ANNUAL REPORTS IN MEDICINAL CHEMISTRY Volume 10

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

Editor-in-Chief: ***RICHARD V. HEINZELMAN***

THE UPJOHN COMPANY
KALAMAZOO, MICHIGAN

ACADEMIC PRESS New York and London 1975
A Subsidiary of Harcourt Brace Jovanovich, Publishers

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Volume 10

CONTRIBUTORS

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ACADEMIC PRESS RAPID MANUSCRIPT REPRODUCTION

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 66-26843

ISBN 0–12–040510–5

PRINTED IN THE UNITED STATES OF AMERICA

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PREFACE

In this volume of Annual Reports we have continued our established editorial policy of reporting each year the significant developments in the major areas of current interest to medicinal chemists, and discussing less frequently those medical areas which are either in their infancy or have reached their "golden years". Eighteen of the topics in Volume 10 did not appear in Volume 9.

We have attempted to strike a suitable balance between, on one hand, progress in the discovery and development of new active classes of drugs or of more effective analogs in older classes and, on the other hand, the biochemical, biological or medical status of some fields just emerging into prominence. Some of our readers tell us that they clearly prefer the former, "more practical" type of chapter. It is my personal belief that the editor of a volume such as this has a responsibility to open a few vistas and to occasionally jar the medicinal chemist out of his established point of view. One of my former section editors put it this way: "If you project to the future you had better be interested in dreams rather than meat."

Medicinal chemists cannot live by meat or dreams alone. Our aim is to provide a balanced diet. Please let us know how we are doing.

Kalamazoo, Michigan
June, 1975

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SECTION I - CNS AGENTS

Editor: Maxwell Gordon, Bristol Laboratories, Syracuse, New York

Section Editorial

During the first twenty years of the clinical use of antipsychotic agents some considerable gains were made in the treatment of the mentally ill. Mental hospital populations decreased for the first time in modern history; there are only half as many psychiatric beds in state hospitals as there were 15 years ago. In addition, there has been a transition of professional and public attitudes from custodial to therapeutic. As time wore on, however, it became apparent that there was a high readmission rate of former mental hospital patients. This relapse rate led to the development of "half-way houses" and other community centers that could provide outpatient care in the patient's normal home/work setting. These developments have also been assisted by recognition of the importance of early diagnosis and outpatient treatment of the mentally ill. The development of long-acting antipsychotic agents has proved useful in both the hospital and outpatient settings,though they are no panacea and the problem of tardive dyskenesias is still unresolved. Inasmuch as fewer than half of schizophrenics released from mental hospitals function adequately as wage earners or housewives, it is clear that more progress is needed in chemotherapy and in the areas of personal, family and community adjustment.

There has been a great deal of research on the biochemical basis for mental disease,and much of it is summarized in this section of the Annual Reports. However,progress in finding new therapeutic agents continues to rely heavily on the classical approaches of the medicinal chemist and the pharmacologist. One straw in the wind of the future is the debt owed in the treatment of Parkinson's disease to biochemical research. The discovery that there is a relative deficit of dopamine in various areas of the brain of Parkinson patients led to the suggestion that the dopamine precursor, dopa, could have therapeutic potential.

Research studies now under way have demonstrated that CNS agents often have effects on neurotransmitter substances, and it is hoped that this kind of research can lead to more systematic therapeutic advances in the future.

Another research area that offers promise for the future is in the use of CNS agents to treat the withdrawn, apathetic geriatric patient. The management of this type of patient is such a serious medical and social problem that the advent of useful new drugs would be a major milestone in medicine. This research is difficult, and translation of animal work to man is tenuous at best,but there is some reason for optimism that progress will be made.

Finally,these pages contain encouraging suggestions that antipsychotic agents can be found which have minimal extrapyramidal side effects, that anti-anxiety agents can be found which produce less ataxia and sedation, that hypnotic agents can be found that do not adversely affect REM sleep or produce dependence, and that synthetic analgetic/antitussive agents can be developed that avoid the side effects of opiates and could eliminate the medical profession's dependence on cultivation of opium.

Section I – CNS Agents

Editor: Maxwell Gordon, Bristol Laboratories, Syracuse, New York

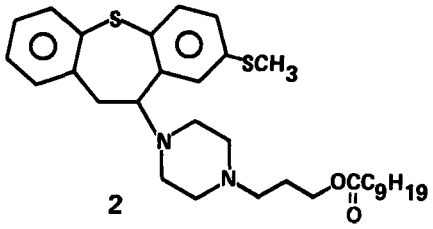
Chapter 1. Antipsychotic and Antianxiety Agents

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

Introduction/Summary – Molindone (Moban^{R}), an antipsychotic drug, was the only new entity introduced in the U.S. in 1974 although several new agents advanced to late stages of development. Clozapine continued to look promising as an antipsychotic drug with a low incidence of extrapyramidal side effects. The demonstration of clinical activity for the new antipsychotic agent butaclamol suggests that it may represent a structural prototype in this area. Evidence in support of the existence of a presynaptic dopamine receptor led to intensive efforts to elucidate its function, and advances in understanding the mechanism of antipsychotic vs. extrapyramidal effects of antipsychotic drugs should permit the rational selection of more effective agents. It has been proposed that benzodiazepines exert their CNS effects by acting at a glycine receptor.

Antipsychotic Agents of Extended Duration – Penfluridol continued to exhibit impressive clinical antipsychotic efficacy as a once-a-week oral medication in a number of trials.[1-5] Sedation and extrapyramidal symptoms (EPS) were rare at effective weekly doses of 160-200 mg[3] although a relatively slow onset of action (four weeks in one study) was noted.[6] Neurological and pharmacological studies of the injectable preparations pipothiazine palmitate (1, IL-19,552)[7] and oxyprothepin decanoate (2,

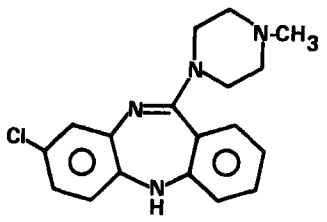
1



2

VUFB-9977)[8] indicate their greater duration of action vs. apomorphine emesis as compared to fluphenazine decanoate. Consistent with this observation is the report that pipothiazine palmitate at doses of 100 to 600 mg i.m. controlled schizophrenic symptomatology for at least 4 weeks.[9]

Tricyclic Antipsychotic Agents – Clozapine (3, Leponex^{R}) continued to demonstrate encouraging clinical activity, with potency equivalent to chlorpromazine (CPZ) and a greatly reduced incidence of EPS even at high doses (800 to 1200 mg/day). Orthostatic hypotension was observed, however, in a small percentage of patients in each of these studies.[10-12] Remission of tardive dyskinesia which recurred upon drug withdrawal was noted in two patients receiving clozapine.[13] Cerebrospinal fluid levels of 3-methoxy-4-hydroxyphenylglycol fell and those of homovanillic acid (HVA) rose significantly in patients treated with clozapine, indicating reduced norepinephrine turnover in brain.[14]



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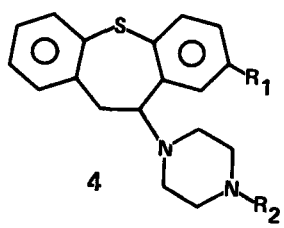

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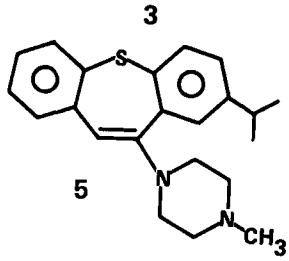
4a) $R_1 = COCH_3$, $R_2 = CH_3$
4b) $R_1 = C_2H_5$, $R_2 = (CH_2)_3OH$
4c) $R_1 = SCH_3$, $R_2 = CH_3$
4d) $R_1 = SC_2H_5$, $R_2 = CH_3$
4e) $R_1 = Cl$, $R_2 = CH_2CH_2$
4f) $R_1 = Cl$, $R_2 = CH_2CH_2$



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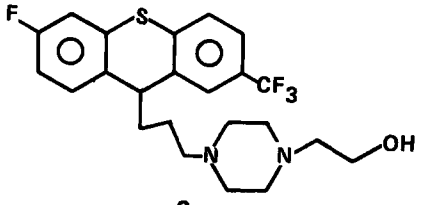

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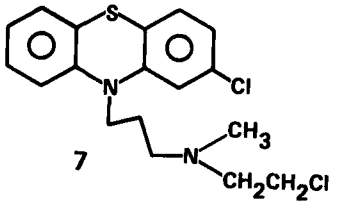

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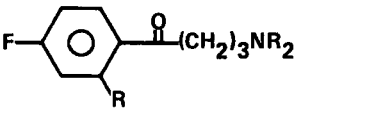
Compounds **4a**[15] and **4b**[16], the most potent of two series of dihydrodibenzo[b,f]thiepins, equalled octoclothepin in their depressant effect (rotating rod (RR), catalepsy) whereas the parent dibenzo[b,f]thiepin (**5**) was somewhat less active. An SAR study of higher thioalkyl homologs of metitepin (**4c**) showed retention of activity in the ethylthio analog (**4d**) only.[17] Cyclic acetals derived from 10-piperazino-10,11-dihydrodibenzo[b,f]thiepins (**4e-f**) were approximately as active as octoclothepin and metitepin as central depressants (mice, RR) and cataleptogenic agents (mice, rats).[18]

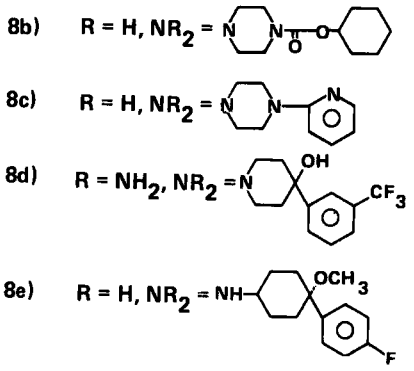
The thioxanthene derivative Lu 10-022 (**6**) resembled haloperidol and fluphenazine in its ability to increase the disappearance of ^{14}C-dopamine in mouse brain and may be longer acting based on its ability to increase accumulation of ^{14}C-catecholamines in mouse brain for an extended period.[19] The N-chloroethyl derivative of CPZ (**7**, P-B 845 Cl) was less potent (¼x) than CPZ in animal behavioral tests, but gave evidence of prolonged duration of action in the anti-amphetamine motor activity test.[20]

The metabolism of CPZ and the biological activity of several of its metabolites have been extensively reported.[21-24] In one study, the ratio of biologically active 7-OH CPZ to the inactive CPZ sulfoxide was significantly greater in responding than in non-responding schizophrenics.[21] Behavioral studies suggest that 7-OH CPZ is less potent than CPZ, but might have useful antipsychotic potential owing to reduced EPS.[23] Other metabolism studies have shown that piperazine- and dimethylamino-substituted phenothiazine neuroleptics with the same nuclear substitution are degraded to identical metabolites (bis-dealkylated amine congeners, excreted as the sulfoxides) in man, rat and dog.[25]

Butyrophenone Antipsychotic Agents – U-32,802A (**8a**) resembled both reserpine and haloperidol in its ^{3}H-NE releasing properties (mouse heart) and HVA-elevating actions, respectively, but differed from these agents in having only a weak effect on brain serotonin (unlike reserpine) and on catecholamine levels (unlike haloperidol).[26] Fenaperone (**8b**) had a characteristic neuroleptic profile in rats and mice and was equivalent to haloperidol in antagonizing epinephrine mortality.[27] Azaperone (**8c**, R 1929) was projected to be a sedative neuroleptic agent in man on the basis of pronounced anti-aggressive (5X CPZ) and anti-shock (125X haloperidol) properties.[28] Preliminary pharmacological results with ID-4708 (**8d**) showed it to be superior to haloperidol and trifluoperidol in a number of neuroleptic endpoints (apomorphine, CAR, catalepsy) predictive of activity in man.[29] The EEG profile of U-35,777A (**8e**) in monkeys was characteristic of major tranquilizing agents suggesting it would have antipsychotic potency between thioridazine and CPZ in man.[30]



8a) R = H, NR_2 =



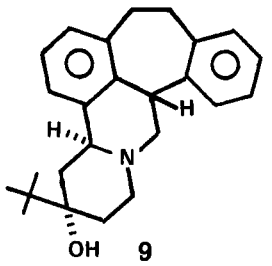
8b) R = H, NR_2 =

8c) R = H, NR_2 =

8d) R = NH_2, NR_2 =

8e) R = H, NR_2 =

Other Compounds Possessing Antipsychotic Activity - Butaclamol (9, AY-23,028) is an interesting new antipsychotic agent of novel structure. Activity resides solely in the (+)-enantiomer which is 2-3X as active as the racemic compound in elevating rat striatal HVA concentrations *in vivo* and blocking dopamine-sensitive adenylate cyclase activity in olfactory tubercle homogenates.[31] Butaclamol was reported to be effective in a preliminary uncontrolled trial in severely ill "drug-refractory" schizophrenics



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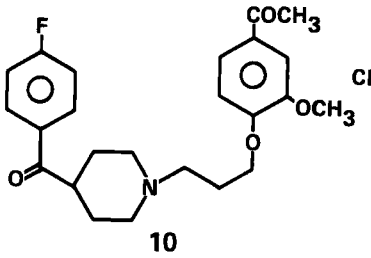

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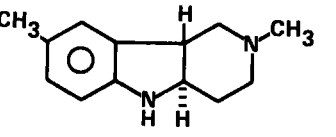

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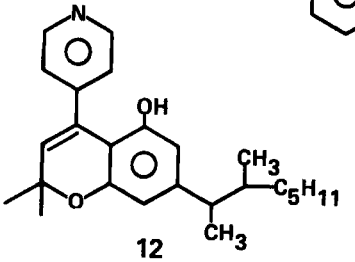
at doses of 20-100 mg/day thereby confirming its activity in animals.[32] Extrapyramidal symptoms which responded satisfactorily to anticholinergic medication occurred. AHR-2244 (10) selectively blocked CAR in mice, rats and cats and suppressed amphetamine lethality at doses intermediate between those of haloperidol and CPZ.[33] Effective antipsychotic activity along with some stimulant properties were reported for carbidine (11) at doses up to 150 mg/day in clinical trials in the USSR.[34] The tranquilizing activity of BRL-4664 (12) was reported to combine elements of both major and minor tranquilizers. It



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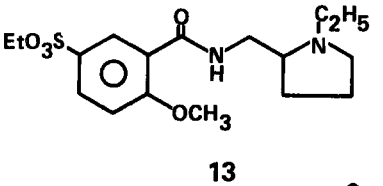

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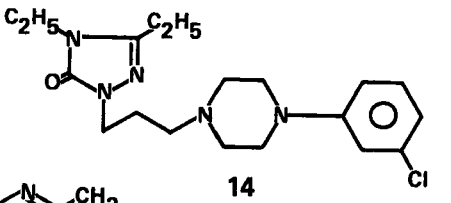

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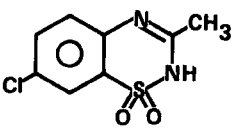

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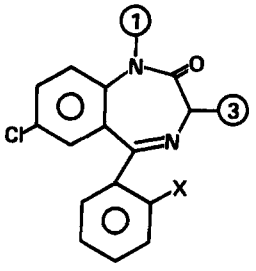
reduced CAR in rats (≈CPZ) and enhanced dopamine turnover in rat striatum like a neuroleptic but also resembled chlordiazepoxide in its ability to impair polysynaptic reflexes in the cat.[35] Reduction of the pyridyl ring of **12** resulted in loss of CNS activity.[36]

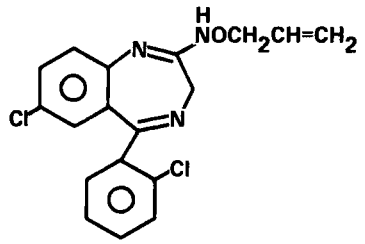


16

16a) 1-CH_3, 3-$OCN(CH_3)_2$, X = H
16b) 1-CH_3, 3-OH, X = H
16c) 1-CH_2CH_2OH, 3-OH, X = F
16d) 1-CH_3, 3-OH, X = F
16e) 1-$CH_2CH_2N(C_2H_5)_2$, 3-H, X = F
16f) 1-$CH{=}CH_2$, 3-H, X = F
16g) 1-$CH{=}CH_2$, 3-H, X = Cl
16h) 1-$CH_2CH_2NC_2H_5$ (OH), 3-H, X = F
16i) 1-CH_3, 3-H, X = F

Sultopride (**13**, Lin 14 18), an analog of sulpiride, exhibited antipsychotic activity accompanied by EPS in a controlled study.[37] Triazolinone (**14**), an analog of trazodone, reduced CAR in rats, abolished aggressiveness and reduced instinctual drives without loss of purposeful behavior in mice (1-10 mpk).[38] Psychopharmacological testing of the anti-hypertensive agent diazoxide (**15**, Hyperstat^R) following unexpected incidences of EPS, disclosed it to have properties characteristic of weak neuroleptics.[39]

Antianxiety Agents - Benzodiazepines and Related Compounds – The pharmacological properties and clinical effects of benzodiazepines were reviewed.[40,41] Camazepam (**16a**, SB 5833) possessed sedative and tranquilizing properties in mice, rats and rabbits and was equal to medazepam and temazepam (**16b**) vs. pentylenetetrazole (PTZ) convulsions.[42] Open[43] and double blind[44,45] clinical trials of this agent demonstrated good to excellent results in both anxious and depressive symptomatology. SAS-643 (**16c**) was 4-10X diazepam in animals (PTZ, muscle relaxation) while SAS-646 (**16d**) was approximately equal to diazepam in the same battery of tests.[46,47] The benzodiazepine-N-oxide **16e**, the 1-vinyl derivatives **16f,g**



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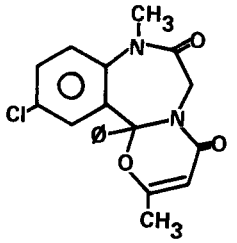

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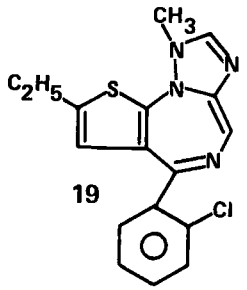

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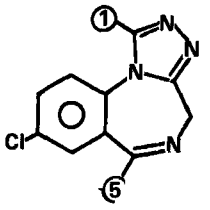
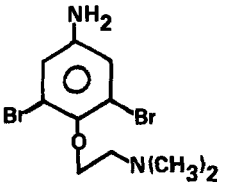
and the related hydroxylamine **16h** demonstrated oral anti-PTZ activity exceeding that of diazepam, chlordiazepoxide and flurazepam.[48] ID-540 (**16i**) was reported to be 3-12X diazepam in anticonvulsant endpoints and 6-7X diazepam in the fighting mice test.[49] Uldazepam (**17**, U-31,920) proved efficacious (≈diazepam) against tension and anxiety in a double blind clinical trial, confirming predictions from pharmaco-EEG studies.[50] Ketazolam (**18**, U-28,774) was shown to be efficacious in an uncontrolled clinical study in anxious patients.[51] Compound **19** (Y-7131) exhibited 2-4X the taming ability of diazepam and was equal to or better than diazepam as a skeletal muscle relaxant.[52]



20a) 1-H, 5-C_6H_5

20b) 1-CH_3, 5-o-ClC_6H_4



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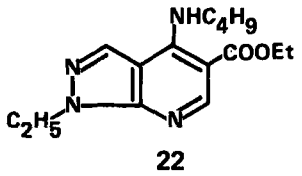
Metabolic studies with the triazolobenzodiazepines estazolam (**20a**, D-40 TA)[53-58] and triazolam (**20b**, U-33,030)[59] indicated that the former is hydroxylated at positions 1 and 4 (in addition to the C_6H_5-ring) whereas the 1-hydroxymethyl and 4-hydroxy metabolites predominate in the latter.

Non-Benzodiazepine Tranquilizing Agents – The phenetidine derivative PM-33 (**21**) exhibited statistically significant improvement in anxiety in a small open clinical study at 50-600 mg/day with only moderate side effects.[60] SQ 65,396 (**22**), a potent cyclic-AMP phosphodiesterase inhibitor, caused a significant reduction in anxiety and tension with minimal side effects (up to 50 mg q.i.d.); however, any preexisting schizophrenic symptomatology tended to worsen.[61] Glaziovine (**23**) was found to possess anxiolytic properties similar to diazepam in a double blind clinical trial without causing asthenia or drowsiness.[62] Abbott-40656 (**24**, SP-106) reduced SMA in several species and blocked aggressiveness in mice and monkeys at 0.5 to 5.0 mpk suggesting its possible utility as a sedative-tranquilizer.[63]



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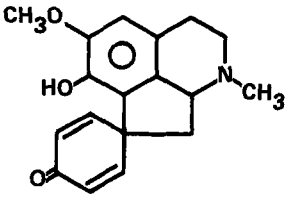

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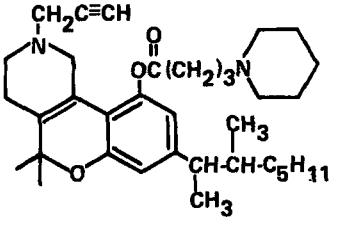

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Mechanism and Sites of Action of Antipsychotic Drugs – This topic has been reviewed recently.[64] It is generally assumed that 1) antipsychotic drugs exert their CNS effects by dopamine (DA) receptor blockade,[65] 2) antipsychotic effects are mediated by receptor blockade in the mesolimbic DA neurons[66] and 3) extrapyramidal effects result from DA receptor blockade in the nigrostriatal system.[66,67] Recent biochemical studies[68,69] are consistent with regional differentiation of antipsychotic and extrapyramidal effects, although at least one group has questioned the relationship of mesolimbic function to

antipsychotic efficacy.[70] It has also been suggested that DA receptor blockade may correlate with extrapyramidal activity rather than antipsychotic activity.[71]

Evidence that the low incidence of extrapyramidal side effects associated with clozapine and thioridazine is related to their central anticholinergic properties continued to appear. Snyder and coworkers[72,73] demonstrated the presence of a muscarinic cholinergic receptor in rat and monkey brain (highest density in the corpus striatum) for which the affinity of antipsychotic drugs is inversely proportional to their propensity to cause extrapyramidal effects in man.[72,74] Evidence that dopaminergic and cholinergic systems are mutually antagonistic in the corpus striatum, but not in mesolimbic brain regions,[69,75] suggests that the anticholinergic properties of antipsychotic drugs such as clozapine and thioridazine fortuitously reduce their extrapyramidal effects but not their antipsychotic activity. Comparison of antimuscarinic properties with conventional measures of DA receptor blockade may therefore provide a rational basis for selecting antipsychotic drugs likely to have reduced extrapyramidal effects.[72,74] Another technique of potential value in selecting antipsychotic candidates is extracellular single unit recording from mesolimbic and nigrostriatal DA neurons which has been reported to enable differentiation of antipsychotic from extrapyramidal effects.[76]

Mechanistic studies have shown that clozapine enhances DA content in rat striatum unlike most neuroleptics,[77] and support for the concept of surmountable DA receptor blockade by this drug has been reported.[78] Also, it was postulated that clozapine may not act directly on the postsynaptic DA receptor, but rather at a presynaptic inhibitory site.[79]

A DA-sensitive adenylate cyclase believed to be associated with the DA receptor has been demonstrated in mesolimbic regions (olfactory tubercle and nucleus accumbens) and the caudate nucleus of mammalian brain.[80,81] Inhibition of this enzyme by tricyclic antipsychotics correlates well with clinical potency[80,82,83] although butyrophenones and diphenylbutylpiperidines are considerably less active than would be predicted. This latter discrepancy may reflect a site of action other than the DA receptor,[83] but a more plausible explanation is that the selective *in vivo* distribution of these agents into dopaminergic brain regions more than counterbalances their low intrinsic *in vitro* activity. Inhibitory potencies of several antipsychotic drugs, including clozapine, were similar whether the enzyme was isolated from mesolimbic regions or from the caudate nucleus,[80,81] making it unlikely that this system alone will aid in the selection of antipsychotic drugs with reduced extrapyramidal side effects. Additional studies with DA-sensitive adenylate cyclase have shown that it is preferentially activated by the trans-conformation of DA[84] which is the same conformation demonstrated previously by Horn and Snyder[85] to be superimposable with the solid-state conformation of chlorpromazine. Greengard et al[86] presented a unified hypothesis for the role of DA-sensitive adenylate cyclase in the DA neuron which is highly recommended to interested readers.

Regulation of Dopamine Synthesis in Dopaminergic Neurons – It has been assumed that DA turnover in dopaminergic neurons is governed by an intraneuronal compensatory feedback mechanism that is triggered by levels of DA at the postsynaptic receptor.[87] There is now evidence that neuronal activity and transmitter synthesis in dopaminergic neurons are autoregulated by both presynaptic and postsynaptic receptors, although the precise mechanisms involved remain to be elucidated. In this respect DA neurons appear to differ from other monoaminergic systems, particularly under conditions of reduced impulse flow.[88] Aghajanian and Bunney[89] postulated the existence of a *presynaptic* DA receptor based on observations that cell bodies of central DA neurons were responsive to microiontophoretic application of DA and apomorphine. They further suggested that this receptor might play a key role in regulating DA synthesis and release (see discussion below). Christiansen and Squires[90] proposed that striatal synaptosomal preparations provide "a pure *in vitro* presynaptic system" for pharmacological studies and observed a correlation between the activity of several antipsychotic drugs in this system with their *in vivo* activity.[91]

Recent research has clarified factors regulating short-term DA synthesis, particularly at the level of tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of DA. These studies have shown that short-term alterations in DA synthesis result from allosterically-induced changes in the affinity of the enzyme for tyrosine, pteridine co-factors and DA.[92] For example, antipsychotic drugs stimulate DA synthesis by causing an immediate increase in the affinity of tyrosine hydroxylase for pteridine co-factors, an effect readily antagonized by apomorphine.[93] This process is probably regulated by a presynaptic DA receptor[94] and has been shown to involve calcium ion[95] and cyclic nucleotides.[92,96] Tyrosine hydroxylase is also activated by an increase in impulse flow in dopaminergic neurons.[97] Since it has been reported that clozapine and thioridazine selectively stimulate tyrosine hydroxylase isolated from the limbic system vs. tyrosine hydroxylase from striatum,[98] measurement of the regional effects of antipsychotics on DA synthesis may provide a useful index for extrapyramidal side effect liability. For additional background on the regulation of tyrosine hydroxylase activity, the interested reader should consult a recent review.[99]

Clinical and Biological Correlates of Schizophrenia – The search for possible endogenous psychotogens in schizophrenics continued as did biochemical studies of enzyme systems capable of producing these putative endotoxins. Although administration of psychoactive doses of N,N-dimethyltrypamine (DMT) to humans revealed that blood levels parallel the subjective "high", it was concluded that levels of endogenous DMT, if produced in schizophrenics, would likely be undetectable.[100] It was suggested that the search for this alleged psychotogen should instead focus on detection of its metabolites. Contrary to earlier reports, 5-methyltetrahydrofolic acid (5-MTHF) was found not to serve as a methyl donor for the conversion of biogenic amines to their corresponding N-methylated congeners. The reaction of the 5-MTHF-enzyme system with tryptamines and catecholamines leads instead to the formation of cyclized tetrahydro-β-carbolines[101-103] and tetrahydroisoquinolines,[104] respectively. The precise mechanism of these reactions is not known at this time and the possible physiological or pathological roles of these cyclic products remains to be elucidated (see also Chapter 5).

