase activity, but it irreversibly blocks the

stimulatory effect of the natural vasoactive hormones. Specificity is inferred from the fact that BrOXY has no effect on parathyroid hormone stimulation of adenyl cyclase in the kidney cortex.

Several bradykinin derivatives, bearing N-terminal nitrogen mustard-type alkylating agents, cause permanent potentiation of bradykinin activity in pig ileum.[54] A possible mechanism involves the irreversible inactivation of kininases by alkylation. Similar angiotensin mustard derivatives act as irreversible antagonists to angiotensin II action in ileum,[55] and thus appear to be promising reagents for affinity labeling studies.

Serum albumin has at least two binding sites for oligopeptide analogs of gastrin.[56] Irradiation of photoreactive derivatives of the pentapeptide X-GlyTrpMetAspPheNH$_2$ (X = 4-azidobenzoyl, 2-nitro-5-azidobenzoyl, 4-acetylbenzoyl, and 4-benzoylbenzoyl) results in incorporation of up to two moles of derivative per mole protein.[57] Irradiation of three of the derivatives in radiolabeled form in the acinar cell suspensions results in incorporation of label.

Other Binding Sites of Interest: (1) Nucleotide Binding Sites - The preparation of three photosensitive diazomalonyl derivatives of adenosine 3':5'-cyclic monophosphate (c-AMP) has been described.[58,59] In the dark, 5 is an allosteric activator of phosphofructokinase. Irradiation at 254 nm, in the presence of ^{3}H-5, results in an incorporation of up to 0.7 moles of reagent per mole enzyme;[60] incorporation is blocked by c-AMP and the derivatives 6 and 7 are ineffective in labeling.[58] An affinity labeling study of the catalytic site of phosphofructokinase, using a 6-mercaptopurine ribonucleoside triphosphate, has appeared.[61]



	R_1	R_2
5	$-COCN_2CO_2Et$	- H
6	$-COCN_2CO_2Et$	$-COCN_2CO_2Et$
7	- H	$-COCN_2CO_2Et$

Derivative 5 was also used in a study of c-AMP receptors in human erythrocyte ghosts.[62] In the dark, it acts as an activator of protein kinase activity and competes with c-AMP for binding. Irradiation causes incorporation of label which is partially blocked by c-AMP. Gel electrophoresis reveals a prominent radioactive band which corresponds in mobility to the endogenous substrate of the membrane protein kinase.

A number of nucleotide derivatives bearing alkylating functions were prepared by Baker[4-7] and others[63-68] as specific inhibitors of enzymes involved in nucleotide metabolism (e.g., thymidine kinase and phosphorylase, guanine and adenosine deaminase). Related derivatives have been

used to label the effector binding site in rabbit muscle phosphorylase b.[69-71] The synthesis of several mercurated nucleosides has been described.[72] These derivatives appear to be quite stable, and they should prove to be interesting reagents for further studies on nucleotide binding sites.

(2) Neurotransmitter Binding Sites - Affinity labeling studies on neurotransmitter receptors were reviewed last year.[73] In a preliminary study,[74] the irradiation of two (p-azidobenzyl)trialkyl ammonium salts was reported to irreversibly inactivate the acetylcholine (ACh) esterase of red blood cells and the ACh receptor of frog sartorius muscle. Specific protection by unreactive ligands seemed to imply that a photoaffinity labeling mechanism was operative. However, subsequent work revealed that the photogenerated species was sufficiently long-lived to be in reversible equilibrium with the binding site before reaction; so, reaction was actually occurring by a conventional affinity labeling process (termed pseudo photoaffinity labeling).[75]

Diazoacetylcholine has been prepared,[76] and its pharmacological activity has been shown to be similar to that of acetylcholine (ACh).[77] Irradiation causes an irreversible depolarizing block of the ACh receptor in mouse diaphragm.[77] Its affinity for ACh esterase is low.[78]

Despite considerable interest in the use of irreversible α-adrenergic blocking agents of the β-haloethylamine type, as affinity labeling reagents for adrenergic receptors, little success has been achieved to date.[79,80] Most studies have used radiolabeled reagents with low specific activity, and non-specific labeling has been extensive.[73]

Catechol-O-methyltransferase is irreversibly inactivated by N-iodoacetyl-3,5-dimethoxy-4-hydroxyphenylethylamine.[81] Inactivation shows a rate saturating effect and is blocked by 3,4-dihydroxybenzoate. Iodoacetate and iodoacetamide also inactivate this enzyme, but at a much slower rate.

(3) Drug Receptors - ^{3}H-N-β-(p-azidophenyl)ethyl norlevophanol (APEN)[82] was used in a preliminary affinity labeling study of the opiate receptor. Although irradiation of APEN caused incorporation of radiolabel into rat brain homogenates and guinea pig ileum, the propensity of APEN for indiscriminant attachment resulted in high levels of non-specific labeling.

The NaK-ATPase activity of bovine rat brain homogenates appeared to be irreversibly blocked by cardiotonic steroid derivatives, strophanthidine bromoacetate (SBA)[83] and hellebrigenin iodoacetate (HIA),[84] and ^{3}H-HIA appeared to label the sites with considerable selectivity. However, a recent study suggests that the action of SBA on the NaK-ATPase is not irreversible.[85]

A variety of reactive anileridine[86] or benzomorphan[87] derivatives have been prepared as potential non-equilibrium inactivators of the analgetic receptor. Certain long-term activity of some of these compounds

may be ascribed to receptor alkylation.

Recent Developments in the Methodology of Photoaffinity Labeling - The potential of a thiocarbonyl group as a photoattaching function is evident from a recent, intriguing photoaffinity labeling study of E. coli RNA polymerase using mono and poly 4-thiopyrimidines.[88] Irreversible inactivation of polymerase activity was observed when the enzyme was irradiated in the presence of 4-thiouridine triphosphate and polydeoxy-4-thiothymidylic acid, and studies with (^{32}P)-derivatives have shown incorporation of label into the β and β' subunits.

The use of an iodopyrimidine as a photoattaching function is described in an elegant study of the photoinactivation of E. coli thymidine kinase.[89] 5-Iodo-2'-deoxyuridine (IdUrd), a substrate analog of thymidine, enhances the rate of photodegradation of the enzyme; the sensitizing effect is saturable, is protected by substrate, and is also affected by the allosteric ligands dTTP and dCDP. Incorporation of label occurs upon irradiation with ^{14}C-IdUrd, but not with ^{125}I-IdUrd, implicating a pyrimidine radical as the active species.

The potential of aryldiazirines as photoattaching functions has been suggested by a recent model study.[90] The chemical stability and accessible chromophore (λ_{max} 370 nm, ε = 70-100)[91] of these derivatives and the high reactivity of the resulting carbene make them particularly attractive.

Photoexcited carbonyl functions (presumably the reactive n-π* state) appear to have considerable potential as photoattaching functions.[15,43,57] The benzophenone triplet was shown to react with AcGlyOMe in benzene or water to give the addition product $Ph_2C(OH)CH(NHAc)CO_2Me$.[92] A series of studies by Elad[93] on the photolytic modification of proteins using toluene or 1-butene and triplet sensitizers (diacetyl/di-tert-butyl peroxide) contains much work of relevance to photoaffinity labeling.

A curious photoactivation of chlorocyanoethyl colchicine (CCCol) has been observed.[94] In the dark, its interaction with the Col binding site on the 120,000 MW microtubule protein dimer is reversible; however, UV irradiation results in irreversible attachment of labeled CCCol to a protein with an apparent MW of 10-15,000.

Conclusion - Although the application of the technique of affinity labeling to hormone binding sites is relatively new, the rapid proliferation of reports is a testament to its current popularity, if not its general utility. However, most of the work to date in which highly-specific labeling has been achieved, is confined to relatively well-defined and homogeneous binding systems. This leaves for the future the delineation of the ultimate selectivity of the conventional, and particularly the photoaffinity labeling techniques, and the successful application of these techniques to the many receptor systems which are available only as heterogeneous preparations.

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Chapter 24 Mechanism-Based Irreversible Enzyme Inhibitors

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Introduction - This review discusses a general approach to the design of irreversible enzyme inhibitors of extraordinary specificity. These inhibitors are so constructed that they require chemical activation by the target enzyme. Upon activation, a chemical reaction ensues between the inhibitor and enzyme resulting in the irreversible inhibition of the latter. Thus, the enzyme by its specific mode of action catalyzes its own inactivation. These inhibitors are "k_{cat} inhibitors" both because they require catalytic conversion by the target enzyme and because it is to this term of the enzymatic process that they owe their great specificity[1]. In this review examples of synthetic as well as naturally occurring inhibitors which act by this mechanism are described.

Examples of Synthetic "k_{cat}" Inhibitors - The paradigm of this kind of inhibitor is to be found in studies on the irreversible inhibition of β-hydroxydecanoyl thioester dehydrase by $\Delta^{(3,4)}$ decynoyl N-acetylcysteamine[2]. This enzyme, which is required for unsaturated fatty acid synthesis in E. coli, catalyzes the reversible interconversion of hydroxydecanoyl thioesters with their α,β trans and β,γ cis counterparts. (Fig. 1)

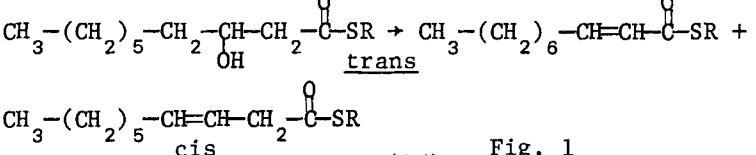


Fig. 1

The "k_{cat} inhibitor" of this enzyme, $\Delta^{(3,4)}$ decynoyl-NAC, is treated as a substrate by the enzyme. However, unlike the β,γ olefinic substrate, the acetylene is converted into the highly reactive conjugated allene which immediately alkylates an active site histidine residue[3]. (Fig. 2)

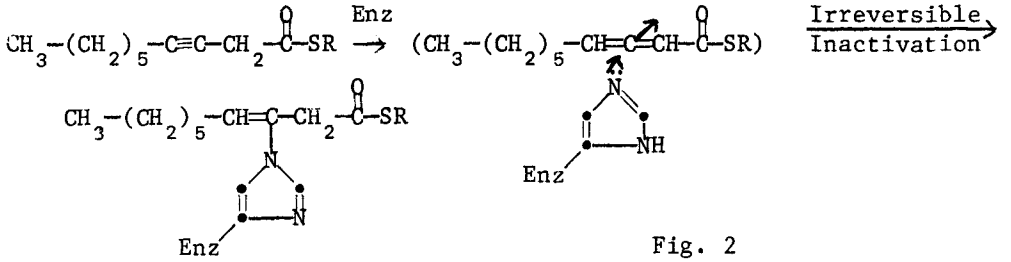


Fig. 2

Since the enzyme can be totally inactivated by a stoichiometric amount of this acetylene, a single turnover is all that is required for irreversible inhibition to occur. The enormous specificity of this inhibitor in multi-component systems and in vivo was demonstrated in the classic experiments of Kass and Bloch[4,5]. These experiments showed that β-hydroxydecanoyl thioester dehydrase was absolutely required for unsaturated fatty acid synthesis in E. coli.

Flavin linked monoamine oxidases are also irreversibly inhibited by molecules that contain an acetylenic moiety .[6] Compounds such as pargyline 1 , chlorgyline 2 , and deprenyl 3 are irreversible inhibitors of the enzyme and in certain instances they have been used clinically[7]. (Fig. 3)

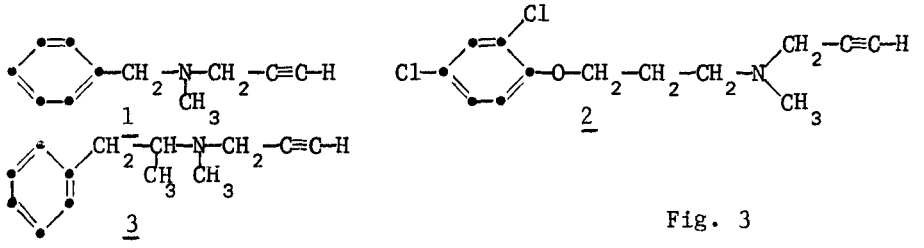


Fig. 3

Studies demonstrate that the acetylenic moiety must be β to the amino group for inhibition to occur and that covalent attachment to the flavin results. Furthermore, we have found that cis-3-haloallyl amines are irreversible inhibitors of this enzyme[8]. As expected, the primary acetylenic amine, propargyl amine, also irreversibly inactivates the enzyme. All of these results can be understood in terms of a plausible mechanism for flavin cocatalyzed reactions[9]. (Fig. 4)

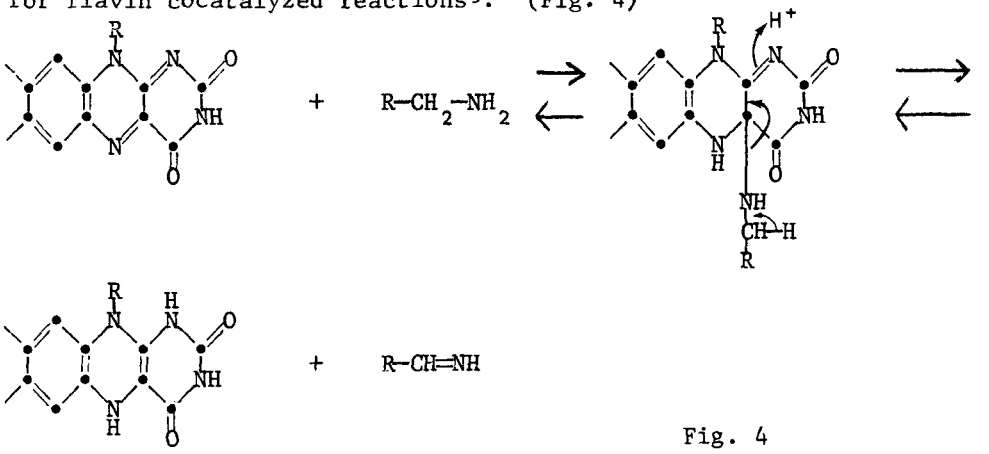
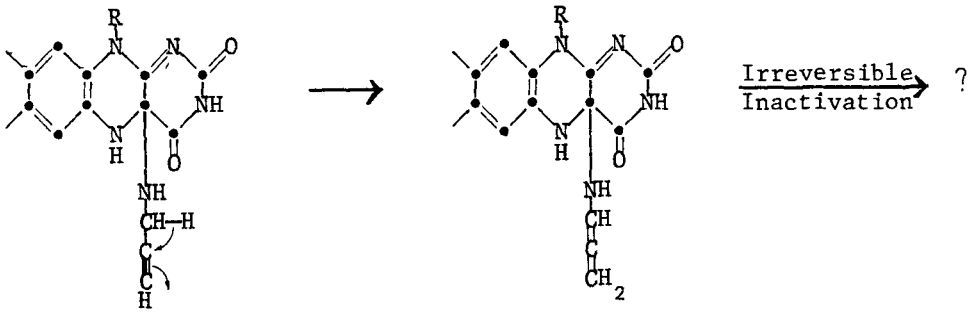


Fig. 4

When the R group is an acetylene or vinyl halide, the following conversions can occur. (Fig. 5)



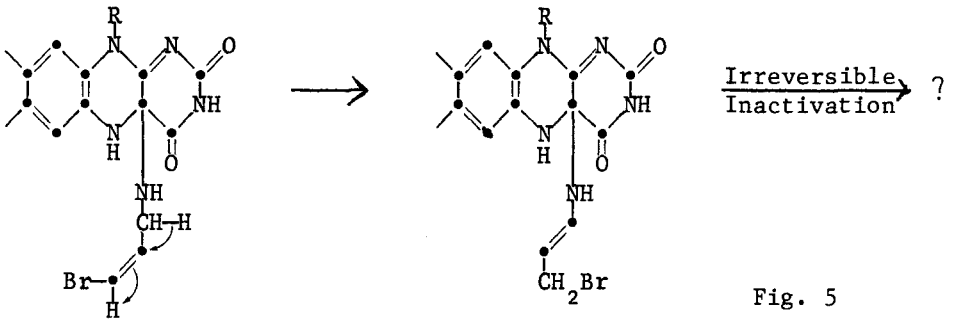


Fig. 5

In both cases, the α-proton is labilized by the enzyme, but instead of the electrons flowing toward the flavin, they are subverted with resultant isomerization of the molecules. Thus, the generation of highly reactive allenes and allylic halides is achieved from their unreactive counterparts.

Other flavin linked enzymes are also susceptible to irreversible inhibition by acetylenes. Abeles and coworkers have demonstrated that flavin linked lactate dehydrogenase is irreversibly inhibited by 2-hydroxy-3-butynoic acid[10]. In this case also, the inhibitor reacts with the flavin cofactor. The great specificity of this inhibitor was used to advantage in studies by these authors designed to determine the physiological role of membrane bound lactate dehydrogenase in E. coli[11]. In addition, the non-flavin linked plasma monoamine and diamine oxidases suffer irreversible inhibition in the presence of the acetylene, propargyl amine[12]. These enzymes require two cofactors for their activity, the first has been positively identified as cupric ion and the second might be a pyridoxal derivative[13,14]. This latter cofactor is capable of forming a Schiff base with the primary amino substrates and, as expected, the enzyme is inhibited by standard carbonyl reagents such as hydrazine[15]. The following conversion could mediate the actual irreversible inhibition step. (Fig. 6)

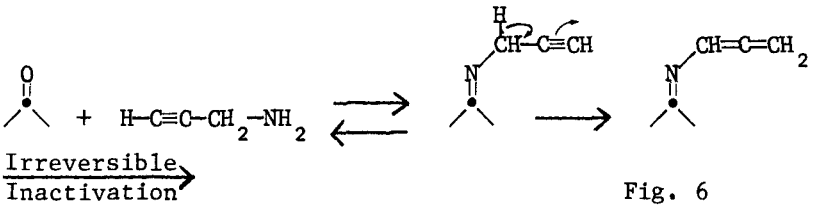


Fig. 6

Consistent with this interpretation is the observation that propargyl amine has no effect on the enzyme when the cupric ion is absent[12]. Therefore, the acetylene is not inhibitory in and of itself but must be enzymatically converted into the active substance.

Enzymes which use the coenzyme pyridoxal phosphate are also quite susceptible to these inhibitors. In 1949, Gunsalus and Wood showed that bacterial threonine dehydratase was irreversible inhibited by serine[16]. The mechanism of action of this enzyme requires abstraction of the α-proton of the amino acid with concommitant elimination of H_2O to form the

aminoacrylate intermediate (AA). (Fig. 7)

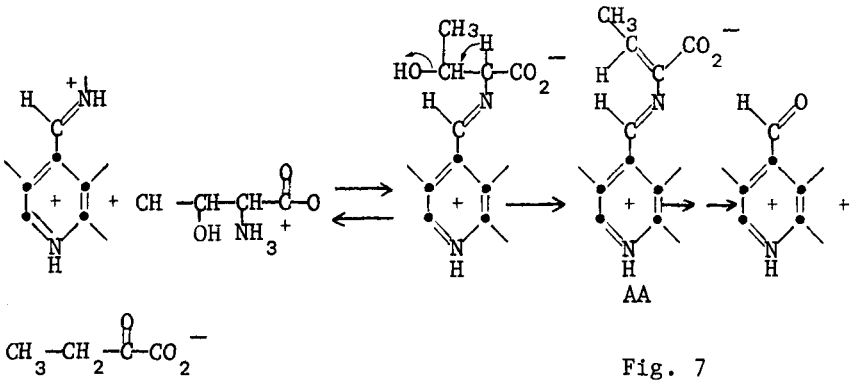


Fig. 7

When serine is the substrate the highly reactive aminoacrylate that is generated apparently engages in a Michael reaction with an active-site amino acid residue resulting in the inactivation of the enzyme[17]. Along similar lines, L-serine sulfate has been shown to be an irreversible inhibitor of soluble aspartate amino-transferase[18] and β-chloroalanine irreversibly inhibits both this enzyme[19] and L-aspartate-β-decarboxylase[20]. The mechanisms of action of these enzymes involve the initial cleavage of the α-C-H bond. Once this occurs, with the inhibitors as substrates, the reaction sequences depicted in Fig. 8 can intervene. (Fig. 8)

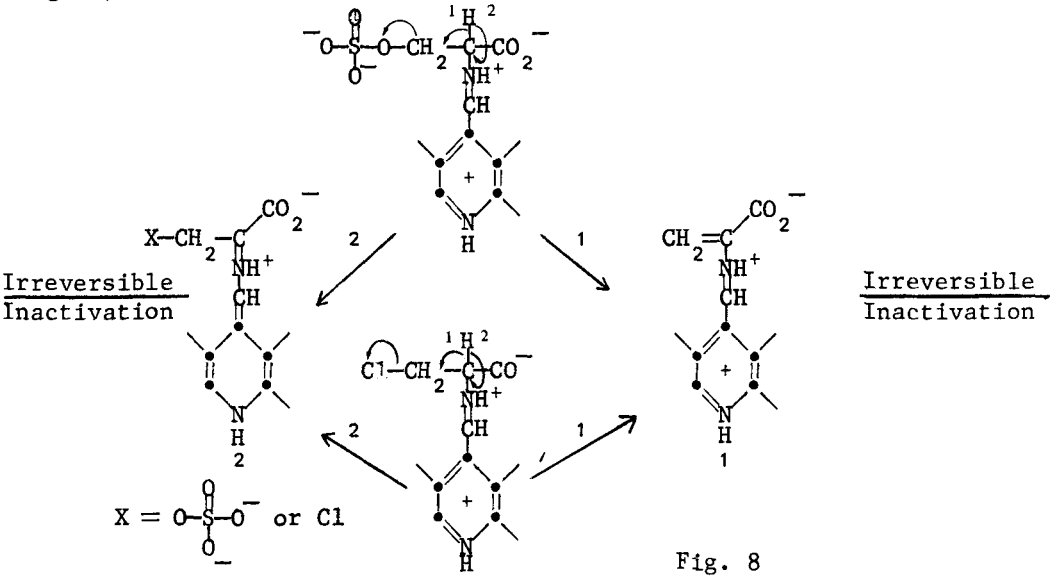


Fig. 8

The subsequent reaction of either of these molecules with critical amino acid residues results in the inactivation of the enzyme. These observations would lead one to expect that β,γ-unsaturated amino acids ought be potent irreversible enzyme inhibitors since their enzymatic conversions would lead to similar highly reactive intermediates. (Fig. 9)

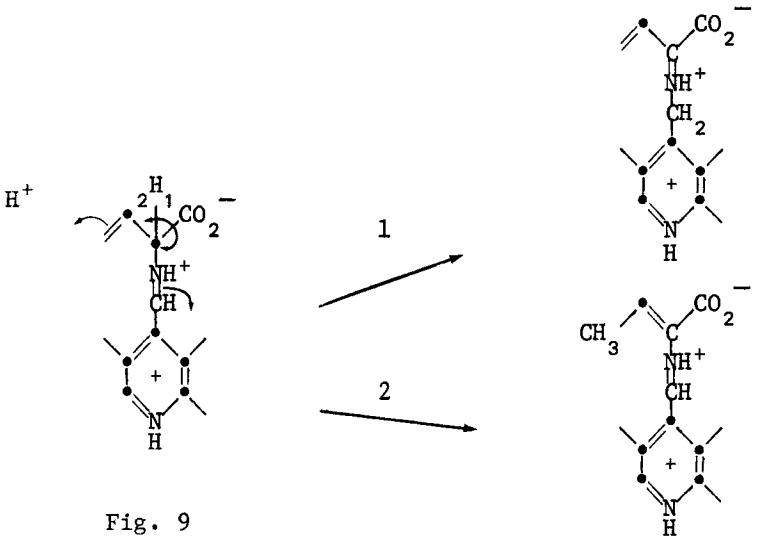


Fig. 9

We have found that 2-amino-3-butenoic acid is an irreversible inhibitor of soluble, pyridoxal phosphate linked aspartate amino transferase[21]. Finally, propargyl glycine has been reported to be an irreversible inhibitor of pyridoxal-linked γ-cystathionase. It is assumed that an isomerization of the acetylene to the reactive allene follows the initial α-C-H bond cleavage[22].

Examples of Naturally Occurring "k_{cat}" Inhibitors - Two naturally occurring β,γ-unsaturated amino acid toxins have been reported in the literature which are of interest here. Rhizobitoxine (2-amino-4-(2-amino-3-hydroxypropoxy)-trans-but-3-enoic acid) (Fig. 10a) is an irreversible inhibitor of β-cystathionase[23,24] from bacteria and plants. The compound shows strong antimetabolic activity, as expected, and resembles the natural substrate for the enzyme, cystathionine. (Fig. 10b)

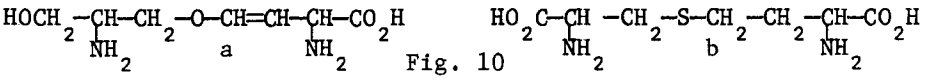
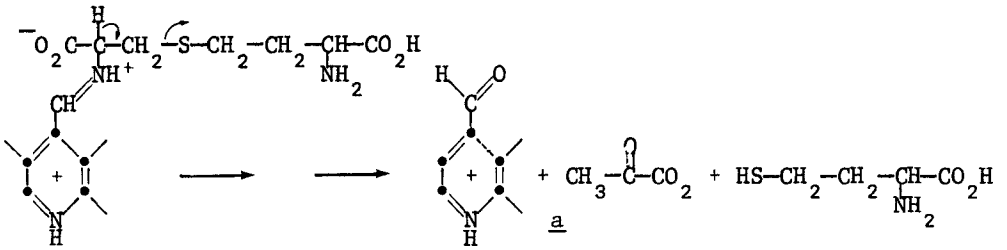
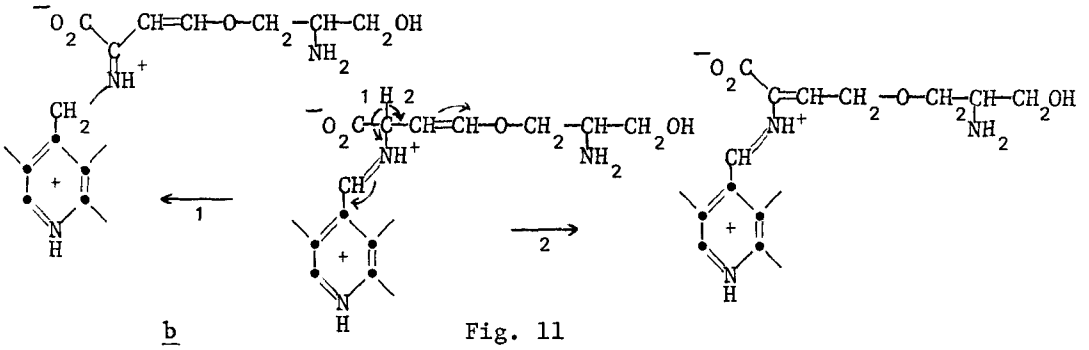


Fig. 10

This pyridoxal-linked enzyme degrades cystathionine by the mechanism shown in Fig. 11a. We propose that rhizobitoxine is an irreversible inhibitor of this enzyme by virtue of the fact that it can be enzymatically converted into the reactive intermediate shown in Fig. 11b.



a



b Fig. 11

In this instance a chemical reaction ensues between the rizobitoxine and the pyridoxal phosphate cofactor. The second compound of this type is L-2-amino-4-methoxy-trans-3-butenoic acid (AMB)[25]. (Fig. 12)

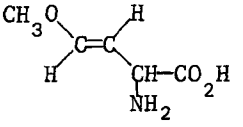


Fig. 12

This compound has been shown to exhibit strong antimetabolic activity against bacteria. We have found that this compound is an irreversible inhibitor of the pyridoxal phosphate-linked aspartate amino transferase[26]. A mechanism similar to the ones described above can account for this. (Fig. 13)

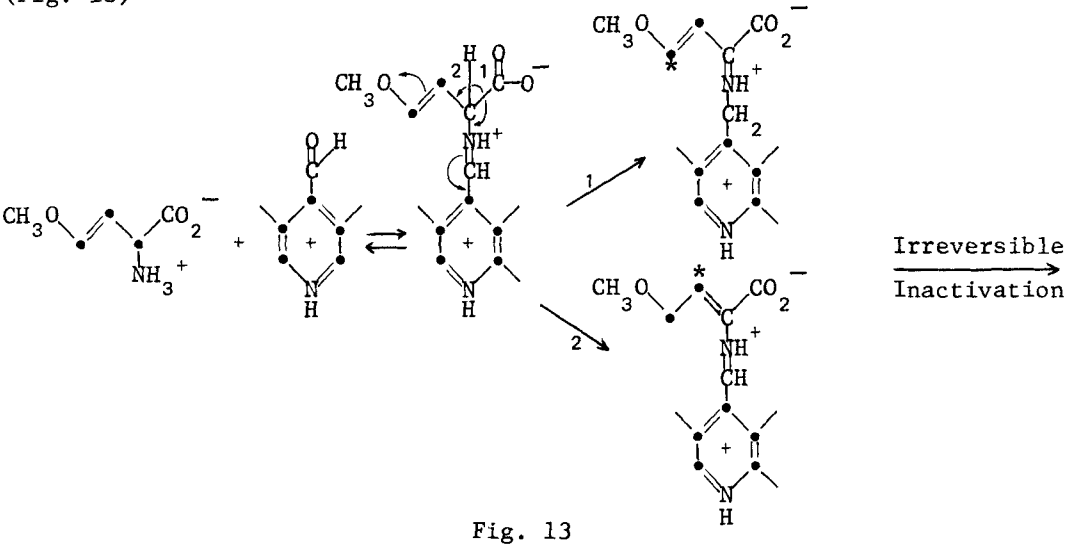
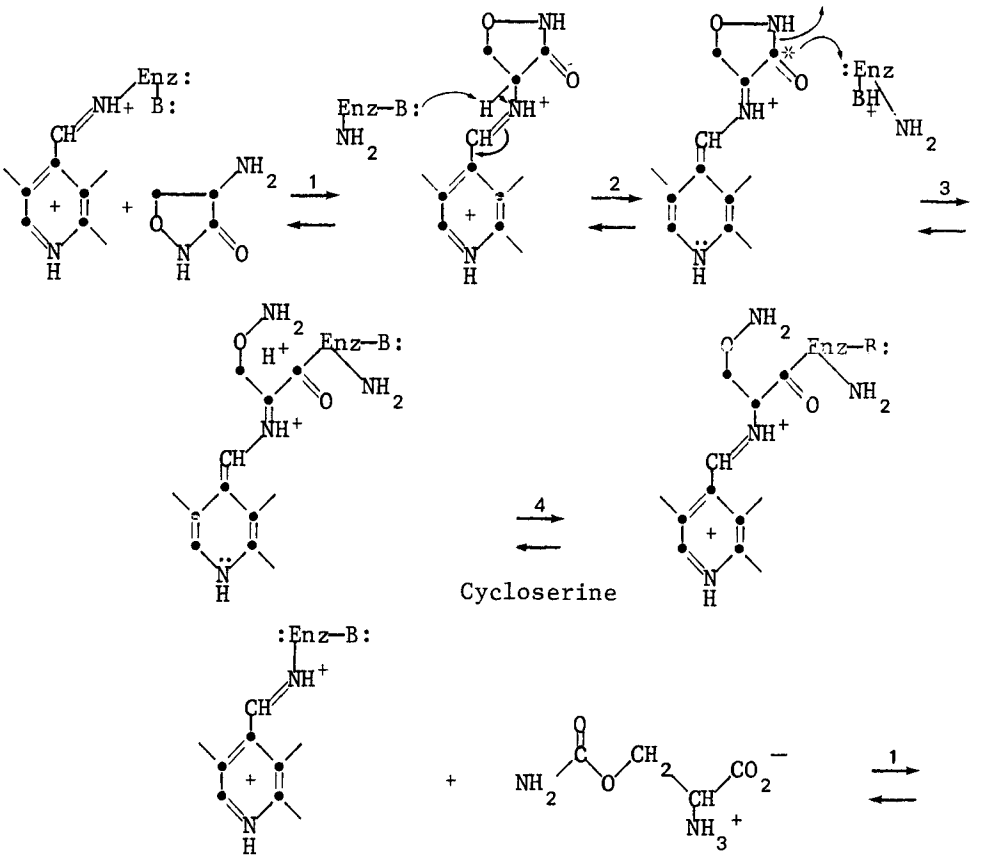
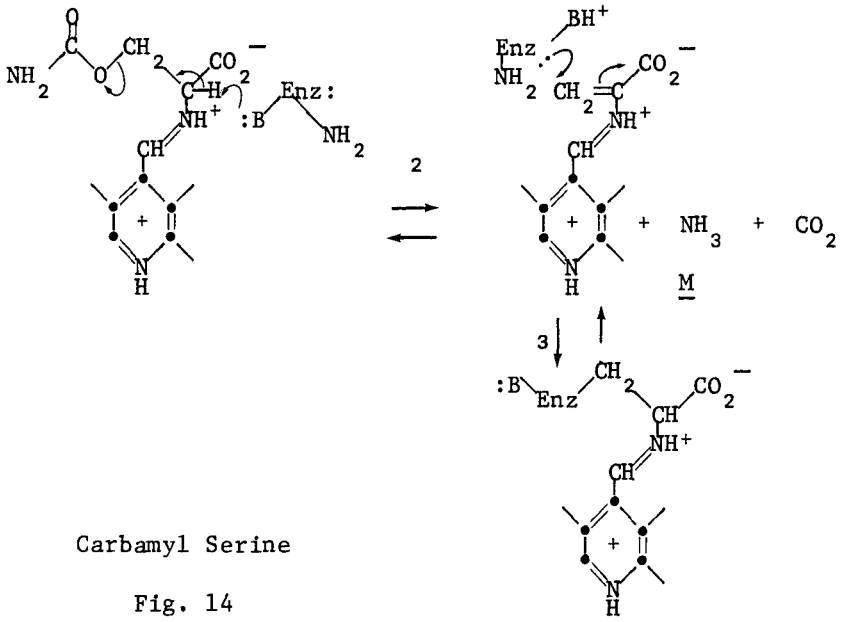


Fig. 13

The antibiotics D-cycloserine and O-carbamyl D-serine are irreversible inhibitors of certain pyridoxal phosphate containing enzymes[27,28,29]. Their utility as inhibitors of cell wall biosynthesis in bacteria is prob-

ably a reflection of the fact that both are irreversible inhibitors of alanine racemase, an enzyme crucial in cell wall biosynthesis[30]. The mechanisms of action of D-cycloserine and O-carbamyl-D-serine are summarized in Fig. 14.



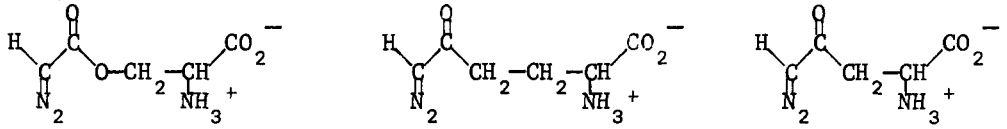


Carbamyl Serine

Fig. 14

In the case of cycloserine the highly reactive acylating agent 1 is enzymatically formed whereas in the case of carbamyl serine the highly reactive Michael acceptor 2 is formed. The latter intermediate is, of course, precisely the same as the active inhibitor of threonine dehydratase with serine as substrate.

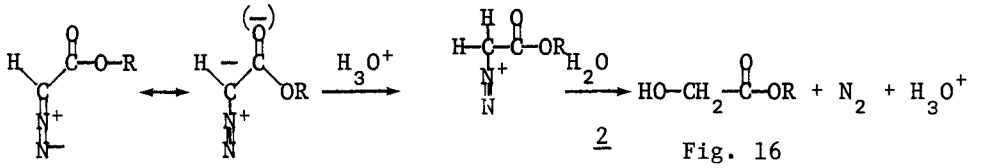
The diazocarbonyl containing antibiotics, azaserine, 6-diazo-5-oxo-L-norlevcine (DON), and 5-diazo-4-oxo-L-norvaline (DONV) also function by a k_{cat} mechanism[31]. (Fig. 15)



Azaserine DON Fig. 15 DONV

These molecules function as irreversible enzyme inhibitors, with the diazo moiety being crucial to their mechanisms of action. In general, the drugs function *in vivo* by irreversibly inhibiting certain enzymes involved in the biosynthesis of purines and pyrimidines and hence the drugs have been used as anti-cancer agents[31].

Since the diazo grouping, as such, is inert towards nucleophilic attack it must first be protonated by the target enzyme to generate the highly reactive diazonium ion[32]. (Fig. 16)



2 Fig. 16

Once generated this ion reacts with an active-site nucleophile at an essentially diffussion controlled rate. For example, azaserine which is an irreversible inhibitor of the enzyme that converts formylglycinamide ribotide (FGAR) to formyl glycinamidine ribotide (FGAM), is protonated by this enzyme prior to alkylating it[33,34]. This protonation is required to activate the diazo compound to the highly reactive diazonium ion. (2 Fig. 17)

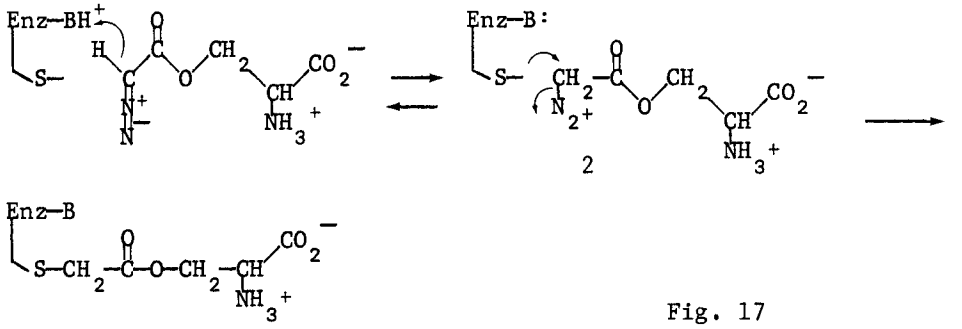


Fig. 17

Once activated, the diazonium compound undergoes an alkylation reaction with an active-site cysteine[33,34].

Conclusion - This article has demonstrated that irreversible enzyme inhibitors which require activation by the target enzyme are of a general nature. Inhibitors of this type have been found amongst both synthetic and naturally occurring molecules. They are exceedingly specific as a consequence of their requiring chemical conversion to the active form by the target enzyme. Thus, unwanted reactions with foreign biomolecules are circumvented. Because of their great specificity inhibitors of this type ought be a useful starting point in the rational design of drugs.

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Chapter 25. Adjuvants to the Immune System

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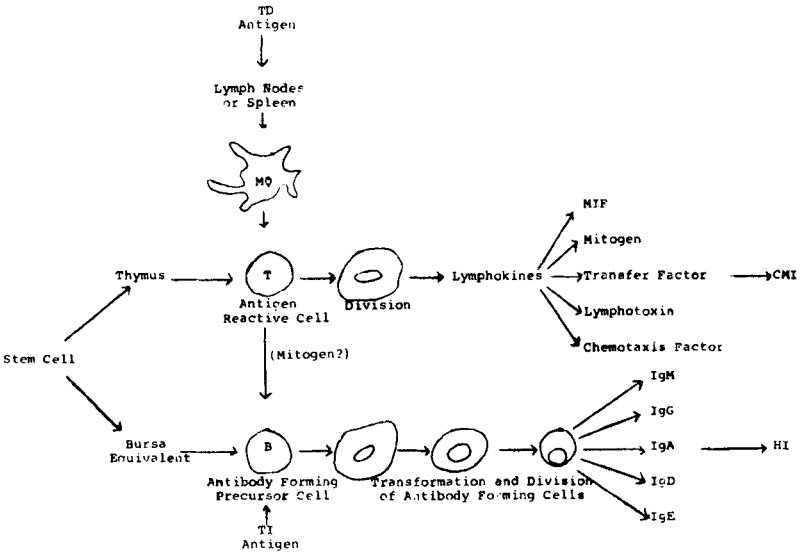
The Immune System - Immune adjuvants (L., Fr., adjuvare, to assist) are defined as substances or procedures which aid in the development or manifestations of the immune system. The protection conferred by this system against material foreign to the host (antigens) ranges from microorganisms to tumors, and its complexity is now beginning to be appreciated.

Two major limbs of the immune response, termed humoral and cell mediated, are defineable with present day knowledge[1]. Either can be enhanced by adjuvants. The two limbs are differentiated primarily on the basis of humoral immunity (HI) being transferrable with serum or plasma from an immune donor to a non-immune recipient, with the active factor(s) being one or several of the five defined immunoglobulin molecules. On the other hand, cell mediated immunity (CMI), functional primarily against grafts, tumors and intracellular dwelling microorganisms, is postulated to occur via soluble mediators, termed lymphokines, secreted over a short range by thymus influenced lymphocytes. The latter are found mainly in peripheral blood leucocyte, spleen, lymph node, or gut associated populations. Contrarywise, in each of these two limbs specific unresponsiveness or tolerance to antigens, such as we exhibit to our own self molecules (and would like to achieve with respect to antigens on organ transplants) can be induced experimentally. These inhibitory procedures also can be reversed by several adjuvants[2,3], providing a model whereby the basic biologic principles involved in recognition of self or non-self by the immune system, might be uncovered. In addition, abnormalities in both limbs are being recognized in the human being, and provide the etiologic basis for a number of important clinical conditions[4].

Recently, the development and manifestations of both humoral and cell mediated immunity have been shown to result from an as yet undefined interaction amongst several different cell types[5,6]. Implicated are a cell in the monocyte-macrophage series (MØ), which is thought to prepare or concentrate the foreign antigen; a thymus influenced lymphocyte (T cell), which is necessary for antibody synthesis but does not itself produce antibody, accordingly termed a "helper" cell; and a bone marrow derived lymphocyte (B cell) which has been shown in chickens to be under the control of a central lymphoid organ, the bursa of Fabricius. The mammalian equivalent of the latter is as yet unknown. Following union of antigen with specific immunoglobulin receptors on the surface membrane, the B cell transforms into a large blast cell and synthesizes antibody during maturation into the plasma cell series. Differentiation and division are characteristic of the response of T and B cells and are the focus of interference by immunosuppressive drugs in transplantation. Two types of antigens have been recognized, thymus dependent (TD) antigens which require the helper effect of the T cell, and thymus independent (TI) antigens, which stimulate the B cell directly. A proposed scheme for the immune system is diagrammed as follows:



Although not depicted in the scheme, the whole complex process of inflammation in many instances is integrated into the immune system, inasmuch as the union of antigens with several antibodies adsorbs, and thereby activates a proesterase, the first of a series of nine substances known as the complement system[7]. A sequential chain of events is set in motion, resulting in enzymatically cleaved split products which act in one sense as adjuvants by being selectively chemotactic for polymorphonuclear leucocytes, monocytes, lymphocytes or eosinophils. By this means, defensive cells are mobilized in the area of antigen (microorganism) deposition, each bringing into the arena its own complex array of physiological mediator substances.

Adjuvants - The very complexity of the immune system introduces the possibility of multiple ways and targets by which its activities might be assisted. The fact that it can be enhanced readily indicates that under normal conditions immunity operates suboptimally. Areas of immediate concern are the potential for elevating the weak immunity engendered by tumor antigens and the utilization of lesser amounts of viruses difficult to isolate in quantities sufficient for mass vaccination. Prior to the recent explosion of knowledge in Immunology, three major classes of practical adjuvants were in use: (a) water-in-oil emulsions, (b) aluminum salt carriers, and (c) mixed vaccines containing Gram negative organisms. Detailed information available to 1966 on the action of each of these has been summarized in one of the few symposia held on this subject[8].

Water in Oil Emulsions - Freund has documented in detail the marked

increase in HI and CMI occurring when antigens are incorporated into water-in-oil emulsions[9]. Complete and incomplete Freund adjuvants have been designated, with the former containing any of several species of killed Mycobacteria in the oily phase. Antigen is suspended in water or saline, and the droplets dispersed uniformly throughout the oil phase by emulsification. The resulting antibody response after one or two injections intramuscularly or subcutaneously, is elevated and high levels persist over many months. The mode of action of water-in-oil emulsions appears to involve the retardation of destruction and elimination of the antigen resulting in a slow, prolonged stimulus[10]. This is supported by the finding that minute amounts of antigen alone given daily for 350 days, mimic the high and prolonged antibody titers seen with this adjuvant[11]. The addition of tubercle bacilli into the oily phase of the mixture (Freund's complete adjuvant) potentiates further these effects as well as CMI. Responsible for the potentiation exerted by the killed Mycobacteria is a wax fraction, termed Wax D, which has been isolated and shown to contain a peptido-glycan linked to a mycolate of an arabino-galactan. White, et al. have documented a striking action of the wax in stimulating macrophage proliferation at the site of injection[12]. The particulate droplets are phagocytized by wandering polymorphonuclear leucocytes and macrophages and are transported to other immunocompetent lymphoid tissues of the body remote from those draining the site of injection. Unfortunately, abscess formation is frequently associated (up to 75% in one study) with this type of adjuvant, restricting severely its usage in man[13]. The toxicity of Freund's adjuvant may reside in part with free fatty acids and degradation products of the emulsifier[14]. In addition, delayed hypersensitivity, as well as auto-immune conditions may be induced readily in experimental animals by this adjuvant[15]. Substitution of vegetable or other more metabolizable oils may circumvent this contraindication. Their efficacy with influenza virus has already been demonstrated[16].

Recently, a non-toxic water soluble adjuvant fraction (WSA) with an approximate molecular weight of 20,000 has been isolated following lysozyme, trypsin, and chymotrypsin treatment of delipidated cell walls of Mycobacteria smegatis. Similar findings were made with the attenuated form of M. bovis, Bacillus Calmette Guerin (BCG)[17]. This material had a stronger adjuvant activity than equal amounts of whole bacteria, cell walls, or the Wax D fraction when added to a water-in-oil emulsion[18]. Antibody titers to PR 8 influenza virus, Columbia SK virus and purified egg albumin were elevated. Furthermore, intial studies showed this product to be non-toxic, non-pyrogenic and unable to sensitize animals to tuberculin or produce allergic arthritis in rats, all of which were facets of the reaction to the intact Mycobacteria. Preliminary evidence indicates WSA is effective in saline, independent of water-in-oil emulsification, increasing its potential for human usage[19].