Mechanisms and Sites of Action of Antianxiety Agents – Corticosteroids, brain monoamines, cyclic nucleotides, γ-aminobutyric acid, glycine and prostaglandins have been implicated in the etiology of anxiety and in its treatment by antianxiety agents. It has been proposed that anxiety results from neurochemical changes induced by corticosteroids and modifications in serotonin pathways in the midbrain tegmental region,[105] consistent with the observation that antianxiety drugs, but not other psychoactive agents, block stress-induced elevations in rat plasma corticosteroids.[106] Support for the hypothesis[107] that antianxiety activity is due to effects on serotoninergic systems came from the report[108] that chlordiazepoxide and benzoctamine decrease serotonin turnover in brain. Others have suggested that this effect may more accurately reflect sedative activity.[109] A review on the peripheral and central role of catecholamines in anxiety appeared recently.[110]

Diazepam elevated basal and stimulated levels of cyclic AMP in guinea pig and rat cerebral cortical slices.[111] A similar study showed that benzodiazepines are potent inhibitors of both cyclic AMP and cyclic GMP phosphodiesterase in supernatant fractions of tissue from various brain regions, but expected regional specificity was not seen.[112] The antiaggressive effects of chlordiazepoxide correlated closely with its ability to inhibit cyclic AMP phosphodiesterase in brain.[113]

Based on the close correlation between the antianxiety and anticonvulsant effects of the benzodiazepines and their ability to displace labeled strychnine from the glycine receptor, it has been concluded that benzodiazepines exert their activity by mimicking the effect of the neurotransmitter candidate glycine in the CNS.[114] The fact that many of the most potent benzodiazepines contain a masked glycine residue in the 7-membered ring was cited as support for this conclusion. Mephenesin has also been reported to mimic the action of glycine and to elevate the concentration of this amino acid in nervous tissue.[115]

Beta-Blockers as Antipsychotic and Antianxiety Agents – The use of β-blockers in psychiatry was extensively reviewed.[116,117] To date there has been no firm evidence of antipsychotic activity for these agents, although a recent report that propranolol controls schizophrenic symptoms[118] deserves followup in a controlled trial. Evidence for antianxiety activity is mixed, but it seems likely that β-blockers may find use in the treatment of anxious patients with manifest somatic symptoms.[110] It has been suggested that propranolol's CNS effects in man may be due to its glycol metabolite, which is structurally related to mephenesin, a known anticonvulsant and antianxiety agent.[119]

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Chapter 2. Narcotic Antagonists and Analgesics

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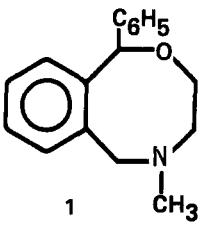
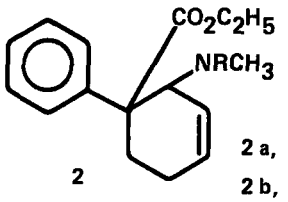
Introduction – The characterization of the opiate receptor and the discovery of a PGE sensitive adenylate cyclase inhibited by morphine represent major advances in the biochemical and pharmacological understanding of opiate mechanisms. While synthetic emphasis has continued to focus on refinements of SAR studies with known agents, the discovery of a cannabinoid derivative with potent analgesic activity may represent a significant chemical development. The biochemical pharmacology of cannabinols and opiates has been reviewed[1] as have the clinical and pathophysiological aspects of pain.[2] The annual evaluation of advanced agents for physical dependence in monkeys was issued.[3a]

Clinical Studies – As in the past, few advanced studies with new agents appeared in 1974. Several new non-steroidal antiinflammatory agents have been reported to possess analgesic activity in the propoxyphene range (these agents are discussed in detail in Chapter 18).

Nefopam 1 (Acupan^{R}) was claimed to possess activity in the codeine range against musculoskeletal pain.[4-6] Additional pharmacology and clinical studies of propiram fumarate (Bay 4503) were the subject of an extensive report.[7] In a U.S. clinical trial, 50 mg (po) of tilidine (**2a**) was shown to be comparable to 100 mg (po) of meperidine.[8] Animal studies with tilidine further demonstrated that its analgesic activity correlates with the plasma and brain levels of active metabolite **2b**.[9] A review of clinical experience with oral pentazocine concluded that it is an effective analgesic in both acute and chronic pain[10] although sedative side effects were reported to be limiting in one study reported during 1974.[11] Butorphanol (3) reported on last year continues to be investigated.[3b,c]

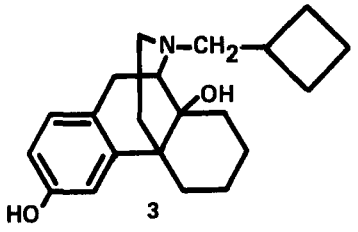


1



2

2 a, R = CH_3
2 b, R = H



3

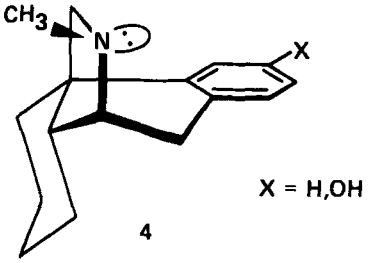
The trend toward fixed combination analgesics accelerated in 1974. Combinations of aspirin with codeine, pentazocine and oxycodone showed significant superiority over aspirin (650 mg) alone.[12] A double blind study of acetaminophen and codeine in severe episiotomy pain demonstrated that a combination of the two agents (600 mg acetaminophen, 30 mg codeine) was superior to either drug used alone.[13] Trials claiming analgesic superiority over aspirin for ethoheptazine-aspirin combinations were also reported.[14,15]

Structure Activity: Strong Analgesics

Once again research efforts focused primarily on elucidation of detailed stereostructural relationships aimed at providing information regarding analgesic receptor topology. Although additional SAR details on previously reported analgesics were disclosed, very few new classes of compounds were reported which were structurally distinct from the classical narcotic analgesics and antagonists.

Opiates, Morphinans and Benzomorphans – The five membered D ring 4-methylmorphinans (**4**) were synthesized and found to be inactive. X-ray analysis revealed that the lone pair of electrons in 4 (X=H) is oriented toward the ring whereas the nitrogen lone pair is oriented away from the ring in the active six

membered ring opiates.[16] Furthermore, opiate binding to the receptor is apparently not governed by basicity since 5 (X=H) was 1.6 times more active (mouse writhing) than **6a** while **6b** was 1.6 times more active than its non-deuterated form.[17] The importance of nitrogen lone pair orientation to analgesic activity led to the proposal of *clastic binding* on the opiate receptor complex itself – a process which requires the stereospecific orientation of the nitrogen lone pair. This contrasts with earlier proposals that the N-protonated form is the active species and provides an alternate forum for elucidating opiate receptor interactions.



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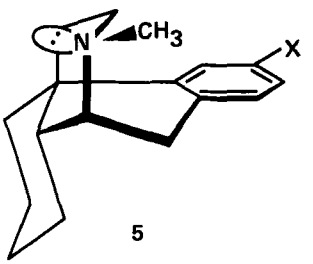

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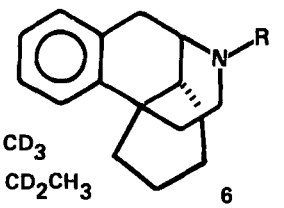
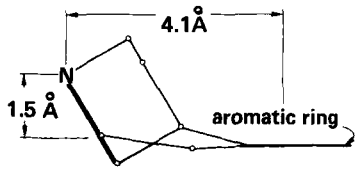


6 a, R = CD_3
6 b, R = CD_2CH_3

6

The simplifying definition of benzomorphan SAR as points within a three dimensional matrix provided the basis for the synthesis of a series of compounds **8** that retained the proper distance from the aromatic ring to the out of plane nitrogen (schematically depicted in 7) but lacked other features known to be important in benzomorphan SAR.[18] These compounds were inactive as analgesics.



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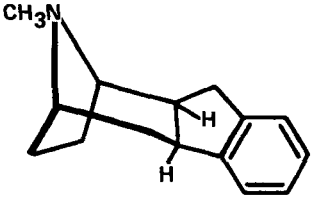

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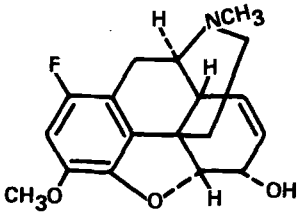
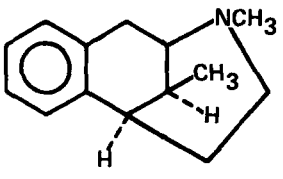


9

The observation that 1-fluorocodeine (**9**) was as active as codeine in the hot plate test provides the first conclusive evidence that the reduction in analgesic activity for 1-substituted codeines is caused by the bulk and not the inductive effect of these substituents.[19] Compound **10** was reported to be as active as codeine in unspecified animal tests.[20]



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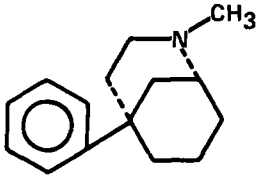
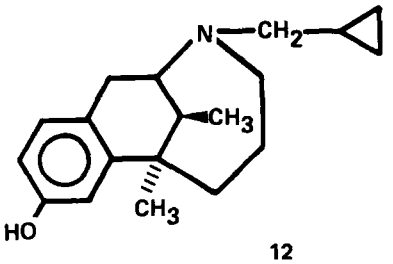


11



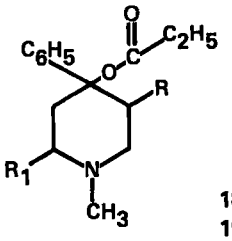
12

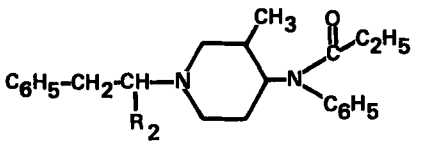
Analgesic agonist-antagonist **11**, whose absolute configuration was established as 1R, 5S,[21] was reported to have low physical dependence capacity (PDC) and toxicity thus rendering it attractive for further study.[22]

In a new series of homobenzomorphans, the most active member TA-414 (**12**) was 1/5 naloxone in narcotic antagonist activity and had no analgesic activity even at high doses.[23]

Prodines and Related Structures – The 4S, α-enantiomers, **13** and **14**, were the most active members (equal to morphine, mouse hotplate) in a series of prodine analogs.[24] Analgesic activity was also reported for the racemic α- and β-diastereomers **13-17**.[25] In the trimeperidine series, the (+) isomer **17** (2S, 4S, 5R) was claimed to be equipotent with morphine and nine times more potent than its enantiomer.[26] These studies provide support for the proposal that the analgesic receptor can discriminate between enantiomeric edges of the piperidine ring.

	R =	R_1 =
13	C_3H_7	H
14	C_2H_5	H
15	$CH_2-CH=CH_2$	H
16	CH_3	H
17	CH_3	CH_3

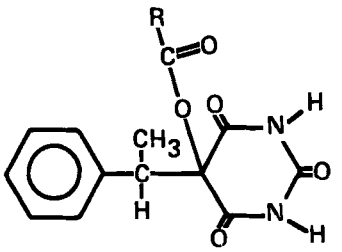




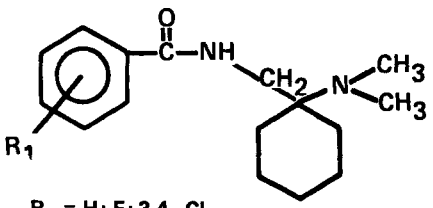
18 $R_2 = H$
19 $R_2 = CH_3$

Significant analgesic activity was reported for methylated diastereomers in the fentanyl series (**18** and **19**).[27] The most active derivative, (+)-*cis*-**18**, was claimed to be 6684 times morphine in the tail withdrawal test, with a faster onset, shorter duration of action and a higher safety margin.

Miscellaneous – A new class of 5-phenethyl barbiturates (**20**) was reported to possess potent analgesic activity with the most active derivatives exceeding codeine orally in the hot-plate test and equalling morphine subcutaneously.[28,29] Furthermore, it was reported that neither **20a** nor **20b** exhibited significant physical dependence capacity in morphine dependent monkeys. Analgesic activity in the codeine/morphine range was found in another series of compounds **21** whose structural features were distinct from morphine. These derivatives were characterized as having good oral activity and little addiction potential (Straub Index).[30] This latter claim contrasts with an earlier report that a member of this family, AH 7921 (**21**, R_1 = 3,4-Cl_2), possesses significant addiction liability.[31]



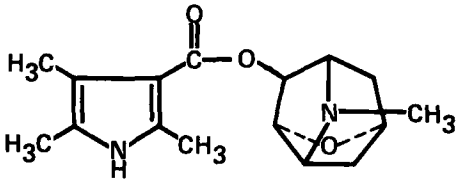
20 a, $R = C_2H_5$
20 b, $R = CH_3$
20 c, $R = c\text{-}C_3H_5$



R_1 = H; F; 3,4-Cl_2

21

The 2,4,5-trimethylpyrrole-3-carboxylic acid ester of scopoline (**22**) was found to be more active than codeine (hot plate).[32] This is surprising since scopoline itself was inactive as an analgesic and the corresponding pyrrole ester of codeine was only 1/3 as active as codeine.

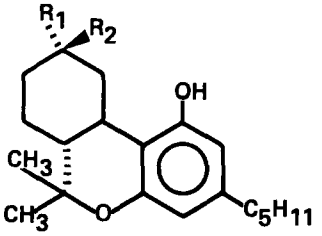


22

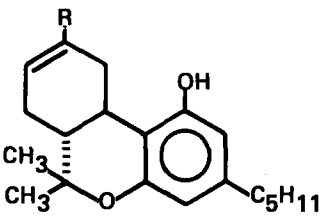
Cannabinols as Analgesics

Existing reports of analgesic activity for the cannabinols can best be described as equivocal.[33a] While the analgesic activity of Δ^9-THC (**27**) was recently demonstrated in the dog,[34] divergent reports regarding the clinical potential of cannabinols as analgesics continue to appear.[35,36] The chemistry, pharmacology, metabolism and clinical activity of cannabinols have recently been reviewed[33] and the early SAR studies of Adams and Todd have been updated and expanded.[37,38] Additional reports of one-step syntheses of Δ^9-THC from olivetol should serve to facilitate additional SAR studies of the cannabinols.[39,40]

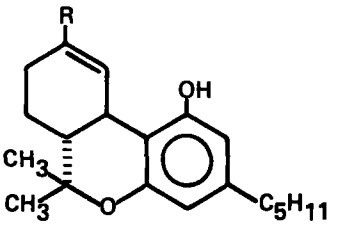
May and Wilson[41] postulated that the analgesic activity of Δ^8 and Δ^9-THC resides primarily in their 11-hydroxy metabolites (**28** and **29**). Their conclusion was based on the observation that 9-*nor* derivatives **30** and **31**,[42] which cannot be transformed into the 11-hydroxy metabolites, lack significant analgesic activity but exhibit dog ataxia and cardiovascular profiles nearly identical to Δ^8- and Δ^9-THC. This finding led to the preparation of (-)-9-*nor*-9-β-hydroxyhexahydrocannabinol (**23**) which proved to be an analgesic with activity in the mouse hot plate test nearly equal to that of morphine. Table I illustrates the regio- and stereospecificity demonstrated for this series of compounds. The importance of the phenolic hydroxyl and trans geometry for analgesic activity in cannabinols has also been reported.[43]



	R_1 =	R_2 =	ED_{50}
23	H	OH	(-) = 1.6
24	OH	H	(-) = B
25	O=C		(-) = B



	R =	ED_{50}
26	CH_3	(-) = 8.8
28	CH_2OH	(-) = 1.9
30	H	(±) = A

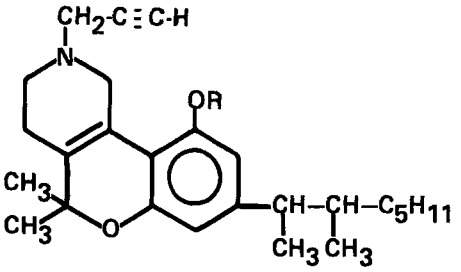


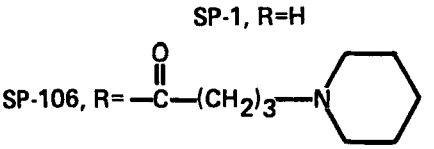
	R =	ED_{50}
27	CH_3	(-) = 9.6
29	CH_2OH	(-) = 1.9
31	H	(-) = B

Table I. Structures and analgesic activity for cannabinols. ED_{50} (s.c.) for mouse hot plate test given in mg/kg. A = inactive at 50 mg/kg. B = inactive at 20 mg/kg.

The key issue of addiction liability for the cannabinols, and in particular **23**, remains unresolved. Naloxone (1 mg/kg) completely antagonized the analgesic effect of **23** suggesting an opiate mechanism with concomitant dependence liability.[41] However, it was recently disclosed that **23**, unlike classical narcotic agonists, fails to suppress withdrawal symptoms in morphine addicted monkeys and also does not act at the opiate receptor *in vitro*.[44] These findings suggest that **23** may be a mechanistically novel analgesic.

Biological selectivity is a further issue critical to any development of cannabinols as analgesics. The variety of biologically diverse activities found for the naturally occurring cannabinols and their analogs was highlighted in a recent symposium. Described were heterocyclic modifications of the cannabinoid nucleus possessing narcotic antagonist, anticonvulsant, antidepressant, hypotensive and psychotherapeutic profiles.[45] Two cannabinoids (SP-1 and SP-106), currently undergoing clinical evaluation, are reported to exhibit both sedative-hypnotic and analgesic activity.[47] In addition, other closely related analogs are reportedly long acting narcotic antagonists.[48]





Biochemistry, Neurophysiology and Pharmacology

The isolation and characterization of the opiate receptor and the discovery of a PGE sensitive adenylate cyclase (AC) inhibited by morphine stand out as important advances which promise to provide new direction and impetus to research in this area. Similarly, the demonstration of a functional overlap between opiate and electrically stimulated analgesia provides additional new insight into nociceptive mechanisms. These discoveries may provide a new and possibly more productive reference point for studies of the specific neurotransmitter mechanisms involved in analgesia and addiction, so dominant in recent years.

The Opiate Receptor – In 1971 Goldstein proposed that opiate stereospecificity might provide the basis for opiate receptor isolation.[50] A series of elegant studies by Snyder,[51] Goldstein and others,[52] has now demonstrated the existence of an opiate receptor in the midbrain and peripheral nervous tissue of vertebrates which stereospecifically binds morphine, other potent agonists, and opiate antagonists such as naloxone. Competition for the opiate receptor by various opiates and antagonists closely parallel their respective pharmacological potency. Distribution of the opiate receptor is confined to nervous tissue, specifically the synaptic membrane[53,54], and parallels the putative sites of action of the opiates.[55] The opiate receptor is present in all vertebrates, but has not been detected in invertebrates. This makes it problematical to involve the biogenic amines mechanistically as they are present in both vertebrates and invertebrates[56] and, in fact, no recognized neurotransmitter has yet been demonstrated to compete for binding to the opiate receptor.

A number of groups are actively seeking the identity of the putative opiate receptor neurotransmitter. Recently, Terenius[57] reported the isolation of an oligopeptide (m.w. ≤1200) which competes with morphine for the receptor. It is interesting to note that a CNS active undecapeptide, substance P, has been reported to block the morphine abstinence syndrome in dependent mice.[49] The α-adrenergic blockers are the only non-opiates which have been reported[58] to compete for binding to the opiate receptor. They have

also been reported to have some intrinsic activity and to suppress narcotic abstinence symptoms (*vide infra*).

No increase in the number or binding affinity of opiate receptors was found in dependent animals[59,60] which thus rules out one mechanism which had been widely advanced to explain opiate dependence. The failure to observe an increase in receptor binding and the discovery of a PGE stimulated AC system which is inhibited by morphine (*vide infra*) is consistent with the alternate proposal that tolerance results from a homeostatic mechanism operating in opposition to a morphine induced response.[60] Supporting this proposal is a recent demonstration that the RNA precursor, orotic acid, accelerates tolerance to morphine analgesia which is consonant with earlier conclusions that RNA and protein synthesis are required for the development of tolerance.[98]

Work on the opiate receptor has been reviewed[61,62] and several procedures amenable to screening have been described.[63,64] Attempts to further characterize the opiate receptor have shown that binding is inhibited by the sulfhydryl reagents N-ethylmaleimide and p-chloromercuribenzoic acid. This suggests the presence of a thiol residue at or near the receptor.[65] Opiate binding to a partially purified proteolipid and to brain cerebrosides has been reported.[66,67] This is consistent with enzymatic experiments which suggest that the opiate receptor is a membrane-bound complex requiring the integrity of proteins and phospholipids.[68]

PGE Sensitive Adenylate Cyclase – Collier[69] has identified a prostaglandin E_1 and E_2 (PGE) sensitive AC in whole brain that is stereospecifically inhibited by narcotic analgesics. Naloxone reverses morphine's inhibitory activity in this AC system *in vivo* and yet has no effect itself except at high concentrations. Based on these experimental findings and four related lines of evidence, Collier proposed that the primary actions of the narcotic analgesics result from inhibition of this adenylate cyclase system. The specificity of this AC system is indicated by the failure of morphine to block either the non-specific rise in cAMP levels due to NaF or the activity of the dopamine sensitive AC.[70,71] This unifying hypothesis provides for homeostatic mechanisms of tolerance development, rationalizes much of what is known about the interactions of cAMP, phosphodiesterase (PDE) and PGE with pain and morphine analgesia, and implicates PGE in morphine tolerance and dependence mechanisms. Inter-relationships between cAMP, PGE and opiate analgesia are also supported by recent reports that PGE and cAMP antagonize morphine analgesia[72] and that the PDE inhibitor theophylline causes hyperalgesia and a quasi morphine-abstinence syndrome in the presence of naloxone.[73,74] Morphine inhibition of PGE stimulated AC activity has also been tentatively identified at other sites known to bear the opiate receptor, such as the guinea pig ileum.

Using neuroblastoma cell lines, two groups of workers[75,76] have confirmed Collier's basic observation and further showed that the PGE antagonist 7-oxa-11-prostynoic acid blocked the PGE sensitive AC in a fashion qualitatively similar to morphine. More important, studies with different cell lines provide evidence for a functional relationship between the AC system and the opiate receptor thereby suggesting a place for both the AC and opiate receptor systems in the understanding of opiate mechanisms.

Neurophysiology – Whereas previous mechanistic speculation has focused on activation of the reward system to explain attenuation of pain following focal electrical stimulation of CNS structures, Mayer and Liebeskind[77,78] have now described a stimulation-produced analgesia in the rat which they speculate shares a common mechanism with morphine and is not subserved by self-stimulation mechanisms. Direct mechanistic overlap between morphine and stimulation produced analgesia is suggested since both modalities were: (1) specifically reversed by naloxone; (2) antagonized by monoamine depletion; (3) subject to the development of long lasting tolerance; and (4) mutually cross tolerant.[78] Only stimulation of the mesencephalic central and periventricular gray areas of the brain significantly reduced responsiveness to all noxious stimuli. These same areas of the midbrain also have been identified as the major site of

morphine analgesic activity in the monkey.[79,80] As originally proposed by Melzack and Wall these authors concluded that stimulation-produced analgesia may result from activation of a neural substrate in the brain which, like morphine, produces an inhibitory action on the transmission of nociceptive stimuli at the spinal level. It seems likely that this substance is the same or similar to the putative neurotransmitter being sought for the opiate receptor.

The Involvement of Neurotransmitters – While new reports[81-84] do not yet allow a unified mechanistic understanding of the role of specific neurotransmitters in opiate analgesia, there have been encouraging advances. The concepts of stereoselectivity and specific antagonism have been employed to show a stereospecific inhibition by morphine of 5-HT stimulated neuronal firing[84b] and to differentiate a nonspecific effect of d- and l-pentazocine on NE from a specific effect on DA.[85] Probes into the involvement of the noradrenergic system in analgesia and dependence indicate that α-adrenergic blockers and dopamine-β-hydroxylase inhibitors[81,87,89] generally produce enhanced antinociceptive activity. Reports with β-adrenergic blocking agents are conflicting.[88] A marked strain-dependent divergence in brain 5-HT levels and morphine analgesia was reported.[84c] The involvement of specific neurotransmitters in analgesia and dependence has recently been reviewed.[86]

Tolerance and Addiction – The description of a sensitive and reproducible primary physical dependence model in the rat will hopefully provide the basis for more uniform experimental definitions of tolerance and physical dependence. Although the development of alternative models has continued,[90,91] the recently reported rat infusion model seems to offer advantages in terms of standardization and sensitivity.[92] Increased sensitivity to naloxone reversal, seen after morphine pretreatment, has been proposed as an early indicator of the initiation and development of tolerance.[93] As previously cited for the opiate receptor work, no increase in specific receptor binding affinity or saturation was found[94] in these studies.

Recent reports suggest that among others, lithium,[95] dopamine-β-hydroxylase inhibitors,[89] haloperidol,[96] and immunization with morphine antibodies[97] are all capable of reducing tolerance and dependence. Enhanced physical dependence on chronic coadministration of morphine and Δ^9-THC, and alleviation of morphine abstinence by acute Δ^9-THC have been observed. No cross tolerance was noted.[99]

The CNS stimuli produced by narcotics and other abused drugs which are discriminable by addicts have recently been shown to serve as learning cues in behavioral studies with laboratory animals. Thus application of this phenomenon appears to provide a unique method for probing subjectively experienced drug effects in laboratory animals[100] and recently has been used to show that the new antidiarrheal, loperamide, does not possess narcotic cue producing activity in rats.[101] State dependent learning and drug discrimination have been reviewed.[102]

Screening Methodology – Several studies relevant to the differentiation of potent and mild analgesics appeared.[103-105] Following earlier work, it has been shown that the hot plate procedure can be modified to detect the antinociceptive activity of previous inactives, such as narcotic antagonist analgesics (e.g. pentazocine), and mild analgesics, such as aspirin and paracetamol, by reducing the temperature to 50° from the standard 55°. However, it has been suggested that the low temperature results can be misleading and that the use of multiple temperatures is primarily useful in providing a clinically relevant measure of maximum intrinsic analgesic potency not relative potency. The work of Mayer and Liebeskind (*vide supra*) reinforces the importance of measuring antinociceptive activity in a diverse battery of procedures. There were several citations[80,106] of the electrically induced flinch-jump procedure including the suggestion that its more highly integrated response and supraspinal circuitry makes it more susceptible to interruption at a number of levels and, hence, to the detection of false positives[77] (e.g. neuroleptics). The intraarterial injection of bradykinin was cited as particularly effective in differentiating true analgesic activity for a series of benzomorphans.[19]

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Chapter 3. Pharmacological Approaches to Maintaining and Improving Waking Functions

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Introduction. Studies of pharmacological agents that affect learning and memory processes are complicated by numerous intervening variables. For example, learning is measured indirectly by changes in behavior, which in turn are dependent upon other drug sensitive variables such as reinforcement, motivation, attention, sensory/motor processes, and drug state. These complications may be minimized by post-trial administration of drugs and appropriate controls.[1,2] Learning and memory research is also hindered by the absence of standard reference agents and behavioral assays For an agent to be considered effective in enhancing learning-memory processes in a general sense, it is expected to alter negatively and positively reinforced behavior, as well as affecting responses that are active and passive in character. A simple extrapolation of experimental data from animals to humans may not be appropriate, since the basic datum in humans is often verbal rather than motor in nature. Although the phenomena of learning and memory in humans and animals are probably mediated by similar mechanisms, there are conceptual difficulties in equating drug effects on cognitive functions in humans and those on relatively simple tasks used in animals. Thus, certain CNS stimulants, such as strychnine, pentylenetetrazol, and amphetamine have been shown to enhance acquisition-retention processes in animals.[1,2] However, conclusive evidence for pharmacological enhancement of these processes in humans is still lacking. Caffeine and amphetamine are generally accepted as performance enhancers in man, particularly in cases of fatigue-induced decrements in performance.[3] Despite the initial enthusiasm for magnesium pemoline as a general performance enhancer in man,[4] a qualitative improvement over stimulants such as caffeine and amphetamine has not been established.