Adsorption onto Aluminum Salts - The powerful adsorptive qualities of the aluminum ion have been utilized with antigens as a second physical procedure which enhances the immune system[20]. Antigens are adsorbed firmly during or after the formation of aluminum hydroxide gel following treatment of aluminum salts with alkali. As with water-in-oil emulsions,

retention and wider dissemination of antigen appear to be important functional aspects of this adjuvant when deposited subcutaneously or intramuscularly. However, it is not as effective as Freund's adjuvant in this regard. White, et al. have demonstrated antibody production in the local granuloma developed at the site of injection, as well as within the regional lymphatic glands[21]. Toxicity and side reactions of aluminum hydroxide or phosphate gels are low, and these mineral salt carriers have proven useful with diphtheria and tetanus toxoids in pediatric human and animal vaccination[22].

Combined Vaccines - Combined vaccines, employing killed Salmonella typhosa (typhoid fever) or Hemophilus pertussis (whooping cough) as one of the components together with diphtheria and tetanus toxoids, give rise to higher antitoxin titers than when fluid toxoids are given singly[23]. The active adjuvant has been shown to be a lipopolysaccharide component of the bacterial cell wall, termed LPS or endotoxin[24]. It is a poly-disperse molecule with an average molecular weight generally over a million. Its action is dramatic, 1-5 μg shortening the induction period and elevating peak antibody titers, but its toxicity is well known, with pyrogenicity, renal damage and shock among its characteristics[25]. In contrast to Freund's adjuvant or aluminum carriers, lengthy prolongation of antibody titers is not a feature of this adjuvant. A strong stimulus of memory cells does occur, however, and on re-exposure to antigen alone, rapid and elevated responses are observed.

B Cell Mitogens - Following definition of macrophage-T-B cell interactions, several substances were described which stimulated selectively either T or B cells to transform into large blast cells and divide[26]. The lipid moiety of the LPS discussed above, termed Lipid A, consisting of phosphorylated glucosamine dissacharide units with ester and amide linked fatty acids, when properly dispersed was shown to be one of the most powerful mitogens for antibody synthesizing B lymphocytes in vitro[27, 28]. Associated with this property was the secretion by the B cells of polyclonal antibodies directly without the injection of antigens. No mitogenic effect was exerted on T cells, although the helper effect of T cells on B cells was necessary for LPS treated animals to exhibit their more profound adjuvant action on TD antigens in vivo[29]. Insertion of the lipid A into the lipid bilayer of the B cell membrane has been postulated to trigger these responses, and the adjuvant property of lipid A is lost following removal by alkali treatment of the ester linked fatty acids[30]. Covalent linkage of several TD antigens to the LPS eliminated their thymus dependency, and when attached to this mitogen they became capable of activating the B cell directly to synthesize specific antibody, independent of the T cell helper effect[31]. It is surmised therefore, that the normal function of the T cell that is replaced by the mitogenic action of the LPS is itself a mitogenic principle, and that the property of mitogenicity for B cells may be a mandatory characteristic of thymus independent antigens. Thus, 7 different TI antigens (lipopolysaccharides from Gram negative bacteria, pneumococcal polysaccharides, bacterial flagellin, levan, polyvinylpyrrolidone, dextran, dextran sulfate) were found to stimulate the uptake of DNA synthesis by B lymphocytes and induce polyclonal antibody

synthesis to several unrelated antigens[32]. As a result of these findings, it has been postulated that all TI antigens possess 2 different reactive sites on the same molecule. One is an antigenic determinant group which focuses the molecule preferentially on the specific clone of antibody forming B cells reacting only to that antigen via the cell's immunoglobulin membrane receptor, and the second is the mitogen (adjuvant) which gives the actual signal to the cell to divide and synthesize antibody[33]. Although binding of TD antigens or haptens directly to B cells does occur via the specific Ig receptor, this binding event alone is incapable of triggering the cell to make antibody until the signal from the mitogen is received. For these thymus dependent antigens, the mitogenic signal can come from the T cell, released in some as yet unknown fashion by specific antigen or by non-specific T cell adjuvants as discussed below, or by concomitant mitogenic stimulation of B cells with TI antigens such as LPS.

T Cell Mitogens - Two plant substances selectively mitogenic over a narrow dose range for T cells are (a) phytohemagglutinin (PHA), isolated from the bean Phaseolus vulgaris, having an affinity for N-acetyl-D-galactosamine[34], and (b) Concanavalin A, obtained from the jack bean, Canavalia ensiformis, which binds to α-D-mannopyranosyl, α and β-D-glucopyranosyl and β-D-fructofuranosyl groups[35]. Responsiveness to these mitogens is a selective marker for T cells although subpopulations within the T cell family are becoming evident[36]. When added in doses of less than 1 μg with antigen to spleen cell cultures containing T cells, Con A is weakly stimulatory of antibody synthesis. In high concentrations, it is inhibitory[37]. On the other hand, the helper effect of T cells is increased strongly following incubation of T cells for 24 hr with Con A. The mechanism of activation is not known, but may be related to a Con A induced release of the mitogenic factor endogenous to T cells. The appearance of a mitogenic factor in the supernatant fluids of cultures of T cells has been described under a number of conditions besides the addition of antigen to sensitized T cells[5]. Thus, mixtures of immunocompetent lymphoid cells from genetically different individuals, termed allogeneic effect, or mixed lymphocyte reaction, releases a mitogenic factor which acts non-specifically on B cells to stimulate them to antibody synthesis. This factor appears to substitute for the T cell helper effect required by TD antigens and permits direct triggering of B cells by antigens which were previously thymus dependent. Thus, adjuvants exerting an effect on the T cell may function by stimulating the secretion of the same mitogenic factor which acts non-specifically on B cells signalling them to initiate antibody synthesis.

Nucleic Acids - The use of nucleic acids as adjuvants to the immune response was first indicated in the demonstration of a restorative capacity of nucleic acid-rich tissues in animals rendered immunodeficient following x-irradiation[38]. Their ability to act as adjuvants to the normal immune response to unrelated antigens has also been documented and shown to be attributable to low molecular weight oligonucleotides[39]. Extended study over the past 5 years has shown the more well-defined polynucleotide complexes (poly A:U and poly I:C) to be powerful adjuvants to many antigens in several animal species[40]. Each of the polynucleotides alone were ineffective and complexing was required. Quantities of antigen as low as 1

nanogram were rendered immunogenic by poly A:U[41]. The polynucleotide action was exerted on several cell types (e.g., macrophage, memory cell and in particular, as a restorative agent in animals rendered deficient in T cells)[42]. T cell cytotoxicity for tumor cells was also enhanced[43], as was protection of mice against rabies virus challenge[44]. Although amplification of T cell activity is one way in which poly A:U and other adjuvants appear to activate the immune system[45], it remains to be delineated whether this occurs by a mitogenic activity increasing T cell numbers or an enhancement of individual T cell effector action. Conversion of normal macrophages into cytotoxic cells capable of killing cultured lymphoma cells has also been demonstrated to be a property of the polynucleotides as well as LPS[46].

A role for nucleic acids as adjuvants is also indicated by many studies documenting a dramatic increase in both limbs of the immune response by nucleic acid-antigen complexes isolated from macrophages[6, 47]. In these instances, antigen was exposed in vitro to thioglycollate induced peritoneal macrophages from normal animals. Following incubation and washing, RNA was extracted and injected into normal animals previously unexposed to the antigen. Remarkably high titers of specific antibody have been induced to many antigens using this technique. The nature of the unique RNA is under investigation. Its effectiveness as an adjuvant has potential for the weakly foreign tumor antigens or antigens such as viral components which are difficult to isolate in large quantities.

The striking potency of ribosomal vaccines protective against the bacteria from which they have been isolated underscores with a different system the adjuvant action of nucleic acids. Thus, in 1965, Youmans and Youmans demonstrated that ribosome-rich material isolated from Mycobacterium tuberculosis protected mice against this organism as efficiently as the whole organism vaccines[48]. The effective material was heat labile, ribonuclease sensitive and had the characteristics of double-stranded RNA[49]. In addition, the ribosomal vaccine acted as an adjuvant to unrelated antigens, as well as in the experimental induction of auto-immune disease[50]. These findings have been extended to other organisms such as Salmonella typhimurium[51], Pneumococcus[52], Staphylococcus[53], and Pseudomonas[54]. A plausible explanation for the efficacy of such preparations lies in the retention of minute quantities of cell wall antigens attached to the RNA, with the latter responsible for stimulating the immunogenicity of such low amounts of antigen. Whether it is due to a mitogenic or another form of stimulus is as yet an unanswered question. In this context, subpopulations of T cells have been demonstrated which suppress rather than help immunological responses[55, 56]. Whether or not some adjuvants might function by removal of, or interference with this suppressor population, is an important aspect of this question.

Non-specific Tumor Regression Induced by Adjuvants - Recently, non-specific regression of tumors has been accomplished by adjuvant procedures involving the inducement of specific cell mediated immunity against non-tumor associated agents which are highly effective at promoting this limb of the immune system[57]. For example, when human beings bearing tumors

were sensitized with either BCG, purified protein derivative of tubercle bacilli, or dinitrochlorobenzene, all potent antigenic inducers of CMI, and subsequently provoked into expression of cell mediated immunity by homologous antigen challenge, tumor regression was observed. This was heightened by deposition of the provoking dose of antigen directly into the tumor, but sensitization and challenge at sites distant from the tumor also effected regression. Mechanistically, the challenge dose of antigen is postulated to provoke the liberation of lymphokines following specific interaction with lymphocytes sensitized by the first dose of antigen. Once released, these effector molecules act non-specifically resulting in the destruction of the tumor tissue as "innocent bystander."

Conclusion - Intelligent, controlled interference with the immune response is a foreseeable goal within the next few decades. Pertinent to this achievement will be chemical definition of the adjuvant induced molecular signals which amplify MØ, T and B cell activity. In this regard, chemically defined molecular adjuvants are beginning to be identified. Thus, the phenylimidothiazole salt, levamisole, has been shown recently to be effective against a syngeneic mouse sarcoma[58], and aid anti-bacterial immunity[59]. Characterization of cell receptors for adjuvants should prove to be particularly revealing, as should the intracellular signals activating the cell. The cyclic AMP system has already been implicated in the latter[60,61].

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Chapter 26. The Cannabinoids: Therapeutic Potentials

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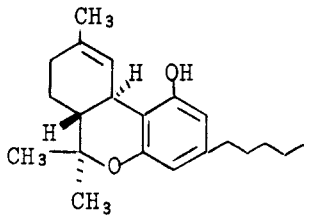
This review will survey the literature of the past year with particular reference to those articles which indicate the therapeutic potentials for cannabinoids[1]. For a comprehensive, up-to-date survey of cannabis chemistry, pharmacology and clinical studies, the reader is referred to a new book[2] edited by R. Mechoulam.

In regard to marijuana or its active component, Δ^9-THC, the following potential medical applications have been listed[3] as areas still to be explored: analgesia, antihypertensive activity, treatment of migraine, management of dying patients and sexual stimulation.

Considering the large number of publications in the marijuana area, few of them examine structure-activity relationships. To date, very little has appeared to show whether the Δ^9-THC molecule can be chemically modified to achieve an enhancement of one particular activity present in Δ^9-THC itself. Thus, any therapeutic possibility for the cannabinoids rests mainly on pharmacological and clinical evidence gathered on Δ^9-THC or marijuana itself.

New activities were discovered last year. It is still too early to decide which of the following areas represents the best possibility for a useful drug in the cannabinoid area.

Anti-Edema, Analgesic, Antipyretic and Anti-Inflammatory Effects - Δ^9-THC inhibits the *in vitro* biosynthesis of prostaglandin E_2 (PGE_2) from arachidonic acid[4] and the biosynthesis of PGE_1 from 8,11,14-eicosatrienic acid[5]. The inhibition of PGE_2 biosynthesis has been postulated as a mechanism of action for such non-steroidal anti-inflammatory agents as aspirin and indomethacin[6]. Some *in vivo* data[7,8] appear to correlate. In the carrageenan-induced rat paw edema, a dose of 10 mg/kg Δ^9-THC caused a 40% inhibition of paw swelling. Daily administration of 20 mg/kg Δ^9-THC inhibited the developing adjuvant-induced polyarthritis in rats. In several tests of analgesic activity (acetic acid-induced abdominal constriction, Haffner's tail pinch, hot plate test and Randall-Selitto test) Δ^9-THC showed effectiveness with ED_{50} of 0.9 to 11.6 mg/kg. At doses up to 20 mg/kg Δ^9-THC did not affect yeast-induced pyrexis in rats.

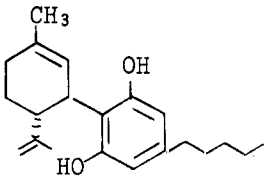


Δ^9-THC

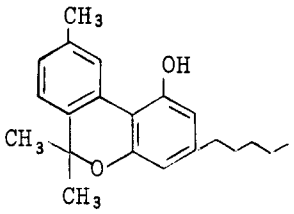
In another study[9] of the effects of Δ^9-THC on yeast-induced pyrexis, carrageenan-induced edema and yeast-induced rat paw hyperesthesia, the following results were obtained: (1) The oral antipyretic potency of Δ^9-THC is approximately 2 times that of phenylbutazone, (2) Δ^9-THC was essentially devoid of antinociceptive activity except at elevated doses which produced a marked catatonic-like state, and (3) Δ^9-THC at doses up to and including 100 mg/kg was totally ineffective in reducing or preventing the edematous response.

Anti-Fertility Activity - At a dose of 2 mg in rats, Δ^9-THC suppressed the cyclic surge of luteinizing hormone secretion and also suppressed ovulation (a characteristic shared by other CNS-active drugs)[10]. However, mating and fertility indices were similar for control and all treatment groups in a study of the effect of Δ^9-THC on reproduction in rats[11]. At doses of 2 and 3 mg/kg, Δ^9-THC caused a deterioration in the sexual performance of male rats (interpreted as reflecting a decreased motivation to copulate)[12].

Anti-Epileptic, Anticonvulsant Action - The anticonvulsant properties of marijuana and Δ^9-THC have been recognized for several years. Now cannabidiol and cannabinol have also been found to be effective anticonvulsants using a maximal electroshock test in mice[13]. (ED_{50}'s: Δ^9-THC, 80 mg/kg: CBD, 105 mg/kg and CBN, 230 mg/kg).



Cannabidiol (CBD)



Cannabinol (CBN)

Treatment of freely moving cats with Δ^9-THC (0.25-0.5 mg/kg) temporarily reduced seizure activity induced by electrical stimulation of subcortical structures[14,15]. One study concluded that THC closely resembles acetazolamide rather than diphenylhydantoin[16].

Antihypertensive, Cardiotonic Effects - A previous review[17] suggested that "the tetrahydrocannabinols warrant evaluation in the treatment of essential hypertension". Δ^9-THC has since been found to significantly lower the blood pressure of rats and block the appearance of hypertension in immobilized rats[18]. At 20 mg/kg s.c., the hypotensive effect persisted over a 96 hr. period and completely counteracted the pressor effects of repeated immobilization stress.

In female rats showing adrenal regeneration hypertension, a dose of 3 mg/kg of Δ^9-THC was capable of lowering blood pressure[19] (this moderate dose for a rat does not produce somatic side effects).

One study[20] draws attention to the fact that doses of marijuana

which produce marked psychological alterations in man, do not alter blood pressure in a systematic fashion. The same paper notes that tolerance to the hypotensive effects of Δ^9-THC (5-25 mg/kg/day) develops rapidly in the spontaneously hypertensive rat.

The mechanism of these blood pressure and other cardiovascular effects has been the subject for much study. Reduction in blood pressure in anesthesized dogs caused by administration of 2.5 mg/kg i.v. of Δ^9-THC is accompanied by a decrease in cardiac output and an increase in local vascular resistance[21]. The fractional blood flow to the vital coronary, cerebral and renal beds was unchanged[21]. The reduction in cardiac output appears to result from the action of Δ^9-THC on the heart rate (bradycardia) as well as venous return[22,23]. A maximal degree of bradycardia is produced only when both sympathetic and parasympathetic innervation of the heart is intact[24]. Δ^9-THC appears to be devoid of any ganglionic or β-adrenergic blocking properties[24]. Interestingly, Δ^9-THC produces a dose-related tachycardia in man[25].

While both Δ^9-THC and propanolol delay the onset of ventricular ectopic extrasystoles, Δ^9-THC does not delay the onset of ouabain-induced arrhythmias[26].

Pulmonary Effects - Asthma - Three groups published last year on the pulmonary effects of marijuana. In one study[27,28] of nine, normal volunteers with previous marijuana smoking experience, airway resistance (measured in a body plethysmograph) fell 38% and specific airway conductance increased 44% at a dose of 84 µg/kg. This effect was accompanied by a 28% increase in heart rate. At a dose of 32 µg/kg, there was no increase in heart rate but there were significant changes in airway dynamics.

In another paper[29] thirty-two experienced male marijuana smokers were given Δ^9-THC by smoking or orally (20 mg dose). Again specific airway conductance increased immediately (48% at 15 min. after smoking marijuana assayed at 2% Δ^9-THC; 45% at 180 min. after ingestion of 20 mg Δ^9-THC). A study[30] suggested that these effects operated via a mechanism other than deep breathing or β-adrenergic stimulation, the dilation being due to, most probably, relaxation of the smooth muscle of the tracheobronchial tree. No data were available on isolated bronchial smooth muscle.

These data strongly suggest a therapeutic potential for Δ^9-THC in the treatment of pulmonary congestion; e.g., asthma[31]. Nevertheless, one paper[32] has pointed out that these pulmonary effects are not reflections of changes in the clinically important peripheral airways (<2 mm diameter).

Potentiation of Barbiturates and Anesthetics: - Pretreatment of mice for six successive days at 20 mg/kg of Δ^9-THC, caused a decrease in the duration of sleeping time after dosing with zoxazolamine and hexobarbital[33]. However, under the same experimental conditions, the duration of barbital sleeping time was enhanced. The authors suggest an induction by Δ^9-THC of the hepatic enzyme systems involved in the metabolism of zoxazolamine and hexobarbital, but no metabolism studies are offered in support of this

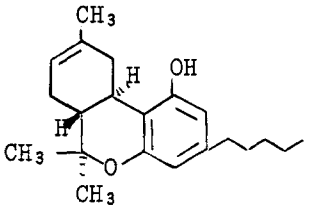
possibility. Another paper[34] from the same laboratory reports that pretreatment of mice with doses of Δ^9-THC (0.625 to 40 mg/kg) caused a significant reduction in the onset to and duration of sleeping time of most of the sedative-hypnotic drugs tested. These studies cast some degree of doubt on the therapeutic potential of Δ^9-THC to potentiate barbiturate sleeping time, unless it is done on an acute dosing basis rather than after chronic treatment.

Two studies[35,36] report on the interaction of Δ^9-THC with anesthetics. The minimum alveolar anesthetic (MAC) requirements for cyclopropane in rats[35] and halothane in dogs[36] were significantly decreased by pretreatment with Δ^9-THC. Analgesia, sedation and prolonged barbiturate sleeping time after acute Δ^9-THC injection may reflect the potential additive anesthetic-like action of the drug. Alternatively, drugs that deplete norepinephrine in the CNS decrease halothane MAC[36].

Δ^9-THC and Sleep - Δ^9-THC continues to be studied as a sedative-hypnotic. One study[37] of the sleep-wakefulness cycle in rabbits was stimulated by the effects of Δ^9-THC on 5HT metabolism in the brain, an effect hypothesized to play an important role in the production of slow wave sleep. No evidence was found that Δ^9-THC in doses of 0.5 and 1.0 mg/kg increased total REM sleep time or prolonged the duration of single episodes[37]. In another study[38] in cats, slow wave sleep was significantly decreased.

A clinical study[39] of Δ^9-THC as a hypnotic found that: (1) Δ^9-THC significantly decreases the time it takes to fall asleep in physically healthy insomniacs. (2) The most significant effect of an oral dose of 30 mg was a "hangover" phenomena or continued "high" the next day. (3) This "hangover" seemed severe enough to eliminate the 30 mg dose range for clinical use as a hypnotic. A comparison study of Δ^9-THC at doses around 20 mg with a standard hypnotic is in progress.

Δ^8-THC when administered to cats at doses of 1-10 mg/kg, i.p. or i.v., induced sedation and caused a trend toward fewer and longer REM sleep episodes following initial suppression of paradoxical sleep[40]. In rats tolerance developed to both the suppression of paradoxical sleep and to behavioral effects (principally excitation)[41].

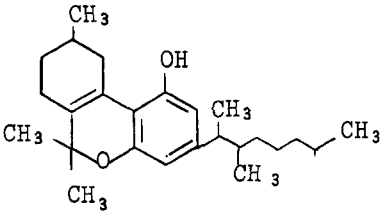


Δ^8-THC

Other CNS Effects: Biogenic Amines - With several standard procedures commonly used to detect antidepressant activity, Δ^9-THC was virtually inactive by i.p. administration at doses up to 20 mg/kg[42]. Clinically, Δ^9-THC (0.3

mg/kg, p.o. twice a day for 7 days) in 8 hospitalized depressed patients failed to produce significant euphoria or antidepressant response[43].

The mechanism of the central effects of cannabinoids continues to be investigated. Δ^9-THC, Δ^8-THC and DMHP all increased the amounts of noradrenaline and dopamine accumulated from tyrosine in rat whole brain[44]. The hypothalmus region was most susceptible to these changes in catecholamine biosynthesis. In spite of the observed increase in catecholamine biosynthesis in the brain and adrenals, the endogenous content of NA and DA in the brain and DA and NA in the adrenals remained unchanged[45].



DMHP

Repeated administration of Δ^9-THC to rats leads to an increase in activity of tyrosine hydroxylase[46]; tryptophane hydroxylase and DOPA decarboxylase were not significantly affected.

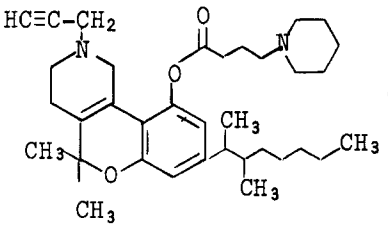
Because of the possible implication of PEA as one of the adrenergic ergotropic modulators, alterations in its disposition may be responsible for the euphoric effects of Δ^9-THC in man[47]. Acute administration of 3 mg/kg of Δ^9-THC increased 4-fold the brain levels of PEA and daily administration of 0.3 mg/kg for 8 days doubles PEA brain levels. In contrast Δ^9-THC induces only relatively small changes in the brain levels of serotonin, catecholamines and acetylcholine.

Confirming earlier work on ingested marijuana, smoked cannabis has been found to produce little changes in the urinary excretion of epinephrine and NE but decreases VMA excretion by 25% from baseline value at 2 hrs. in humans[48].

Summary - Even a cursory look at the literature on cannabis would lead one to the conclusion that marijuana is "a drug for all reasons". Perhaps the many biological activities of marijuana impeded its therapeutic use during the past 40 years. As recently as 1971 the natural material or THC appeared to offer little advantage over currently used medications used as sedatives, analgesics, antidepressants and antihypertensive drugs[49].

With the identification in 1964 of Δ^9-THC as the "active" ingredient in marijuana, a new chapter in marijuana research was opened. The possibility of structural modification to enhance activity was again explored. Undoubtedly during the past decade many investigators (especially those in the pharmaceutical industry) have pursued the possibility of modification of the Δ^9-THC structure to delineate activities.

An indication of the efforts along these lines has been reported[50] by Abbott Laboratories. Compound, ABBOTT 40656, SP106, a water-soluble benzopyran derivative is presently under Phase I clinical evaluation as a sedative-hypnotic.



ABBOTT 40656 (SP106)

Time will tell whether the pursuit of structure-activity relationships will lead to therapeutically useful drugs in any or all of the disease areas mentioned in this review. Also new activities might be discovered.

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Section VI - Topics in Chemistry

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Lawrence, Kansas

Chapter 27: Reactions of Interest in Medicinal Chemistry

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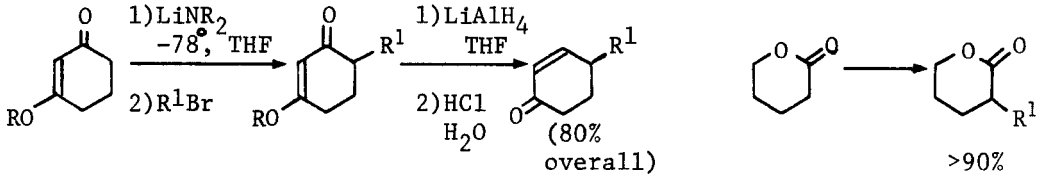
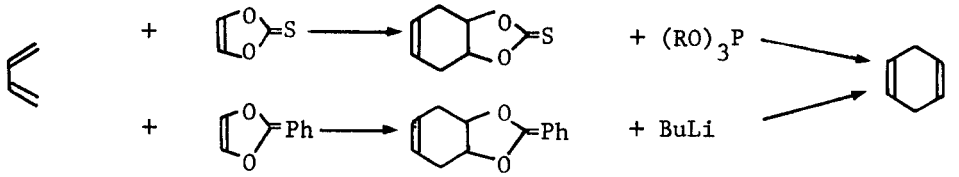
Several useful reference books of general interest were published in 1973 including: Annual Reports in Organic Synthesis-1973, the third of the annual series by J. McMurry and R. B. Miller (covering the literature to March, 1973); Protective Groups in Organic Chemistry by J. F. W. McOmie; and Volume 27 of Synthetic Methods of Organic Chemistry by William Theilheimer. In Theilheimer's preface is included an interesting summary of "Trends in Synthetic Organic Chemistry" citing eighty-one references to the synthetic literature 1971-1973.

Computers - The possibilities and limitations of using *computer literature searches* to find particular kinds of synthetic reactions has been reviewed.[1]

C-C Bond Formation - Tertiary phosphines have been shown to be *good catalysts for Michael reactions*, offering a useful alternative to alkoxide or hydroxide catalysis.[2] *Lithium "amides"* have been used to form enolates and carbanions at -78° for a variety of synthetic purposes cited throughout this chapter, including for C-C bond formation. The alkylation of cyclohexenones[3] (or α-diketones[4]) and of lactones[5] is illustrative:

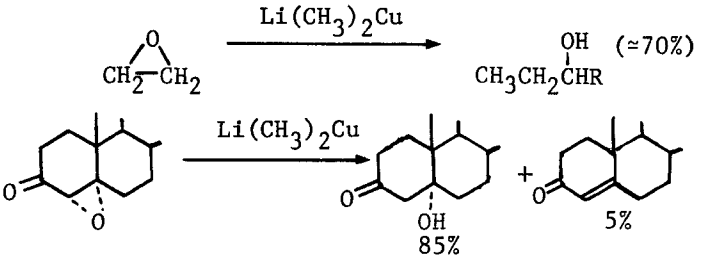


Acetylene is normally a very sluggish dienophile in *Diels-Alder* reactions, but two *acetylene synthons* have been reported to be very reactive -- vinylene thionocarbonate and 2-phenyl-1,3-dioxol-4-ene.[6]

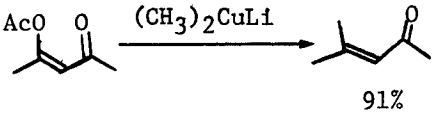


Enamines to be used in C-C bond formation may be prepared under very mild conditions by treatment of the aldehyde or ketone with the trimethylsilyl derivative of the appropriate amine.[7]

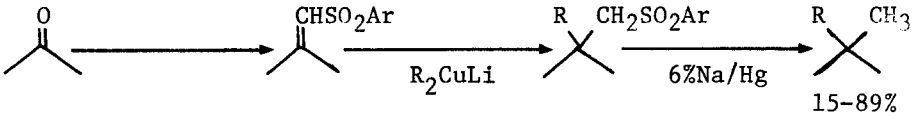
Organocopper Reagents - Many new organocopper reagents and uses of these reagents have been reported in 1973--including one report of lithium di-n-butyl cuprate acting as a reducing agent in certain situations.[8] The following examples illustrate recent advances. The *electronic and structural requirements* for addition of organocopper reagents to α,β-unsaturated carbonyl compounds have been studied in detail.[9] *Epoxides treated with organocopper reagents* form simple alcohols[10] and even α-hydroxyallenes.[11] On the other hand, treatment of lithium dimethyl copper with some α,β-epoxy ketones failed to yield any alkylated product.[12]



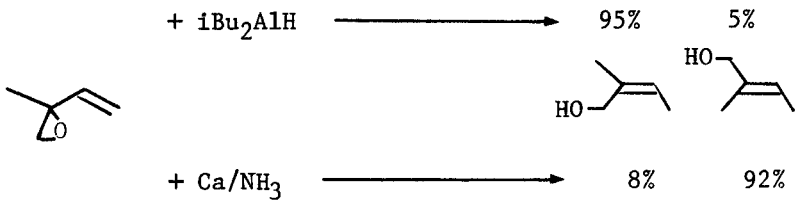
Lithium dialkyl cuprates react with enol acetates of β-diketones to give the corresponding β-alkyl-α,β-unsaturated ketone.[13]



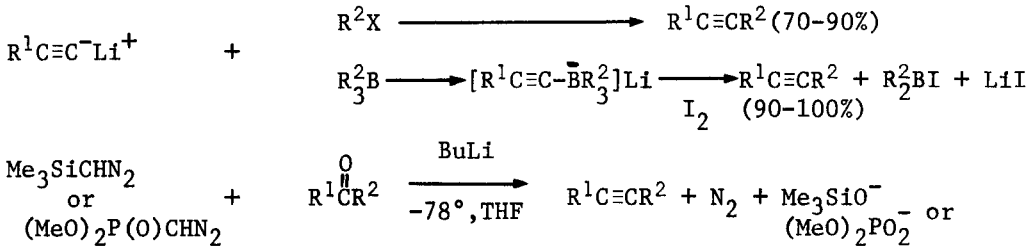
A new class of mixed cuprate (I) reagents, Het(R)CuLi, allows *selective alkyl group transfer.*[14] Lithium diorganocuprates *add to primary tosylates in high yields* (70-100%), but secondary tosylates give somewhat lower yields (10-75%).[15] α-*Alkyl ketones* have been prepared by treatment of dimethylcuprate (I) on α,α'-dibromoketones. Yields are good, and the reaction appears to proceed via a cyclopropanone intermediate.[16] Reaction of α,β-unsaturated sulfones with lithium dialkylcuprates has been used to prepare gem-dialkyl products.[17]



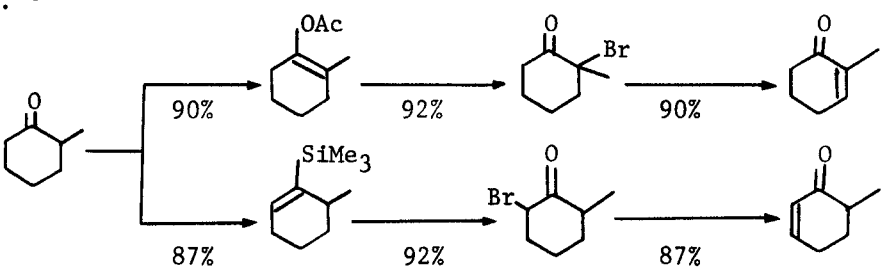
Preparation of C=C and C≡C - *Epoxides* have been used as starting materials for several olefin syntheses. Olefins may be prepared by addition of trifluoroacetic acid to a solution of the epoxide and *triphenylphosphine selenide.*[18] *Trisubstituted allylic alcohols* may be stereoselectively prepared in good yield by the acid catalyzed ring-opening of cyclopropyloxiranes[19] and by conjugate reduction of α,β-unsaturated epoxides.[20]



Intramolecular Wittig reactions have been used to form new C=C bonds in high yields.[21] *Disubstituted acetylenes* may be obtained in high yields by treating lithioalkynes with alkyl halides;[22] by treating carbonyl compounds in a one-step, base-induced reaction with trimethylsilyldiazomethane or dimethylphosphonodiazomethane;[23] or by treatment of lithium 1-alkynyltriorganoborates.[24]

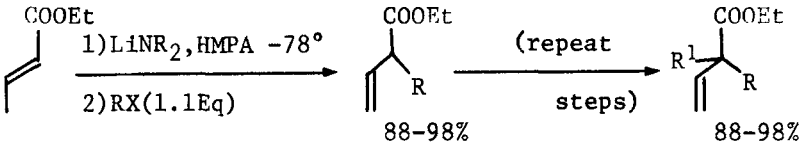


Unsaturated Carbonyl Compounds - A *two carbon-homologation* of ketones, aldehydes, or halides to α,β-unsaturated aldehydes has been achieved using 2,3,4,6-tetramethyl-4H-1,3-oxazine.[25] Ketones have been brominated selectively on one side of the carbonyl group with specifically generated enolates. Dehydrobromination then yields *position-specific α,β-unsaturated ketones*.[26]



α,β-*Unsaturated ketones and esters* may be obtained from the corresponding saturated compounds by treatment with $PhSe^-Na^+$, PhSeCl, or PhSeBr followed by oxidation with H_2O_2 or sodium periodate.[27] New syntheses of α-*methylene lactones* continue to be reported. For example, lactones may be treated with a *lithium amide at -78°* and carbon dioxide to form the α-carboxy-lactone, and then with formaldehyde[28] (or in a longer sequence with iodomethyl methyl sulfide[29]) to form the α-methylene product.

Alkylation of α,β-unsaturated carbonyl compounds usually occurs in the α-position, but it has been shown that *deconjugative alkylation* can occur in the presence of hexamethylphosphoramide (HMPA).[30]



Protective Groups and Their Removal - *Lactones and esters* may be protected as *1,3-dithiolanes.* The ester or lactone is treated with bis(dimethylaluminum) 1,2-ethanedithiolate, and deprotection is achieved with mercuric oxide and BF_3-Et_2O in 15% aqueous THF. The dithiolanes were shown to be stable to acetic acid, to KOH-aqueous methanol, and to $LiAlH_4$ or methyllithium in ether.[31]

Another report appeared describing the use of the *t-butyldimethylsilyl protecting group for alcohols,*[32] and it was also found to be useful for protecting *deoxynucleosides*--especially the 5' hydroxyl.[33] *Ylids containing silyl protective groups* have also been successfully employed in Wittig reactions to synthesize complicated olefins under very mild conditions. However, the ylid must be pure and salt free, and a precise stoichometry must be used.[34]

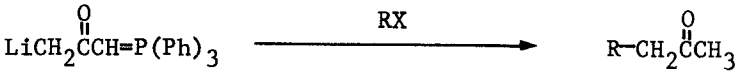
A review of new amino function protecting groups was published[35] and the *4-isopropyloxycarbonyloxybenzyloxy carbonyl group* has appeared as a *new alkali-labile amino protecting group.*[36]

S-substituted mercaptoethanols can serve as *phosphate protective groups* in nucleotide synthesis,[37] and 1-oxopyridin-2-yldiazomethane has been reported to be a water soluble phosphate blocking group.[38] Tetrahydropyranyl ethers and 4-methoxy-THP *ethers may be prepared in only five minutes* by treatment of the parent alcohol with dihydropyran in anydrous dioxane, using p-toluenesulfonic acid as a catalyst. Yields are 80-90%.[39] *Bromoketals* have been investigated to meet the requirements of *protective group removal under neutral conditions.* Ketalization is carried out by treatment of the carbonyl compound with bromoglycol and p-toluenesulfonic acid as a catalyst. Deketalization is accomplished by refluxing the bromoketal in methanol containing zinc dust.[40]

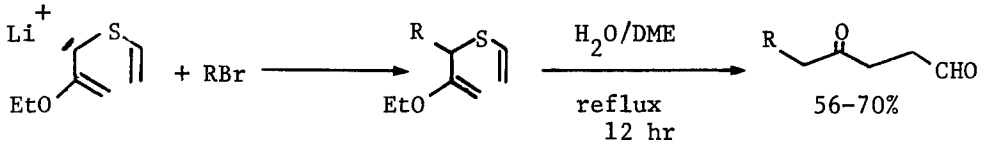
Saturated Aldehydes and Ketones - Both primary and secondary alcohols can be oxidized in 94-98% yield to their corresponding carbonyl compounds with *dimethyl sulfoxide-chlorine complex* at -45° to -10°,[41] a procedure which is reportedly superior to previous oxidation methods. *Terminal alkynes are converted to ketones* by alkylation or protonation of their lithium alkynyltrialkylborate salts, followed by H_2O_2 oxidation.[42]

$$R^1_3B + LiC{\equiv}CR^2 \longrightarrow [R^1_3\bar{B}\text{-}C{\equiv}C\text{-}R^2]Li \xrightarrow[2)H_2O_2]{1)R^3X} R^1C(=O)CHR^2R^3 \quad 76\text{-}90\%$$

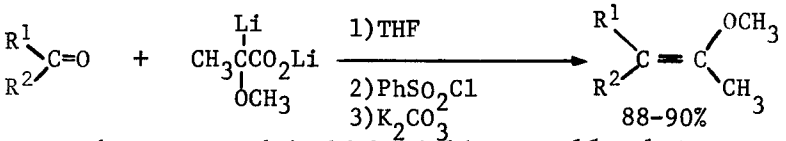
Acetonyl functions can be prepared from the readily available lithium salt of acetylmethylene triphenylphosphorane by reaction with alkyl halides.[43]



β-*Ketoesters* are produced in 40-90% yield by condensation of O-silylketone acetals with acid chlorides followed by hydrolysis.[44] γ-*Ketoaldehydes* result from alkylation of the lithium salt of 2-ethoxyallyl vinyl sulfide, followed by *thio-Claisen rearrangement* and hydrolysis.[45]



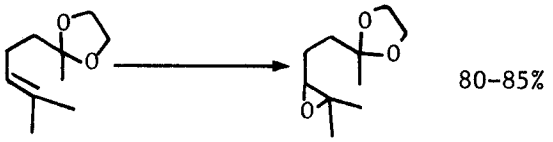
High yields of *trisubstituted enol ethers*, readily convertible to methyl ketones, result when ketones are treated with dilithium O-methyl lactate followed by decarboxylation.[46]



Olefins may be converted in high yield to α-*chloroketones* by treatment with chromyl chloride in acetone.[47] α-*Carboxyketones*, generally relatively inaccessible, can be prepared by treatment of ketones with lithium 4-methyl-2,6-di-*tert*-butylphenoxide and carbon dioxide.[48] *Cyclic ketones* result from *ring expansion of methylene cycloalkanes with cyanogen azide* followed by hydrolysis, a procedure which complements the standard diazomethane-ketone approach.[49] Functionalized *cis-bicyclic ketones* can be synthesized by haloketal cyclization followed by hydrolysis.[50]

Epoxides - A modified method for the conversion of *ketones to epoxides* via β-hydroxysulfides has been developed.[51]

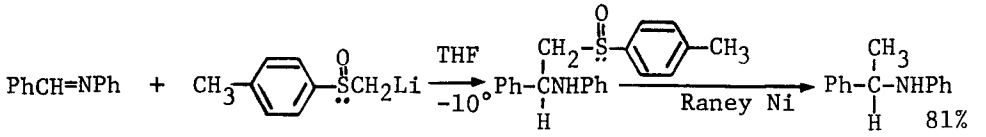
Acid-sensitive olefins may be epoxidized in high yield using a two-phase system of dichloromethane and aqueous sodium bicarbonate to which is added *m*-chloroperbenzoic acid.[52]



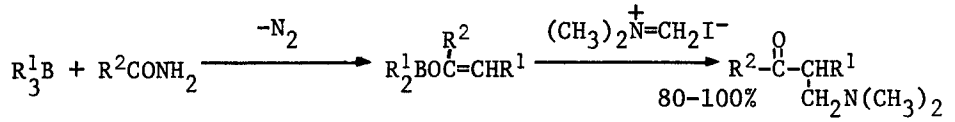
Two new routes to *arene oxides* of carcinogenic aromatic hydrocarbons have been reported, which employ OsO_4 or NBS sequences to prepare the required 1,2-diol or α-bromo alcohol intermediates for epoxidation.[53,54]

Amines and Nitriles - Primary and secondary amines may be *methylated* in high yield by treatment with formaldehyde in methanol followed by reduction with sodium borohydride.[55] Similarly, aromatic primary amines may be monomethylated by *sodium borohydride reduction* of their N-aryl-amino methylsuccinimides, generated by treatment of the amine with aqueous alcoholic formaldehyde and succinimide.[56] A general, *stereospecific synthesis of secondary amines* can be achieved in high yield by treatment of alkyl or aryl azides with alkyl- or aryl-dichloroborane ($RBCl_2$) followed by base hydrolysis. The reaction proceeds with retention of configuration of the alkyl group in the alkyldichloroborane.[57]

Optically active amines can be prepared in 80-90% yield (90% optical purity) by reaction of imines or nitriles with optically active *p*-tolylsulfinyl carbanion followed by reduction with Raney nickel.[58]



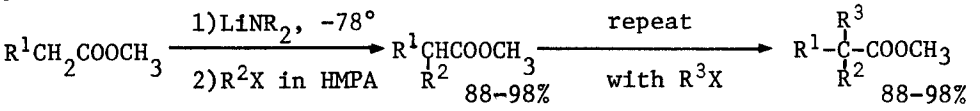
A new β-*arylethylamine* synthesis by aryl aldehyde homologation has also been developed.[59] A procedure has been found for the specific *ortho alkylation of aromatic amines*.[60] A review of the use of *quaternary ammonium compounds in organic synthesis* has recently appeared.[61] *Mannich bases* may be prepared regiospecifically using the reaction of enol borates with dimethyl-(methylene)-ammonium iodide.[62] A very complete review of



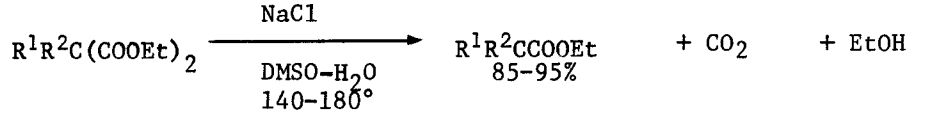
synthetic uses of Mannich bases has been published.[63]

It is now possible to convert *ketones into nitriles* directly using tosylmethylisocyanide.[64] *Nitriles* may also be prepared using the action of in situ generated dichlorocarbene on amides, thioamides or aldoximes.[64] Under these same conditions ureas yield *N-cyanoamines*.

Carboxylic Acids and Derivatives - A convenient one-flask method for *ester alkylation* also uses lithium diisopropylamide at low temperature.[65]

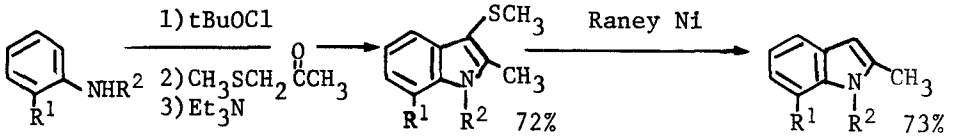


Decarbalkoxylations of geminal diesters can be effected by treatment with sodium chloride in hot, wet dimethylsulfoxide.[66] Similar yields are obtained in the decarbethoxylation of both β-keto and α-cyanoesters.

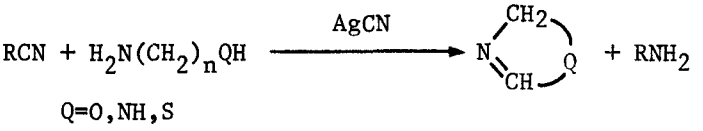


Alkyl halides (R^1X) may be converted to the corresponding aliphatic carboxylic acids (R^1COOH), esters (R^1COOR^2) or amides ($R^1CONR^2R^3$) using *disodium tetracarbonylferrate* followed by oxidation with O_2 or NaOCl, or halogenation on the presence of the appropriate alcohol or amine.[67] Primary and secondary *alcohols* (R^1R^2CHOH) may be converted in good yields *to amides* ($R^1R^2CHNHCOR$) using chlorodiphenylmethylium hexachloroantimonate in nitrile solvents.[68] *α-Amino acid derivatives* may be prepared from malonic acid half-esters using diphenylphosphorylazide. Substituted glycine, valine, phenylalanine and tryptophan were generated, among other amino acids.[69] Finally, there is an excellent review of modern approaches to *β-lactam* syntheses.[70]

Heterocycles - Alkyl or aryl dichloroboranes react with 2-iodoalkyl azides to give stereospecifically *N-alkyl and N-aryl aziridines* in yields of 73-94%.[71] The ring closure of anilines to *2-substituted indoles* may be accomplished in the following manner,[72] and *oxindoles* may be obtained with a change in the sulfide reagent of step 2 to $CH_3SCH_2C(=O)OC_2H_5$.[73]



A direct synthesis of *substituted pyridines* in yields of about 50% is found in the treatment of acetylene and an alkyl nitrile in the presence of a cobalt catalyst.[74] A general method for the synthesis of 3- and 3,4-substituted *furans from ketones* has been developed.[75] A new route to various heterocycles with *2 or more heteroatoms* has been presented, as well as a procedure involving 1,3- or 3,3-cyclization on difunctional nucleophiles, such as hydrazine, hydroxylamine, alkyl and aryl-diamines.[76,77]

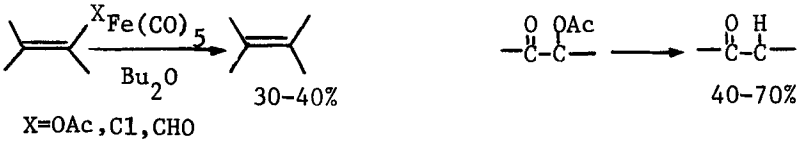


Sulfur Chemistry - Two facile methods of the heretofore difficult *sulfoxide to sulfide reduction* have been accomplished with diisobutyl aluminum hydride[78] and dichloroborane in THF at 0°.[79] With the latter reagent, ketones, esters, and amides remain unaffected. A review on *sulfoximes* and derivatives as synthetic reagents presents some new methods for the preparation of various *oxiranes, aziridines, alcohols, cyclopropanes, and alkenes.*[80] *Allylic sulfoxide anions* have proven useful for the *synthesis of allylic alcohols,* including trisubstituted olefinic allylic alcohols.[81] Transesterification between a dialkylacylphosphonate and a sulfonic acid yields *sulfonate esters.*[82] The oxidation of aliphatic *mercaptans to sulfinic acids* with the use of *m*-chloroperbenzoic acid is especially useful in that the excess perbenzoic acid is removed by precipitation at -80°.[83]

Reduction - *Ether soluble* AlH_3 may be made by treatment of $LiAlH_4$ with

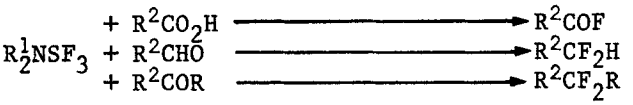
$BeCl_2$ or $ZnCl_2$ (with concomitant precipitation of $LiBeH_2Cl_2$ or LiCl).[84] The Birch reduction has now been applied to Ar heterocycles[85] yielding, for example, N-substituted 1,4-dihydroquinolines from quinoline. A variety of reductive uses for $LiEt_3BH$ have been reported.[86,87]

Lithium-ethylamine reduction of 1,4-dialkylbenzenes gives the corresponding 1,4-dialkylcyclohexa-1,4-diene.[88] Among the many new applications of metal carbonyls are to be found the reductions of enol acetates, vinyl chlorides, and α,β-unsaturated aldehydes to olefins; and α-acetoxyketones to ketones.[89]

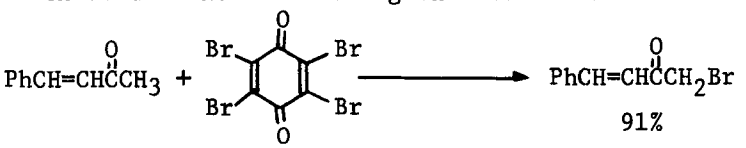


Removal of aromatic *phenolic* groups is accomplished in 65-90% yield by conversion to the sulfonate, followed by catalytic hydrogenation.[90] Aromatic *amines* are deaminated by aprotic diazotization with pentyl nitrate in yields up to 89%.[91]

Useful Reactions (not elsewhere classified) - *Fluorination* of 12 types of compounds has been simplified and improved by the use of *dialkylamino trifluorides*, resulting in faster reaction times (15 to 30 min.), lower temperatures (0 to 80°), and better yields (55-89%) than with SF_4.[92]



The difficult task of selective α-bromination of α,β-unsaturated ketones may be achieved without affecting the double bond.[93]



Reviews (not mentioned elsewhere) - *Organoboron chemistry*,[94] *Metal π-allyl complexes*,[95] *organic electrochemistry*,[96] *free-radical preparative organic chemistry*,[97] and *asymmetric syntheses* (utilizing π-allyl nickel halides, Lewis acids, and phosphoranes to introduce chiral centers,[98] have been reviewed.