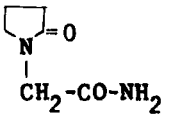
Recent Trends. Since the last review of this topic,[5] there has been an explosion of activity in the memory-learning area, as reflected by the large number of books, reviews and symposia, which are partially cited in the following categories:

a) Biochemical, physiological and neurological bases of memory.[1, 6-10]
b) Cognitive functioning and aging.[8,11-16]
c) Experimental approaches in man and animals.[1,8,11,12,17,18]
d) Pharmacological effects on memory processes.[1,3-6,10,11,15,19]
e) Mediation of memory processes by putative neurotransmitters.[1,19-23]
f) Mediation of acquisition-retention phenomena via macromolecules, and molecular theories of memory.[6-8,24-27]

The reader is also referred to a periodic literature survey bulletin.[138]

There are a few new structures with possible facilitatory effects on performance and acquisition. Piracetam 1 was reported to be active especially in anoxic environments.[28] The hydrochloride of 3-[2-(benzylmethylamino)ethyl] benzoic acid methyl ester 2 was reported to facilitate acquisition in rats and improve retention in man.[29] Another compound,G-130

3 was reported to facilitate acquisition of avoidance by rats, as well as producing a psychomotor stimulation and anorexia similar to amphetamine.[30]



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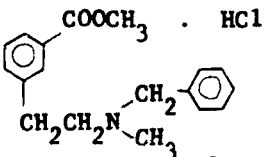

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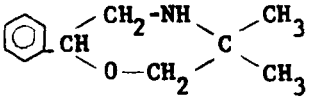

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In the last five years there has been a gradual change in the direction of research with pharmacological agents and memory and learning. That is, there is a noticeable trend toward improving the behavior of specific patient populations during their waking state. Thus, pharmacological agents are being developed for use as therapeutic adjuncts in patient populations having identifiable deviations from some "normal" performance level. It is to these new trends that this review will address itself.

Performance Deficits in the Elderly. It is generally assumed that there are decreases in cognitive functioning with advancing age.[11] However, the general observation that aged individuals retain older material better than more recent events is not completely valid.[31] Nevertheless, the behavioral deficits of old age are associated with numerous changes in the CNS, such as an increase in the ratio of glial to neuronal cells, changes in enzymatic activity, and alterations in the disposition of putative neurotransmitters.[32] In many cases, the behavioral differences between younger and older humans appear related to performance variables, rather than differences in intellectual capacity.[11,33,34] Likewise, performance deficits have been noted in studies comparing younger and older rats,[35,36] mice[37] and monkeys.[38] Such variables should be considered when investigating drug effects on learning-retention in animals of different ages.

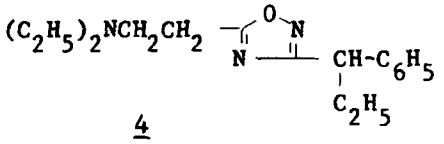
One approach to the development of pharmacological agents for use in elderly patients is based upon the possibility that cellular respiratory homeostasis is altered due to cerebrovascular insufficiency and/or inefficient oxygen utilization in neuronal tissue. It is well established that oxygen deprivation due to high altitudes results in cognitive deterioration and amnesia.[39]

Recently, it was reported that cognitively impaired elderly patients were treated successfully with oxygen therapy.[40-42] However, improvement was not noted in psychotic aged patients.[43] In another study, it was found that cognitive changes persisted beyond the increases in arterial pO_2 and that the changes were accompanied by EEG alterations and increased levels of lactate and pO_2 in the cerebrospinal fluid.[44] Normalized performance in aged animals using oxygen therapy has, to our knowledge, not been reported, but there is ample evidence that hypoxic conditions interfere with memory consolidation, retrieval processes[45,46] and performance.[47,48]

One area of possible success in the alleviation of oxygen deprivation -induced performance deficiencies is the development of pharmacological

agents that protect against anoxic conditions. Of recent interest in this regard is 1, a nonstimulant, nonadrenergic agent that enhances avoidance acquisition in normal rats, possibly by improving registration processes.[49] 1 has been reported to protect rats against amnesia produced by hypoxia.[28,50] However, the effects on memory storage or consolidation remain unclear since it is ineffective in protecting against electroconvulsive shock-induced amnesia[51] and in facilitating acquisition of avoidance when given post-trial.[52] 1 has also been reported to reduce lethalities and associated EEG changes induced by barbiturate intoxication or anoxia in rabbits.[53] Similar protective effects have been noted following interruption of circulation to rabbit fetuses.[54] In initial clinical trials, 1 increased alertness, counteracted fatigue and improved verbal interactions in aged patients.[55,56] 1 was also reported to attenuate decreased vigilance associated with slow heart rates controlled by artificial pacemakers.[57] However, another group of investigators found only minimal improvement with 1 in hospitalized geriatric patients.[58] Other types of pharmacological agents to counteract effects of oxygen deficiency need to be developed. Of possible therapeutic interest are agents that mimic or induce 2,3-diphosphoglycerate, which is a constituent in red blood cells facilitating the dissociation of oxygen and hemoglobin.[59,60]

Treatment of performance decrements due to cerebrovascular or cerebrometabolic insufficiency with cardiovascular agents has shown promise. Hydergine^{R}, a combination of hydrogenated ergot alkaloids, reportedly improves cognitive function and social interaction of geriatric patients with cerebrovascular deficits.[61-65] However, the effects of long-term therapy with Hydergine^{R} are not yet established and experimental designs could be improved. Although Hydergine^{R} exhibits peripheral vasodilatory properties, the mechanism by which it improves post-ischaemic metabolism is not yet clear.[66] In addition, proxazole 4 and cyclandelate 5 reportedly improve the cognitive state of patients with cerebrovascular deficits.[67,68] A related observation is that elderly hypertensive patients exhibit a deterioration in cognitive and intellectual functions.[69] Thus,



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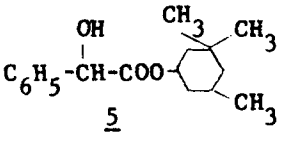

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one line of unexplored research lies in the possible use of vasodilators and hypotensives in animals exhibiting cardiovascularly mediated performance deficits.

Pharmacologic intervention in the aged with conventional agents (analeptics, sedative-hypnotics, antipsychotics, etc.) has not been promising. Stimulants such as amphetamine, methylphenidate and caffeine are contraindicated in the elderly because of the side effects of these agents.[11] Although substantial improvement in elderly patients has been reported following the use of magnesium pemoline,[70] only minimal effectiveness has been observed by others.[71] In a recent six week, double blind study of aged patients, magnesium pemoline and methylphenidate did not enhance

memory or intellectual functioning.[72]

Hyperkinesis and Minimal Brain Dysfunction (MBD). MBD is a generic name for a cluster of behavioral traits characterized by overactivity, distractability, impulsivity and learning deficits in children.[73] In general, amphetamine and methylphenidate are reported effective in increasing performance measures and cognitive functioning in some patients diagnosed as hyperkinetic.[74-76] In addition, d-amphetamine appears to be more effective than l-amphetamine in calming overanxious, hyperkinetic subjects, while both isomers are equally effective in treating the symptoms of aggression and hostility.[77] There is also recent evidence that magnesium pemoline[78] and caffeine[79] may be useful in treating hyperkinetic children. Drugs of other classes have been tried with less clinical efficacy than the stimulants.[80] Finally, there are unanswered questions concerning the long-term effects of chronic drug administration, the extension of therapeutic effects into the postdrug phase, and the possible development of behavioral tolerance and dependence.[74,81,82]

There is increased interest in the development of animal models for testing agents to be used in hyperactive subjects with poor learning abilities. For example, the prenatal or postnatal chronic administration of lead to rats,[83-85] mice[86] and lambs[87] produced learning deficits associated in some cases, with hyperactivity. Similar deficits in rodents have been reported following exposure to methylmercury.[88] In addition, the ability to learn in adulthood has been shown to be dependent upon nutritional factors[89,90] and the presence of certain inorganic ions such as zinc[91] during critical stages of development. Learning ability during adulthood is also dependent upon the environmental conditions during rearing. Thus, isolation[92] or overcrowding[93] could be used to produce learning-deficient subjects for pharmacological studies. Excessive intake of lysine or phenylalanine produces reversible retention deficits in mice[94] and impairs shuttle box avoidance in rhesus monkeys,[95] respectively. Learning deficits and activity changes have been noted in rats surviving encephalic viral infection.[96] Hyperkinetic, untrainable dogs are reportedly responsive to treatment with stimulants like hyperkinetic children.[97] However, genetically "nervous" dogs showed heightened avoidance responding and were more susceptible to behavioral disruption from amphetamine than control dogs.[98]

One final approach to the development of animal models for the psychopharmacologic study of learning-retention deficits lies in the utilization of individual or strain differences. For example, differences in accuracy of responding have been noted in "bright" and "dull" apes.[99] It has also been reported that there are "poor" and "good" performers within certain strains of rats.[100] In addition, there are differences in the learning ability and pharmacologic responsivity between strains of rodents.[101,102]

Relationship Between Sleep-Wake Cycles and Learning-Memory. It has been shown that there is a decrease in performance in man following sleep deprivation, inversion of sleep-awake cycles, or change in relative proportions of sleep-awake periods.[103] Furthermore, there are indications that the paradoxical sleep (PS) cycle may be associated with memory or consolida-

tion processes. An increase in PS has been reported in animals following learning situations and PS deprivation prior to training tends to impair long-term retention of acquired behavior.[104,105] Deprivation of PS following training tends to disrupt short-term retention and consolidation.[106] These data suggest that performance deficits in animals may be induced by alterations in the sleep-awake cycle, and these deficits may be reversible by pharmacological means.

Electrophysiological Correlates of Learning. Electrical stimulation of reticular mesencephalic and hippocampal sites reportedly facilitates acquisition of behavior in rodents.[107] It was also reported that strychnine or pentylenetetrazol applied locally at these same brain sites also facilitate learning. The hippocampus is also believed to be involved in various aspects of memory processing.[108,109] The presence of regular, low frequency (6-8 Hz) and high voltage theta EEG patterns in the hippocampus appears to be associated with consolidation processes.[110] In addition, compounds that tend to disrupt memory consolidation suppress, whereas purported facilitators of learning enhance theta wave activity. Thus, replication and extention of these EEG observations may eventually lead to a tool in the prediction of a drug's effect on learning and memory.

Role of Brain Peptides. Short chain polypeptides originating from the intermediate and posterior hypophysis have been reported to have CNS activity. Melanocyte stimulating hormone (MSH) and various fractions of the adrenocorticotrophic hormone (ACTH) facilitate several types of conditioned behaviors in animals[111,112] and enhance short-term visual memory in humans.[113] Recent evidence in normal adults suggests that the heptapeptide fraction $ACTH_{4-10}$, which is also found in MSH, is the CNS active component.[114] It would be informative to establish whether or not $ACTH_{4-10}$ would be effective in selected patients such as aged individuals or in cases with endocrine imbalance. Scotophobin, a pentadecapeptide occurring naturally in the brain and prepared synthetically, reportedly augments only dark-light avoidance in the rat.[115] Synthetic scotophobin-like peptides, e.g., desacetylscotophobin, were reported to affect other types of aversively controlled behaviors.[116] These results must be evaluated critically in view of recent comments concerning the possible activity of the scotophobin-like polypeptides.[117]

Role of Neurotransmitters in Learning-Memory. The agents known to affect learning-memory processes have varying effects on the neurochemistry of the brain. Thus, there are correlative, but not necessarily causative changes in the function of putative neurotransmitters that may aid in the development of compounds facilitating learning-memory processes.

The cholinergic system has long been suspected as being involved in memory consolidation.[22,23] However, the cholinergic system also appears to mediate selective inhibition of responding,[23] and this effect on performance output obfuscates the interpretation of data on agents that interact with the cholinergic system. In spite of the mass of contradictory data, there appears to be a time-dependent change in the sensitivity of cholinergic functioning after learning which is responsive to pharmacological intervention.[21] Recent reports have indicated differences in acetylcholine (ACh) metabolism in the temporal lobes of good and poor perfor-

ming mice.[118] Newly synthesized ACh in the hippocampus is also implicated in the acquisition of passive avoidance.[119] It is worthy of note that acquisition of conditioned behavior can be depressed by peripheral administration of both physostigmine and neostigmine.[120] Since only physostigmine is active centrally these data suggest that peripheral actions of the anticholinesterases also play a role.

5-Hydroxytryptamine (5-HT) is believed to be a neurotransmitter having inhibitory effects on memory processes.[20] Conditions such as hypothermia, hypoxia, and electroconvulsive shock (ECS) increase brain 5-HT levels and also interfere with subsequent retention of previously acquired tasks.[19] Recent evidence suggests that the retrograde amnesic effect of 5-HT may be related to its inhibitory effect on protein synthesis.[121] In addition, lowering of brain 5-HT by midbrain raphe lesions[122], 5,6-dihydroxytryptamine[123] or p-chlorophenylalanine[124,125] improves avoidance responding. An improvement in delayed discrimination responding by monkeys and reversal learning by rats has been reported following administration of very low doses of LSD and other psychotomimetics.[126,127] However, LSD has also been reported to disrupt delayed responding in monkeys,[128] casting some doubt on whether the behavioral effects of LSD can be attributed to a singular effect on 5-HT receptors.

Unlike 5-HT, the catecholamines (CA), norepinephrine (NE) and dopamine (DA), appear to be involved as facilitators of acquisition and memory. Decreasing releasable CA impairs acquisition of avoidance and memory formation,[129-131] although the exact role that NE and DA play in mediating these processes is not clear.[132] Increasing the availability of CA at the synapse, as with amphetamine, appears to facilitate acquisition of active tasks, although the facilitation is most likely due to alterations in active responding.[133] A recent paper has indicated that administration of L-DOPA to depressed patients improved long, but not short term memory.[134]

In humans, aging was correlated with increases in monoamine oxidase activity and progressive decrements in hindbrain NE, while 5-HT was not affected.[135] Changes in brain CA metabolism as a function of age is dependent upon numerous factors, but it is interesting to note that acute cerebral infarction in humans is associated with an increased accumulation of extracellular NE and 5-HT in CSF.[136] In addition, long-term exposure to hypoxia has been reported to decrease the biosynthesis of brain monoamines.[137] Thus, the performance decrements observed in the elderly may be due, in part, to a decline in brain monoamine function. The possibility that these decrements in neurotransmitter function may be associated with local hypoxic conditions in neuronal tissue may provide a basis in the development of new agents for geriatric and related patient populations.

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Chapter 4. Sedatives, Hypnotics, Anticonvulsants, General Anesthetics

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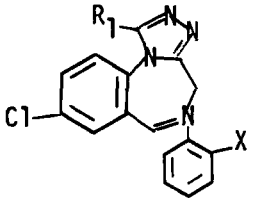
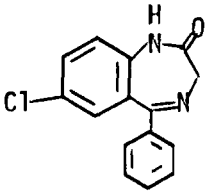
Introduction - The highlights of a literature survey of pertinent research reported during 1973-4 are discussed below. Some of the literature cited, such as that describing benzodiazepine derivatives, may also be in other chapters, since these compounds have many overlapping pharmacological effects. The papers cited were chosen primarily to indicate the direction of current activity in the area of central nervous system depressants.

Sedatives and Hypnotics - The usefulness of benzodiazepine derivatives as hypnotic agents was reviewed by Oswald et al[1] and Greenblatt and Shader[2]. Triazolam (I), a triazolobenzodiazepine, was shown to be an effective hypnotic agent in several human studies[3,4,5]. A closely related compound, D40TA (II), showed similar activity in laboratory animals[6]. The sleep-promoting effects of N-desmethyl diazepam (III), a metabolite of diazepam, were studied by Tansella et al[7] in human subjects. The compound compared favorably with amobarbital as a hypnotic.



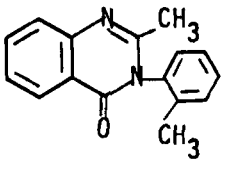
I $R_1 = CH_3$, X = Cl

II R_1 = X = H

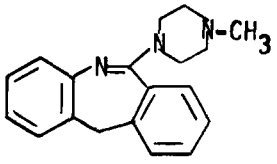


III

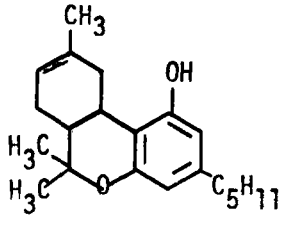
The pharmacology of methaqualone (IV) and closely related structures was reviewed by Brown and Goenechea[8]. Perlapine (V), a tricyclic compound devoid of antipsychotic action, gave a favorable profile as a sedative-hypnotic after daily administration to laboratory animals and man for 3-4 weeks[9,10]. Daily administration of marihuana or Δ^9-tetrahydrocannabinol (VI) was found to increase slow-wave sleep in human subjects, but tolerance appeared to develop after 4-8 days[11,12]. Hartmann and James[13] found significant interactions between sleep cycles and several standard psychotropic agents (chlorpromazine, reserpine, chloral hydrate,



IV



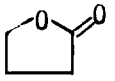
V



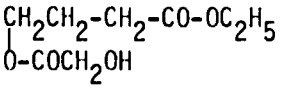
VI

amitriptyline) after long-term administration to human subjects.

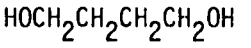
Lien et al[14] studied the central nervous system activity of five lactones in mice. A dose-dependent sedation and hypnosis was produced by γ-butyrolactone (VII), but no loss of righting reflex was seen after administration of α-methyl-γ-butyrolactone, γ-valerolactone, δ-valerolactone, or γ-heptalactone. Laborit et al[15] found hypnotic and muscle relaxant activity after administration of ethyl γ-hydroxybutyrate glycolate (VIII) to mice. Administration of 1,4-butanediol (IX) to rats produced a dose-related sedation and loss of righting reflex[16].



VII

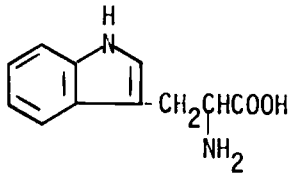


VIII

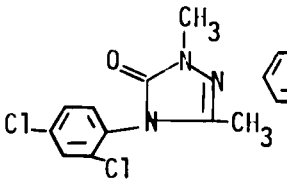


IX

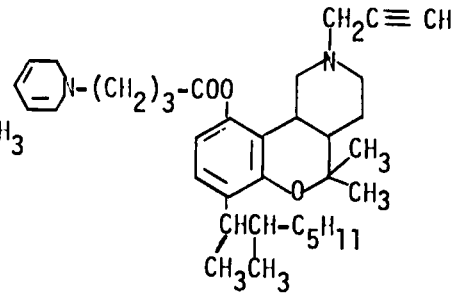
Bed-time administration of L-tryptophan (X) significantly reduced sleep latency in human subjects[17]. Two novel structures showing sedative-hypnotic activity in laboratory animals were AHR 3084 (XI) and SP106 (XII)[18,19].



X

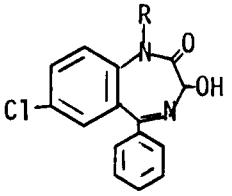


XI

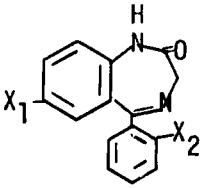


XII

Anticonvulsants - De Angelis et al[20] studied the effects of demethylation and hydroxylation on the anti-Metrazol and anti-strychnine effects of benzodiazepines. Demethylation of diazepam to produce N-demethyldiazepam (III) increased anticonvulsant activity. Methylating and/or hydroxylating diazepam to produce methyloxazepam (XIII) or oxazepam (XIV) reduced anticonvulsant activity. Additional studies[21] on the effect of chlorine substitution indicated that chlordemethyldiazepam (XV) was more active than N-demethyldiazepam. Dechlordemethyldiazepam (XVI) was inactive as an anticonvulsant but still showed sedative and muscle relaxant activity.

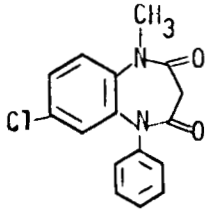


XIII, R=CH_3
XIV, R=H

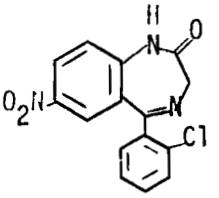


XV, X_1=X_2=Cl
XVI, X_1=X_2=H

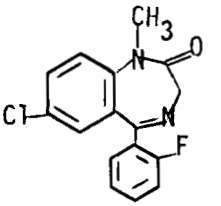
The animal pharmacology of clobazam (XVII) was reviewed by Barzaghi et al[22]. This compound was twice as active against metrazol convulsions as chlordiazepoxide and had a similar behavioral profile. The animal pharmacology of clonazepam (XVIII) was reviewed by Blum et al[23]. Another benzodiazepine, ID540 (XIX), was 4-5 times more potent than diazepam as an anticonvulsant agent.[24]



XVII

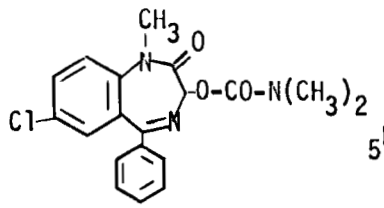


XVIII

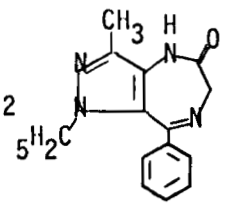


XIX

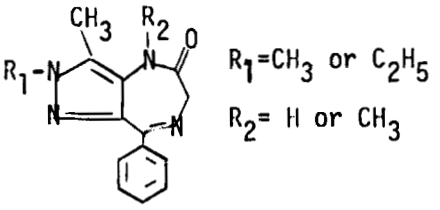
Camazepam (XX) was approximately 1/3 as potent as diazepam against Metrazol convulsions but was a much less active antagonist of either electroconvulsive shock (1/12) or strychnine (1/60)[25]. Behavioral effects of the compound were qualitatively similar to those of diazepam. The animal pharmacology of pyrazapon (XXI) was reviewed by Poschel et al[26]. DeWald et al[27] found that 2,3-dialkyl analogs of pyrazapon also showed good anti-electroconvulsive shock activity (XXII).



XX



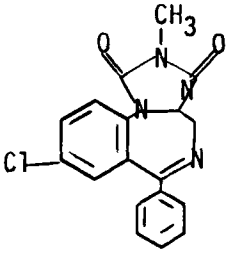
XXI



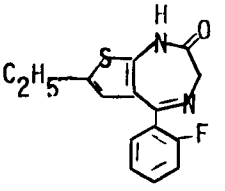
XXII

Synthesis of novel compounds containing a benzodiazepine nucleus continued to be a fertile field for medicinal chemists. Moffett and Rudzik[28] studied a series of benzodiazepines with ureas in the 2 position. The most active compound (XXIII) had anticonvulsant and depressant activity in the diazepam range. The most active member of a series of thieno[2,3-e][1,4] benzodiazepines (XXIV) was active as a Metrazol antagonist at a dose of 0.3 mg/kg, i.p.[29] Steinman et al[30] studied a series of fluoroalkylbenzodiazepines with good anticonvulsant activity. (XXV)

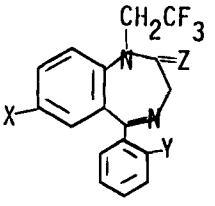
Hester and Rudzik[31] studied a series of 2-oxyaminobenzodiazepines. The most active compound (XXVI) was as potent as diazepam. Ning et al[32] studied a series of 7-azidobenzodiazepines (XXVII). These compounds had good anticonvulsant and taming properties.



XXIII

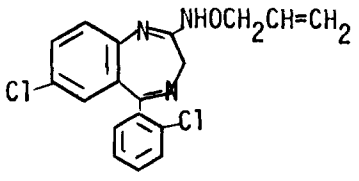


XXIV

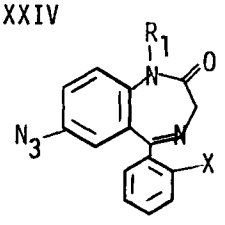


XXV

X = Br, Cl
Y = F, Cl
Z = H, O, S



XXVI



XXVII

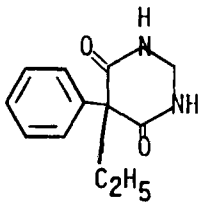
R_1 = H, CH_3
X = H, F

Baumel et al[33] followed plasma and brain levels of primidone (XXVIII) and its metabolites. The time course of protection against metrazol was related to the appearance of the metabolites phenobarbital and phenylethylmalonamide. The time course of protection against electroshock was correlated with levels of primidone.

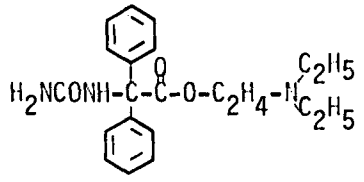
The anticonvulsant activity of cannabis alkaloids was studied by Karler et al[34]. The most potent alkaloid was Δ 9-tetrahydrocannabinol (VI), b tolerance developed rapidly after 3-4 days of drug administration.

A possible solution to the formulation and therapeutic problems posed by the marked water-insolubility of diphenylhydantoin (DPH) was suggested by the development of a pro-drug (XXIX). This compound is reported to have a water solubility 9-15000 times greater than DPH and a bioavailability, in terms of DPH equivalents, approaching 100%[35].

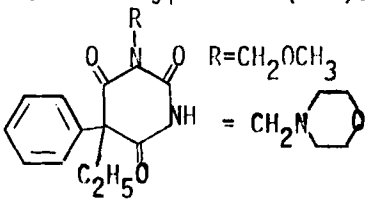
Vida et al[36] reported on derivatives of phenobarbital that had good anticonvulsant activity but were weak or inactive as hypnotics (XXX).