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Chapter 28. The Intramolecular Diels-Alder Reaction in Organic Synthesis

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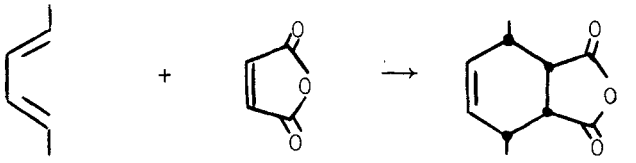
The [4+2]cycloaddition reaction long known to organic chemists as the Diels-Alder reaction is among the most powerful of the many synthetic tools available to chemists interested in the synthesis of complex organic molecules.[1] Its ability to produce with high stereoselectivity, good yield and in a predictable manner a single stereoisomer containing several chiral centers is almost unique among organic reactions. For example the addition of maleic anhydride to trans, trans-2,4-hexadiene gives stereoselectively the adduct 1 containing four asymmetric centers.[2]



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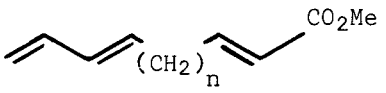
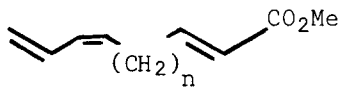
Because of these remarkable assets, as well as a tremendous versatility, the Diels-Alder reaction has been utilized in a large number of total syntheses of complex molecules.

Although the early literature records a number of reactions which can be interpreted as involving intramolecular Diels-Alder reactions, it is only in the last decade that organic chemists have studied the intramolecular Diels-Alder reaction in terms of its scope and limitations and intentionally applied the intramolecular cyclization in the synthesis of complex molecules. This chapter will review some of the more interesting studies of the intramolecular Diels-Alder reactions and its application to the preparation of a variety of classes of natural products. The intramolecular Diels-Alder reaction is of great potential utility because it may be used to solve orientation problems that might be encountered in the corresponding intermolecular reaction. It is also likely that there will be enhanced reactivity in some systems because the entropy of activation will be appreciably lower than that of the analogous bimolecular reactions.

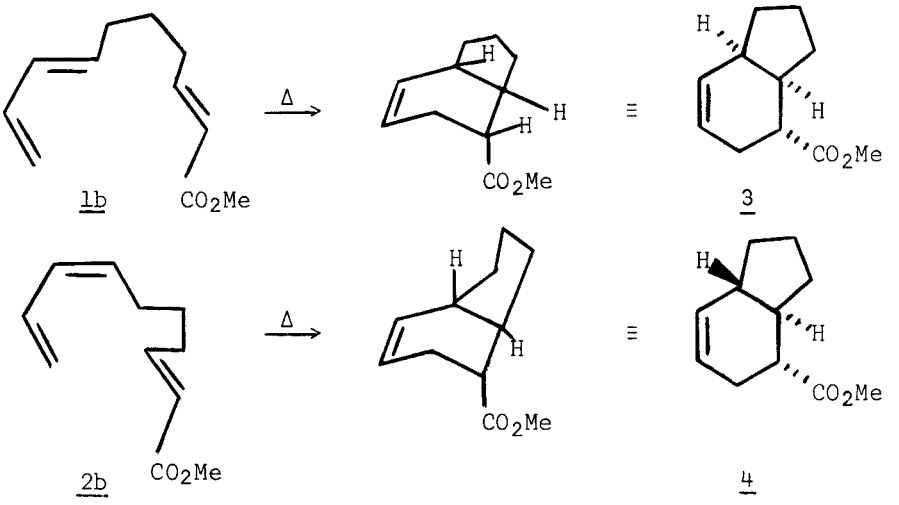
House[3] reported in 1965 the first systematic study of the stereochemistry and structural limitations of the intramolecular Diels-Alder reaction. In this study the thermal reactions of the isomeric triene esters 1 and 2 were examined. With both 1a and 2a the intramolecular Diels-Alder reaction failed, but with three carbon atoms separating the diene and dienophile portions of the molecule both the cis- and trans-isomers (1b and 2b) underwent stereoselective cyclization to produce the hydrindane derivatives 3 and 4.



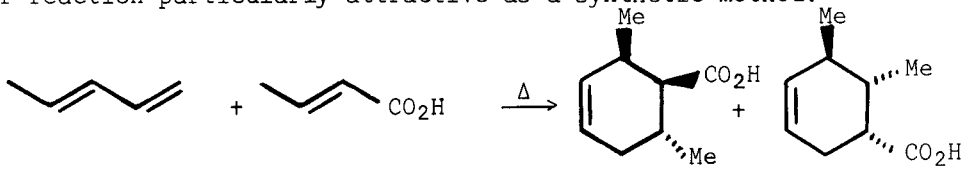
1a, n=2
b, n=3



2a, n=2
b, n=3



Of particular interest was the finding that the cis-1,3-pentadiene derivative 2b underwent ready cyclization because systems of this type often fail to undergo bimolecular Diels-Alder reactions. Secondly, the intramolecular reaction dictates a specific orientation of the diene and dienophile which is different from that observed in the analogous intermolecular reaction. It is these two features which make the intramolecular Diels-Alder reaction particularly attractive as a synthetic method.



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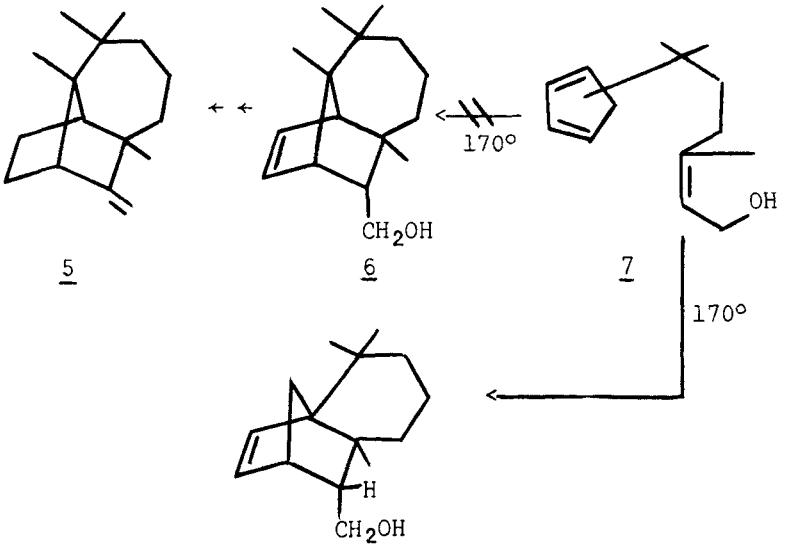
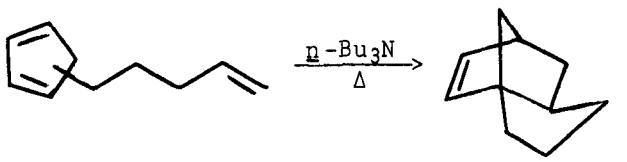
One of the earliest attempts to use the intramolecular Diels-Alder reaction for the synthesis of a natural product was reported in 1963 by Brieger who attempted to prepare longifolene (5) by a route involving an intramolecular Diels-Alder reaction.[4] The synthetic strategy was based upon the consideration that a compound such as 6 could be readily converted to longifolene (5) by reduction of the double bond followed by dehydration and that 6 could potentially be prepared by an intramolecular cyclization of 7. The required intermediate 7 was prepared as a mixture of isomers by the coupling of cyclopentadienyl magnesium bromide with the hydrogen chloride addition product of geranyl acetate. Unfortunately,



5 6 7 8

7 did not cyclize to give the product with the longifolene skeleton but instead gave the tricyclic isomer 8 as the product of the intramolecular Diels-Alder reaction.

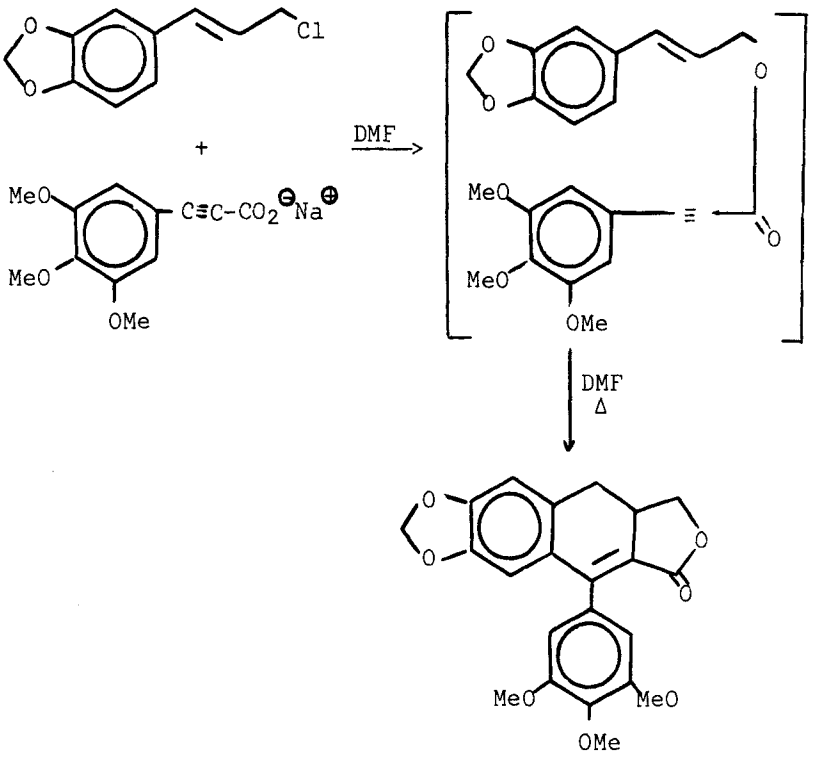
A similar cyclization has been exploited by Corey for the preparation of the tricyclic system 9.[5]



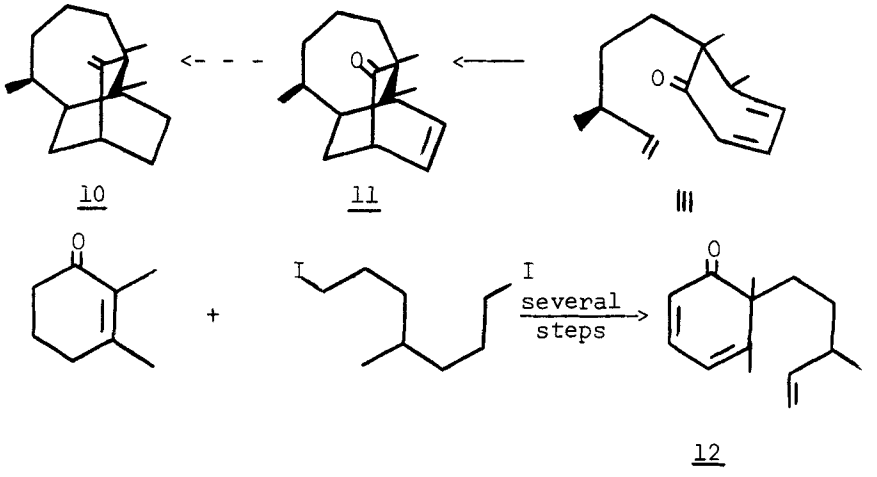
9

Klemm has published an extensive series of papers describing his work which has taken advantage of the intramolecular Diels-Alder reaction for the preparation of a series of lignan lactones, podophyllotoxins and picropodophyllins.[6] In these reactions an aromatic ring contributes one of the double bonds of the diene portion of the molecule. A typical example is given below.[6b] Although the yields in these cyclizations are often low the intramolecular Diels-Alder route provides a remarkably short synthetic pathway to these systems from readily available intermediates.

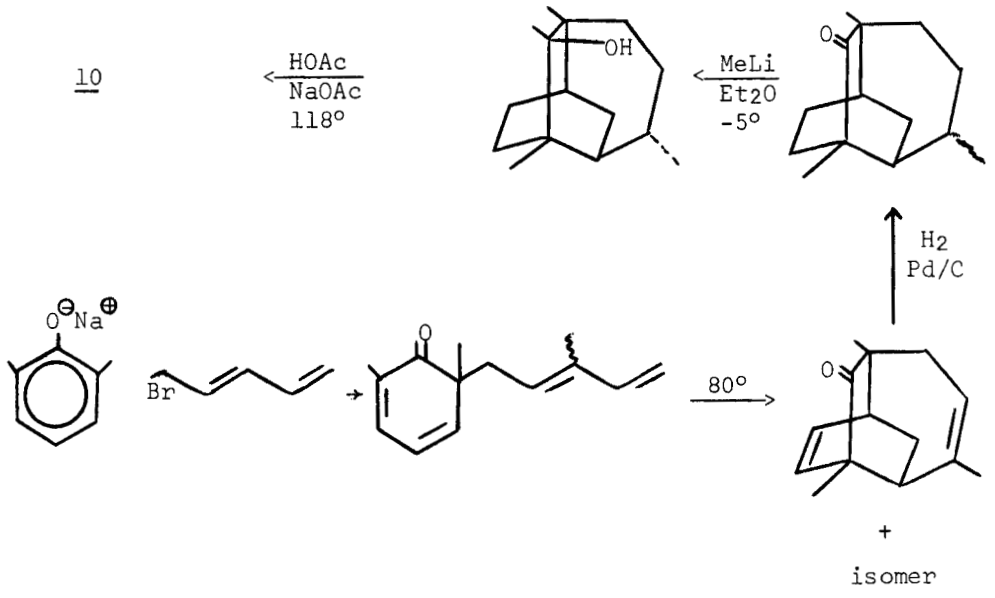
Two novel synthetic approaches to the sesquiterpene seychellene (10) have been based upon the utilization of the intramolecular Diels-Alder reaction as the key step in the generation of the tricyclic carbon skeleton. In the first approach[7] the strategy involved the preparation of the tricyclic intermediate 11 by the intramolecular cyclization of the cyclohexa-



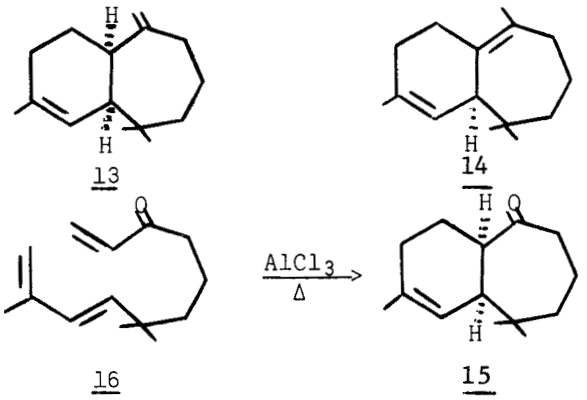
dienone 12. The required cyclohexadienone 12 was prepared in several steps as a mixture of diastereomers from 2,3-dimethylcyclohexenone and generated in situ by an amine oxide pyrolysis. Only a low yield of the internal adduct 11 was obtained and it was easily converted into seychellene.



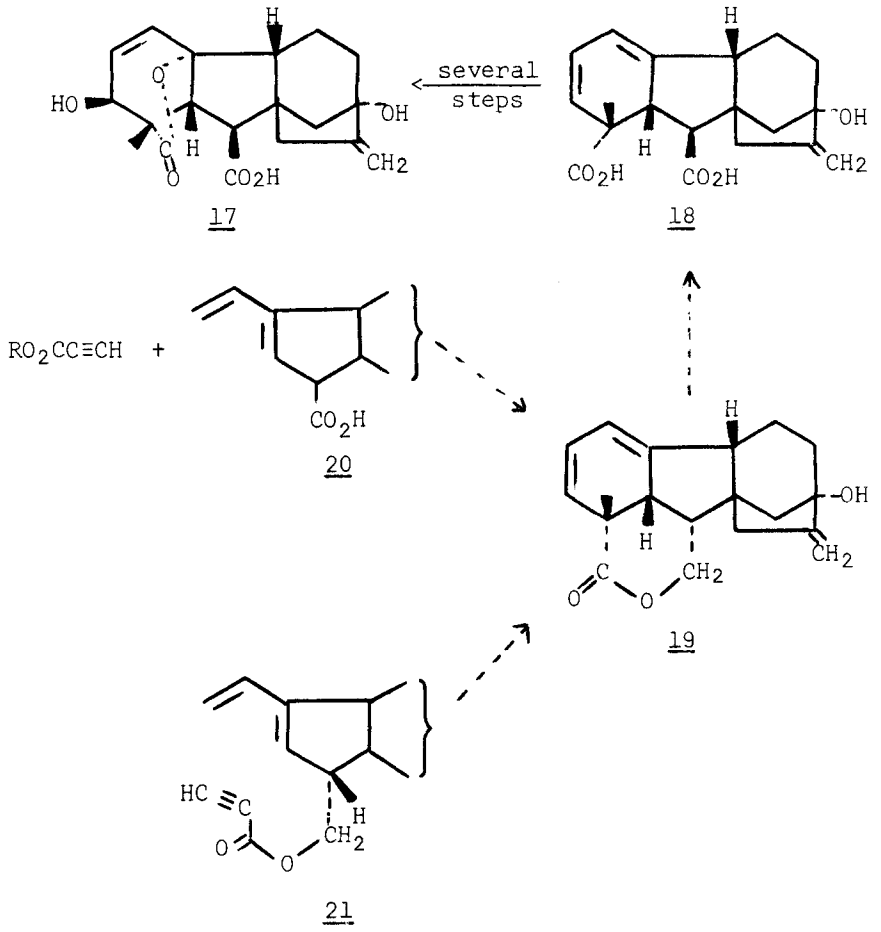
Very recently an alternative utilization of the intramolecular Diels-Alder reaction for the synthesis of seychellene (10) was reported.[8] In this case an isomeric tricyclic intermediate was prepared and subjected to a Wagner-Meerwein rearrangement to give seychellene. As outlined below, the intramolecular Diels-Alder reaction utilized the cyclohexadienone portion of the molecule as the dienophile rather than the diene as had been done in Fukamiya's approach.



Wenkert[9] has developed an interesting synthesis of the α- and β-himalchenes (13 and 14) which uses an intramolecular Diels-Alder reaction as the key step in establishing the proper carbon skeleton. The bicyclic unsaturated ketone 15 served as an appropriate precursor which could be converted readily to either of the two himalchenes 13 and 14 and was itself produced by the cyclization of the triene 16 in unspecified yield.

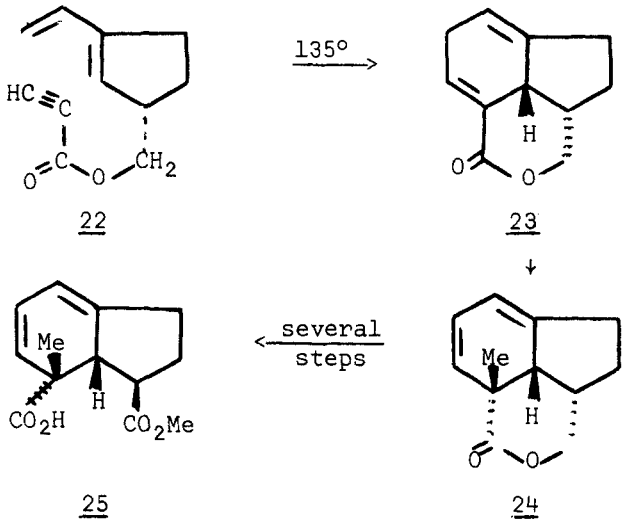


An elegant example of the tremendous potential of the intramolecular Diels-Alder reaction for solving stereochemical and orientation problems was recently provided by Corey[10] in some model studies directed toward a total synthesis of gibberellic acid (17). A previous study[11] had established that the functionality and stereochemistry of ring A could be introduced in a series of several steps from 18. A possible route to 18 would involve the preparation and alkylation of a cyclohexadienic ester of the type 19 by a Diels-Alder reaction (cf., 20). The intermolecular reaction would likely give products arising from both possible orientations as well as products resulting from approach of the propargyl ester to both sides of the diene molecule. However, by constructing the system in such a way



that the A ring is introduced by an intramolecular Diels-Alder reaction both of these problems can be solved. In order to test the feasibility of this process the model system 22 was prepared. On heating at temperatures above 135°, 22 cyclized to give the tricyclic lactone 23 in yields of up to 70%. The stereochemistry can be unambiguously assigned as the only pos-

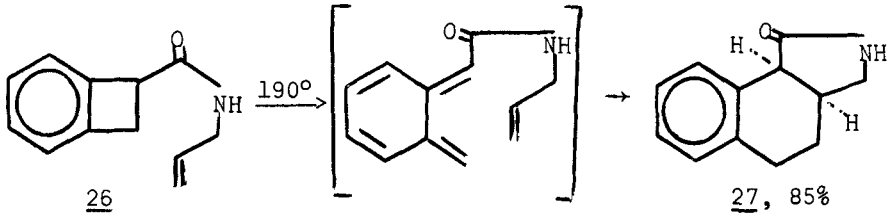
sible stereochemistry for the product of this intramolecular Diels-Alder reaction.



22 23 25 24

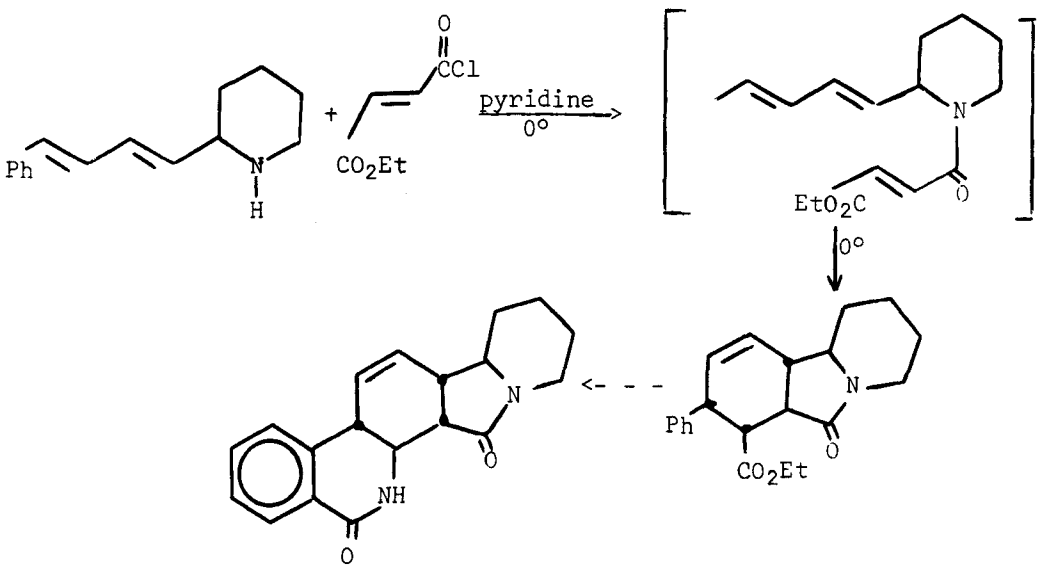
An added advantage of this approach is that lactone 23 can be alkylated stereoselectively with lithium isopropylcyclohexyl amide and methyl iodide to give 24, which was converted in several steps to 25 containing stereochemistry analogous to that present in gibberellic acid.

Several examples have recently appeared which demonstrate the utility of the intramolecular Diels-Alder reaction for the construction of heterocyclic systems. Oppolzer[12] has established that thermally generated o-quinodimethanes will undergo intramolecular Diels-Alder reactions to produce polycyclic amides (e.g., 26 → 27). The required amides of the type 26 are readily obtainable from benzocyclobutanecarboxylic acid. This method has been applied to the total synthesis of the alkaloid dl-chelidonine.[12b]

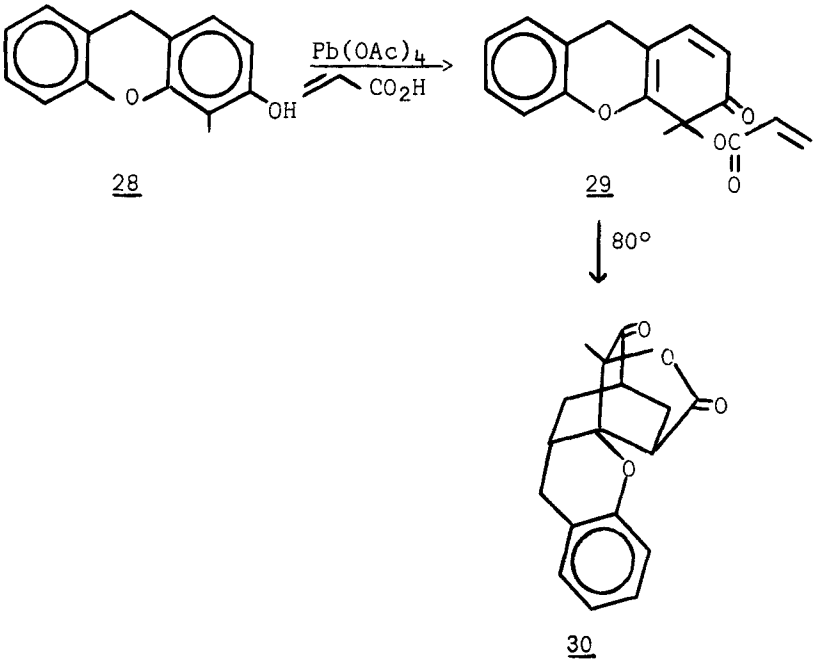


26 27, 85%

Gschwend has examined the intramolecular cyclization of pentadienylacrylamides for the preparation of perhydroisoindolines[13a] and has applied this reaction to the preparation of aza- and diaza-steroid analogs.[13b] A typical example is shown below. A number of these cyclizations take place under exceedingly mild conditions.



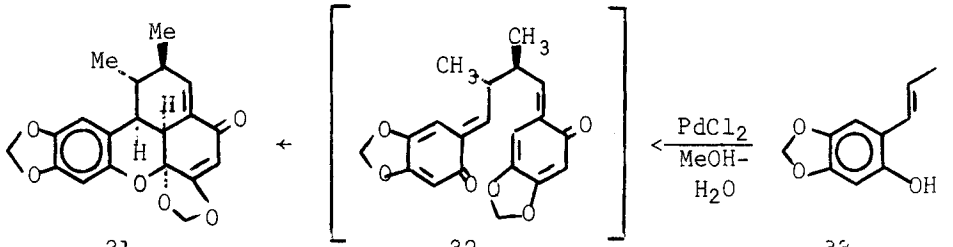
Yates[14] has applied the intramolecular Diels-Alder reaction to the synthesis of the 2,4a-ethano-2,3,4,4a-tetrahydroxanthene system which is present in the naturally occurring compounds morellin and gambogic acid. The dienone 29 was generated by oxidation of the xanthene 28 with lead tetraacetate in acrylic acid to give the desired system 30.



28 29

30

Finally, an intramolecular cyclization of a bis-o-quinonemethide (32) was employed by Chapman[15] in the total synthesis of the lignan carpanone (31). In this remarkably facile synthesis the required o-quinonemethide was generated in situ by phenolic coupling of two molecules of 2-(trans-1-propenyl)-4,5 methylenedioxyphenol (33) with palladium dichloride. This procedure led directly to carpanone (31) in 46% yield and involves the introduction of five asymmetric centers in a single step.



31 32 33

In addition to the foregoing examples, the literature records a number of other examples of intramolecular Diels-Alder reactions. The examples cited, however, give an indication of the ways in which this method can be used in organic synthesis.

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Chapter 29. Drug Binding and Drug Action

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Introduction - The interactions of a drug with a biological system can be divided into two categories, namely specific and nonspecific. For most drugs the specific interaction involves combination with a tissue component called a receptor. Such an interaction usually initiates a chain of events which finally culminates in the expression of the pharmacological activity of the drug. However, before a drug can combine with its receptor, it will interact with many other body components. While these nonspecific interactions do not result in any expression of the pharmacological activity, they are nevertheless important, since they modulate the amounts of drug which finally arrive at its receptor site. It is the purpose of this article to examine the ways in which binding to plasma and tissue proteins can alter the distribution, metabolism and excretion of a drug.

The binding of drugs to plasma proteins. The interaction of a drug (D) with a protein (P) may be described in terms of the Mass-Action Law by the following expression

$$D + P \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} DP \qquad (1)$$

where k_1 and k_{-1} are the rate constants for association and dissociation respectively. If this protein has multiple, mutually independent, binding sites and if all the sites have the same affinity for the drug, then it may be shown that

$$K = \frac{[D_B]}{[D_F]\,(n[P]-[D_B])} \qquad (2)$$

where $[D_F]$ and $[D_B]$ are the molar concentrations of free and bound drug, respectively, K is the association constant for the interaction, n is the number of drug binding sites per mole of protein and $[P]$ is the molar concentration of protein. It may readily be seen from equation 2 that, in the absence of complicating factors, such as the presence of other drugs or endogenous ligands, the degree of drug binding under equilibrium conditions depends on (i) the total concentration D_T (i.e. $[D_B] + [D_F]$ of drug in the system, and (ii) the affinity constant (K) for the interaction. Since, for any given system, the values for n,$[P]$ and K are constant, it is obvious that the total drug concentration is probably the most important factor that determines how much of a drug is bound. The effect of $[D_T]$ on the per cent binding of a hypothetical drug that is bound soley to plasma albumin is shown in Fig. 1.

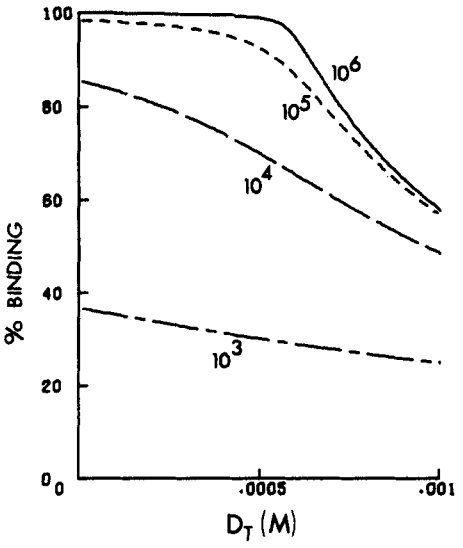


Fig. 1. The effect of drug concentration, $[D_T]$, on per cent binding of a drug in plasma. Each curve represents a different value of K. Calculated from equation 5 assuming that n = 1 and P = 5.8×10^{-4}. Adapted from reference 6.

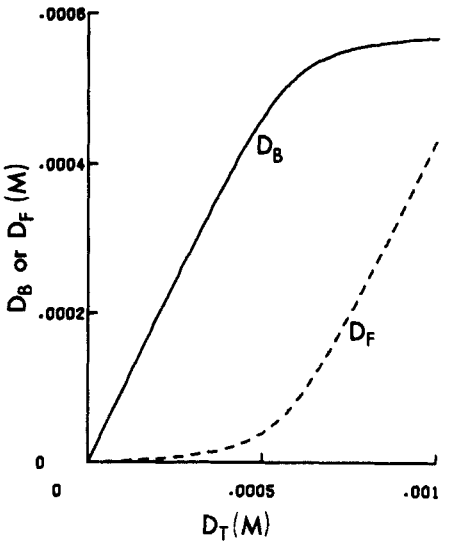
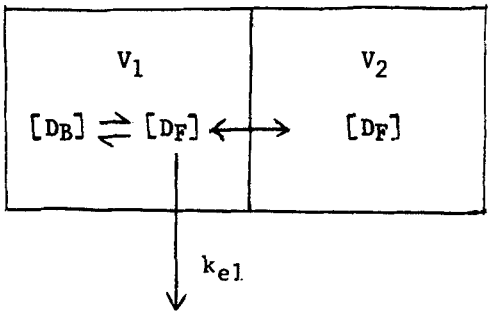


Fig. 2. The effect of drug concentration $[D_T]$ on free $[D_F]$ and bound $[D_B]$ phenylbutazone in plasma. Calculated from equation 5 assuming n = 1, P = 5.8×10^{-4} M and K = $10^{5.2}$. Adapted from reference 6.

It may be seen from Fig. 1 that as the concentration of drug increases the per cent of drug bound decreases. While this effect is quite small for drugs that have a weak affinity ($K \sim 10^3$) for plasma albumin, it can be quite large for drugs that are strongly bound. Brodie and Hogben[1] have shown that the free concentration of phenylbutazone in plasma increases dramatically when $[D_T]$ exceeds the concentration where the plasma proteins become saturated. The theoretical values of $[D_B]$ and $[D_F]$ for phenylbutazone are shown in Fig. 2.

The effects of plasma protein binding on drug distribution and kinetics are best understood in terms of a simple two compartment system.[3-6] This model makes the following assumptions[6]: (i) that the drug is given intravascularly, (ii) that the drug is distributed into two aqueous compartments, i.e. plasma water (volume V_1) and a second compartment (volume V_2) which represents that part of the residual body water which is accessible to the drug, (iii) that protein binding is governed solely by the Mass-Action Law within the plasma water compartment, (iv) that the rate of equilibration between the two compartments is much faster than the rate of drug elimination, (v) that drug metabolism and excretion are first-order processes, (vi) that drug metabolism and excretion are a function of the concentration of unbound drug and (vii) that the rate constants for



metabolism and excretion, k_m and k_e, can be combined to give k_{el}, the rate constant for elimination,

$$k_{el} = k_m + k_e \qquad (3)$$

If these assumptions hold, then the body content at any given time is given by

$$\text{Body content} = V_1[D_T] + V_2[D_F] \qquad (4)$$

Plasma protein binding and drug distribution. Martin[5] has derived the following equation from the relationship given in equation 2.

$$[D_T] = [D_F]\left(1 + \frac{nP}{K^{-1} + [D_F]}\right) \qquad (5)$$

If it is assumed that for a 70 kg man, plasma volume is 3 l, extracellular fluid volume is 9 l and total body water is 39 l, then equation 5 may be used in conjuction with equation 4 to predict the effect of protein binding on drug distribution. In Fig. 3 the relationship between $[D_T]$ and $[D_F]$ and total body content of a drug is shown for drug A which is lipid insoble and is unable to cross cell membranes and thus is restricted to extracellular fluid and drug B which has access to cell water. It may be seen from Fig. 3 that when the total body content is low (about 1 mmole) there is little difference between $[D_T]$ and $[D_F]$ for drugs A and B, because both are almost completely bound to plasma albumin and are thereby confined to the plasma compartment. However, as the body load of drug decreases the plasma protein binding sites become saturated and the unbound drug partitions between the plasma water and the second compartment. At high body loads the free concentration of drug B is about one-third of that for drug A, because B has access to a larger extravascular compartment.

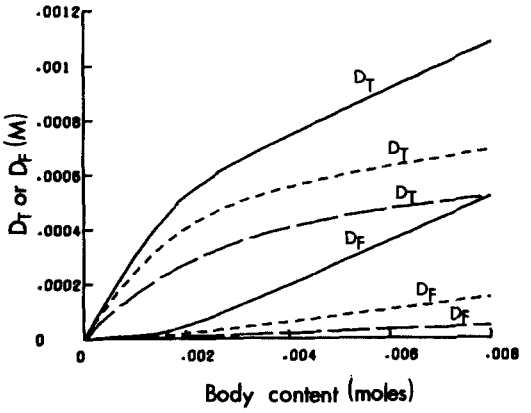


Fig. 3. Relationship between total amount of drug in the body, concentration of drug, $[D_T]$, in plasma and concentration of unbound drug, $[D_F]$, in tissue fluid. Calculated from equations 4, 5 and 10 assuming $K = 10^5$, $n = 1$, $P = 5.8 \times 10^{-4}$ M, $V_1 = 3$ liters. Drug A, (——), $V_2 = 9$ liters. Drug B, (----), $V_2 = 39$ liters. Drug C, (—— ——), $V_2 = 39$ liters, $V_3 = 100$ liters, Adapted from reference 6.

Tissue binding and drug distribution. Before examining the effect of tissue binding on the distribution of a drug, it is necessary to introduce the concept of apparent volume of distribution, V_A, calculated from the following expression,

$$V_A = \frac{\text{Total dose of drug in body water at any time}}{[D_F]} \qquad (6)$$

The apparent volume of distribution may be defined as that volume of body fluids which would hold the drug in solution at the same concentration as in body water. It should be emphasized that V_A is not the same as the generally accepted definition of volume of distribution which is calculated from $[D_T]$ and not $[D_F]$. Equations 4 and 6 may be combined to give

$$V_A = \frac{V_1 [D_T] + V_2 [D_F]}{[D_F]} \qquad (7) \qquad \text{or} \qquad V_A = V_1 \frac{[D_T]}{[D_F]} \qquad (8)$$

It may be seen from equation 8 that if a drug is bound to plasma proteins V_A will be greater than the true volume of distribution. The binding of drugs to tissues will also increase the volume of distribution. In theory it should be possible to introduce terms into equation 8 similar to that for plasma protein binding which would accurately predict the effect of tissue binding on V_A. In practice this is impossible due to the large number of different tissue components that can interact with drugs. In addition, little is known about the nonspecific tissue binding sites, although some attempts are now being made to study this interaction (M. Bickel, personal communication). In the absence of specific binding data, tissue binding can be expressed as a third compartment that is accessible to the drug. Equations 4 and 8 may then be rewritten to give equations 9 and 10 respectively,

$$\text{Body content} = V_1[D_T] + V_2[D_F] + V_3[D_F] \qquad (9)$$

$$V_A = V_1 \frac{[D_T]}{[D_F]} + V_2 + V_3 \qquad (10)$$

The effect of V_3 on the concentration of free and bound drug in plasma is shown in Fig. 3 (drug C).

Drug displacement and drug distribution. It has often been suggested that the displacement of one highly bound drug from its plasma binding sites by another drug can cause a large increase in the plasma concentration of free drug. More recently, however, this postulate has been called into question.[7-10] The two compartment model can be used to predict the effect of such a drug-drug interaction (Table 1). The data in Table 1 indicate that, at the lower body load, drug A would show the largest (8-fold) increase in free drug concentration. In contrast, drug B, which has a larger volume of distribution, shows only a threefold increase in free drug concentration. However, drug C, which is also bound to tissue proteins, would exhibit a sixfold increase in free concentration if displaced from both plasma and tissue binding sites. Displacement of C from just the plasma binding sites would result in a twofold increase in the plasma

concentration of free C, if the displaced drug was taken up by the tissues. At the higher body load, drug C would show a fourfold increase in free drug concentration when displaced from both plasma and tissue binding sites. Very little change would be observed by the displacement of any of the drugs solely from plasma proteins. These calculations show that the change in free plasma concentration of a displaced drug depends upon (i) volume of distribution, (ii) whether or not there is concomitant displacement from tissue binding sites and (iii) the body load of drug. The most dangerous clinical interaction would be one in which displacement occurred from both plasma and tissue binding sites.

Table 1

Effect of drug displacement on $[D_F]$ and $[D_T]$

Drug	Body content							
	1 millimole				8 millimoles			
	$[D_T]^+$	$[D_F]^+$	[D]*	[D]/$[D_F]$	$[D_T]^+$	$[D_F]^+$	[D]*	[D]/$[D_F]$
A	30.3	1.02	8.33	8.17	109.0	52.4	66.60	1.27
B	24.4	0.69	2.38	3.45	79.6	15.2	19.00	1.25
C	16.1	0.37	2.38‡	6.43	52.3	4.6	19.00‡	4.13
	16.1	0.37	0.70**	1.89	52.3	4.6	5.63**	1.22

+Drug concentrations (M x 10^5) calculated from Fig. 3
*Plasma drug concentration (M x 10^5) assuming complete displacement from binding sites.
‡Calculated assuming complete displacement from plasma and tissue binding sites.
**Calculated assuming complete displacement from plasma binding sites only.

The effect of drug binding on kinetics. If, as is suggested by the two-compartment model, drug elimination, whether by metabolism or excretion, is a first-order process depending on $[D_F]$ and having a rate constant k_{el}, then we can write

$$[D_F] = [D_F]^o \, e^{-k_{el}t} \qquad (11)$$

where $[D_F]^o$ is the value of $[D_F]$ at the time of administration assuming instantaneous equilibration. Since elimination is a first-order process, it may be expressed in terms of clearance where clearance is that volume of plasma water which is effectively cleared of a drug in unit time. Furthermore, Butler[11] has pointed out that

$$k_{el}(min^{-1}) = \frac{\text{Clearance (ml/min)}}{V_A \text{ (ml)}} \qquad (12)$$

From equation 11 it can be shown that

$$t_{\frac{1}{2}} = \frac{\ln 2}{k_{el}} \qquad (13)$$

When equations 13 and 14 are combined, the following relationship is obtained

$$t_{\frac{1}{2}} = \ln 2 \frac{V_A}{\text{Clearance}} \qquad (14)$$

It has been previously shown that binding to plasma and tissue proteins, by reducing $[D_F]$, will increase V_A. If the rate constant for dissociation of the drug protein complex (k_{-1}) is very rapid, the drug will be eliminated as though it were all free in one large component of volume V_A. Under these conditions the two-compartment system behaves kinetically as a pseudo one-compartment model.

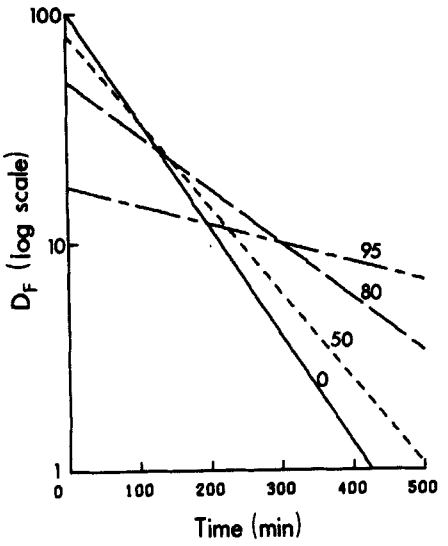


Fig. 4. Potential effect of unbound drug concentration, $[D_F]$, in tissue fluid with time. Each line represents a different value of per cent binding which is assumed not to change with time. The intercept on the ordinate, $[D_F]^o$, was calculated from equation 4. Slope of line calculated from equations 6 and 14. Dose = 10^{-3} moles, V_1 = 3 liters, V_2 = 9 liters, clearance = 0.13 liters/min. Adapted from reference 6.

The effect of various degrees of protein binding on the plasma decay curve following a standard dose of drug is shown in Fig. 4. It will be seen that protein binding has two effects: (i) it reduces the initial concentration of free drug, $[D_F]^o$, and (ii) it increases $t_{\frac{1}{2}}$ so that $[D_F]$ falls less rapidly. The fall in drug concentration with time will be linear (Fig. 4) only if V_A has the same value at all drug concentrations. However, as is shown in Fig. 1, the binding of drugs with a high affinity for plasma albumin changes with $[D_T]$ so that at high values of $[D_T]$ the value of V_A will decrease and elimination will be more rapid (Eq. 13).

Burns and co-workers[12] have reported that, when a high dose of phenylbutazone is given to a patient who already has a "plateau" blood level of the drug, the rate of elimination, which is initially very rapid, eventually slows to its previous value. Krüger-Thiemer[3] has developed a rate equation for the change of $[D_F]$ with time using the pseudo one-compartment model together with equation 5. This expression was solved with the aid of a digital computer, and the lines generated are shown in Fig. 5. The model accurately predicted the kinetic behavior of a single oral dose of sulfaorthodimethoxine.[3,4]

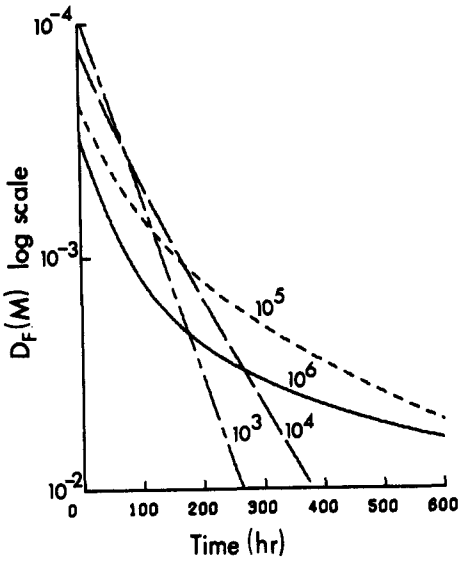


Fig. 5. Potential effect of plasma binding on the change of $[D_F]$ in tissue fluid with time. Model curves generated according to the pseudo one-compartment model assuming an initial drug dose of 5 mmoles, V_1 = 3 liters, V_2 = 40 liters, n = 2, P = 10^{-3} M, kidney clearance = 0.82 liters/hr.[3,4]

Some other factors not considered in the pseudo one-compartment model. This model cannot predict the pharmacokinetics of drugs that are rapidly metabolized by liver enzymes or eliminated by kidney transport systems. Gillette has recently pointed out[8] that, for drugs whose clearance by the liver or kidney approaches the blood flow through that organ, binding to plasma proteins may enhance the rate of elimination. This effect is illustrated in Table 2, where the apparent rate constants of elimination (rate/concentration) at various concentrations of two hypothetical drugs have been calculated. One of the drugs is completely cleared from the plasma as it passes through the liver, while the other is eliminated so slowly that only 1% of the unbound drug is extracted as it passes through the liver. In making these calculations, it was also assumed (i) that the distribution phases had been completed, (ii) that the unbound forms of the drug distributed in body water, (iii) that the kinetic volumes of distribution (V_D) were the same as the equilibrium apparent volumes of distribution (V_A), i.e. that the rate constants for the transfer of drugs from the body compartment to the blood were large compared with the

apparent rate constants of elimination, (iv) that the ratio KnP/(1 + KnP) equals 0.98 and (v) that the blood flow rate through the liver is 1.6 liters/min.[8] When the total concentrations of the drugs in plasma are infinitely greater than the concentrations of the bound forms, V_D would be 50 liters and k_{el} for the rapidly eliminated drug would be 100 times that for the slowly eliminated drug. However, as the plasma levels of the drugs decrease, the ratio of the total amounts of the drugs in the body to $[D_T]$ decreases, whereas the ratio of the total amount of the drugs in the body to $[D_F]$ increases. Thus k_{el} for the rapidly eliminated drug increases as the plasma level of the drug declines (see Eq. 12), whereas k_{el} for the slowly eliminated drug decreases.