XXVIII

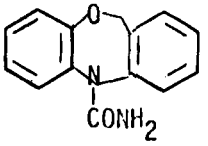


XXIX

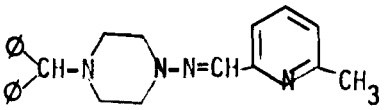


XXX

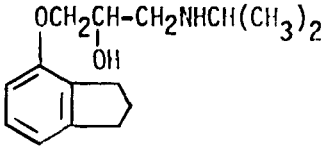
Compound SQ 10996, a dibenzoxazepine (XXXI) had anti-electroshock activity similar to carbamazepine and approximately 1/2 that of diphenylhydantoin[37]. No activity was found against metrazol convulsions. Compound SC 13504 (XXXII) was active in several animal test systems as an anticonvulsant[38,39]. Compound USVC 6524 (XXXIII) showed good activity against electro-convulsive shock in mice[40].



XXXI

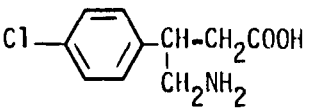


XXXII

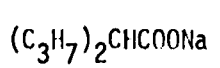


XXXIII

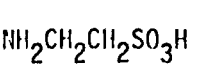
Baclofen (XXXIV), a muscle relaxant compound, protected laboratory animals against Metrazol and thiosemicarbazide convulsions but not those induced by electroshock, strychnine, or picrotoxin[41]. Simler et al[42] reported that the anticonvulsant effect of sodium dipropylacetate (XXXV) appeared to be mediated through an increase in brain GABA levels. The anticonvulsant effect of taurine (XXXVI) in laboratory animals was reviewed by Mutani et al[43]. Taurine was found to be active in human subjects after intravenous infusion of 150-200 mg/kg in patients refractory to standard compounds[44].



XXXIV

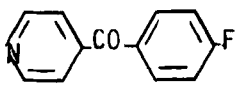


XXXV

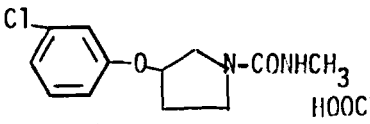


XXXVI

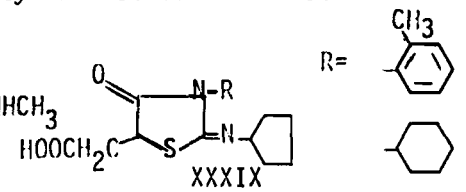
Breen et al[45] studied the anti-electroshock activity of a series of substituted benzoylpyridines. Electron-releasing substituents were found to enhance anticonvulsant activity, the most active being compound XXXVII. Boswell et al[46] studied the anticonvulsant activity of a series of 3-phenoxypyrrolidines. The most active was compound XXXVIII, which had muscle relaxant and anticonvulsant activity similar to that of mephenesin. Nagar et al[47] found anti-metrazol activity in a series of substituted thiazolidones (XXXIX).



XXXVII

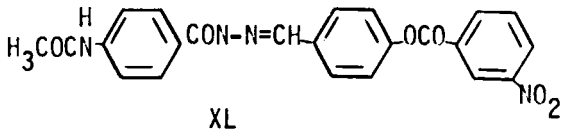


XXXVIII

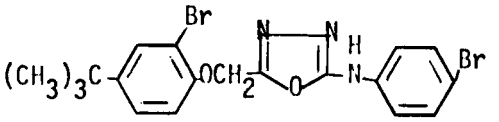


XXXIX

Parmer et al[48] studied a series of substituted benzylidinohydrazines. The most active compound (XL) showed good anti-pentylenetetrazol activity. In another study by Parmar[49] on a series of substituted oxadiazoles, the most active compound against pentylenetetrazol was XLI.

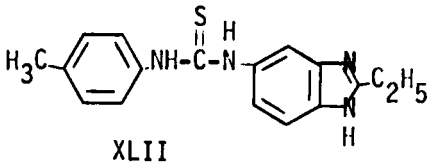


XL

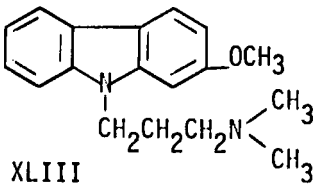


XLI

Gupta et al[50] studied the anticonvulsant properties of a series of benzimidazoles. Compound XLII was the most potent against electroshock and pentylenetetrazol convulsions. Shoeb et al[51] found anti-electroshock activity in a series of N-alkylaminocarbazoles. Compound XLIII was the most potent member of the series.



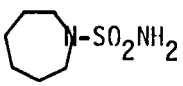
XLII



XLIII

Lien et al[14] surveyed several classes of anticonvulsant compounds and compared three physicochemical parameters: lipophilicity, dipole moment, and steric orientation. Anticonvulsant activity was correlated with a low resultant dipole moment on a polar moieties, while stimulant or analeptic activity was correlated with a high dipole moment.

The use of a new convulsant agent for anticonvulsant testing was proposed[52]. Compound SaH-41-178 (XLIV) was reported to differentiate between trimethadione and diphenylhydantoin. Fowler and Julien[53] proposed a test for antipetit mal drugs which consisted of applying conjugated estrogens to the cerebral cortex of cats. The ability of test compounds to antagonize the estrogen-induced spike potentials were examined. The method was capable of evaluating the efficacy of six standard anti-petit mal drugs. A similar technique was described by Wilkinson and Halpern[54].

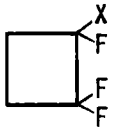


XLIV

General anesthetics - Dittmann and Etschenberg[55] compared the anesthetic activity of several halogenated methane derivatives in guinea pigs. These effects were enhanced with increasing chlorine content and were decreased with increasing fluorine content. Kendig et al[56] found that d- and l-halothane did not differ in their ability to depress synaptic transmission in the isolated cervical sympathetic ganglion of rats. These results were interpreted as supporting the theory that anesthesia by inhalation agents is a physical phenomenon in which the stereochemical configuration of the anesthetic molecule plays only a minor role.

Waddell and Baggett[57] compared the stereoisomers of pentobarbital and found that (-) pentobarbital was a more potent intravenous anesthetic than (+) pentobarbital in mice. In addition, (-) pentobarbital was a much smoother anesthetic, the anesthesia produced by (+) pentobarbital frequently being preceded by hyperirritability and spasticity. Similar results were obtained for other barbiturates[58].

The anesthetic effects of 42M9 (XLV) were studied in beagles[59]. The compound was administered in a concentration of 0.7-1.2% in oxygen or an oxygen-NO_2 mixture. Emergence and recovery were uneventful. A similar compound, 22M13 (XLVI), produced anesthesia in mice at a concentration of 1%[60].



XLV, X = Br

XLVI, X = Cl

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Chapter 5. Biological Factors in the Major Psychoses

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Introduction - The direct data available to support the hypotheses implicating biological factors in the etiology of the major psychiatric disorders is weak in comparison to the systematic data available regarding drug effects in animals and man. Although indirect data such as from studies correlating behavioral and biochemical effects of drugs in animals and man indicate that changes in brain chemistry can influence mood, emotional expression, cognition and motor activity, direct evidence reviewed elsewhere[1-5] has not yet revealed regular changes in the biochemical systems which could be identified as mediating these drug effects in individuals with depression, mania or schizophrenia. Nonetheless, developments in genetic investigations in psychiatry have succeeded, at least in part, in separating psychosocial factors communicated from one family to another from biologically transmitted factors;[6] these studies continue to underlie the need for further searching into the biological antecedents of the psychiatric disorders.

In terms of substantive areas of investigation, we have deliberately limited ourselves to the question of the possible relationships between the neurotransmitter amines and abnormal mental functioning, particularly because of the large number of interactions between these transmitters and the drugs which have specific effects on the various clinical manifestations of affective illness and schizophrenia.

This review will be limited to pharmacological studies. Space does not permit a discussion of the growing body of data on amine metabolites in cerebrospinal fluid and urine. A recent comprehensive review of this field[7] emphasizes that the available amine metabolite data in affective illness and schizophrenia is generally congruent with theories involving some disturbance in central amine systems in these mental illnesses, with the major issue being that of specificity.

The Affective Disorders: The Syndrome of Depression - Before discussing pharmacological studies in affective disorders, it is important to draw a distinction between depression as a symptom and depression as a syndrome. Depressed feelings may simply represent a normal response to the stresses or disappointments of everyday life. However, for some individuals the symptom of depression is only a part of the clinical syndrome of depression. Of particular relevance to a discussion of biological factors is the syndrome of "endogenous" depression. This term is applied to individuals with depressive symptoms of sufficient severity and duration to have seriously interfered with their functioning, often requiring hospitalization. In addition to a depressed mood these patients show psychomotor retardation (or agitation), sleep disturbance, particularly early morning awakening, loss of appetite with weight loss, loss of interest, excessive tiredness, and thought patterns dominated by feelings of helplessness, hopelessness,

and guilt. There is frequently a past history of depressive (and/or manic) episodes, and often a family history of mood disorders. Somatic therapies (antidepressant drugs or electroconvulsive therapy) are usually required for adequate treatment.

The Syndrome of Mania: The typical manic episode may start with a sudden "switch" from a depressive phase or it may develop gradually out of a depression or a normal phase. The initial "hypomanic" phase of the episode is characterized by increased psychomotor activity, including increased initiation and rate of speech and increased physical activity. At this point the accompanying mood is usually labile with a predominance of euphoria, although irritability may become apparent when the individuals many demands are not met. The cognitive state during the "hypomanic" phase is characterized by expansiveness, grandiosity, and over-confidence. Thoughts are coherent though often tangential. As the manic episode progresses the pressure of speech and psychomotor activity increase still further while the mood state becomes more of a mixture of euphoria and dysphoria. The irritability observed initially progresses to open hostility and anger, and the accompanying behavior is frequently explosive and assaultive. Racing thoughts progress to definite flight of ideas with increasing disorganization of the cognitive state. Preoccupations present earlier became more intense with grandiose and paranoid trends now apparent as frank delusions. In some patients the manic episode can progress to an undifferentiated psychotic state experienced by the patient as clearly dysphoric and accompanied by frenzied psychomotor activity. Thought processes which earlier had been only difficult to follow, now become incoherent and definite loosening of associations is often seen. Delusions are often bizarre and idiosyncratic, and some patients in the phase even experience ideas of reference and disorientation. At least superficially this phase of the syndrome is difficult to distinguish from an acute schizophrenic psychosis.

Clinical and Biological Heterogeneity -Recent evidence from clinical, genetic, pharmacologic, and biological studies suggest that major depressions can be meaningfully subdivided into bipolar and unipolar groups on the basis of the presence or absence of a prior history of mania. Unipolar patients have a later age of onset and their depressions have mixed features of agitation and retardation accompanied by a significantly higher frequency of symptoms of anger, anxiety and physical complaints. Family history data indicates a significantly higher frequency of mania in the first degree relatives of bipolar patients compared to unipolar patients. In a series of biological studies in hospitalized depressed patients we have noted a number of significant differences between unipolar and bipolar groups. We have also observed some unipolar-bipolar differentiation in the antidepressant response to tricyclics and to lithium: compared to patients with unipolar depression, bipolar patients tend to respond less well to imipramine; the reverse appears to obtain with the antidepressant effects of lithium - that is, a higher frequency of antidepressant responses in the bipolar group compared to the unipolar group. The above unipolar-bipolar differences have recently been reviewed.[8]

Brain Amines in Affective Illness: Pharmacological Evidence - The major hypotheses and data concerning the biochemistry of affective illness have focused on the possibility that major depression is related to a functional deficit in central norepinephrine or serotonin systems, while mania is related to a functional excess of NE or 5HT. These hypotheses were based largely on the observations that drugs which decreased functional amines (particularly NE) in the synaptic cleft in animals (reserpine, lithium, alpha-methyl-para-tyrosine) either had some depressant potential or had antimanic properties when given to patients, while coversely drugs which could increase amines at the synapse (MAO inhibitors, tricyclics, amphetamines) were stimulants, had antidepressant properties or could precipitate mania in susceptible individuals.

Reexamination of the pharmacological data reveals some observations which do not easily "fit" those theories which imply a simple one-to-one relationship between a given mood state and a unidirectional disturbance in the functional state of one or more central amine neurotransmitters.[9] First, drugs which are stimulants in normal individuals are not generally found to be therapeutic in patients suffering from major depressive illness, while those drugs which do have antidepressant activity are not stimulants in normals. This data on differential response to drugs support the concept that depression may not simply represent a quantitative extension of a normal mood state, but may reflect a qualitatively different psychobiological substrate. Second, the weight of the available clinical evidence suggests that the MAO inhibitors are not very effective antidepressants in patients with more severe, "primary" or "endogenous" depressions[10] - that is, the group that has been the major focus of studies concerned with amine dysfunction. Rather these drugs are most efficacious in outpatients with mixed anxiety-depression syndromes or in "depressives" with atypical features such as anxiety, fatigue, phobia, or somatic complaints.[11] Thus, their spectrum of usefulness does not coincide with the tricyclics which are more likely to benefit depressed patients with classical "endogenous" symptoms.

In relation to the clinical effects of drugs which decrease functional amines in brain, a recent critical review of the original reports of reserpine-induced depressions[12] noted that the incidence of patients who experienced major depressive symptoms (analogous to "endogenous" depressions) across all studies (approximately 6%) was almost identical to the percent incidence of individuals with prior histories of depression. Thus, it appears more likely that reserpine is capable of precipitating depression in susceptible individuals rather than inducing it de novo; this is an important distinction, since depressions can be precipitated by a variety of agents or conditions not directly related to amine function. Lithium, another drug which can decrease amines at the synapse[13,14] should increase depressive symptoms in patients according to the amine hypothesis. However, to the contrary, lithium has been shown in controlled studies to have moderate antidepressant properties in some of the depressed patients[15,16] and to effectively prevent recurrences of depression when used prophylactically.[17,18] Thus, drug-catecholamine relationships in depressed

patients contain a number of findings which are discrepant in relation to the catecholamine hypothesis of depression. In the case of manic or hypomanic reactions, the behavior-amine relationships appears more consistent. Thus, the drugs which increase functional amines can precipitate manic or hypomanic reactions in susceptible individuals, whereas the drugs which decrease functional brain amines have some beneficial effect in mania.

In addition to the above inconsistencies in the clinical data, the mood-altering drugs discussed above not only tend to affect both catecholamines and indoleamines in a similar way, but also have a variety of effects on other neurochemical systems. Because of this relative non-specificity of these drugs, a good deal of recent work has focused on ways to alter specific amine systems in order to study behavioral effects in patients with affective illness.

Studies With Experimental Compounds Affecting Brain Catecholamines - L-DOPA, the amino acid precursor of the catecholamines, when administered in large oral doses can be taken up by brain and there converted to DA and NE. In earlier studies of L-DOPA in depression, the doses employed were small, and thus, the generally negative results were difficult to interpret. A more recent trial[19] in 26 hospitalized depressed patients employed high oral doses (averaging 100 mg/kg) administered over periods ranging from 18 to 45 days with and without a peripheral decarboxylase inhibitor. Eighty percent of the patients failed to show any improvement in depression on L-DOPA; the few patients who appeared to respond all had prominent psychomotor retardation. In the non-responders there was nevertheless consistent evidence of some activation with increases in anger and psychosis ratings in some patients and hypomanic episodes in those patients with a prior history of mania (bipolar). These predominantly negative results with L-DOPA in depression suggest that catecholamine (particularly DA) depletion as defined by the reserpine model (which is reversible by L-DOPA) is not a sufficient explanation for the pathophysiology of the majority of depressions.

Alpha-methyl-para-tyrosine (AMPT) is a potent and specific inhibitor of tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of DA and NE.[20] We administered AMPT in doses up to 4 gm to eight manic patients and noted improvements in five of them. Two of the five responders showed a consistent pattern of relapse following placebo substitution. The levels of catecholamine metabolites in the cerebrospinal fluid were markedly reduced in the patients on AMPT, verifying that brain amines had in fact been reduced. These findings suggest that central catecholamine function may be elevated in at least some manic patients. Recently, fusaric acid, a potent inhibitor of dopamine-β-hydroxylase (which converts DA to NE) in noradrenergic nerve endings), has been tried in mania without beneficial effect, but with a qualitative shift to more psychotic symptoms.[21]

Studies With Experimental Compounds Effecting Brain Serotonin - In an attempt to reverse the hypothesized deficit in brain serotonin, L-tryptophan (the amino acid precursor of serotonin) has been administered to de-

pressed patients with varying results. In one single-blind study,[22] it was reported that L-tryptophan plus an inhibitor of monoamine oxidase was as effective as electro-convulsive shock, while L-tryptophan alone (5-7 gm/day) was somewhat less effective. A recent double-blind study found L-tryptophan alone as effective as imipramine in the treatment of depression.[23] On the other hand, others have been unable to find any significant antidepressant activity for this amino acid.[24,25] Recent evidence that some patients with bipolar depression show improvement on L-tryptophan[26] may provide a partial explanation for the disparity in earlier reports of its efficacy in depression. The immediate precursor of serotonin, 5-hydroxytryptophan, has also been tried in depressed patients; two double-blind studies failed to show any significant antidepressant effects,[27,28] although it has been suggested that a subgroup of depressed patients identifiable by lower serotonin metabolite levels in the CSF may respond to this serotonin precursor.[29] Methysergide, a drug which antagonizes serotonergic function (presumably by blocking receptors) has been examined as an antimanic agent by several groups, including one controlled comparison of methysergide and lithium. Two early trials of methysergide in mania demonstrated antimanic properties of the drug, particularly when given intramuscularly. These results were not able to be duplicated in clinical trials using oral methysergide (reviewed in [30]). On the basis of spinal fluid metabolite data, it has been suggested that decreased central serotonergic function may occur in both mania and depression.[31] Of relevance to this is a recent trial of L-tryptophan in mania, in which some advantage of L-tryptophan over chlorpromazine was reported.[32] A partial antimanic effect was similarly observed in ten patients studied at the NIMH.[33]

Conclusions - In relation to catecholamines, a summary of the data from the mood-altering drugs and from the amine precursors should focus on the fact that drugs which can increase functional catecholamines in brain (MAO inhibitors, tricyclic antidepressants and L-DOPA) can all precipitate mania in susceptible individuals, while drugs which decrease functional catecholamines in brain (reserpine, lithium, AMPT) all have some antimanic properties. However, in relation to the effects of these drugs in depression the picture is more confused. L-DOPA is not an antidepressant in the bipolar patients, with its minimal antidepressant effects confined to a few of the unipolar group. A finding of major importance has been that when L-DOPA is given to bipolar depressed patients, almost all of them experience mania or hypomania without a concomitant decrease in depression. From the data it would appear that the level of activation (particularly psychomotor activation) might correlate more closely with central catecholamine function than the complex mood state itself. Some relationship between the onset of manic symptoms and increased central catecholamine function is supported by the data.

In relation to serotonin, the pharmacologic data do not support the notion that central serotonergic function is increased in mania. In regard to the hypothesized involvement of serotonin in depression, the tryptophan data is controversial, although it appears that there may be a subgroup of depressed patients responsive to a serotonin precursor. The evidence that tryptophan may have some antimanic properties as well is interesting, and raises the possibility that a dysfunction in serotonergic

systems may be involved in the underlying pathology common to both mania and depression. Alternatively, a disturbed balance between the different neurotransmitter systems may be present in the different states of the affective disorders.

Schizophrenia - It has become more common to speak of "the schizophrenias" rather than schizophrenia in recognition of the many different patterns of behavior, albeit with some common underlying features, which have come to be recognized as forms of "schizophrenia". Symptom presentation has yielded some different subtypes (e.g., paranoid, undifferentiated, catatonic, borderline, and pseudoneurotic or ambulatory schizophrenia), and this grouping has been supported by suggestive evidence that symptom subtypes may be reflections of familial or genetic influences.[34] Chronicity and differential response to drugs have also served to define other subtypes, principally the acute, undifferentiated patients who manifest rapid improvement with anti-psychotic agents from the chronic schizophrenic group who often require long-term mental hospital placement. These wide differences in symptomatology and clinical course make difficult the postulation of a single biochemical factor as involved directly in the etiology of "schizophrenia", although many research strategies in the field seem directed towards this goal.

Similarly, the focus on "model psychoses" elicited by drugs has often been made with the implied assumption of a generalizable form of schizophrenia. For example, LSD and other psychotomimetic substances may produce cognitive disorganization, hallucinations and delusions with some resemblance to acute schizophrenic episodes. Similarly, high dose amphetamine administration produces symptomatology closely resembling an acute paranoid psychosis. There are important differences, however, between these states and the state of the chronic schizophrenic individual with thought disorder and a life style discordant with the rest of the world that have yet to be reconciled with the still frequent assumptions underlying studies searching for "the biochemical basis of schizophrenia".

As in the affective disorders, it seems equally, if not even more important, to frame hypotheses which are open enough to include a multi-factorial etiology of "the schizophrenias" with contributions of varying weights in different individuals to a final behavioral syndrome with some common features but many differences. The contributions to each form of schizophrenia may be from various biochemical sources, and perhaps from psychosocial factors. Kety[35] and others have advocated the interpretation of biochemical and genetic contributions to schizophrenic behavior in terms of factors resulting in increased vulnerability to the schizophrenia syndrome. This concept of vulnerability would seem to provide a better strategic construct for considering biochemical and biological factors (as well as other contributory events) which are less likely to be directly related to psychosis as a qualitatively different state, but which may be dimensions of normal biochemical changes characteristic of cognitive and emotional function, and of behavior which only at the extremes of a continuum may be contributory to schizophrenic behavior. A construct of this kind would also seem to be more compatible with the genetic and family

study evidence which indicate a higher incidence of other less severe behavioral abnormalities besides schizophrenia (including neurotic disorders and criminality) in the near relatives of schizophrenic individuals. Thus, biochemical factors would not be required to provide an all-or-none "schizophrenia psychosis factor", but rather could be seen only as providing some contributory element which under certain environmental pressures or with certain coincident events might result in one or another of the forms of abnormal behavior recognized as schizophrenia or as a schizophrenia-related psychiatric or psychosocial disorder. These constraints principally derived from genetic studies would seem to require reevaluation of the biological hypothesis of schizophrenia. In the following sections of this chapter, several of the current formulations of possible biological contributions to schizophrenia will be examined briefly. Other approaches to the biology of schizophrenia from the viewpoint of psychotomimetic drug models,[4,36] psychophysiology[37] and other aspects included in recent reviews of the syndrome[37,38] will not be considered in this chapter.

Altered Methylation Processes in Schizophrenia - Some methylated derivatives of the catecholamines (e.g., mescaline, the 3,4,5-methoxy derivative of phenylalanine) and indoleamines (e.g., DMT, N,N-dimethyltryptamine) have psychotomimetic properties. The suggestion that these or similar substances might be formed or abnormally metabolized in schizophrenic individuals and might contribute to schizophrenic behavior was first elaborated by Osmond & Smythies.[39] Although many studies have been carried out using different strategic approaches to examine this hypothesis, our focus here will be in the pharmacological approaches.

The Precursor-Load Strategy - This approach has been utilized to determine whether catecholamine or indoleamine precursors including phenylalanine, dihydroxyphenylalanine (L-DOPA), tryptophan, or 5-hydroxytryptophan (5-HTP) would elicit or exacerbate schizophrenic behavior in normals or schizophrenic individuals.[40] L-tryptophan has been described in a few instances to produce behavioral effects including euphoria and "drunken" behavior, but these effects have not been observed in many other studies. 5-HTP in small doses has also generally been found to be without behavioral effects, although large doses in combination with a peripheral decarboxylase inhibitor have been suggested to have antipsychotic effects.[41] While phenylalanine apparently does not change human behavior, L-dihydroxyphenylalanine has some psychosis-inducing properties in Parkinsonian patients,[42,43] and has been demonstrated to increase psychosis ratings in depressed patients with pre-existing psychotic symptomatology, while other non-psychotic depressed patients did not develop psychotic symptomatology.[44] L-DOPA has also been shown to exacerbate symptomatology in schizophrenic patients when the L-DOPA was given in an attempt to reverse phenothiazine drug-induced Parkinsonian symptoms.[45] It is not clear in any of these instances whether the increased symptomatology in the different patient groups represented a specific change in psychotic symptomatology or simply reflected the general behavioral activation produced by L-DOPA in which many different behaviors apparently become more overtly expressed.[42]

Methionine Loading - A somewhat different variant of the precursor-load strategy has also been used in studies of schizophrenic individuals. Methionine, a methyl group donor which is capable of increasing the levels of S-adenosylmethionine in brain has been administered to psychiatric patients (predominantly chronic schizophrenic individuals) and to a few normal individuals in studies recently reviewed elsewhere.[46] In almost all of the studies a monoamine oxidase inhibiting drug has been used concurrently, because the original study by Pollin, et al.[40] indicated that methionine used alone produced no behavioral effects in the patients. In almost all of the double-blind studies in schizophrenic patients some individuals developed increased symptomatology, often including apparent exacerbation of psychotic phenomena. Some elements of confusion, somnolence and disorientation were observed in the studies, and the difficulties in determining whether the deleterious effects of the methionine-MAOI combination represented an activation of the schizophrenic process or the superimposition of drug-induced CNS toxicity state have been debated. The few non-schizophrenic psychiatric patients and normal controls included in these studies have generally shown minimal responses to combined methionine and MAOI treatment, suggesting that schizophrenic patients are more susceptible to the deleterious effects of this drug combination, whether or not it actually represents the same type of process which occurs endogenously in schizophrenia.

Phenothiazine-Dopamine Relationship - Matthysse[47] has recently reviewed the data indicating a close relationship between the phenothiazines which have anti-psychotic properties and the ability of these drugs to increase the rate of dopamine turnover in brain, an alteration most likely reflecting a dopamine receptor blockade produced by these drugs. Studies indicating an elevation in homovanillic acid (HVA) levels in cerebrospinal fluid after phenothiazine administration in man[48] suggest the likelihood of a similar dopamine receptor blockading effect of these drugs in man. On the basis of this evidence, it has been postulated that there may exist an over-activity of the dopamine neurotransmitter systems in schizophrenia, perhaps particularly in those limbic system areas containing dopaminergic neurons. Although most phenothiazines "fit" in that their anti-psychotic potency roughly corresponds to their effects on dopamine in a variety of systems, there are some exceptions as noted below.

Amphetamines and Paranoid Schizophrenia - Recent studies have demonstrated that not only psychiatric patients but also normal individuals regularly develop changes in thought, mood and activity which closely resemble acute paranoid schizophrenia when d- or l-amphetamine is administered in large doses over a period of several days.[49,50] Amphetamines in animals produce compulsive, stereotyped behavior; these effects can be blocked by phenothiazines and seem to be primarily mediated by brain dopamine.

A variety of evidence from studies of schizophrenic individuals has suggested that some schizophrenic patients manifest hyperarousal and appear psychophysiologically to be in a state of central over-activation.[51] Evidence reviewed elsewhere is compatible with the postulation that a similar

state may be directly produced by L-DOPA administration[45] and may be mediated by increased brain dopamine.[43]

There exists related data which does not directly support or which conflicts with the series of statements made above. For example, phenothiazine drugs have many different biological actions, such as membrane stabilization effects[52] which may contribute to their clinical efficacy, even though these effects are partially shared by other structurally similar drugs which do not have prominent anti-psychotic activity. Furthermore, some phenothiazines such as thioridazine which are clinically very effective have relatively little dopamine receptor blocking properties in primates,[47] while another phenothiazine, thiethylperazine is probably an effective dopamine receptor blocking agent as measured by its stereotypy blocking effect but has little anti-psychotic activity[53]. However, some inconsistencies of this sort may become interpretable in terms of differences in the absorption, distribution and metabolism of the drugs in man under clinical conditions, or the possibility that dopamine receptors in the limbic area may have some different properties from those elsewhere in brain; for example, it appears that acetylcholine function effects DA synthesis predominantly in the striatum[54]. However, the phenothiazine and dopamine-related effects can, at present, only be considered to provide indirect, inferential support for a "dopamine hypothesis of schizophrenia".

The Noradrenergic Reward System and Schizophrenia - In early work, Stein (1968) delineated the presence of an apparent noradrenergic reward system in brain which may contribute to goal-directed behavior. Stein and Wise (1971) have postulated that a continuing process of destruction of the norepinephrine nerve terminals in brain may occur in schizophrenic individuals. The possibility of this damage occurring via endogenous production of a 6-hydroxydopamine-like agent was suggested. Recently, Stein and Wise[55] have evaluated some aspects of this hypothesis by measuring human brain dopamine-β-hydroxylase (DBH), which is localized exclusively in noradrenergic nerve terminals, using this enzyme as a marker for intact vs. destroyed nerve terminals. They demonstrated a 40% reduction in DBH in several brain areas from schizophrenic patients compared to normal controls, with appropriate concern for factors such as age, sex, time before autopsy sampling, and phenothiazine treatment effects. This apparent reduction in brain DBH activity in schizophrenia had not been found to be reflected in the periphery[56]. This hypothesis is of special interest because it endeavors to explain the longer-term features of the schizophrenic individuals in terms of a loss of goal-directed, integrative behavior.

Monoamine Oxidase in Schizophrenia - This enzyme exerts regulatory functions in setting the level of free amines in nerve endings and in limiting the accumulation of amines and amine metabolites synthesized elsewhere which can be taken up by the non-specific transport processes and stored in nerve terminal vesicles or cytoplasm (e.g., some so-called "false transmitter amines"). Measurement of monoamine oxidase in platelets from psychiatric patients has demonstrated markedly reduced MAO levels in chronic but not acute schizophrenic individuals, and moderately reduced levels in bipolar manic-depressive patients compared to unipolar depressed

patients and normal controls.[57] There are many mechanisms by which reduced MAO could contribute to abnormal behavior, but it is not yet known whether the altered platelet MAO activity reflects MAO activity in other tissues, including brain.[58]

Creatinine Phosphokinase (CPKase) in Schizophrenia - This enzyme is elevated in plasma in association with various kinds of tissue injury, and also, unaccountably, in acute psychotic states, particularly schizophrenia. Meltzer[59] has carefully studied this phenomena and demonstrated that the source of the increased enzyme is probably muscle, and that histologic evidence of muscle damage is present in almost half of the acutely psychotic patients. Chronic schizophrenic patients and non-psychotic psychiatric patients do not manifest CPKase abnormalities, whereas a related tissue enzyme, aldolase, is often similarly elevated in acutely psychotic patients.

Conclusion - It is obvious that the role of specific biologic process in "schizophrenia" or in subgroups of schizophrenic individuals is not yet explicable. As indicated in the introduction, it seems necessary to indicate that different hypotheses and different models seem to underly different study approaches to "schizophrenia". For example, the hypothesis postulating abnormal methylation processes in schizophrenia seems more to be directed towards an explanation of some psychotic symptoms found in a subgroup of schizophrenic patients; in contrast, other approaches (e.g., the theories regarding dopamine, the destruction of norepinephrine terminals, and MAO) are processes which may be more reflective of longer-term characteristics of some schizophrenic individuals. It should be noted that no schizophrenic subgroup was found to be different from any other in the DBH and MAO studies, although perhaps this lack of differences may only reflect the small number of individuals included in each study. Also, the possible relevance of the kinds of abnormalities discussed above to other individuals with schizophrenia spectrum disorders in the close relatives of schizophrenic patients has not yet been addressed in these hypotheses. Vast information gaps remain between our understanding of the relationships between neurotransmitter systems in brain, normal behavior, and psychotic, schizophrenic, and other forms of abnormal human functioning.

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Section II - Pharmacodynamic Agents

Editor: Frank H. Clarke, CIBA-GEIGY Corp., Ardsley, New York

Chapter 6. β-Adrenergic Blocking Agents

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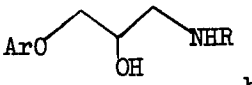
Introduction - The concept of using a specific β-adrenoceptor antagonist to protect the ischemic or threatened ischemic myocardium from sympathetic drive as treatment of angina pectoris and myocardial infarction was pioneered by Black some 15 years ago.[1] The validity of the concept was soon proven and these agents have now become accepted as the treatment of choice for angina.[1] Their role in the management of myocardial infarction is less clear cut[2] although evidence of their prophylactic value is emerging.[3,4] Although it was generally envisaged that β-blocking drugs would be useful for the treatment of those diseases characterised by excess sympathetic activity (such as pheochromocytoma, thyrotoxicosis, etc.) no one could have anticipated the range of clinical conditions against which the compounds are now being used or studied. These range from hypertension,[5,6] specific arrhythmias,[7] glaucoma,[8] migraine[9] through to a range of psychiatric disorders such as anxiety,[10] essential tremor,[11] alcoholism, drug dependence,[12] and possibly schizophrenia.[13]

The continued emergence of new compounds, coupled to the difficulties in relating laboratory pharmacological profiles to clinical effect, tends to obscure real advancement. The table compares the profiles of some of the newer β-blocking drugs with those of the well established ones. Only tolamolol, metoprolol (H93/26) and ICI 66082 have a novel profile. In almost all conditions efficacy appears to relate directly to cardiac β-blocking effect. Thus potency becomes significant in relation to dosing convenience to maintain a 24 hour inhibition (although duration of action is probably of more value in this respect) and in the minimization of non-adrenergically related side-effects. Some authors continue to associate the absence of membrane stabilizing properties with increased safety,[21,22] although the evidence that these non-specific depressant properties have no clinical significance is now overwhelming.[23] Even with propranolol, the maximum blood levels achieved are well below those needed to have any effect on the myocardial membrane. Heart failure is precipitated by β-blocking drugs in those patients requiring a high level of sympathetic drive to maintain a compensated myocardium - i.e. it is a consequence of β-blockade alone.

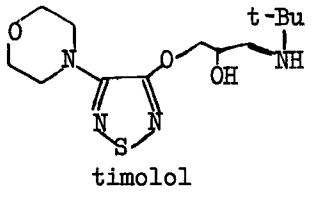
Of the remaining two parameters, cardioselectivity is clearly important in a drug to be given to patients prone to bronchospasm. However, the clinical cardiovascular consequences of both cardioselectivity and of i.s.a. are still not fully understood, particularly in relation to the precipit-

ation of cardiac failure.

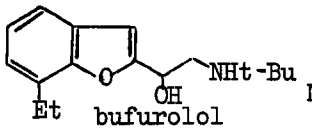


Name	Ar[a]	R	Potency[b]	i.s.a.[c]	m.s.a.[d]	sel.[e]	ref.
propranolol	1-naphthyl-	i-Pr	1	-	+	-	7
S2395	8-thiochromanyl-	i-Pr	10-12	-	(+)	-	15
alprenolol	2-allyl-Ph-	i-Pr	1	+	+	-	7
oxprenolol	2-allyloxy-Ph-	i-Pr	2	+	+	-	7
pindolol	4-indolyl-	i-Pr	20	+	+	-	7
bufuralol	(see below)		1	+	(+)	-	25
bunitrolol	2-cyano-Ph-	t-Bu	15-20	+	+	-	16
bufetolol	2-furfuryloxy-	t-Bu	0.9	+	(+)	-	20
sotalol	(see below)		0.1	-	-	-	7
timolol	(see below)	t-Bu	4	-	-	-	14
practolol	4-acetamido-Ph-	i-Pr	0.4	+	-	+	7
acebutolol	4-propionamido-2-acetyl-Ph	i-Pr	0.2	+	-	+	7
H87/07	4-$MeOCH_2CH_2O$-Ph	i-Pr	0.4	+	-	+	17
tolamolol	2-methyl-Ph	A	0.8	-	-	+	18
metoprolol	4-$MeO.CH_2.CH_2$-Ph	i-Pr	0.5	-	-	+	17
ICI 66082	4-$H_2NCO.CH_2$-Ph	i-Pr	0.9	-	-	+	19

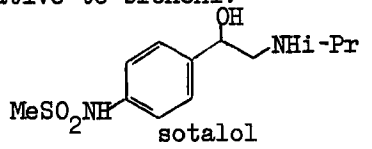
a) Ph represents phenyl; b) in vivo blockade of isoproterenol tachycardia - rough guide relative to propranolol; c) intrinsic sympathomimetic activity; d) non-specific membrane stabilising activity (symbols in parentheses indicate estimates); e) cardioselective relative to bronchi.



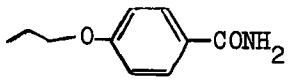
timolol



bufurolol



sotalol

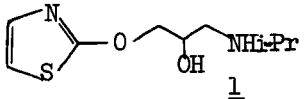


A

New Drugs - Four new drugs have had a limited release in the past year. Acebutolol ('Sectral') was launched by May and Baker in South Africa; bufetolol ('Adobiol') by Yoshitomi in Japan; timolol ('Blocadren') by Merck Sharp and Dohme in the U.K. and sotalol ('Beta-cardone') by Duncan Flockhart from Mead-Johnson in the U.K. The launching of sotalol so many years after discovery is surprising particularly in view of the recently announced decision to discontinue clinical studies because of lack of commercial interest in the U.S.A.[24]

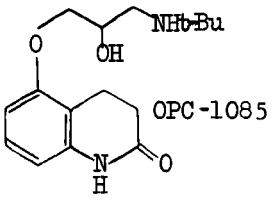
New clinical studies - Initial clinical studies on four new compounds have been reported in the past year. Bufuralol (Ro 3-4787) (see table) was found to be equipotent with propranolol in antagonizing exercise tachycardia in healthy volunteers but did not reduce the resting heart rate whereas propranolol did, a fact attributed to the i.s.a. of the former.[25] Adolfsson and co-workers[26] studied the effects of a non-selective β-blocker (alprenolol) and two selective β-blockers (H87/07 and H93/26 [metoprolol]) on exercise tolerance in anginal patients and found that all three drugs had an approximately equal effect on angina despite their differing pharmacological profiles (see table). ICI 66082 is a cardioselective β-blocker, devoid of i.s.a. or m.s.a. which is being studied mainly in the treatment of hypertension.[6] In patients with severe angina, ICI 66082 was as potent as propranolol in its blocking action on exercise heart rate.[27]

New chemical types - No structural type has emerged which offers any improvement over existing β-blockers. Perhaps the most interesting novel compounds reported in the past year are a series of thiazolyloxypropanolamines.[28]

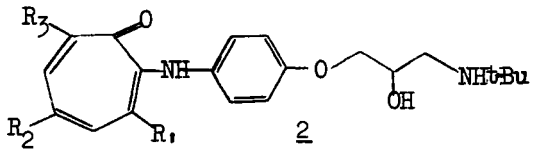


1

Compound 1 is a selective potent myocardial stimulant with selective β-receptor blocking activity which, in anaesthetized dogs, increased myocardial contractility, cardiac output, coronary blood flow and heart rate and moderately decreased total peripheral resistance but had little or no effect on blood pressure. Some structure-activity relationships showed that β-stimulant potency went hand in hand with potency as a β-blocker. It is reported that clinical studies using 1 for the treatment of heart failure in humans have begun.[29] From a series of dihydrocarbostyrils in which the quinolone ring was substituted by the oxypropanolamine side chain in positions 5,6,7 and 8, the 5- substituted t-butylamino analogue (OPC-1085) was the best compound.[30] OPC-1085 is a non-selective potent (equipotent with pindolol i.e. ca 30 times more active than propranolol) β-blocker with the same level of i.s.a. as pindolol but is not effective in reducing blood pressure.[31] Replacement of the 4'-acetamido function of practolol with an aminotropine system is reported to give compounds 2 which are more potent than practolol as β-blockers (isolated guinea pig ileum) and more effective than standard α-blockers (e.g. tolazoline) in lowering the blood pressure in spontaneously hypertensive rats.[32]

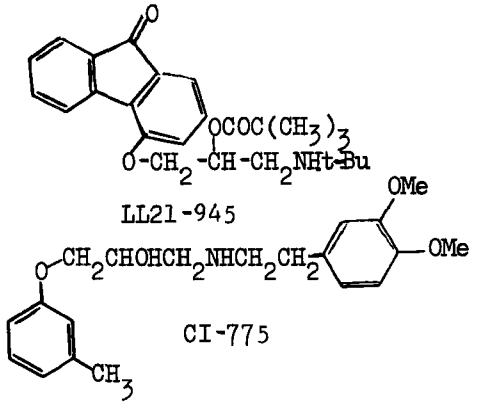


OPC-1085



2

Esterification of the β-hydroxy group of the oxypropanolamine side chain is reported to give β-blockers with a long duration of action. Ester groups were chosen which were slowly hydrolysable in-vivo and bulky radicals such as pivaloyl were the most effective groups in retarding hydrolysis. LL21-945 was as potent as propranolol in blocking the cardiac β-receptors in isolated atria and showed i.s.a. but not to the same extent as pindolol. In anaesthetized dogs the time of onset of action was ca 2.5 hours compared with 5 minutes for both propranolol and pindolol but whereas the activities



of the 'normal' blockers decreased over a 5 hour test period, LL21-945 still inhibited the heartrate increase caused by isoproterenol by 30-50% after 39 hours.[33] In isolated guinea pig atria and trachea, CI-775, was 32 times more potent in antagonizing isoproterenol responses on the atria than on the trachea. The 3,4-dimethoxy phenethylamino substituent was considered to be necessary for this cardioselective activity and its incorporation into other β-blockers was claimed to enhance their selectivity.[34]

Structure Activity Relationships - Further evidence to support Levy's postulate[35] that the lipophilic properties of β-blockers are major determinants of their m.s.a. (i.e. their non-specific pharmacological effects) has been reported in three separate studies using different biological models. In a series of β-blockers Hellenbrecht and co-workers[36,37] found a correlation between the degree of local anaesthetic activity (measured in frog nerve) and myocardial conduction velocity (frog heart), with lipophilic parameters of the molecules. It was proposed that the m.s.a. of β-blocking drugs could be predicted from their octanol/water partition coefficients or even by calculation of their $\Sigma\pi$ values according to Hansch's method.[38] In a study on the rate of calcium uptake in the sarcoplasmic reticulum vesicles isolated from rabbit skeletal muscle, Temple and co-workers[39] found that inhibition of calcium uptake was greater with β-blockers that had m.s.a. (e.g. propranolol) than those which did not (e.g. sotalol) and the observed activities were related to the lipophilicities of the compounds.

There is still much controversy about the nature of the β-receptor and Lands'[40] original classification of the β-receptor into β_1 (cardiac adipose tissue) and β_2 (uterus, bronchial and vascular muscle) is being questioned. Some evidence in support of Lands original classification has appeared.[41] However, in a study of the β-blocking actions of practolol, propranolol and butoxamine in anaesthetized dogs, the relative potencies of the compounds on the cardiac, bronchial and vascular responses to isoproterenol led the author[42] to the conclusion that in the dog each of these tissues has a different β-receptor sub-type. In anaesthetized dogs, H64/52 (para analogue of alprenolol) was shown[43] to have similar β-blocking activity on cardiac and bronchial smooth muscle but had much less activity on vascular smooth muscle. From this study, the classification of bronchiolar and vascular smooth muscle as β_2 was untenable. Using a series of "bis" aryloxypropanolamines as antagonists of isoprenaline in isolated atria (guinea pig), trachea (guinea pig) and adipose cells (rat), Zaagsma and co-workers[44] found only a poor correlation between the pA_2 values for antagonism of lipolysis and atrial stimulation but a significant correlation between the pA_2's for antagonism of lipolysis and tracheal stimulation. They suggested that the lipolytic β-receptor is not of the β type nor of the β_2 type but is a sort of 'composite' β-receptor having certain proper-

ties of both β_1 and β_2.

Frog erythrocyte adenylate cyclase has the characteristics of a β_2 adrenergic receptor and the responses of this model to various agonists and antagonists correlate well with the responses observed in mammalian tissue preparations.[45]

The molecular features affecting the relative antagonist potency on β-receptors from various tissues continues to receive much attention. Increasing the size of the amino substituent from H through to t-Bu in a series of o-cyanophenyloxypropanolamines, not only increased the potency of the compounds as cardiac β-blockers but also increased their selectivity for β_2 receptors; thus the t-Bu analogue (bunitrolol) was 4 times more potent in antagonizing the vasodepressor response to isoprenaline than the cardiac chronotropic response whereas the primary amino analogue (K8 1439) was 6 times more potent on cardiac than on vascular β-receptors.[46] This latter effect parallels that usually found with β-agonists[47]. In a series of substituted oxypropanolamines the relatively increased cardioselectivity of the p-methoxy (R=i-Pr) relative to its o methoxy analogue[48] adds further support to the hypothesis[49] that an appropriate substituent in the aromatic ring para to the oxypropanolamine side chain is an important factor in determining cardioselectivity. However, the relatively high cardioselectivity of tolamolol and the reported cardioselectivity of CI-775 (previous section) show that para substition is by no means the sole determinant of cardioselectivity. Very few structure/activity guidelines have emerged to help a chemist control the degree of i.s.a. in a β-blocker. The structural features necessary to eliminate i.s.a. from a molecule are very difficult to define. Of the "pure" β-blockers (i.e. no i.s.a.) ICI 66082 and H93/26 have the common feature of a para methylene group but two other non i.s.a. compounds, propranolol and tolamolol, have no such substituent.

Clinical and mode of action studies - The mechanism whereby β-blockers control the blood pressure of hypertensive patients remains unresolved but appears to be related to β-blockade, as blocking drugs are effective in doses related to their relative cardiac blocking potencies.[6] In a double-blind crossover study the β-blockers propranolol, timolol, pindolol and alprenolol produced a fall in blood pressure which is not significantly different from each other.[50] A good correlation has been shown, irrespective of the etiology, between the antihypertensive effect of propranolol and both the pre-existing level of plasma renin activity and the extent to which the drug produces an absolute reduction in renin.[51] The reduction with propranolol of the plasma renin activity response to tilting is however not significantly correlated with the fall of blood pressure observed.[52] Further evidence against a mechanism of antihypertensive action involving renin is provided by pindolol which has high intrinsic activity and can maintain the fall in blood pressure produced by chronic administration of propranolol but without maintaining the reduction in renin activity.[53] Furthermore, the cardioselective drugs practolol[54] and ICI 66082[55] have been shown to exert an antihypertensive effect without lowering recumbent plasma renin activity. The effect of ICI 66082 on renin release is not clear as it has also been

reported to reduce renin activity in association with its antihypertensive effect.[56] In animal experiments this drug which is of similar potency to propranolol on cardiac β-receptors, is only about 1/5 as potent in inhibiting the release of renin by renal nerve stimulation.[57]

That a central mechanism may be responsible for the antihypertensive activity of β-blockers must be considered as propranolol,[58,59] alprenolol, pindolol, practolol, ICI 66082, sotalol and oxprenolol[59] produce a prolonged fall of mean arterial pressure after intracerebroventricular administration in conscious animals. This effect appears to be related to β-blockade as it is not seen with d-propranolol[58,59] and the maximum response coincides with maximal inhibition of the tachycardia response to intracerebroventricular isoprenaline.[59] These drugs differ however in their distribution following systemic administration and practolol and ICI 66082 do not readily cross the blood-brain barrier.[27]

In anginal patients, acute administration of ICI 66082 increases maximal working capacity to a greater extent than propranolol (+9%), possibly as a result of the unaltered peripheral resistance during exercise; propranolol increases peripheral resistance under these conditions.[27] Heart rate and cardiac output are depressed both at rest and during exercise, whereas stroke volume is unaltered and aortic blood pressure is decreased only during exercise. The cardioselectivity of ICI 66082 is also evident in its lack of effect on airways resistance. Tolamolol resembles propranolol in its haemodynamic effects in anginal patients except that it causes significantly less increase in systemic vascular resistance.[60] Tolamolol has been shown to depress heart rate, cardiac output and also cardiac contractility when the heart is paced at a constant rate, without altering left ventricular end-diastolic pressure.[61] A comparison of acute administration of alprenolol and cardioselective compounds with (H87/07) or without (H93/26) intrinsic activity in anginal patients shows H87/07 to reduce resting heart rate to a significantly lesser extent than H93/26 but the exercise heart rate although tending to be higher for H87/07, is not significantly different. Total work, time for appearance of pain or ST changes during work and systolic blood pressure at rest and work are similar for all drugs but the time for disappearance of ST depression after work is significantly longer for H87/07 than H93/26.[26]

The hypothesis has been put forward that one mechanism contributing to the efficacy of propranolol in angina pectoris may be enhancement of oxygen availability.[62] In the <u>in vitro</u> situation, high concentrations of propranolol (0.5mM) reduce oxygen binding by hemoglobin in intact red cells.[64] cells.[63] The dextro isomer is however even more potent than the racemate in this effect which is uninfluenced by adrenaline but prevented by chlorobutamol, suggesting that β-receptors are not involved.[63] In healthy subjects, propranolol has been shown to be without significant acute effects on oxygen hemoglobin affinity[64,65] but this is not so for patients with coronary artery disease on chronic propranolol and a significant increase in P_{50} (decrease in affinity) has been observed from control level, sufficient to increase systemic oxygen delivery by 20-30%.[65] A review of the literature[62] provides evidence of a rightward shift of the oxygen

hemoglobin dissociation curve in certain patients with ischemic heart disease with or without evidence of coronary obstruction, which may be a compensatory mechanism to sustain oxygen delivery. Similarly the decrease in affinity with propranolol[65] may be secondary to changes in cardiac or pulmonary function rather than a direct action on the red cell. Further work is required to clarify the role of propranolol in the relief of angina.

The mortality rate of patients discharged from hospital after myocardial infarction is high. In a double blind study involving patients selected as being suitable for receiving β-blockers, the total mortality in a two year follow-up period in the placebo group is 12%. Prophylactic treatment with alprenolol reduces the total mortality but not significantly. However, the number of sudden deaths (within 24 hours of first symptoms) is significantly reduced.[64] In this study the number of reinfarctions is not affected but in an open investigation, reinfarctions are significantly reduced.[63] Possible mechanisms for this protection are the prevention of development of malignant arrhythmias during the initial phase of infarction and an influence on the area of ischemia. The extent and severity of myocardial ischemic injury can be altered substantially even several hours after coronary occlusion[66] and moderate increases in heart rate leading to increased myocardial oxygen consumption have a deleterious effect on infarct size in conscious dogs.[67] Thus in hypertensive patients, reduction of cardiac afterload by ganglion blockade is effective in reducing myocardial injury post infarction.[68] Intravenous administration of propranolol reduces blood pressure, heart work, heart rate, coronary blood flow and myocardial oxygen uptake but increases lactate extraction by the heart, indicative of reduced ischemia.[69] The incidence of an inappropriately rapid heart rate during movement of patients with acute myocardial infarction is effectively controlled by intravenous sotalol and atropine (to prevent tachycardia) but practolol, with intrinsic activity, is of only limited value.[70] Practolol has however been shown to reduce heart work and chest pain in uncomplicated acute myocardial infarction.[71]

The manifestations of anxiety can be grouped as either psychic (fear, tension, apprehension, difficulty in concentrating) or somatic (trembling, palpitations, tachycardia, sweating, nausea). The latter appear to be a result of autonomic inbalance involving excess sympathetic activity. The use of β-blockers in anxiety requires further evaluation but in review, they appear to be of value.[10,12,72] β-Blocking drugs can control some of the somatic features of anxiety and are of particular benefit where somatic symptoms are prominent, in the management of neurocirculatory asthenia, hyperkinetic heart syndrome, anxiety tachycardia and tremor. Some investigators have reported that the mental as well as the physical state of anxiety in patients is benefited by β-blockade,[9,10] and there is controversy as to the mechanism involved. It may be the result of direct actions on the central nervous system as can be demonstrated in animals[11,12] or a consequence of control of physical distress. This latter is supported by the findings that d-propranolol with weak β-blocking activity is inactive as an anti-anxiety drug while practolol, which does not cross the blood brain barrier to any extent, is active.[10,12] Further argument against a central action is provided by the observation that in chronic anxiety, if

the symptoms are mainly psychic, diazepam but not propranolol is effective as an anti-anxiety drug, but if somatic features predominate, propranolol is also effective.[73] Propranolol is without effect on the EEG of these patients but reduces heart rate and finger tremor in both somatic and psychic anxiety patients. Diazepam also reduces finger tremor.[74] In normal subjects exposed to anxiety-provoking situations, diazepam is effective in reducing subjective anxiety but propranolol is not, suggesting that attention is directed toward the source of anxiety rather than the bodily symptoms.[75]

The value of β-blockers in psychic disorders, treatment of alcoholism and heroin abuse remains to be resolved[12,72] but they do appear to have a place in the treatment of action tremors of familial, senile or essential nature.[12] Use for the treatment of tremor of Parkinson's disease is less promising.[12]

Migraine is thought to be a functional rather than an organic disorder which may well be associated with the autonomic nervous system and induced by a variety of causes including stress. It has been confirmed that prophylactic treatment with propranolol under double blind crossover conditions, significantly reduces the mean frequence of headache attacks.[8,9] It may also favorably influence the severity and duration of attacks. Two patients responded to propranolol but not practolol[8,9] (pindolol also is ineffective in preventing migraine attacks).[76]

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Chapter 7. Cardiovascular Agents

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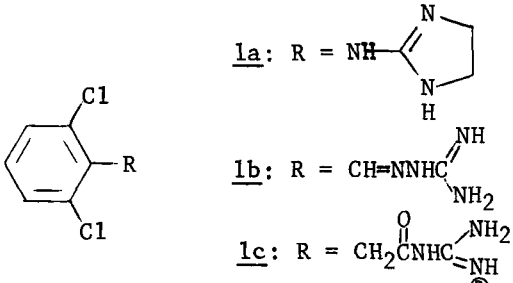
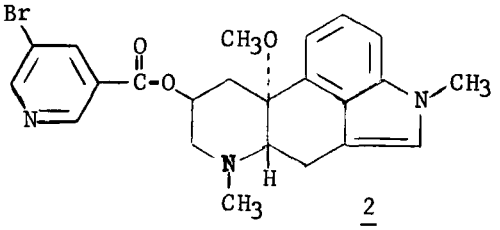
This review covers recent advances in new or potentially important non-diuretic hypotensive, antiarrythmic and antianginal agents excluding β-blockers and their derivatives, which are covered in chapter 6.