Table 2

Relationships between protein binding of drugs in plasma, the extraction ratio in liver, and the apparent rate constant of elimination*†‡**

Drug concentration (M)	100% of Total amount in plasma is extracted			1% of Unbound amount in plasma is extracted		
	Bound in plasma (%)	k (min^{-1})	$t_{1/2}$ (min)	k (min^{-1}) X 10^3)	$t_{1/2}$ (min)	(days)
00/Ka	0	0.032	21.6	0.320	2160	1.5
100/Ka	32.6	0.045	15.4	0.304	2280	1.6
10/Ka	82.0	0.118	5.9	0.215	3220	2.2
1/Ka	96.0	0.221	3.14	0.0865	8000	5.5
0.1/Ka	97.8	0.245	2.84	0.0543	12,750	8.8
0.01/Ka	97.98	0.250	2.80	0.0505	13,700	9.5

*Unbound drug distributed with body water; V_D = 50 liters.
†KnP/(1 + KnP) = 0.98.
‡Blood flow rate = 1.6 liters/min.
**Taken from reference 8.

Gillette[8] has also examined the effect of tissue binding on the elimination of a drug that is rapidly metabolized by the liver. In Table 3 the half-lives of a series of hypothetical drugs that have different kinetic volumes of distribution (V_D) but are completely cleared by the liver are shown. The minimal half-life of a drug that is eliminated by the liver and has a V_D/kg of 20 liters/kg in man would be about 10 hr. If only 50% of the drug were cleared as it passed through the liver, the biological half-life would be 20 hr. Thus it would be a mistake to assume that the clearance of a drug is always slow when it has a long biological half-life.

Table 3

Relationships between V_D, liver plasma flow, extraction ratios, and $t_{1/2}$*†

V_D (liters)	$\frac{V_D}{kg}$ (liters/kg)	Extraction ratios			
		100% $t_{1/2}$ (hr)	50% $t_{1/2}$ (hr)	25% $t_{1/2}$ (hr)	10% $t_{1/2}$ (hr)
5.0	0.071	0.036	0.072	0.14	0.36
15	0.214	0.11	0.22	0.44	1.1
50	0.71	0.36	0.72	1.4	3.6
70	1.0	0.5	1.0	2.0	5
140	2.0	1.0	2.0	4.0	10
280	4.0	2.0	4.0	8.0	20
700	10.0	5.0	10.0	20.0	50
1400	20.0	10.0	20.0	40.0	100
2800	40.0	20.0	40.0	80.0	200

*For these calculations, it was assumed that the blood flow rate was 1.6 liters/min in a 70-kg man, which is equivalent to 96.0 liters/hr and to 1.37 liters/kg/hr.
†Taken from reference 8.

In considering the possible factors that affect the metabolism of highly bound drugs, it would appear that the rate of dissociation of a drug-protein complex might limit the elimination of the bound form of the drug. However, Gillette has shown[8] that for this mechanism to be important

Table 4

Rate constants for the formation and dissociation of drug-protein complexes[14]

Drug	Protein	k_1 (M^{-1} sec^{-1})	k_{-1} (sec^{-1})	$K\left(\frac{k_1}{k_{-1}}\right)$ (M^{-1})
4[(5,7-Disulfonic acid naph-2-ol)-1-azo] benzene sulfonamide (I)	Carbonic anhydrase	5.8×10^5	0.075	7.7×10^6
1-Naphthol-2-sulfonic acid-4-[4-(4'-azo benzene-azo)]-phenyl-arsonic acid (II)	Plasma albumin	3.6×10^5	2.5	1.4×10^5

the half-time for dissociation of the complex would have to be equal to or greater than the mean transit time of the blood through the sinusoids of the liver. In the dog this transit time is about 10 sec[13] so that about

50% of a drug-protein complex could dissociate if k_{-1} is approximately 0.07 sec. The rate constants for two drugs that interact with carbonic anhydrase and plasma albumin are shown in Table 4. If the transit time of blood through the human liver is about 10 sec, the clearance of drug II, bound to albumin, would not be limited by k_{-1}, whereas only about 50% of drug I, bound to carbonic anhydrase, could be removed. It should be emphasized, however, that it is impossible to predict from the association constant (K) alone that k_{-1} for a given drug-protein interaction is rate-limiting. A complete kinetic analysis of the system must be made.

Conclusions - Binding to plasma proteins can have a profound effect on the distribution of a drug and on the rate at which it is eliminated from the body. Plasma binding has a lesser effect on a drug that is able to enter cells than on one that is restricted to extracellular fluid. Plasma binding has a marked effect on the properties of a drug only if the K for the drug-protein interaction is greater than 10^4. Plasma binding may increase the rate of elimination of drugs that are completely cleared by organs such as the liver or kidney. The effect of displacement of one drug by another on the free concentration of first drug is governed by the volume of distribution of the drugs, the body load of the first drug, whether the first drug is also bound to tissues and whether the second drug displaces the first from both tissue and plasma binding sites.

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Chapter 30. Cytochrome P-450 Monoxygenases and Drug Metabolism

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Introduction - Oxidative metabolism of foreign compounds has been known to occur in living organisms for more than one hundred years.[1, 2] However, it has only been during the past twenty years that a group of unusual hemoproteins designated by the term cytochrome P-450 have been established as the terminal oxygenases in the respiratory chain responsible for many of these oxidations. One such cytochrome P-450, which catalyses the oxidation of camphor, has recently been isolated from soil bacteria and crystallized.[3] In higher organisms the P-450 enzymes are membranal proteins and display a wide spectrum of oxidative activity towards drugs, pesticides and other xenobiotic compounds. Their solubilization and isolation as pure enzymes have proven difficult. High levels of P-450 are found in microsomal fractions from liver and adrenal cortex and in mitochondrial fractions from the latter tissue. These hemoproteins have also been implicated in the biosynthesis of cholesterol, steroid hormones and bile acids.[4] The present article highlights certain aspects of the chemistry of mammalian P-450 monoxygenases with emphasis on their role in drug metabolism.

Spectral Properties of Cytochrome P-450 Monoxygenases - Liver microsomal fractions contain two major hemoproteins: cytochrome b_5 and cytochrome P-450. Both exhibit an absorbtion maximum near 420nm, but the latter hemoprotein is atypical in that the spectrum of the reduced carbon monoxide-complexed pigment shows a strong absorbance band at 450nm; hence the origin of the designation P-450. The cytochrome monoxygenases present in microsomes from animals pretreated with polycyclic hydrocarbons differ substantially from the P-450 monoxygenases that are present in untreated or phenobarbital-treated animals.[5-13] Since the spectrum of the reduced carbon monoxide complex of this new class of cytochromes has a maximum at 448nm instead of 450nm, the term cytochrome P-448 was introduced. This new class of cytochromes has also been termed P_1-450.[6]

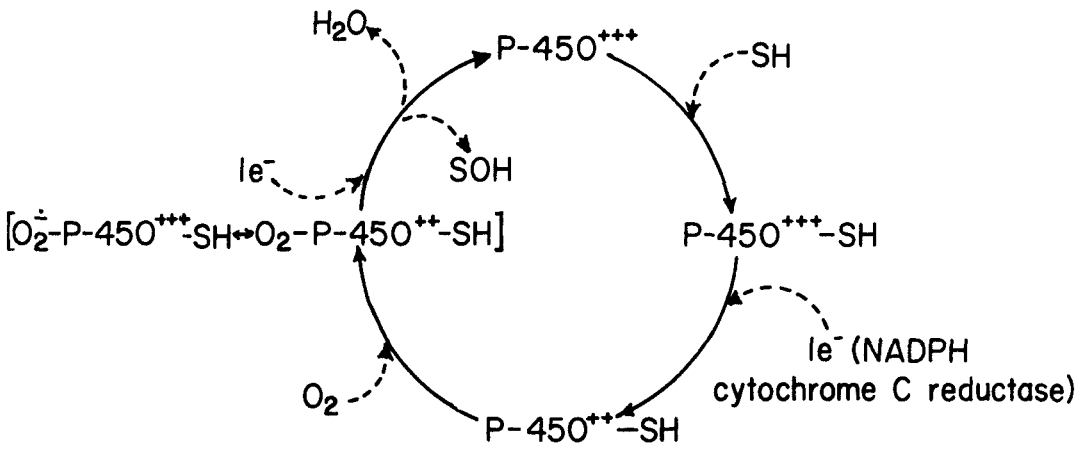
Distinctive spectral changes[14-16] have been noted after addition of various compounds to the oxidized form of cytochrome P-450. The compounds which elicit such spectral changes have been placed in two categories: Type I substrates such as hexobarbital, ethylmorphine and testosterone which cause loss of absorbance at 420nm with a concomitant increase at 385nm, and Type II substrates such as aniline, pyridine and acetanilide which cause an increase in absorbance at 430nm and a decrease at 400nm. Such alterations in the heme chromophore reflect interaction at or near the active site of the enzyme. Type II substrates have nonbonding electron pairs which are thought to interact with the heme iron. Three types of EPR spectra have been seen for oxidized cytochrome P-450, two low spin forms and one high spin form.[17] One of the low spin forms is associated with substrate-free oxidized P-450, while the other is observed

only upon interaction with a Type II substrate.[18] When a Type I substrate is added to oxidized P-450, the low spin form is converted to the high spin form.[19] Evidence for another spectral species of cytochrome P-450, presumably a ternary complex of reduced P-450, oxygen and substrate has been reported for bacterial and mammalian systems[20, 21] (see however ref. 22). Spectral evidence for P-450-product complexes generated during substrate metabolism has also been obtained.[23]

Characterization of Cytochrome P-450 Monoxygenases - Pretreatment of animals with barbiturates or polycyclic aromatic hydrocarbons results in substantial increases of the levels of P-450 in liver. Alterations in product distribution and/or changes in substrate specificities after such treatments provide evidence for more than one form of cytochrome P-450.[5-7, 24-26] Data on competitive inhibitions provide corroborative evidence for the presence of more than one monoxygenase in microsomal membranes.[27-29] Although a number of cytochrome monoxygenases thus appear to be present in liver, each enzyme probably can accept a variety of substrates and each is capable of catalyzing various oxidative transformations including epoxidations, aryl and alkyl hydroxylations and oxidations at nitrogen and sulfur.

Efforts to solubilize and isolate the P-450 monoxygenases from the microsomal membranes have been hampered by the facile conversion to a catalytically inactive form, cytochrome P-420. In 1968, however, a successful solubilization of catalytically active P-450 from hepatic microsomes was reported.[30, 31] The solubilized system, which effected w-hydroxylation of fatty acids, required three components for catalytic activity; cytochrome P-450, NADPH-cytochrome c reductase, and a heat-stable, chloroform-soluble factor, the active component of which was identified as the microsomal lipid phosphatidylcholine.[32] Further studies with this solubilized and reconstituted system have indicated that the cytochrome P-450 and P-448 fractions have different catalytic activities[33-35] and that the terminal oxidase activity resides in the b-type cytochrome (P-450 or P-448) fraction rather than the cytochrome c reductase or lipid fractions.[33, 36, 37] The cytochrome P-450 and P-448 were found to compete for reductase when present together.[38] Cytochrome b_5 did not appear to be an obligatory component of the reconstituted system.[39] Cytochrome P-450 and P-448 fractions from rat liver were found to contain high levels of an epoxide hydrase, which can convert intermediate oxides to vicinal diols.[40] Further purification has afforded fractions relatively free of cross-contamination of monoxygenase and hydrase enzymes.[41] Recently, differential solubilization of monoxygenase activity towards a Type I substrate (naphthalene) and a Type II substrate (aniline) was reported.[42]

Mechanism of Electron Transport and Oxidation - The generally accepted mechanism for electron transport during oxidative reactions catalyzed by cytochrome P-450 is shown below.[43] The oxidized cytochrome reacts rapidly with substrate (SH) to form a P-450-substrate complex. One

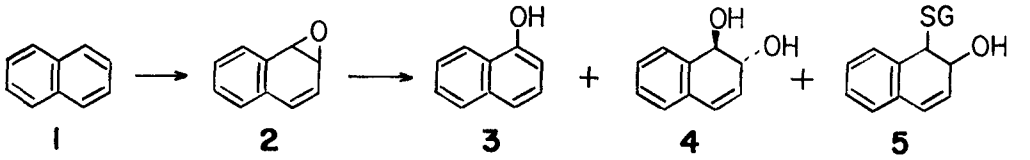


electron reduction of the iron to the ferrous state is catalyzed by the NADPH-dependent flavoprotein, cytochrome c reductase. This reduced enzyme-substrate complex binds molecular oxygen. The question as to which of the two extreme formalisms, Fe^{++}-O_2 versus Fe^{++}-$O_2^{\cdot-}$, best describes the resulting complex is unclear. However, superoxide ($O_2^{\cdot-}$) has been implicated in the reaction.[44] Transfer of a second electron is followed rapidly by release of product and water without detectable intermediates. The exact role of cytochrome b_5 in this sequence is unknown. However, reduction of cytochrome b_5 is an NADH-dependent process and the oxidation of various substrates with hepatic microsomes is known to be stimulated by the presence of NADH.[45] A number of studies have indicated that the rate determining step of the above sequence is the reduction of the enzyme-substrate complex and that the rate of this reduction is strongly dependent on the nature of the substrate.[46-48]

Criteria for the involvement of the P-450 system in the metabolism of specific drugs or xenobiotic compounds include: i) enhancement of metabolism after pretreatment of animals with phenobarbital and in some instances after treatment with polycyclic aromatic hydrocarbons ii) localization of enzyme activity in microsomal fractions with high levels present in liver iii) inhibition by carbon monoxide, piperonylbutoxide, β-diethylaminoethyl-3,3-diphenylpropylacetate (SKF 525a) and certain other compounds iv) requirement for NADPH as a source of reducing equivalents. The following sections provide representative examples of types of drug metabolism which appear to be catalyzed by P-450 monoxygenases.

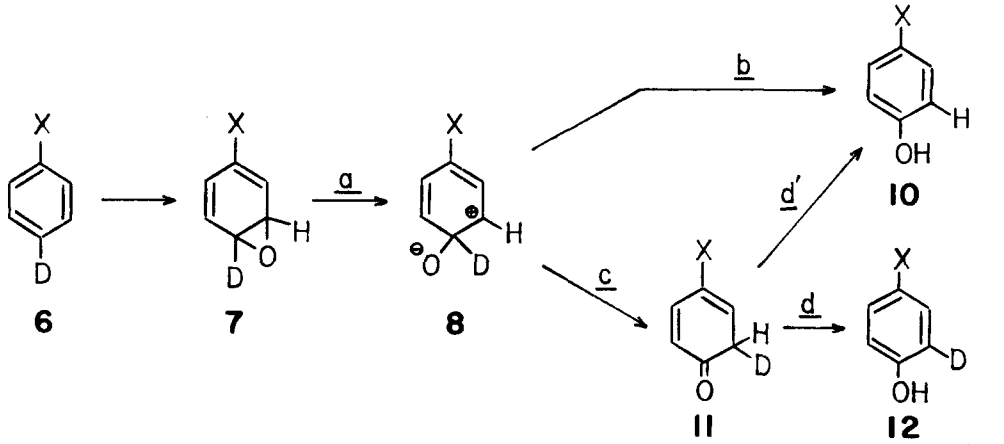
Aromatic Oxidation - Mammalian metabolism of aromatic compounds appears to proceed via an initial P-450-catalyzed formation of relatively labile arene oxides which lead to phenols, dihydrodiols, catechols and premercapturic acids. The conversion of a typical arene oxide, benzene oxide, to a dihydrodiol and a premercapturic acid with hepatic enzymes

and to phenol by isomerization[49] was consonant with this view. However, it was not until naphthalene 1,2-oxide 2 was identified as the obligatory intermediate in the hepatic metabolism of naphthalene 1 that an arene oxide was isolated in a biological system.[50, 51] Subsequently, tentative evidence has been presented for the formation of arene oxides from a number of other polycyclic aromatic hydrocarbons (see reference 52).



1 2 3 4 5

Isomerization of appropriately deuterated arene oxides to phenols is accompanied by migration and retention of the deuterium in an adjacent position, as in the isomerization of 1-deutero-naphthalene 1,2-oxide to 2-deutero-1-naphthol.[53] Similar migrations of ring substituents occur during aromatic "hydroxylations" and have been called the "NIH Shift." Because of its widespread and characteristic nature, the "NIH Shift" is now used as a criterion for monoxygenase-catalyzed phenol formation.[54, 55] The only apparent exceptions are during "hydroxylations" of phenols and aniline in which retentions are very low probably due to the reactive nature of the ring. The proposed mechanism for the "NIH Shift" is as shown. The substrate 6 is converted to the arene oxide 7, which then



undergoes a spontaneous heterolytic opening of the oxirane ring (path a). The resultant zwitterionic species 8 can either lose the deuterium substituent directly (path b) or form the ketone 11 with concomitant migration of deuterium (path c). The ketone then enolizes to the phenol with preferential retention of deuterium (path d). The extent of overall deuterium retention will depend on the relative importance of paths b and c and the isotope effect in the enolization (path d vs d'). A more complete discussion of arene oxides and the "NIH Shift" can be found in recent reviews.[55, 56]

The magnitude of the "NIH Shift" observed during formation of

4-hydroxyacetanilide from 4-deuteroacetanilide was significantly less with cytochrome P-448 as compared to that with P-450.[57] This provided evidence for differences in the active site of these two classes of monoxygenases. The extent of retention of deuterium in "catecholic" metabolites from 4-deuterochlorobenzene was explicable in terms of intermediate dihydrodiols, rather than with pathways involving conversion of 4-chlorophenol to the catechol by a second "hydroxylation."[58] Phenols in certain instances also appear to be formed by a direct oxygen-insertion pathway similar to that involved in aliphatic hydroxylation.[59] An example is the formation of 3-nitrophenol from nitrobenzene.

Aliphatic Oxidation and O-Dealkylation - Cytochrome P-450 monoxygenases appear to be involved in a variety of aliphatic hydroxylations. One common pathway involves an initial w-hydroxylation as with pentobarbital,[60] followed by further metabolism to the aldehyde and acid by dehydrogenases. Hydroxylation also frequently occurs at the penultimate carbon as in amobarbital[61], at benzylic and allylic positions and at carbons bearing a heteroatom. Many examples of such oxidations are cited in a recent review.[62] Hydroxylation at carbon bearing a heteroatom such as oxygen, nitrogen or sulfur leads to the so-called dealkylation reactions so common in drug metabolism.

The mechanism of aliphatic hydroxylation by cytochrome monoxygenases would appear to involve a direct oxygen-insertion with retention of configuration. For example, when S-(+)-1-^{2}H-ethylbenzene was incubated with liver microsomes, the 1-phenylethanol produced in the reaction consisted of 92% of the R isomer and only 8% of the S isomer.[63] Phenobarbital pretreatment diminished the stereoselectivity of this reaction.[63, 64] The reaction of the α,α-dideutero-derivative exhibited an isotope effect of 1.8 when compared to the undeuterated substrate, consistant with an oxygen-insertion mechanism. In certain examples of aliphatic hydroxylation, however, it appears from the absence of an isotope effect with labeled substrates that the insertion reaction is not the rate-limiting step.[65-67]

Another class of aliphatic oxidations catalyzed by microsomal monoxygenases is O-dealkylation. The reaction involves cleavage of the alkyl-oxygen bond as shown by the lack of incorporation of oxygen-18 into the phenolic product, 4-hydroxyacetanilide, when 4-methoxyacetanilide was treated with microsomes in the presence of $H_2{}^{18}O$ and $^{18}O_2$.[68] The mechanism of such O-dealkylations appears to involve hydroxylation of the alkyl moiety to form a hemiacetal or hemiketal which decomposes to form the O-dealkylated product and, respectively, either an aldehyde or a ketone.[69] An isotope effect of 2.0 for the demethylation of 4-nitroanisole containing a trideutero-methyl group[70] is consonant with a mechanism in which oxygen-insertion is rate-limiting.

N-Dealkylation and Oxidative Deamination - Formally, N-dealkylation, a common metabolic pathway for xenobiotic compounds, would appear quite

analogous to O-dealkylation reactions. However, the N-dealkylation reaction can proceed via either of two mechanisms: direct formation of an unstable carbinolamine[69, 71] or the formation of an N-oxide which can then rearrange to the carbinolamine.[72] The results for the majority of the compounds studied favor the first mechanism, but they do not rule out the second entirely. Formation of the carbinolamine would appear to be catalyzed by P-450, while N-oxide formation is catalyzed by hepatic flavoproteins. There are now many examples where carbinolamines, carbinolamides and carbinolimines have been implicated or isolated as intermediates in N-dealkylation reactions.[62, 73-76] Oxidative deamination, as for example in the conversion of amphetamine to phenylacetone[77], may be considered as a variant of the N-dealkylation reaction, where the substrate is a primary amine. Carbinolamines, oximes, imines, and hydroxylamines have all been implicated in the metabolism of amphetamine.[78-80]

Reactions at Sulfur - Methylmercapto-compounds undergo metabolic demethylation presumably similar in mechanism to that of O-dealkylations.[81] Oxidation at sulfur represents another major pathway of metabolism, as in the conversion of chlorpromazine to the sulfoxide[82] and in the conversion of phenylmethylsulfide, a metabolite of the pesticide Dyfonate, to both the sulfoxide and the sulfone.[83]

Reductions - Cytochrome P-450 monoxygenases have been implicated in the reduction of nitro- and azo-functions under anaerobic conditions.[84] An example is the conversion of 4-nitrobenzoic acid to 4-aminobenzoic acid. In vivo such reductions are probably catalyzed by other enzymes.[85]

Oxidative Model Systems - The chemical nature of the oxidizing species which is generated from molecular oxygen by cytochrome P-450 is unknown. Current reviews on this problem are available.[86, 87] In general most authors agree that an "oxene" or "oxenoid" mechanism is operative, whereby the net equivalent of an oxygen atom transfer occurs during the oxidation. Similarity between the reactions of carbenes and nitrenes (addition to double bonds, C-H bond insertions) with the oxidations catalyzed by cytochrome P-450 (epoxidation, arene oxide formation, aliphatic hydroxylation) have led to the "oxene" terminology.