New Products - One of the oldest known hypotensives, sodium nitroprusside was marketed in the U.S.A. by Hoffmann-LaRoche as Nipride® in 1974.[1a] It is indicated for rapid reduction of blood pressure in hypertensive crisis and is given only as an intravenous infusion because its effect is evanescent. Clinically it was also used to produce controlled hypotension during anaesthesia and renal angiography and to manage patients with acute myocardial infarction and chronic heart failure.[2-4] Clonidine (1a) marketed in Germany in 1966, was released on the U.S. market this year by





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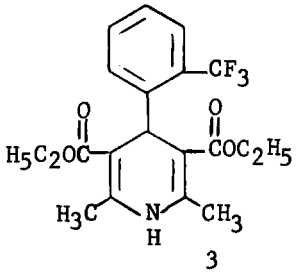
Boehringer-Ingelheim as Catapres®. A recent study of patients with mild essential hypertension treated only with clonidine (300-600 μg/day) for a year indicated a similar hemodynamic mechanism to that of α-methyldopa with similar side effects.[6] Utility of intravenous clonidine in acute hypertensive crisis was reported in another study.[7] Problems of "hypertensive rebound" from discontinuation of the drug[8,9] associated with raised catecholamine levels can be reversed by α-or β-blockade[8,10] or by reserpine pretreatment.[8] Recent studies supporting clonidine's effect of lowering plasma renin activity have appeared,[11,12] its effect of increasing renal blood flow in rats[13] and in man[14] was reported and its ability to lower exchangeable sodium in severe hypertensive patients was claimed.[14] Clonidine and other hypotensive 2-aminoimidazolines were reported to exert their effects in the anterior hypothalamus of rats whereas other potent peripheral α-stimulants differing structurally, such as oxymetazoline, failed to cause hypotension, even when injected into that area of the brain.[15,16] This suggests a difference between central and peripheral α-adrenergic receptors. The higher affinity of clonidine for pre- vs. postsynaptic adrenoceptors in the rabbit pulmonary artery[17] also suggests such a difference. Two recent successful trials on Combipres® (clonidine-chlorthalidone) have also been reported.[18,19] Nicergoline (2) has been marketed in Italy by Farmitalia as Sermion® . This α-blocker[20] is used as a peripheral and cerebral vasodilator and as adjuvant therapy for arterial hypertension. It can be used parenterally in hypertensive crisis

and is said to be well tolerated and to produce a gradual fall in blood pressure with no undesirable cardiac effects?[20] Prazosin[5] was marketed in 1974 in Great Britain and Switzerland by Pfizer as Hypovase® and by Carlo-Erba as Sinetens®.[1b] Conclusions from many reeent clinical reports show prazosin as a potent (1 to 36 mg/day[21]) orally active drug, especially for mild to moderate hypertension[22] with few side effects.[23-26] It is especially useful in combination with a diuretic[25] or a β-blocker[24] and the combination of all three is useful in patients with renal insufficiency.[27-29] Its difference from other vasodilators was demonstrated in renal hypertensive dogs, where p.o. and i.v. administration caused hypotension without tachycardia or significant increase in plasma renin.[30] Its mechanism is said to be twofold: a direct relaxation of vascular smooth muscle and α-blockade.[31]

Antihypertensives in Clinical Investigation - Recent clinical studies supported the utility of minoxidil[5] in combination with a β-blocker and a diuretic in severe hypertension.[32-34] This combination was helpful in improving renal function in some patients with nephrosclerosis.[35] Addition of propranolol to minoxidil appears necessary not only to reduce tachycardia and plasma renin activity[36] but also to reduce the incidence of myocardial anoxia.[37] A similar myocardial effect could be induced in dogs[38-40] and likewise improved by propranolol.[40,41] SKF 24260 (3) is hypotensive in man



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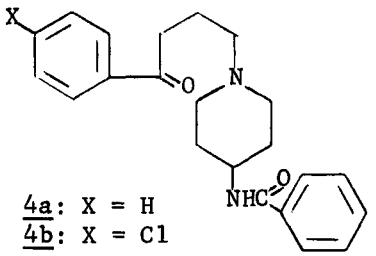
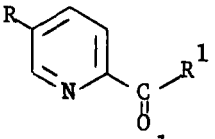


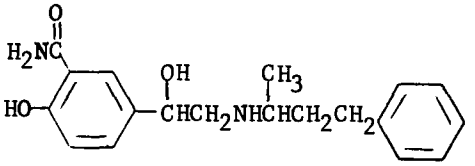
4a: X = H
4b: X = Cl



5a: R = C_4H_9, R^1 = OH
5b: R = C_4H_9, R^1 = NH_2
5c: R = $Me_2C(S)S$-, R^1 = OH

probably by direct action on vascular smooth muscle.[42] Studies on dogs indicate hypotension is caused by selective dilation of precapillary resistance vessels in both skeletal muscle and intestine. The reflux tachycardia is abolished by propranolol in rats but only partly in dogs.[43] A series of 98 analogs has been synthesized and compared with SKF 24260.[44] None of the metabolites of this compound is active.[45] Indoramin[5] lowered blood pressure effectively in several clinical trials when given orally[46-48] and increased digital blood flow in patients with Raynaud's syndrome when given intravenously.[49] Addition of desmethylimipramine caused no reversal of antihypertensive effects.[48] Structure-activity relationships for a number of indoramine analogs have been published[50-52] and several variants showed favorable activity in hypertensive rats. The best analog appeared to be 4a but the most potent α-antagonist was 4b. The hypotensive and dopamine β-hydroxylase inhibiting activity of fusaric acid (5a) was demonstrated in man at 300-600 mg/day.[53] Fusaric acid amide (bupicomide, Sch. 10595, 5b) also active in man at 300-1800 mg/day, showed hemodynamic effects like hydralazine.[54,55] The amide is metabolized to the acid in man.[56] The reduced acid is claimed to be active in spontaneous hypertensive rats.[57] The analog 5c, YP-279, is also hypotensive in rats but has no

effect on brain norepinephrine biosynthesis, unlike 5a or dibromofusaric acid[58,59] Fusaric acid is also claimed to increase 5-hydroxytryptamine in the brain and to inhibit the binding of tryptophan to serum albumin peripherally[60] The combination of phentolamine (an α-blocker) and oxprenolol (a β-blocker) gave very good control in patients with severe hyperten-



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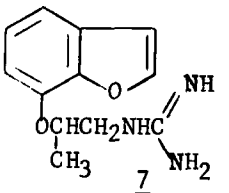

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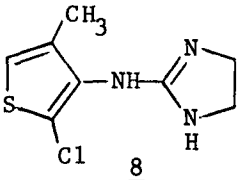

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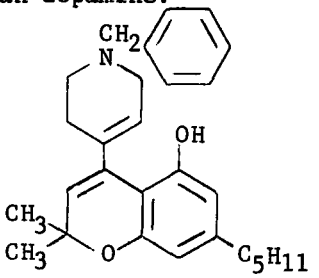
sion.[61] AH-5158 (6), a competitive antagonist of both α- and β-adrenoceptors, produced hypotension in six normotensive humans.[62] Guanabenz (Wy-8678, 1b) was shown to be safe and effective in clinical trials (16-32 mg/day) without orthostatic hypotension and diarrhea.[63] The Labaz compound L-6569 (7) was dropped from clinical trial due to an insufficient margin between the effective hypotensive dose and the dose producing orthostatic hypotension.[64] The Sandoz compound BS 100/141 (1c) shows a mechanistic profile in animals like clonidine.[65] It is said to be active in moderately severe hypertensive patients at 3-8 mg/day without orthostatic hypotension and hypertensive rebound. Tolerance development required an increase in dose.[66] Tiamenidine (Hoe 440, 8), in the clinic in Europe,[67] is less potent in renal hypertensive rats than clonidine but shows less sympathomimetic effects in dogs and cats.[68]

Natural Products and Related Compounds - SQ 20,881 (Pyr-Trp-Pro-Arg-Gln-Ile-Pro-Pro), an angiotensin converting-enzyme inhibitor, lowered blood pressure and raised aldosterone levels in hypertensive patients at 1-4 mg/kg i.v. The effect occurred even in patients with normal renin levels and was strongly augmented by sodium depletion.[69-72] Malignant hypertension in rabbits of the Goldblatt type was prevented provided the peptide injections were started at the time of the hypertension-inducing operation.[73] Saralasin acetate (P-113, Sar^1-Ala^8-angiotensin II), a competitive antagonist of angiotensin II,[74] which lowers blood pressure on infusion in patients with high renin hypertension[75,76] is also more effective with concurrent sodium depletion. This peptide apparently produces no hypotension and few, if any, side effects. Activity of this inhibitor appears to be central as well as peripheral.[77,78] The analog Sar^1-Gly^8-angiotensin II increased the potency of three vasodilators in dogs, indicating that a rise in plasma renin activity interferes with the hypotensive effect of these drugs.[79] One study in hypertensive patients indicated that infusion of Sar^1-Ile^8-angiotensin II, unlike P-113, was largely ineffective in lowering blood pressure.[80] Activity of these compounds has led to the synthesis[81-85] and evaluation[86,87] of many analogs. In rat tests, Sar^1-N-methylphenylalanine^8 angiotensin II[88] and Sar^1-Ile^5-C-phenylglycine^8-angiotensin II[89] appear to have the most potential as angiotensin II antagonists. The structure of neurotensin, the vasoactive peptide from bovine hypothalami, has been defined by synthesis as pGlu-Leu-Tyr-Glu-Asn-Lys-

Pro-Arg-Arg-Pro-Tyr-Ile-Leu?[90] Other peptides which show hypotensive properties are substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Me),[91,92] dextran derivatives of eledoisin analogs;[93] two octapeptides claimed to be renin inhibitors[94] and the vasoactive intestinal peptide (VIP) isolated from hog small intestine?[95,96] The role of prostaglandins in blood pressure control was recently reviewed?[97,98] Cases of the utility of intravenous PGE_1 in lowering blood pressure in various types of hypertension were reported[99] and its use in nonocclusive mesenteric ischemia suggested.[100] Success of PGA_1 in hypertensive crisis has also been reported[101] but it appears to be of no use in high renin hypertensive subjects.[102] A neutral lipid from renomedullary interstitial cells which lowers the blood pressure of hypertensive rats has been found.[103] Variation of the cannabinol structure has led to some separation of hypotensive and CNS effects. The best of these appear to be BRL 6155 (9)[104] and compound 10.[105,106] Coenzyme Q_{10}, given orally (1-10 mg/kg/day) suppresses the development of hypertension or reduces advanced hypertension in experimental hypertensive rats and dogs.[107] The aporphine analog 11a reduces blood pressure in hypertensive rats and dogs[108] and the analog 11b is a more potent and prolonged renal vasodilator than dopamine.[109]



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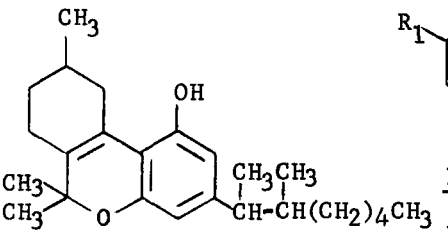

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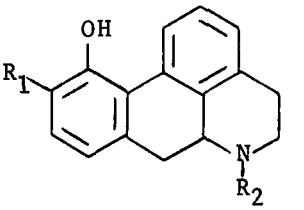
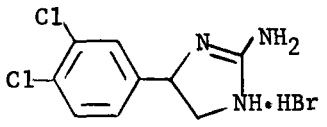


11a: R_1 = H; R_2 = CH_3
11b: R_1 = HO; R_2 = C_3H_7

Other Antihypertensives under Investigation - MJ-10459-2(12) shows potent antihypertensive effects in several animal models, probably by a mechanism of attenuated sympathetic function as a result of peripheral norepinephrine depletion.[110,111] A number of related compounds have been patented.[112] The mandelamidine (13) caused hypotension in rats comparably to



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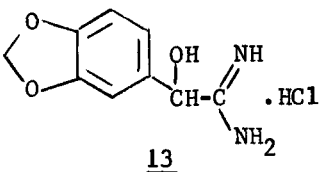

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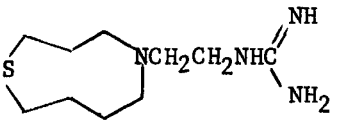

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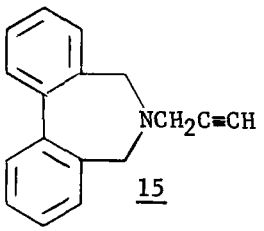
bethanidine and guanethidine with some lessening of side effects.[113] The analog 14 showed a similar profile to guanethidine but was less potent in renal hypertensive rats.[114] The azapetine analog 15 showed strong hypotensive activity in dogs by α-blockade at sympathetic neuroeffector sites.[115] L-8142 (16) lowers blood pressure for a prolonged period in several rat models.[116] The amiquinsin analog 17 was less active than the quinoline compound in dog models.[117] Compound 18 appears to be one of the best of a series of benzofurans with hypotensive (cats, Grollman rats) and diuretic



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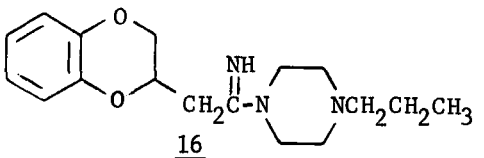

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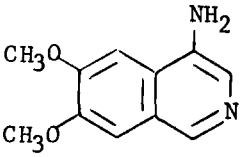

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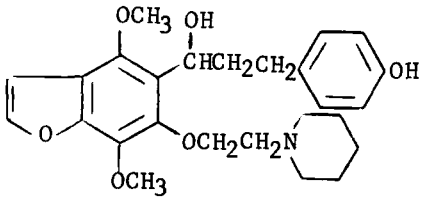
properties.[118] ISF 2123 (19) appears qualitatively similar to hydralazine but more potent in rats and dogs.[119] DJ-1461 (20), though only one-fourth as potent as hydralazine in lowering blood pressure, is one-hundredth as



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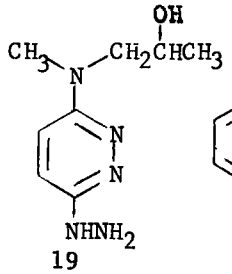

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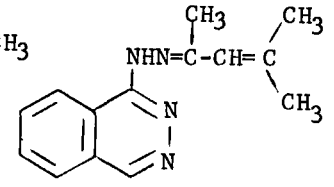

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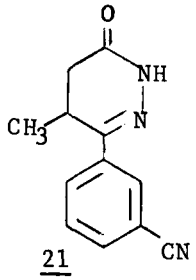
potent in increasing heart rate in rat studies.[120] Compound 21 was the most potent of a large series of pyridazinone derivatives in rats, but preliminary toxicology in several species revealed hemorrhagic patches in the heart area.[121] Abbott 31699 (22) caused a delayed, long-lasting hypotensive effect in hypertensive dogs[122] which did not correlate with plasma levels



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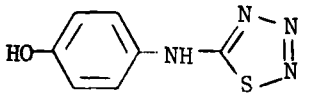

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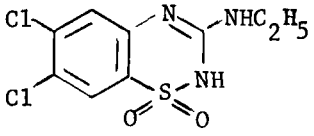
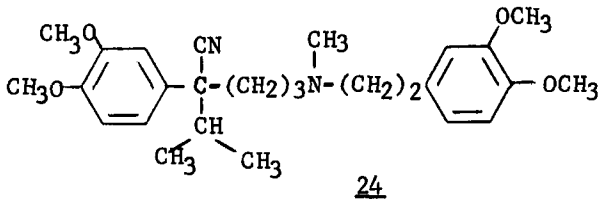


23

of the parent drug or a metabolite. This suggests that more than one agent may cause the activity.[123] The diazoxide analog 23 reduced blood pressure and heart rate in rats but caused ECG changes.[124]

Antianginal and Antiarrhythmic Agents - Verapamil (24) normalized sinus rhythm at 10 mg.i.v. in a number of patients with supraventricular tachycardia.[125] Its mode of action, which likely results from the ability to block calcium transport across the myocardial cell membrane[126,127] sets it apart from other well-known antiarrhythmic agents.[128,129] It is claimed to be not contraindicated in obstructive airways disease,[130] possibly useful in therapy-resistant hypertension or hypertensive crisis[131,132] and well suited to the treatment of angina pectoris.[126] Aprindine (AC 1802, Lilly 99170, 25) was effective in the treatment of chronic stable ventric-

ular extrasystoles in two recent studies.[133,134] The drug slows conduction



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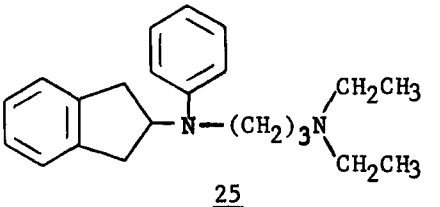
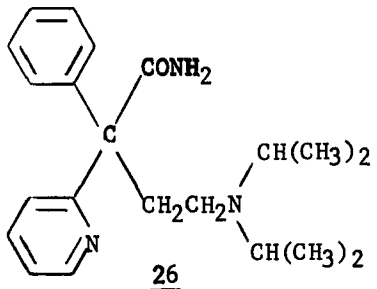
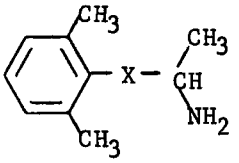


25

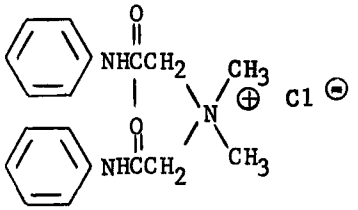
velocity in the AV node as well as in the His-Purkinje system and the sinus node recovery time is prolonged.[135] Activity may depend on certain calcium-dependent membrane processes and sodium conductance changes.[136] The metabolism of aprindine in man was recently reported.[137] Attempts to modify the carboxamide groups of the promising clinical candidate disopyramide (26)[138] did not produce a superior compound.[139] Mexiletine (Kö 1173, 27a) reduced ventricular extrasystoles or ventricular tachycardia in twelve patients in a recent study.[140] Clinical trials were suggested for a related compound, N36095 (27b).[141] QX-572 (28) was effective after i.v. infusion in twelve patients with therapy-resistant ventricular arrhythmias.[142]



26

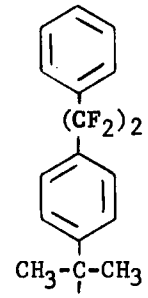


27a: X =-OCH_2-
27b: X =-NHCO-



28

The tetrafluoro compound 29 was orally effective in dog and baboon models



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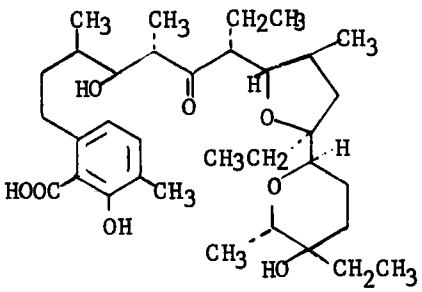
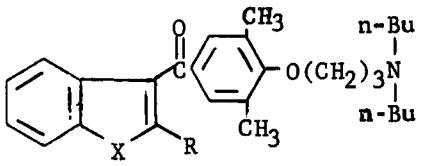


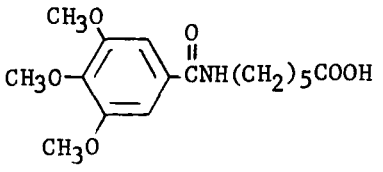
30



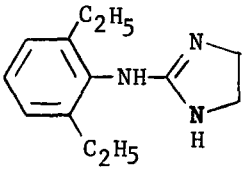
31a: X = O; R = CH_2CH_3
31b: X = S; R = CH_3

and was selected for clinical trial as an antiarrhythmic agent.[143] Replacement of the tetrafluoroethane bridge with ethyne gives a compound of comparable activity.[144] Studies in various animal models revealed that the antibiotic lasalocid (X-537A, Ro2-2985, 30) appears to be a prototype of a new class of inotropic drugs.[145] The amiodarone analog L 8412 (31a),[146] now in clinical trial as an antianginal agent has been followed by L 9146

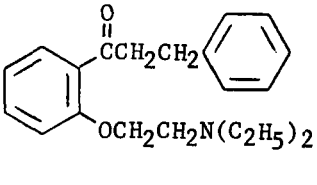
(31b) having hemodynamic effects similar to amiodarone.[147] Capobenic acid (C-3, 32) was reported to give good results in 57 cases of acute myocardial



32

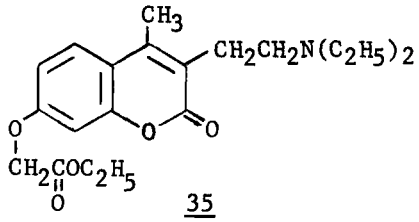


33

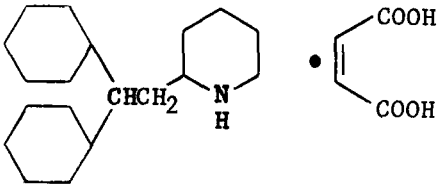


34

infarct and 10 cases of coronary insufficiency.[148] ST-91 (33) caused pronounced bradycardia in man and prolonged exercise time for anginal patients, however, with α-stimulant produced side effects.[149] Etafenone (34) is now



35



36

said to give favorable antianginal effects via inhibition of calcium influx and phosphodiesterase.[150] A broad clinical investigation of chromanar (35) indicated that the drug reduced the frequency of anginal attacks and nitroglycerin requirements with minimal side effects.[151] Two recent clinical studies on perhexilene maleate (Pexid®, 36) demonstrated its effectiveness in anginal patients.[152,153] In dog studies, it appeared to have no significant effect on the cardiac conduction system.[154]

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Chapter 8. Diuretics

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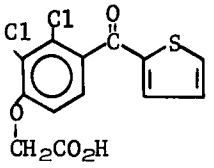
Since the advent of the thiazide diuretics, which practically replaced the mercurials, much research has been devoted to the synthesis of purely organic diuretic compounds. This research has resulted in more potent diuretics, such as furosemide, ethacrynic acid and bumetanide. However, the search still goes on in this field, for the goal is no longer mere potency but elimination of the undesirable attributes that have continued to accompany drug-induced diuresis.

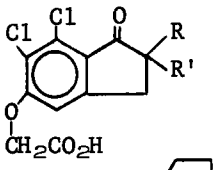
Of the several undesirable side effects of the commonly used diuretics, hypokalemia and hyperuricemia stand out as ubiquitous and concerning problems. Amiloride and triamterene, two antikaliuretic compounds, have gained favor, particularly in combination with thiazides, for the control of the former problem; however, until recently, little progress has been reported in solving the problem of uric acid retention, an undesirable attribute of all non-mercurial diuretics.

Uricosuric Diuretics - Within the past year, two classes of uricosuric diuretics have been reported. The synthesis of tienilic acid (SKF-62698), several of its congeners, and their diuretic efficacy in mice and dogs have been described.[1] More recently, a clinical investigation[2] of tienilic acid in the treatment of patients with moderately severe arterial hypertension showed an average reduction of 27 mmHg in systolic pressure and a decrease in diastolic pressure of 2 mmHg. Most notable in the report, however, was that tienilic acid effected reductions of from 35 to 50% in plasma urate. Tienilic acid appears to have its major site of action in the diluting portion of the distal tubule.[3]

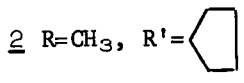


SKF-62698

Cragoe and co-workers[4-6] have reported on a series of (1-oxo-2-substituted-5-indanyloxy)acetic acids as potent orally active agents possessing both uricosuric and saluretic activity. The nature of the substituents at the 2-position markedly influenced the saluretic activity as measured in the rat, dog and chimpanzee as well as the uricosuric activity in the chimpanzee. Thus, 1 displayed modest activity as a diuretic in the three species and marked uricosuria in chimpanzees, as determined using established testing procedures.[7-9] Replacement of the 2H with methyl, 2, gave a profound increase in both biological parameters. Then substitution of an aryl group for the cyclopentyl moiety, 3, optimized the saluretic effect of the series.[10] Thus, uricosuric saluretics were synthesized which were comparable to probenecid and the loop diuretics in their respective intrinsic pharmacodynamic responses. Clinical studies



1 R=H, R'=

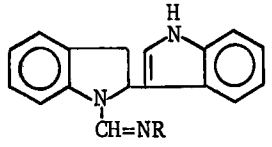


2 R=CH_3, R'=

3 R=CH_3, R'=C_6H_5

have confirmed the marked diuretic, saluretic and uricosuric activities observed in chimpanzees.

Heterocyclic Compounds - Structure-activity comparisons of a series of 1-imidoyl-2-(2-and 3-indolyl)indolines revealed that the indoline nitrogen must be maintained in an amidine configuration if diuretic activity is to be retained. The t-butyl compound in the 2,3-subseries (MJ-8592-1) was the most active compound in either group. MJ-8592-1 and the corresponding isopropyl compound, 4, produced substantial increases in the levels of volume excreted and natriuretic response with minimal kaliuresis.[11] In adrenalectomized rats, MJ-8592-1 produced dose related electrolyte excretion responses in both the presence and absence of exogenous DOCA. Thus, MJ-8592-1 counteracted mineralocorticoid-induced $Na^{\oplus}$ retention in the distal tubule but was also capable of increasing $Na^{\oplus}$ excretion by a mechanism independent of interactions with the adrenal corticol steroids or the tubular sites influenced by those hormones.[12]



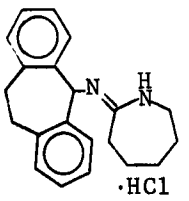
R=C(CH$_3$)$_3$ MJ-8592-1
R=CH(CH$_3$)$_2$ 4

Grisar et al[13] prepared a series of lactamimides for evaluation as hypoglycemic and diuretic agents. Two compounds in particular, RMI 11842 and RMI 11749, showed a favorable $Na^{\oplus}/K^{\oplus}$ excretion ratio when tested by the method of Lipschitz;[14] however, when evaluated in mannitol infused dogs by the method of Beyer[15] the lactamimides caused considerably less $Na^{\oplus}$ and $Cl^{\ominus}$ excretion.

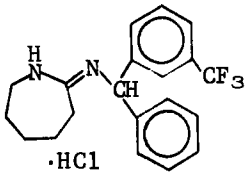
A number of 2,3-disubstituted-1,6-naphthyridines were synthesized by Hawes and co-workers[16] based on their structural relationship to triamterene. One compound, 5, was as natriuretic as triamterene and hydrochlorothiazide (HCT) at 15 mg/kg p.o. but was devoid of anti-kaliuretic activity.

The potassium-sparing attributes of azolimine (Cl 90748) and clazolimine (Cl 88893) have been reported.[17] In combination with HCT, the $Na^{\oplus}/K^{\oplus}$ excretion ratio was markedly increased.

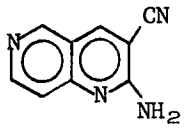
Cl 90748 R=H
Cl 88893 R=Cl



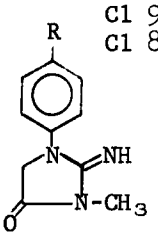
RMI 11749



RMI 11842

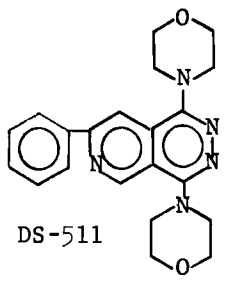


5



Yurugi and co-workers prepared a series of pyrimido(4,5-d)-,[18] pyridazino-(4,5-c)-[19] and pyrido(3,4-d)pyridazines[20] as diuretic agents. Several members of each series displayed marked diuretic activity, and one member of the latter series, DS-511, was studied extensively in rat, dog and man.[21] The diuretic profile of DS-511 was similar to that of HCT, however, DS-511 was more water diuretic and significantly less kaliuretic. Studied

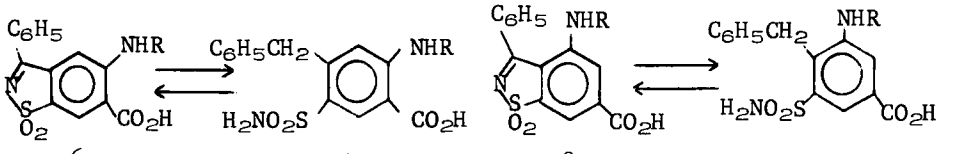
in combination with known diuretics (e.g., HCT, acetazolamide and triamterene), DS-511 produced a synergistic natriuretic effect without modifying the kaliuretic effect of the co-administered diuretic, leading to the conclusion that the two compounds were acting at different sites in the renal tubule.



DS-511

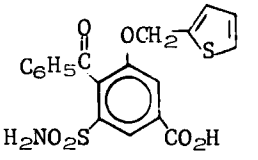
Sulfamoyl Benzoic Acids and Hydrazides - Continuing to explore the effects of modifying the ring substituents of m-sulfamoylbenzoic acids, Feit and colleagues have reported extensively on 2- and 3-substituted amino-4-benzyl-5-sulfamoylbenzoic acids and their related 1,2-benzisothiazole-1,1-dioxides.[22,23] The high diuretic and saluretic potency of the benzisothiazoles was explained as being due to an equilibrium existing in aqueous solution between these compounds, 6, 8, and the corresponding ring opened sulfonamides. The dependence of diuretic potency on structural changes was more pronounced in the anthranilic acid, 6, 7, than in the 3-aminobenzoic acid, 8, 9, series. In analogy with the relative potencies of furosemide and bumetanide, furfurylamino appears to provide maximal potency in the anthranilic (furosemide) series, whereas butylamino and benzylamino are optimal in the m-aminobenzoic acid series. One compound (9c, besunide) was chosen for further investigation.

In two subsequent papers, it was demonstrated that certain 3-alkylthio-4-phenoxy- and 3-alkylthio-4-phenylthio-5-sulfamoylbenzoic acids possessed equipotent diuretic activity to their progenitor 3-alkylaminobenzoic acids.[24,25] Concurrently, it was concluded that the influence of aryl moieties attached by NH, O, S, SO, SO_2, CO, or CH_2 to the 4-position of the sulfamoylbenzoic acid diuretics was largely a steric factor rather than physicochemical in nature. Thus, compounds (e.g., 10) were designed that were saluretic at doses as low as 1 µg/kg i.v. in dogs.



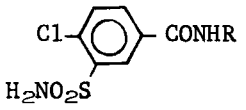
6 7 8 9

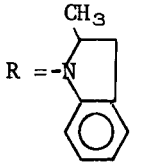
R = a, $CH_2C_6H_5$
b, $CH_2C{=}CHCH{=}CHO$
c, n-butyl



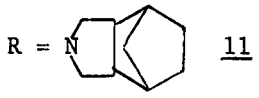
10

Considerable clinical data have been obtained in normal,[26,27] hypertensive[28] and edematous[29] subjects on the diuretic indapamide (S-1520). In comparison with furosemide,[30] S-1520 was shown to be a longer acting diuretic. S-1520 and furosemide provoked similar increases in diuresis,





S-1520



11

but the former drug induced a larger increase in sodium and chloride excretion, which lasted more than 24 hours. The studies suggest, however, that S-1520, in common with many other diuretics, may produce hypokalemia, hyperglycemia and hyperuricemia upon extended administration. Another closely related structure, 11, has been reported to be a potent antihypertensive diuretic in oral rat studies.[31]

Natriuretic Hormone - For two decades it has been known that expansion of plasma volume by administration of saline increases natriuresis.[32-34] The investigations of de Wardener et al,[35,36] which seemed to indicate that the natriuretic effect was independent of dilution effect, renal nerve stimulation, or a rise in arterial pressure and, therefore, must be due to change in concentration of some circulating factor, lent impetus to the search for a natriuretic hormone. In the opinion of some, there is considerable evidence for the existence of such an entity.[37]

This evidence rests on the fact that plasma or urine fractions taken from volume expanded or salt loaded animals show the presence of an inhibitor of toad bladder sodium transport or will cause natriuresis upon intravenous administration to a non-expanded animal. This has been demonstrated in many experiments of which a few examples suffice to show the procedure. Urinary extracts prepared from human subjects on high and low salt intakes were injected into rats. The extracts from the first group increased natriuresis while those from the latter did not.[38] When dogs were administered desoxycorticosterone (DOCA) (a sodium retaining steroid), at first sodium was retained but eventually there was "escape" of sodium (natriuresis). Upon the onset of natriuresis, but not before, ultrafiltrates of jugular plasma tested on toad bladder show inhibition of sodium transport.[39]

Since all clinically used diuretics have well-known side effects, a hormone producing natriuresis and only natriuresis would provide an approach to therapeutic management. The isolation and identification then becomes a key to any progress in this direction. The chemical evidence suggests that it is a peptide of a molecular weight variously thought to be 10,000 to 50,000,[40,41] 1000 to 1500,[42] or even less than 1000.[43] There is also evidence that it arises in the posterior lobe of the pituitary.[44]

In a preliminary manner, Kaplan et al[43] report the isolation of at least seven peptide peaks by gel filtration of urine or plasma from uremic patients. Only two of these have natriuretic activity; one is highly acidic and the other basic. Separation and characterization of these two entities is in progress to determine if a sample of the long-elusive natriuretic hormone has been isolated.

Other investigators doubt the existence of a natriuretic hormone.

After an investigation of synthetic and natural polypeptides related to the pituitary, Cort[45] concludes that there is probably no such molecule as "natriuretic hormone", at least in the pituitary, in the sense of a single, highly specific, hitherto unknown molecule of high natriuretic potency, but that there can be a "natriuretic potentiator", which, when released, can, in combination with known peptide hormones (e.g., vasopressin, oxytocin), produce an altered renal response.

The status of natriuretic hormones has been reviewed in depth by Levinsky.[46] It is his conclusion that plasma volume expansion triggers a series of events that leads to intrarenal vasodilation due to intrarenal release of bradykinin (or less likely, a prostaglandin). Subsequent increased renal blood flow is depicted as being the fundamental step in a process by which sodium excretion is increased. In his opinion, the involvement of any "natriuretic hormone" other than circulating catechols and renal vasodilators is open to question.

Prostaglandins - Extensive investigations destined to ultimately unravel the fundamental physiological functions played by the renal prostaglandins (PGE_2, PGA_2 and $PGF_{2\alpha}$) in the renally mediated maintenance of body fluid homeostasis have continued at an accelerated pace during the past two years and constitute the subject of several excellent, recent reviews.[47-50] Although often conflicting, experimental evidence continues to mount suggesting that prostaglandins A and/or E may function as intrarenal natriuretic hormone(s). Prostaglandins A, E and F were detected recently in ten-week old fetal human kidneys.[51]

Attalah and Lee[52] recently observed that in saline loaded rabbits, low sodium excretion levels (~12 meq/day) were paralleled by extremely high PGA_2 levels in papilla trailing off to relatively low PGA_2 levels in outer medulla and, ultimately, to extremely low PGA_2 levels in the renal cortex, whereas, in animals excreting high amounts of sodium (~56 meq/day), the opposite PGA_2 concentration trend was found. In addition, administration (i.m.) of indomethacin, a potent PG biosynthesis inhibitor, led to both lowered PGA_2 tissue levels (except in the cortex) and a decrease in sodium excretion (~57.6 ± 5.0 to 34.9 ± 4.2 meq/day). Since Larsson and Änggård[53] have shown that 15-hydroxy PG dehydrogenase levels are high in the rabbit cortex and low in the outer medulla and papilla and Lee *et al*[54] recently confirmed that the cortex is the major site of PGA_2 degradation *in vivo* via injection of ^3H-PGA_2 directly into the rabbit renal artery, the above observations led Lee to postulate, but yet to prove, that PGA_2 may be an important factor mediating natriuresis accompanying saline loading. Lafferty *et al*[55] demonstrated that PGA_2, but not PGE_2, markedly inhibits oxygen consumption and $Na^{\oplus}/K^{\oplus}$-ATPase activity in both renal cortex and outer medulla slices; hence, this evidence suggests that PGA promoted natriuresis may result from metabolic inhibition of the energy sources required for tubular sodium reabsorption.

The genesis of PGA_2 (medullin) remains unresolved to date. McGiff *et al*[47,48] believe that PGE_2, biosynthetically generated via endoperoxide isomerase mediated heterolysis of the endoperoxide PG precursor,[56] must

give rise to PGA_2 via an as yet unknown mechanism, since he has consistently failed to demonstrate[57] PGA_2 biosynthesis in both rabbit and dog renal medullary preparations after addition of either labelled arachidonic acid or PGE_2. These observations coupled with his earlier observation[58] that the renal vasodilator-diuretic activity of PGA_2 is only one-fifth that of PGE_2 led McGiff[47] to conclude "that PGA_2, if present in the kidney, is present in amounts which are not readily detected and that this problematic renal production of PGA_2 could not account for the high peripheral blood levels reported[59] for PGA compounds in man." In addition, McGiff does not believe that PGA_2 of renal origin functions as an antihypertensive substance.

Montgomery and colleagues[60] recently investigated the relative effects of PGA_1, sodium ethacrynate and placebo (5% dextrose injection) in normotensive human volunteers maintained on restricted sodium diets and, contrary to earlier investigations[61-64] in patients with hypertension or various edematous states, found that PGA_1 failed to elicit diuresis. Since Zusman et al[65] had demonstrated previously that restricted sodium diets resulted in elevated PGA plasma levels in normal volunteers, Montgomery and colleagues feel that their failure to observe diuresis following exogenous PGA_1 administration suggests that PGA_1 may exert its diuretic effects by a mechanism yet to be defined and not operant in normal physiologic states.

Recently, Williamson et al[66] reported that the well documented increase in renal blood flow (RBF) induced by "loop diuretics" (ethacrynic acid and furosemide) may be mediated by PGE release. This postulate is based on studies in dogs which revealed increased PGE levels (determined by radioimmunoassay) in renal venous blood post loop diuretic administration via direct injection into a renal artery but no increase in either PGE levels or RBF, if the animals were pretreated with indomethacin. However, these results, as well as much of the published literature, must be viewed with caution, since, as aptly pointed out by McGiff et al,[47] amongst others, experimental observations based upon radioimmunoassays may be deceiving due to insufficient inherent discrimination amongst closely related chemical structures. Likewise, the use of non-specific PG biosynthesis inhibitors, such as indomethacin, i.e., agents inhibiting biosynthesis of all PG's rather than a specific class, such as PGE's, etc., fail to provide information necessary to implicate a specific PG(s) as the active agent(s).

In conclusion, although the information cited herein undoubtedly indicates that PG's are intimately involved in normal kidney function and, probably in the mechanism of various renal disorders as well, determination of their precise physiological role(s) in the kidney and subsequent utilization of this knowledge by medicinal chemists for design of future diuretic agents, awaits development of major advances in cellular and physiological endocrinology research, more reliable and sensitive PG assay techniques and as yet unavailable specific PG biosynthesis inhibitors or PG antagonists to facilitate their thorough investigation at truly endogenous, rather than artificially high, exogenous levels.

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Chapter 9. Pulmonary and Antiallergy Drugs

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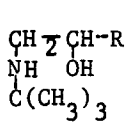
Asthma may be defined as a type of reversible, obstructive pulmonary disease due to widespread narrowing of the bronchial airways and edema of the bronchial mucosa, caused by specific allergic and/or nonspecific irritative stimuli.[1,2] While the common endpoint of the asthmatic response is airway narrowing and difficulty in breathing, several factors may cause or predispose to the condition. Several theories proposing autonomic imbalance in the lung of the asthmatic have been suggested. These include a generalized decrease in beta-adrenoceptor responsiveness,[3] an elevated reflex cholinergic activity[4] and an elevated alpha-sympathomimetic activity[5,6]; such factors could contribute in varying degree to the asthmatic response in different patients. This proposed autonomic imbalance need not be confined to the smooth muscle of the lung but may involve other cells such as lymphocytes[3] and basophils[7] as well as other tissues, such as the adrenal medulla and liver.[3] The responsiveness of bronchiolar smooth muscle to histamine or slow reacting substance of anaphylaxis (SRS-A), mediators released following antigenic provocation, is enhanced in the asthmatic and the total response may also include the discharge of mucus and inflammation. Therapy for asthma may be directed against any or all of these factors.

Beta-Adrenoceptor Agonists - Several beta-adrenoceptor agonists which selectively stimulate beta-2 receptors (mediating bronchodilatation) relative to beta-1 receptors (mediating cardiac stimulation) are being studied.[8] Pyrbuterol (1) has been tested in man and was active orally at doses of 2, 5, or 10 mg for up to 6 hr after administration.[9] Further work on isoetharine, a selective beta-adrenoceptor agonist known since 1950, has been directed toward a separation of erythro and threo isomers.[10] The racemic erythro and (-) erythro isomers were the most active forms tested for relaxation of trachea, stimulation of atria and reduction of rat passive cutaneous anaphylaxis.

A series of 2-, 5-, and 6- chloro substituted analogs of isoproterenol was prepared in an attempt to find potent and broncho-selective bronchodilators with a long duration of activity.[11] Substitution of position 2 of isoproterenol and several N-substituted derivatives generally afforded compounds with greater potency than their nonchlorinated counterparts whereas substitution in the 5 or 6 position decreased beta-adrenoceptor potency as determined in vitro using relaxation of guinea pig trachea and increased rate of right atrial contraction. A high degree of tracheobronchial vs. cardiac specificity was not obtained. Chlorination of the 2 position of isoproterenol did not alter duration of bronchodilator activity. Schwender, et al.[12], reported on the bronchial selectivity of (2). In vivo tests in dogs and guinea pigs as well in vitro

evaluation using guinea pig trachea and atria indicated that this compound possessed a selective action for bronchiolar vs. cardiac beta receptors. While the potency of this new selective beta agonist was 1/100 that of salbutamol, its maximum efficacy was similar.

Several papers reported on the bronchoselectivity in man of NAB365 (clenbuterol, 3) which at an oral dose of 10 or 20μg was found to be similar in bronchodilating potency to a p.o. dose of 2.5 mg of terbutaline or 2.0 mg of salbutamol.[13-15] There was a significant bronchodilator response following administration of each drug. Following an oral dose of 20μg, the bronchodilator response was evident after 15 minutes, reached a maximum after 2-4 hours and began to fall within 5-7 hours. With regard to cardiovascular effects, one study indicated that no increase in pulse or arterial pressure occurred after long periods of treatment.[14] Another study indicated a minor decrease in heart rate 10 and 30 minutes after p.o. administration of either NAB365 or fenoterol.[15]



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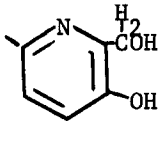

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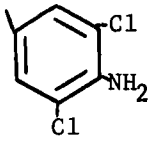

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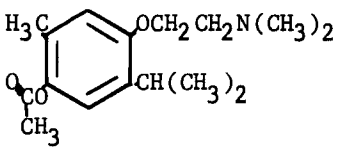


thymoxamine

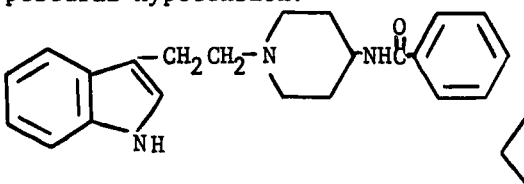
Although several β-adrenoceptor agonists have selectivity for bronchiolar, beta-2 (relative to cardiac or beta-1) adrenoceptors, these newer agents given p.o. tend to produce tremor. Incidence of tremor may be as high as 40%.[16] The tremor response is due to direct stimulation of beta-adrenoceptors which appear to be of the beta-2 type. Separation between bronchodilator and tremorogenic response has not been reported.

Finally, since decreased beta responsiveness has been implicated in asthma for certain cell types such as lymphocytes[3] as well as for smooth muscle and since histamine synthesis[17] and release from lung tissue following antigenic challenge[18] is decreased by beta agonists, the beneficial role of these drugs may be due to several mechanisms.

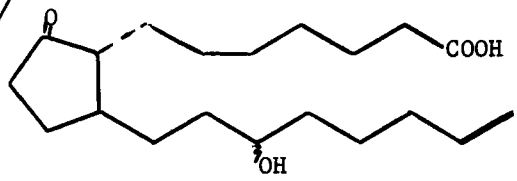
Alpha-Adrenoceptor Antagonists - The presence of alpha-adrenoceptors mediating bronchiolar smooth muscle contraction has been reported in both humans and animals.[19,20] The therapeutic utilization of this concept was first reported by Marcelle,[21] who treated six refractory asthmatics with phentolamine (5 mg by inhalation). There was improvement in lung function which lasted for 18 hours. Inhibition of histamine-induced or exercise-induced bronchospasm has been reported following administration of alpha-adrenoceptor blocking agents such as phenoxybenzamine,[7] indoramin,[22] phentolamine,[7,23] or thymoxamine.[24] Also, although a direct bronchodilator response may not be observable, alpha-adrenoceptor blockade may enhance the response to beta-adrenoceptor agonists. Thus, Palmer, et al.,[25] reported that a significantly greater increase in forced expira-

tory volume in one second ($FEV_{1.0}$) was obtained when alpha blocking drugs were administered with salbutamol, than when salbutamol was given alone; the alpha-adrenoceptor blocking drugs had no greater effect than did the placebo. Apart from alpha blockade in smooth muscle, it has been reported that alpha-adrenergic stimulation (phenylephrine or norepinephrine in the presence of propranolol) enhanced immunologic release of histamine and SRS-A from human lung tissue.[26]

It is well known that alpha-adrenoceptor blocking agents have several pharmacologic activities. For example, phenoxybenzamine has antihistaminic and weak anticholinergic properties and blocks uptake of catecholamines; phentolamine increases circulating catecholamines; and thymoxamine and indoramin have antihistaminic properties in the same order of potency as their alpha-blocking effects.[27,28] All of these pharmacologic actions could contribute to the observed beneficial response in histamine- or exercise-induced asthma. A prominent side-effect of alpha-blockade is postural hypotension.



indoramin



AY 22093

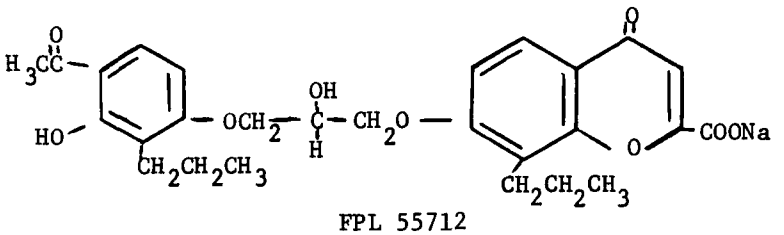
Anticholinergics - The exact role of anticholinergics in the therapy of asthma is still not defined. While bronchoconstriction, probably reflex in nature, is blocked by atropine or vagotomy in experimental animals and by atropine in man, the drying effects of anticholinergics remain an important consideration. Sch 1000 (isopropyl atropine) has been evaluated in several acute tests. Ulmer[29] reported that Sch 1000 had a "better therapeutic range" than atropine methyl nitrate or certain beta-adrenoceptor stimulants in "chronic obstructive airways disease". Sch 1000 was reported to be a more effective bronchodilator than salbutamol in bronchitics,[30] while the reverse relationship was reported in asthmatics.[31] Kaik[32] reported that Sch 1000 and fenoterol (Th 1165a) had approximately the same average bronchodilator effects in 20 patients, but that the two drugs did not produce similar bronchodilatory responses in the same individual. This finding suggests that only certain patients may benefit from anticholinergic therapy.

The acute effects of Sch 1000 on parotid secretion of saliva in human volunteers has been reported. Sch 1000 was similar in potency to atropine in blocking salivation[33] due to acetyl-β-methylcholine. Knowledge of the effects of long term therapy with anticholinergics such as Sch 1000 in different types of obstructive lung disease would be valuable.

Prostaglandins - Since the lung is capable of rapid synthesis of $PGF_2\alpha$ and since asthmatics are extremely sensitive to the bronchoconstrictor effects of this prostaglandin, it is tempting to speculate on the importance of this substance in the pathogenesis of asthma. The major metabolite of $PGF_2\alpha$ in the plasma, 15-keto-13,14-dihydro-$PGF_2\alpha$, increased by up to 8-fold in peripheral venous blood following antigen-induced asthmatic attacks in 5 subjects.[34] The increase in the $PGF_2\alpha$ metabolite could be demonstrated within a few minutes after commencing the antigenic provocation and "seemed to be correlated to the severity of the attack"[34] However, other investigators reported that indomethacin in doses reported to suppress over 90% of endogenous prostaglandin synthesis in man has no effect on either antigen-induced or exercise-induced asthma.[35] In guinea pigs sensitized to ovalbumin, elevated urinary levels of a $PGF_2\alpha$ metabolite, 5α, 7α-dihydroxy-11-ketotetranor prostanoic acid, are found during the anaphylactic response.[36] Indomethacin (25-50 mg/kg p.o.) reduced the basal excretion of the PGF metabolite and also abolished the expected rise on challenge with antigen. However, the guinea pigs were not protected from respiratory distress and convulsions; no pulmonary mechanics were measured. In contrast, in cats, inhibition of prostaglandin biosynthesis with aspirin reduced pulmonary vasoconstrictor and bronchoconstrictor responses to hypoxic breathing and it is reported that hypoxic ventilation of isolated perfused cat lungs caused the appearance in pulmonary perfusates of prostaglandin-like substances.[37] It appears that although $PGF_2\alpha$ is liberated during bronchospastic conditions due to several initiating factors, its role as a primary mediator of asthma is not yet substantiated.

A new bronchodilator compound, a prostanoic acid derivative (AY22093) was compared to isoproterenol and PGE_2 in several pharmacologic tests predictive of clinically efficacious bronchodilatation.[38] AY22093 was similar in profile to PGE_2 but was less potent.

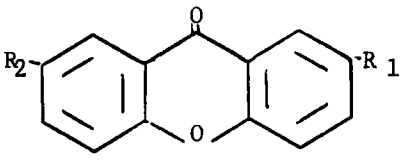
Specific Inhibitors of Anaphylactic Mediators - Eosinophilia is a common occurrence in atopic individuals, especially allergic asthmatics. While early work suggested that antigen-antibody complexes caused eosinophil migration,[39] later studies showed that soluble mediators from anaphylaxis were responsible.[40] A specific chemotactic factor for eosinophils (ECF-A) has been identified[41,42] from human or guinea pig lungs. Wasserman, et al., have shown that the factor exists preformed in the mast cell granule.[43]



FPL 55712

FPL 55712, a compound that had been shown to be a specific blocker of SRS-A[44], has now been shown to be an inhibitor of eosinophil chemotaxis by ECF-A[45] with an IC_{50} also of 3.8×10^{-7}M. Whether such a compound will have clinical utility in asthma is still unknown but might help to define the role of SRS-A and ECF-A in this disease.

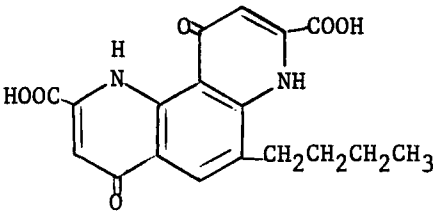
Anaphylactic Inhibitors - With the discovery of new mediators of immediate hypersensitivity almost every year it is clear that the mechanism of allergic asthma is more complex than the concept of histamine impinging on bronchial smooth muscle. An active research area is the search for agents that inhibit the allergic reaction at its inception. Cromolyn sodium (a compound that inhibits the release of allergic mediators from sensitized tissues but does not interfere with the combination of antigen and antibody) is the prototype for this type of drug but suffers from a lack of oral activity and from a limited solubility which precludes aerosolization. Three xanthones with similar antiallergy profiles have been studied in various animal models and man.[46] They are AH 7725, AH 7079, and AH 6556.



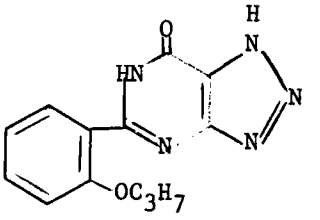
	R_1	R_2
(4)	-COOH	$CH_3S(=O)_2$-
AH 7725	-COOH	$HOCH_2CH_2O$-
AH 7079		CH_3O-
AH 6556	-COOH	CH_3O-

All three compounds inhibited antigen-induced histamine release from chopped human lung *in vitro* at about 10^{-4}M.[47] They also inhibited γ_1 antibody-mediated release from guinea pig lung *in vitro* ($IC_{50} \simeq 10^{-5}$M). AH 7079 was about equipotent to cromolyn *in vitro* against antigen-induced histamine release from rat peritoneal mast cells. When bee venom was used to release histamine from rat mast cells AH 7725 was equipotent to cromolyn but AH 7079 was markedly less so. AH 7079 as an aerosol preparation (15 and 30 mg/ml) was effective in allergic bronchial challenges in

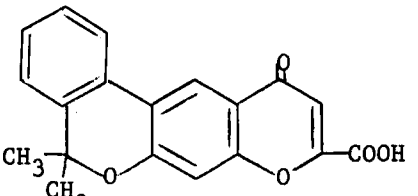
man[48] while AH 7725 was orally active at a dose of 500 mg[49] in 30 ml of water 2 hours before antigen challenge. Clinical data on the oral activity of a similar compound (4) at 8 mg/kg, 2 and 5 hours before testing, showed a statistically significant inhibition of exercise-induced asthma; the clinical utility of this agent is still under study.[50]



ICI 74,917



M&B 22,948

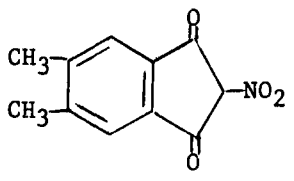


PR-D-92-EA

A tricyclic quinolone acid ICI 74,917 was found to be about 300 times as potent as cromolyn sodium in rat passive cutaneous anaphylaxis (PCA) and devoid of antimediator effect. In the guinea pig it would not block PCA but would inhibit allergic bronchoconstriction while lacking direct bronchodilating activity[51] (suggesting tissue specificity for the compound in this species). Another compound, M&B 22,948 an azapurinone, antagonized allergen-induced release of histamine in passively sensitized human lung, PCA in the rat and reagin mediated anaphylactic bronchospasm in the guinea pig.[52] This compound does have some direct antimediator activity against histamine, SRS-A and prostaglandin F2α.