Three general types of oxidants[86] are capable of effecting the range of reactions catalyzed by cytochrome P-450. Molecular oxygen in the presence of a metal ion and a source of reducing equivalents, in some instances the reduced metal ion, is one of these. Such reactions are typified by the Udenfriend system[88, 89] which employs Fe(II), O_2 and ascorbic acid. A second class consists of the reactions of H_2O_2 and organic peroxides. Examples from this class include the metal-catalyzed decomposition of H_2O_2[90, 91] or organic peroxide[92] and the reactions of peroxy-acids.[93-95] Reducing equivalents are not consumed in these reactions. Finally, there are non-peroxidic oxidants which transfer oxygen. These include photoexcited azaromatic-N-oxides[92] and inorganic

reagents such as chromyl acetate.[96] The most serious problems encountered with such model systems have been the complexity of the reactions and the question of what criteria should be applied when comparing models to the enzyme-catalyzed reactions. Advent of the "NIH Shift" has provided an invaluable criterion for such comparisons. Models which do not display this phenomenon must be considered inadequate, despite how attractive they might appear from other standpoints. At present a suitable model which involves the reduction of molecular oxygen is, therefore, unavailable.[86] Hydroxylations by organic peroxides[92], peroxy acids[93, 97] photoactivated N-oxides[92], and chromyl acetate[96] do show the requisite migrations. These oxidants indicate the type of oxygen atom transfer which is necessary, but afford no evidence for how such a species could be generated from molecular oxygen.

Summary - Cytochrome P-450 monoxygenases have now been implicated in major oxidative metabolisms for most foreign compounds in mammals. Such metabolisms usually result in the formation of polar products (alcohols, phenols, secondary amines, etc) which are more readily conjugated and excreted. These metabolisms, thereby, appear to accomplish the primary function of such monoxygenases, i.e., detoxification via enhanced rates of elimination. In certain instances, however, such as in the conversion of imipramine by N-demethylation to desmethylimipramine,[98] the metabolism may result in the formation of a compound with higher biological activity. In other cases, metabolism may result in the formation of "bioactivated" metabolites which upon reaction with tissue constituents elicit cytotoxic or carcinogenic sequelae. Examples of such bioactivations include the conversion of halobenzenes and polycyclic hydrocarbons to reactive arene oxides (for a current review see ref. 52). It is thus clear that a complete understanding of the P-450 monoxygenase system assumes prime importance with respect to both pharmacokinetics and toxicity of medicinal agents.

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Chapter 31. Use of Chemical Relationships in Design of DDT-Type Insecticides

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General - The pharmacology of insecticides is of broad general interest in that it involves details of action and detoxication both in target insects, in man and domestic animals, and in the thousands of non-target species in the environment. Thus comparative pharmacology is rapidly becoming a recognized area of study. For the past 25 years new candidate insecticides were selected largely on the basis of insecticidal effectiveness and long-term persistence. Those most successful in meeting these requirements are now being phased out[1]. Many of the less persistent organophosphate and carbamate replacements are extremely toxic to man and possess little or no intrinsic selectivity. There is an urgent need to develop biodegradable and pharmacologically selective insecticides which are also relatively persistent on surfaces. Restudy of the DDT-type molecule provides an avenue for meeting these objectives.

Mode of Action of DDT - Despite thirty years of intensive study and the utilization of more than 4×10^9 lb. of DDT, there is little definitive knowledge about the critical lesion produced in DDT poisoning. In both insects and mammals, DDT produces hyperexcitability, violent tremors, convulsions, prostration, and death. Physiologically, the tremors result from initiation of repetetive after-discharge in the nerve axon, so that a single efferent nerve impulse produces a prolonged volley of afferent impulses. These may have the effect of literally stimulating the insect to death that may result from metabolic exhaustion, or from endogenously produced neurotoxin[2]. This axonal effect is associated with an increase in negative after-potential and results in prolongation of the action potential of the nerve[3] presumably through inhibition of K^+ efflux. The molecular mechanisms for this action are the subject of substantial conjecture.

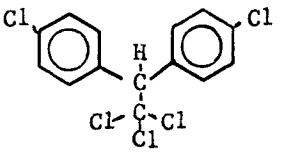
DDT has a high electron affinity and Matsumura and O'Brien[4] suggested charge-transfer complex formation between DDT and nerve membrane and have isolated a DDT protein combination that appears to have these properties. The well known negative temperature coefficient of DDT intoxication[5] supports this idea. Gunther et al[6] suggested that DDT must bond with a cavity in the protein matrix, and Mullins[7] proposed a generalized receptor site in the regular interstices of cylindrical lipoprotein strands forming the membrane lattices of the nerve axon. The lipoprotein strands were estimated to be about 40 A° in diameter and when packed in hexagonal array with a 2 A° separation, provide hypothetical pores into which the DDT molecule should fit snuggly in end-on position, distorting the membrane and presumably producing ion-leaks causing excitation. Holan[5] interwove these ideas into the concept of the DDT molecule acting as a molecular wedge, entering into the lipoprotein of the nerve membrane so that the dichlorodiphenylmethane moiety is locked into the overlying protein-layer through molecular complex formation, and the trichloromethyl group fits into a

pore in the membrane, the "sodium gate", keeping it open to Na^+ ions and delaying the falling phase of Na^+ ion potential. Weiss[8] has explained the implied paradox of DDT blockage of the membrane pore as leading to Na^+ leakage, by proposing "molecular springs" of helical phospholipids which when expanded prefer hydrated Na^+ to K^+ and when contracted prefer unhydrated K^+ to Na^+. Absorption of DDT molecules between the expanded springs would prevent their closure and facilitate abnormal Na^+ permeability.

From parallel beam projections of a number of toxic DDT analogues, Holan[5] concluded that the van der Waals' diameter of the aliphatic apex of the active molecular wedge should be 6.0 ± 0.5 A°, or close to that of the hydrated sodium ion; and that the maximum allowable distance between the p, p' substituents of the aryl rings is about 14.0 A°. More recent data described later, suggest that the latter measurement will need revision.

Matsumura and Narahashi[9] found DDT to strongly inhibit Na^+, K^+, and Mg^{++} ATP'ase of rat brain and have suggested a correlation between ATP'ase inhibition and the electrophysiological symptoms of DDT poisoning. The strong ATP'ase inhibition by the insecticidally and electrophysiologically inert DDE or 2,2-bis-(p-chlorophenyl)-1,1-dichloroethylene remains difficult to explain.

Critical Fit at Site of Action - The present views of the action of DDT strongly support a precise physical interaction between a macro-molecular element of the nerve axon, the "DDT-receptor", and the DDT-type molecule[5,6,7]. Analogues isosteric with DDT (1) and with appropriate lipophilicity produce typical DDT-like axonal instability. This phenomenon can be best observed by prior inhibition of mixed function oxidase (MFO) detoxication by administration of a synergist such as 3,4-methylenedioxy-6-propylbenzyl n-butyl diethyleneglycol ether (piperonyl butoxide)[10] or, 2-(3,4-methylenedioxyphenoxy)-3,6,9-trioxaundecane (sesamex)[11]. Under these conditions, the completely methylated DDT-isostere 1,1-bis-(p-tolyl)-neopentane (2) (synergized LD_{50} Musca 35 µg/g) approaches the toxicity



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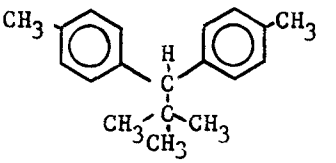
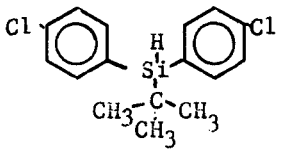


2

LD_{50} values for Musca domestica in µg per g. or Culex pipiens fatigans larvae in ppm will be given subsequently without prefix and are taken from[10,12,13] unless otherwise indicated.

of DDT (LD_{50} Musca 5.5 μg/g) [12]. In general typical DDT-like activity is found in all the isostere combinations of Cl (Van der Waals' radius 1.75 A°) and CH_3 (radius 1.97 A°).[13] There is, however, clearly an optimum size both to the constituent parts and to the molecule as a whole. A series of silicon analogues of DDT including bis-(p-chlorophenyl)-tert-butylsilane (3), bis-(p-chlorophenyl)-dichloromethylsilane (4) and bis-(p-chlorophenyl)-trimethylsilylmethane (5) proved to be nearly inactive as toxicants to Musca and Culex [14] and it was suggested that the nearly



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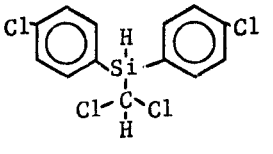

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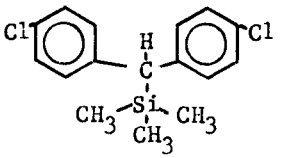

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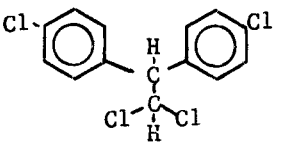
50% larger size of silicon (Si-C bond 1.86 A°) compared to carbon (C-C bond 1.48 A°) caused an increase in size of the molecule in all tetrahedral dimensions so that interaction with the DDT receptor site was prevented, e.g. the van der Waals' radius of $-C(CH_3)_3$ is 2.79 A° vs. 3.3 A° for $-Si(CH_3)_3$ [15].

This narrow range of restriction in the physical size of the active DDT-type molecule is supported by the relative inactivity of 2,2-bis-(p-chlorophenyl)-1,1,1-tribromomethane (Musca 500, Culex 0.55) where the van der Waals' radius of $-CBr_3$ is 3.21 A°; and by comparison of 2,2-bis-(p-chlorophenyl)-2,1,1,1-tetrachloroethane (completely inactive) vs. 2,2-bis-(p-chlorophenyl)-2-fluoro-1,1,1-trichloroethane (Musca 125, Culex 0.092) [13] where the atomic radius of F is 1.47 A° compared to 1.75 A° for Cl [15]. With decreased crowding around the β-carbon, using 2,2-bis-(p-chlorophenyl)-1,1-dichloroethane as a model (6) (Musca 19.7, Culex 0.038), insertion of α-F (7) substantially improved toxicity (Musca 4.1, Culex 0.024), insertion of α-Cl (8) is acceptable (Musca 18.5, Culex 0.24, and toxicity is further decreased with α-Br (2,2-bis-p-chlorophenyl)-2-bromo-



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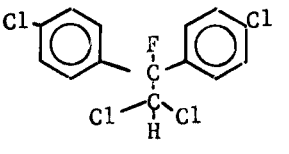

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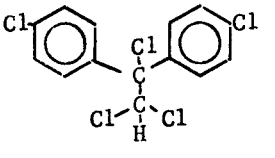

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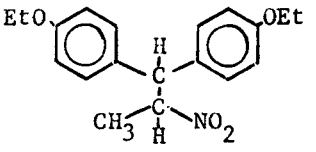
1,1-dichloroethane) (Musca 55, Culex 0.19).

Other Aliphatic Moieties - Other DDT-like compounds include 1,1-bis-(p-chlorophenyl)-2-nitropropane or Prolan®. [11] The analogue 1,1-bis-(p-ethoxyphenyl)-2-nitropropane (9) is very toxic (Musca 5.0, Culex 0.045). Replacement of the C-H with CH_3 in the corresponding 2-nitroisobutane (10) sharply reduced toxicity (Musca 80, Culex 0.2) probably because of the increased Van der Waals' radius ($CH(CH_3)NO_2$ ca 3.23 A°, $C(CH_3)_2 NO_2$ ca 3.00 A°).



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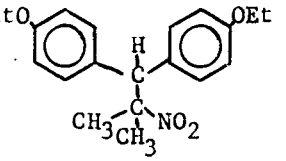

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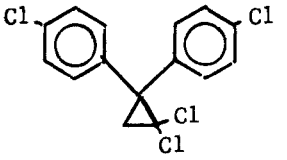

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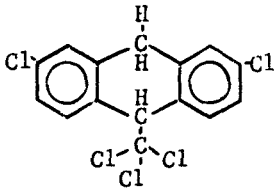
Introduction of the cyclopropane group into DDT chemistry was first tried in 1944 [16] but 1,1-bis-(p-chlorophenyl)-cyclopropane is essentially inactive (Musca 7500, Culex 1.0). However, the corresponding 1,1-di-(p-chlorophenyl)-2,2-dichloro-cyclopropane (11) [5] (Musca 12.0, Culex 0.042) is approximately as toxic as DDD (6), whose configuration it approximates [17]. The corresponding 1,1-di-(p-chlorophenyl)-2-chlorocyclopropane was about one-tenth as active and in agreement with the trihaloethanes; the 2,2-difluorocyclopropane (Musca 415) was of low activity, the 2,2-dibromocyclopropane (Musca 365) only slightly more active, while the 2-chloro-2-fluoro (Musca 60) and 2-chloro-2-bromocyclopropane were intermediate [5].

Essential Configuration - The biological action of DDT (1) and analogues depends upon optimum configuration of the phenyl rings and trichloromethyl-group about the central α-carbon. For maximum toxic action the rings must assume the butterfly or trihedral configuration [18]. The essentiality of this configuration is demonstrated by the low insecticidal activity of 2,7- dichloro-9-trichloromethyl-9,10-dihydroanthracene (12) (Musca>500, Culex 0.35), in which the aryl rings are planar [19,20]. The diphenylmethane moiety is not essential for DDT-like activity and an atom of N, O, or S can be interposed between the two aryl moieties to produce compounds that approach DDT in toxicity to insects [21]. These compounds such as α-trichloromethyl-p-ethoxybenzyl N-(p-ethoxyaniline) (13) (Musca 15.5, Culex 0.19) and α-trichloromethyl-p-ethoxybenzyl p-ethoxyphenyl ether (14) (Musca 27, Culex 0.11 [21], produce characteristic DDT-like symptoms of intoxication [22]. Observations of Fischer-Hirschfelder-Taylor molecular models of these compounds show that the staggered alignment of the groups aryl-X-C-aryl (13, 14) permits the p, p'-substituents to assume configurations very close to those of DDT. The importance of this staggered align-



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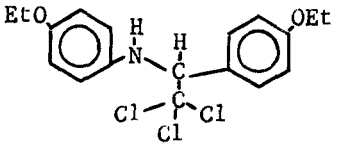

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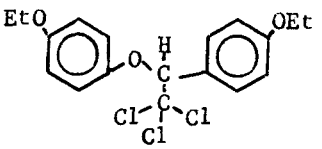

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ment in permitting the α-trichloromethylbenzylphenyl ethers and α-trichloromethylbenzyl anilines to fit the DDT receptor is shown in Holan's[5] results with substituted diphenyldichlorocyclopropanes. The 1,1-di-(p-chlorophenyl)-2,2-dichlorocyclopropane (11) (Musca 12) was highly toxic while its conformer trans-1,3-di-(p-chlorophenyl)-2,2-dichlorocyclopropane (Musca >10,000) was inactive. The rigid cyclopropane ring in the last clearly prevents assumption of the staggered configuration found DDT and in (11).

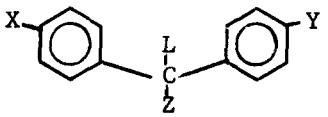
X-ray diffraction determinations of the crystalline structures of DDT, 1,1-bis-(p-ethoxyphenyl)-2,2-dimethylpropane and 1,1-di-(p-chlorophenyl)-2,2-dichlorocyclopropane have recently been completed[17], and it was found that these three compounds were extremely similar in stereochemistry. The studies showed that there is a lack of a mirror plane running through the alkyl chain so that the two phenyls are unevenly twisted.

Asymmetrical Analogues - The earlier literature of DDT toxicology contains many statements that symmetry of structure is an essential feature of all good DDT analogues[24]. A few attempts were made to investigate asymmetrically substituted p,p'-diaryltrichloroethanes with substituents such as Cl-CH_3 (Musca 62.5, Culex 0.032), CH_3O-OC_2H_5 Musca 16, Culex 0.039), and F-OCH_3 (Musca 47, Culex 0.47). Recent study of such asymmetric compounds[10] shows that insecticidal activity is associated with a substantial degree of asymmetry and that the permissible limits for high toxicity vary with the nature of the aliphatic moiety. Thus representative LD_{50} values for p,p'-dialkoxyphenyl-1,1,1-trichloroethanes were: CH_3O-OC_3H_7 (Musca 24, Culex 0.18), CH_3O-OC_4H_9 (Musca 21, Culex 0.18), CH_3O-OC_6H_{13} (Musca 500, Culex 1). However for p,p'-dialkoxyphenyl-2-nitropropanes the LD_{50} values were: CH_3O-OC_6H_7 (Musca 42, Culex 0.11), CH_3O-OC_4H_9 (Musca 21, Culex 0.062), CH_3O-OC_6H_{13} Musca 37, Culex 18), and CH_3O-OC_8H_{17} (Musca 75, Culex >1).

These several sets of data demonstrate conclusively that the overall size and shape of the DDT-type molecule is the critical factor in deter-

mining the efficiency of interaction with the macromolecular axonic receptor. The efficiency of interaction is determined by optimum combination of the substituents on both aromatic rings or in the α-(C_2) and β-(C_1) carbons. Marked asymmetry of structure, e.g. as great as CH_3O-OC_8H_{17}, can be associated with high toxicity. In this regard it is interesting that the most toxic diaryltrichloroethane when given orally to the white mouse contained the p,p'- substituents C_2H_5O-OC_3H_7 (LD_{50} 75), vs. C_2H_5O-OC_2H_5 (300) and Cl-Cl (DDT) (LD_{50} 200) [10].

Multiple Regression Analysis - Quantitative biological data for several hundred DDT analogues supports the simple concept that the biological effect results from maximum complementarity between the DDT-type molecule and the DDT receptor[6] and the view that there is a critical optimum for summation of the van der Waals' forces of the four key substituents X, Y, L, and Z [14]:



Toxic molecules result when these substituents vary over a wide range (e.g. F to $C_8H_{17}O$ for X and Y) implying that the receptor is by no means a rigid structure [5,7] but must have a considerable degree of flexibility or elasticity. Therefore as the summation of size of X, Y, L, and Z increases, interaction with the cavity or pouch of the DDT receptor increases and eventually reaches a maximum beyond which increases in the sizes of the substituents result in decreased complementarity and loss of insecticidal activity [14].

Free energy parameters - the Taft steric substituent constants E_s, have been employed to quantify this idea [14]. E_s values represent solely steric parameters and are directly proportional to van der Waals' radii [15]. A parabolic function was developed to express the relationship of LD_{50} to E_s:

$$\log LD_{50} = \alpha' + \beta' \sum^{i} E_s + \gamma' \sum^{i} \left[E_s\right]^2$$

where i = substituents X, Y, or Z

Using LD_{50} values to Musca for 25 symmetrical and unsymmetrical DDT analogues with variations in X and Y, in multiple regression analyses using E_s, π, F, and R values, it was shown that the correlation coefficient was 0.74 for LD_{50} alone and was increased to 0.87 using synergized LD_{50} values where MFO detoxication was inhibited by prior application of piperonyl butoxide. E_s was the only free energy parameter of significance in the regression equations indicating that van der Waals' interaction forces are primarily responsible for the DDT-receptor interaction[6]. Similar multiple regression analysis of LD_{50} values to Musca for 9 DDT analogues where X and Y remained constant as Cl and L and Z were varied using E_s, π, and σ^*, gave r = 0.86 with the combination of E_s and σ^* as highly significant. Therefore a combination of steric and polar effects contributed to the interaction of L and Z with the receptor[14].

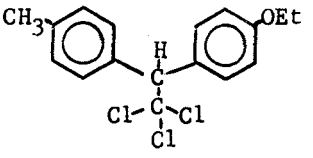
This sort of multiple regression analysis has opened the way to the rational design of effective new DDT analogues based on optimizing the van der Waals' radii of groups X, Y, L, and Z. Several effective new compounds have been designed from these correlations, e.g. 1-(p-fluorophenyl)-1-(p-butoxy)-nitropropane[27]. Using data from several new sets of compounds under evaluation it appears feasible to develop a unified mathematical description of the combined effects of groups X, Y, L, and Z upon toxicity of the DDT type compound, and this is presently under study.

Comparative Toxicology - A substantial amount of precise LD_{50} data to the house fly Musca domestica, to the black blowfly, Phormia regina, to the house mosquito larva, Culex fatigans, and to the malaria mosquito larva, Anopheles albimanus enables us to take a quantitative look at the dimensions of the macromolecular target site of the DDT-type molecule in several species. Additionally, using the synergized LD_{50} to virtually eliminate the effects of in vivo detoxication it is feasible to examine which sort of DDT-type configuration is intrinsically the most active. Surprisingly, DDT itself appears to have the best fit for only Anopheles larvae of the species examined. Based on synergized LD_{50} values, the order of LD_{50} values to Musca for the p,p'-disubstituted aryl trichloroethanes with the indicated substituents was C_2H_5O (1.75) CH_3O (3.5) Cl (5.5). To Phormia the order was CH_3O (4.6) C_2H_5O (7.4) Cl (8.25). To Culex the order was C_2H_5O (0.04) CH_3O (0.067) Cl (0.07), and to Anopheles Cl (0.05) C_3H_7O (0.077) C_2H_5O (0.086)[10]. For the p,p'-disubstituted-diphenyl-2-nitropropanes, the synergized LD_{50} values to Musca were in the order C_2H_5O (1.25) C_3H_7O (2.3) CH_3O (2.75) Cl 7.0; and to Phormia C_2H_5O (1.35) CH_3 (6.0) CH_3O (7.75) Cl (11.70). To Culex and Anopheles larvae C_2H_5O (0.045-0.048) Cl (0.064-0.066)[27].

These data show rather clearly that the optimal size of the aryl substituents is related to the size of the aliphatic portion, e.g. $-CCl_3$ vs. $-CH(CH_3)NO_2$, thus leading back to the hypothesis of Gunther et al[63].

Both methoxychlor and methylchlor show decreased insecticidal activity over DDT in some insects especially lepidopterous larvae due to in vivo degradation in the insect body. However, they may be more toxic to other insects, e.g. methoxychlor to the Mexican bean beetle, Epilachne

varivestis and to Phormia regina, and their activity can be materially improved by synergism with piperonyl butoxide [10]. Asymmetrical analogues such as 2-(p-methylphenyl)-2-(p-ethoxyphenyl) 1,1,1-trichloroethane (15) (Musca 9, Culex 0.13) [10] are insecticidally effective, of low toxicity to fish and mammals, and readily biodegradable [28,29].



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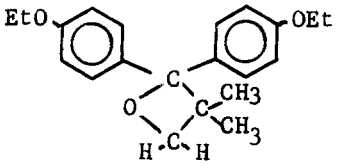

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Alterations of the aliphatic moiety of the DDT molecule to introduce degradophores (easily degraded moieties) have produced compounds such as 1,1-bis-(p-ethoxyphenyl)-3,3-dimethyloxetane (16), (Musca 26) [11,30] which is synergized 50-fold by sesamex to the housefly and is readily degradable.

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