PR-D-92-EA inhibited PCA in rats and blocked Ascaris-induced bronchospasm in Rhesus monkeys. PR-D-92-EA blocked responses produced by bradykinin, serotonin, SRS-A, PGE_2 and $PGF_{2\alpha}$ but not those of histamine on isolated guinea pig ileum.[53]

Of a series of 2-nitro indan-1,3-diones, (5) was the most potent in rat PCA.[54]



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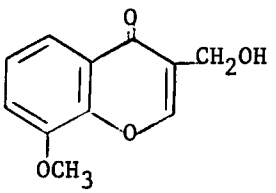
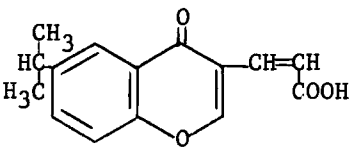


W8011



(6)

New synthetic methods have permitted the exploration of 3-substituted chromones. Of a large series of 3-carboxy,3-hydroxymethyl and 3-formyl chromones the most active was W8011 which had an oral ID_{50} of 2 mg/kg and an i.p. ID_{50} of 1 mg/kg in rat PCA.[55a,b,c] In another series (6) was most potent (ID_{50}</mg/kg i.v.).[55d]

Corticosteroids - Interest remains high on aerosolized steroids for treatment of asthma. Two recent reviews stress the adrenal-sparing effect of these preparations.[56,57] Similar results have been found with aerosolized preparations of triamcinolone acetonide.[58]

In a different approach the macrolide antibiotic troleandomycin (TAO) was used to reduce the dose of oral methyl prednisolone needed by steroid-dependent asthmatics.[59] With 250 to 500 mg/day of TAO, about 70% of the patients significantly reduced their steroid requirements with no untoward side-effects. This activity of TAO is unrelated to its antibiotic activity.

Emphysema - Emphysema is an anatomic alteration of the lung characterized by an abnormal enlargement of the air spaces distal to the terminal, non-respiratory bronchiole, accompanied by destructive changes of alveolar walls. Several agents have been used to produce emphysema-like conditions in animals, with papain being the agent most used.[8]

Progesterone, which prevents papain-induced emphysema in rats, has now been evaluated in an existing emphysema-condition in rats and was found to have a therapeutically beneficial effect.[60] The mechanism of the beneficial response, whether prophylactic or therapeutic, is unknown.

Although progesterone elevates α_1-antitrypsin in humans and a deficiency of α_1-antitrypsin has been associated with panlobular emphysema in man, the mechanism of progesterone prophylaxis in rats does not appear to be due to alterations of serum antitrypsin.[61] In other studies, Martorana, et al.[62] reported that intracardiac administration of human α_1-antitrypsin 1 hour prior to exposure to papain in hamsters did not prevent emphysema-like changes, but that intratracheal instillation of α_1-antitrypsin 1 hour before exposure significantly prevented the development of the lesion. This finding gives further support to the concept of an intra-alveolar (rather than circulating) antiprotease as a factor in the pathogenesis of emphysema. A lung antiproteinase, present at the air-lung interface, has been isolated by saline lavage of freshly excised dog lungs. Immunologic techniques demonstrated that there were differences between this lung antiproteinase and serum antiproteinase. The serum and lung antiproteinase showed differential inhibitions of elastase and trypsin.[63]

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Chapter 10. Agents Affecting Gastrointestinal Functions

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Introduction – Drug research on disorders of the gastrointestinal system continues to concentrate on peptic ulcer disease. Therapeutic management of this malady in general aims at reduction of gastric acid secretion and drug research has therefore focused on approaches and agents that will accomplish this in man. In the two-year period 1973-1974 outstanding developments directed towards peptic ulcer therapy have been (1) the discovery and development of the histamine H_2 antagonists, burimamide and metiamide (2) the clinical testing of several classes of antisecretory prostaglandins and (3) research on the identity and actions of gastrointestinal hormones. Significant developments in these areas constitute the major emphasis of this review.

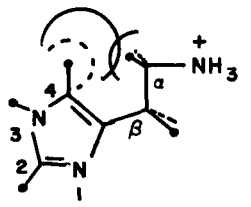
Summaries of gastric antisecretory and antiulcer agents[1] including prostaglandins[2,3,4] and antisecretory antihistamines, have been published. The physiology and chemistry of secretin, gastrin, pancreozymin[5] and gut hormone candidates[6] have also been reviewed. The proceedings of an international symposium on H_2 antagonists[7] should be consulted for a more complete perspective of this area.

Histamine H_2 antagonists – Two types of histamine receptors (H_1 and H_2) have been proposed.[8] Since conventional antihistamines block H_1 receptors but fail to inhibit acid secretion stimulated by histamine at gastric H_2 receptors, Black and his associates at SK&F chose to modify the agonist, histamine, in a search for a competitive H_2 antagonist.[7] Their successful development of the histamine H_2 receptor antagonists, burimamide and later metiamide, is an excellent example of a reasoned approach to drug discovery (see below). The availability of selective histamine antagonists may help define the role of histamine in the gastric acid secretory process and clarify the clinical usefulness of a gastric antisecretory agent in ulcer therapy.

Histamine agonist activity – Initially the SK&F group defined the structural features responsible for H_1, as opposed to H_2, agonist activity in histamine. Employing extended Huckel theory (EHT) calculations, which were in agreement with NMR conformational interpretations,[9] side chain trans/gauche conformer populations were examined for α – and β –methyl and N,N-dimethyl histamine. Although these were quite different no selectivity in H_1 or H_2 agonist action was observed,[10] a result not predicted by Kier's hypothesis that H_1 agonist activity was associated with the trans and H_2 agonist activity with the gauche side chain conformation.[11] Moreover, a decreased trans rotomer preference in α – and β –methyl histamine resulted in reduction of both H_1 and H_2 activity suggesting the involvement of the trans conformer at both types of histamine receptor.

4-Methylhistamine – Since 4-methylhistamine selectively stimulated H_2 receptors (H_2/H_1 ratio = 200) an effect of the methyl group on the orientation of the imidazole ring relative to the side chain was postulated.[12] EHT calculations on the N_3-H tautomer of the 4-methylhistamine monocation indicated that the C_4-methyl influenced the orientation of the imidazole ring relative to the side chain. Coplanarity of the ring with the C $\alpha - \beta$ side chain carbon bond was hindered by steric interaction between the methyl and the α –methylene of the side chain.

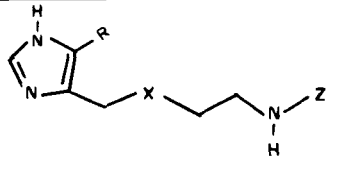
The H_1 conformation – Since conformations unfavorable for the H_2 agonist, 4-methylhistamine, might be associated with effective drug interaction at H_1 receptors Ganellin was able to define an "H_1-essential" conformation for histamine in which the trans side chain is fully extended.[12] The carbon and nitrogen atoms are coplanar with the ring and a maximum distance of 5.1 A separates the charged ammonium group from the ring N_1-nitrogen atom. The Ganellin hypothesis differs from the Kier hypothesis in that the relationship of the imidazole ring to the side chain is emphasized rather than the conformation of the side chain. Furthermore, the Ganellin hypothesis requires a histamine conformation 3Kcal above the calculated energy minimum.



Steric interaction between the C_4-methyl & α-methylene in 4-methyl histamine

	SUBSTRATE	$N_1 - NH_3^{\oplus}$ distance
Ganellin	Histamine H_1 conformation	5.1 Å
	4-methyl histamine	4.37 or 3.14 Å
Kier	Histamine H_1 conformation	4.55 Å
	Histamine H_2 conformation	3.6 Å

Chemistry of H_2 antagonists – An early approach was based on analogy with adrenergic β -receptor stimulants and their antagonists. Removal of the acidic hydrogen atom from the histamine imidazole ring by fusion with a benzene ring[13] or addition of lipophilic substituents to the imidazole ring[7] failed to yield an H_2 antagonist. The SK&F group retained the essential features of the imidazole and 4-methylimidazole ring and modified the histamine side chain. Increase in histamine chain length from 2 to 4 carbons and incorporation of the nitrogen into a non-basic thiourea moiety resulted in the discovery of the H_2 antagonists burimamide and the less potent 4-methylburimamide. Gastric antisecretory activity followed intraperitoneal but not oral administration of burimamide.[14,15] Inhibition of histamine or pentagastrin stimulated acid secretion was observed by intravenous infusion in man[16] suggesting the need for a related orally active compound.



R = H, X = CH_2, Z = $\overset{S}{\overset{\|}{C}}-NHCH_3$ burimamide

R = CH_3, X = S, Z = $\overset{S}{\overset{\|}{C}}-NHCH_3$ metiamide

R = CH_3, X = S, Z = $\overset{N-C\equiv N}{\overset{\|}{C}}-NHCH_3$ cimetidine

Chemical rationale for metiamide — Using pKa arguments, the preferred tautomer (80%) of the histamine monocation in aqueous solution was calculated to be the N_3-H form.[17] Since burimamide was predicted to exist primarily in the N_1-H form, structural modifications such as an increase in ring electron density at C_4 and decrease at C_5 were made to favor the N_3-H tautomer and to enhance activity. The reduced activity of 4-methylburimamide was attributed to protonation of the imidazole ring at physiological pH (7.4), suggesting the advisability of lowering overall electron density in the ring. Based on the known bioisosterism of S for a methylene group and electron withdrawing character of S relative to carbon, a methylene unit in methylburimamide was replaced by S thus favoring the N_3-H tautomer and resulting in an imidazole ring pKa (6.80) somewhat closer to that of histamine (5.90). The resulting compound, metiamide, proved more potent than either burimamide or methylburimamide[18] and was orally active.

Pharmacology of H_2 antagonists — Inhibition of pentagastrin, histamine, food and vagally stimulated acid output by burimamide and metiamide[19,20,21] is consistent either with histamine being the final common mediator of acid secretion for these stimuli or with the hypothesis that H_2 receptor blockade alters receptor properties of the parietal cell for other agonists.[21] Metiamide's antisecretory effect was not associated with inhibition of gastrin release since at antisecretory doses serum gastrin levels were unchanged in dog[22] and man.[23,33] Burimamide[24,25,26] and metiamide[27] but not H_1 antagonists inhibited histamine stimulated adenyl cyclase which supports the role of c-AMP as the mediator in the action of histamine on gastric acid secretion.

Pepsin, in addition to gastric acid, is an "aggressive" factor in duodenal ulcer disease. In dogs[28] and man,[29] metiamide inhibited pepsin output stimulated by pentagastrin. Elevation of gastric pH following metiamide administration, probably also contributed to pepsin inactivation. Metiamide, in contrast to atropine, did not inhibit the secretion of neutralizing pancreatic bicarbonate stimulated by secretin in either dog or man.[30]

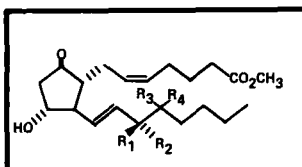
Clinical data on H_2 antagonists — Infusion of metiamide inhibited vagally induced acid secretion in healthy human volunteers[31] and in duodenal ulcer patients inhibited acid secretion and reduced pepsin output stimulated by histamine, pentagastrin, vagal stimulation (by insulin) or a peptone meal.[29] Oral doses of metiamide in the range 100 to 400 mg inhibited basal,[32] nocturnal[23] and food stimulated[33] acid secretion in duodenal ulcer patients and infusion of metiamide reduced pentagastrin stimulated acid secretion in gastric ulcer patients.[34] In a Zollinger-Ellison syndrome patient, a 200 mg oral dose of metiamide effectively reduced acid output from 20 to 4 meq/hr.[35] Following the report of two cases of reversible agranulocytosis,[32] clinical studies with metiamide were restricted in the U.S. and England.[36] Cimetidine, an H_2 antagonist similar in structure and potency to metiamide, has been tested in man. Intravenous infusion of cimetidine inhibited histamine or pentagastrin stimulated gastric secretion with an EC50 of about 2.5 μM and a half-life of about two hours. In high dose chronic toxicity studies in dogs, cimetidine, unlike metiamide, did not produce kidney damage or agranulocytosis.[37]

Prostaglandins – Improved tissue selectivity and metabolic stability has led to progress in the development of synthetic prostaglandins as therapeutic agents for gastric and duodenal ulcer disease. Recent reports also indicate that prostaglandin gastric antisecretory effects are retained in analogues with reduced stereochemical complexity.

Oral activity – Intravenous infusions, but not oral doses, of prostaglandin E_1 (PGE_1) inhibited pentagastrin stimulated acid output in man although basal secretion of acid was not affected by PGE_2 given orally.[38] Failure to observe antisecretory effects with orally administered PGE_2 apparently relates to rapid inactivation in vivo since chemical modifications of the PGE_2 structure that prevent rapid enzymatic inactivation by the enzyme, 15-OH prostaglandin dehydrogenase,[38,39] result in oral activity. Synthetic prostaglandins with this modification include 15(R) and 15(S)-methyl PGE_2, the 16,16-dimethyl PGE_2 methyl esters and the structurally more simple 11-desoxy, 15-methyl and 16,16-dimethyl prostaglandin derivatives.

Side effects – No less troublesome than the rapid metabolism of natural prostaglandins are the untoward systemic effects associated with prostaglandin administration. In the rat both 16,16-dimethyl PGE_2 and 15(S)-15-methyl PGE_2 (C-15 hydroxyl in the natural α configuration) inhibited basal and histamine or pentagastrin stimulated acid output at doses (2 μg/kg) that also caused prolonged increases in intestinal tone and motility.[40] Accordingly, it is likely that continued development of clinically useful antisecretory PGE_2 analogues will emphasize separation of gastric antisecretory from smooth muscle activities.

Clinical results for 15(S) and 15(R)-methyl PGE_2 – In man, 50 μg of intragastrically instilled 15(S)-15-methyl PGE_2 completely inhibited basal acid secretion for 90 minutes but was associated with nausea.[38] The ED50 for inhibition of pentagastrin-induced acid secretion in man was 40 μg whereas abdominal discomfort occurred with 80 μg/doses.[41] In contrast to the 15(S) isomer, a 200 μg dose of 15(R)-15 methyl PGE_2, instilled intragastrically, reduced basal[38] and pentagastrin-stimulated[42] acid secretion for two hours without side effects. This reduction in acid output probably resulted from an effect on acid secretion since no consistent alteration in volume output was noted.



R_1	R_2	R_3	R_4	
CH_3	OH	H	H	(15S)15-CH_3 PGE_2 methyl ester
OH	CH_3	H	H	(15R)15-CH_3 PGE_2 methyl ester
H	OH	CH_3	CH_3	16,16 di-CH_3 PGE_2 methyl ester
H	OH	H	H	PGE_2 methyl ester

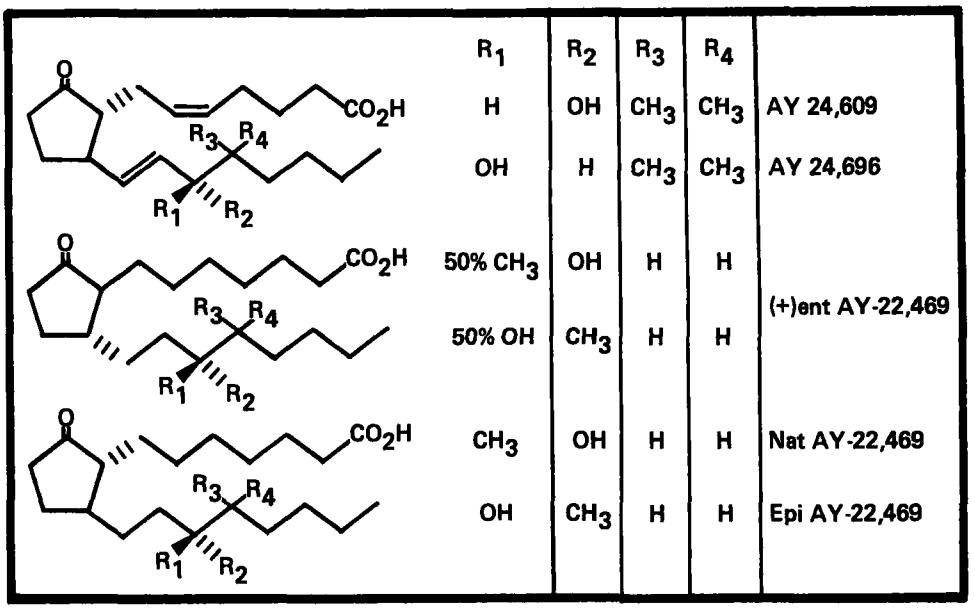
Clinical results for 16,16-dimethyl PGE_2 – Results with the dimethyl substituted PGE_2 analogues are generally similar to those cited with the 15(R) and 15(S)-methyl analogues. The ED50 for inhibition of pentagastrin stimulated gastric acid ouput was 40 μg following intragastric instillation of drug[41] and a 200 μg dose elevated the gastric luminal pH to 7.0[39] The appearance of abdominal cramping at 80[41] and nausea and diarrhea at 100 μg doses[43] suggests a limited usefulness for the 16,16-dimethyl PGE_2 analogue in treating peptic ulcer patients. Attempts to circumvent patient discomfort by giving 16,16-dimethyl PGE_2 intrajejunally resulted in a loss of the gastric acid inhibitory activity suggesting that the

mode of action may be partly topical in nature or that drug degradation is enhanced in the small intestine.[44]

Gastric antiulcer activity of 15(R)-methyl PGE_2 – A statistically significant effect on the rate of gastric ulcer healing, judged by endoscopic observation, was observed in a ten patient study using 150 μg oral doses every six hours for two weeks.[45] These results are important considering the difficulty usually encountered in demonstrating increased gastric ulcer healing in hospitalized patients who have a characteristically high healing rate.[46]

In six patients with acute gastritis, oral doses of 150 μg of 15(R)-15 methyl PGE_2 methyl ester resulted in large amounts of mucin discharging into gastric pits and an increase in viscid mucoid secretion covering the gastric mucosa.[47] The mucotropic effect of the 15(R)-methyl and possibly the 16,16-dimethyl PGE_2 analogue is reminiscent of the increased mucous secretion reported for patients receiving carbenoxolone sodium.[48] These studies suggest that synthetic prostaglandins may have both gastric acid antisecretory and mucous stimulating effects thus possibly providing a broad therapeutic approach to both gastric and duodenal ulcer disease.

Pancreatic effects – 15(S)-methyl PGE_2 inhibited acid secretion but not pancreatic bicarbonate output stimulated by exogenous secretin or duodenal acidification.[49] By way of contrast, in dogs atropine, 16,16-dimethyl PGE_2 methyl ester and PGE_1 inhibited pancreatic bicarbonate secretion,[50] a potentially undesirable action for a duodenal ulcer healing agent since neutralization of acid in the duodenum is deficient in duodenal ulcer patients.[51]



Prostaglandin SAR — In rats the C-15 epimers of 15-hydroxy-15-methyl-9-oxo prostanoic acid (AY-22,469) were similar in oral antisecretory activity, with the epimer mixture having antisecretory and antiulcer ED50's in pylorus ligated rats of 4.9 and 0.85 mg/kg, respectively.[52] Enantiomeric AY-22,469 (side chain stereochemistry reversed) had 2.8 times the antisecretory activity of the nat isomer (normal side chain sterochemistry).[53] The 15-(nat)-hydroxy-16,16-dimethyl-9-oxoprostanoic acid (AY-24,609) with antisecretory and antiulcer ED50's in pylorus ligated rats of 1.0 mg/kg and 0.12 mg/kg, respectively, was more potent than either AY-22,469 or AY-24,696 (the C-15 epimer of 24,609).[54] The high dose required to cause diarrhea in rats (ED50 = 7 mg/kg po) suggests that AY-24,609 may have some tissue selectivity.

Comparisons of potency relative to the 15(R) and (S)-methyl and 16,16- dimethyl PGE_2 methyl esters are difficult because different animal models were used in these studies. However, it is clear that reduction in structural complexity in the 11-desoxy prostaglandins is compatible with oral gastric antisecretory activity.

Gastrointestinal hormones, *gastrin heterogeneity* — The presence of at least eight different forms of the gastrin molecule complicates the interpretation of studies relating serum gastrin levels to disease states since the relationship between the immunochemical and biological properties of the various gastrins remains unclear. Nomenclature describing the different gastrin forms is unsettled; suggested terminology,[55] however, is listed in decreasing order of molecular weight. Nonsulfated and sulfated forms, designated I and II respectively, exist for G-34, G-17, and G-13 but the biological significance of sulfation remains unclear.[56]

Designation	Mol. Wt.	No. Amino Acids	Biologically Active
A. Big, Big Gastrin (BBG)	>20,000	Unknown	Unknown
B. Component I	Unknown	Unknown	Unknown
C. G-34 (Component II, Basic Gastrin, Big Gastrin {BG})	3,839	34	Yes
D. G-17 (Component III, Little Gastrin {LG})	2,098	17	Yes
E. G-13 (Component IV, Mini-gastrin {MG}, HG-5-13)	1,647	13	Yes

Biological activity — BBG, the dominant form of gastrin in fasting porcine, canine and human plasma,[57] has a half-life of 90 minutes[58] and exists as a minor component in the plasma of pernicious anemia and Zollinger-Ellison (Z-E) patients and in extracts of Z-E tumor.[57] Plasma levels of BBG in contrast to G-17 and G-34 did not rise following a test meal.[57] The biological significance of component I which has not been characterized beyond initial chromatographic separations[59,60] is unclear. Infusions of equimolar doses of G-34 and G-17 produced similar gastric acid secretory responses in dogs.[55] However, since the metabolic half-lives of G-34 and G-17 are 15.8 and 3.2 min., respectively, the plasma steady state levels are 5-fold higher for the G-34 form indicating that on an equimolar endogenous basis G-17 is 5 times as potent as G-34.[55]

The tridecapeptides (G-13 I and II), isolated from Z-E tumor tissue,[61] are less potent stimulants of acid secretion. G-13 is identical to the C-terminal (5-17 fragment) portion of G-17, is approximately one-half as potent as G-17 and has a half-time disappearance rate from blood of 1.8 min.[62] A second tridecapeptide, corresponding to fragment 1-13 of G-17 has been isolated from porcine antral mucosa and serum of fasting gastrinoma patients.[63] This N-terminal fragment, designated NT-G-17, does not stimulate acid secretion[64] perhaps because it does not contain the active C-terminal segment G-13. The di-, tri- and tetrapeptide amides of the C-terminal portion of G-17 are capable of stimulating acid output[65,66] but corresponding methyl esters of tetra- and tripeptides are not as potent as the amides.[66] Substitution of leucine for methionine increases the potency of the tripeptide fragment nearly two-fold.[67] The biological significance of the peptide fragments remains uncertain since small peptides will not react with most radioimmunoassay antisera.

Several peptide inhibitors of gastrin release have been identified including secretin,[68] glucagon,[69] calcitonin,[70] vasoactive intestinal peptide (VIP),[71] gastric inhibitory peptide (GIP)[71] and somatostatin.[72] Whether some or all of these peptides affect gastrin release under physiological conditions is unclear. A pH-dependent conformational change of gastrin has been proposed as a physiological mechanism for control of gastrin release.[73] The role of gastrin as a trophic hormone appears increasingly important. Pentagastrin stimulated mucosal cell growth of rat tissue both in vivo[74] and in vitro[75] and elevated the depressed DNA and RNA content in antrectomized rats.[74] Pentagastrin, but not histamine, also stimulated thymidine incorporation into the DNA of stomach, duodenum and ileum but not liver in a dose-dependent manner.[76] Gastrin may also be important to the normal ontogenic development of the gut.[77]

Secretin — Since secretin inhibits gastric acid secretion and stimulates pancreatic bicarbonate release,[78] an appraisal of its physiology is of some importance in studying the pathogenesis of duodenal ulceration. Clinical studies with secretin have been limited, in part, by short-lived in vivo effects (approximately 3 minutes)[79] and lack of oral efficacy.[5] Subcutaneous injections of slow releasing secretin formulations have a prolonged duration of action.[80,81,82] Pancreatic bicarbonate release is stimulated by inhalation of secretin snuff preparations[83] but large dose requirements may limit this approach.[84]

Attempts to measure endogenous levels of secretin by radioimmunoassay (RIA) have been difficult since secretin lacks a tyrosine that can be iodinated.[85] However, the synthesis of 6-tyrosyl porcine secretin[86] and the iodination of the N-terminal histidine[87] are major advances and have made the measurement of endogenous secretin levels possible.[87,88] Although fasting plasma secretin levels vary considerably[85] consistent increases occur following instillation of intraduodenal HCl.[87,89] In dogs, plasma secretin half-life is three minutes,[79] however, the sites and mechanisms for removal from the circulation are unclear. Bile salts also effect secretin release[90] although their physiological role, if any, is not known.

Although duodenal acidification releases secretin and results in stimulation of pancreatic bicarbonate release following a meal, two arguments suggest that secretin does not mediate gastric acid inhibition following duodenal acidification: (1) increases in plasma secretin

following intraduodenal HCl are small (approximately 3-fold) and do not correlate with the degree of acid inhibition observed[91] and (2) exogenous secretin doses that produce plasma secretin levels higher than that observed following instillation of intraduodenal HCl do not affect stimulated acid output.[91] A humoral substance other than secretin has been suggested as mediating the acid inhibition resulting from intraduodenal HCl.[92] Pancreatic bicarbonate release in response to duodenal acid loads may be defective in duodenal ulcer patients.[93] Decreased bicarbonate release could be related to a decreased sensitivity of the bicarbonate secreting cells to stimulation by secretin or a defective release of secretin.[51] It is unclear whether deficient secretin release in duodenal ulcer patients is a cause or effect of duodenal ulceration.[93] Secretin may contribute further to the defense of the intestinal mucosa by stimulating mucous output[94] since a secretin induced mucotropic effect has been shown for both cats[95] and man.[96]

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Chapter 11. Antithrombotic Agents

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In our society, clinical thrombosis is currently regarded as the number one cause of morbidity and mortality among the middle aged and elderly. When one considers that the integrity of the microcirculation is one of the most basic requirements for life itself, as well as the normal functioning of each organ, tissue and cell in the body, the potential far-reaching implications of a thrombotic episode become apparent. In addition to the major problems of coronary thrombosis, stroke and shock, many other conditions ranging from diabetic retinopathy to hyper-acute rejection of organ transplants are thought to result from impaired microcirculation stemming from various forms of intravascular thrombosis.

While anticoagulant drugs have proven useful in treating or preventing venous thrombosis, their efficacy in cases of arterial thrombosis is open to serious question. The role of platelet aggregation in arterial thrombosis has recently received a great deal of attention and a number of agents have been described which are capable of inhibiting platelet aggregation. However, the clinical utility of this class of drugs is just beginning to be explored. Fibrinolysis remains an intriguing concept for both preventing and treating intravascular thrombosis but also has yet to be proven practical for extensive clinical use. This review summarizes some of the past year's most important findings with regard to these classes of drugs.

Platelet Aggregation Inhibitors

Past reviews have described a number of new agents which had been reported as being active inhibitors of *in vitro* platelet aggregation (PA).[1-5] However, recent emphasis seems to be shifting from the use of superficial *in vitro* screening models to the development of more sophisticated *in vivo* models of thrombosis as well as in depth study of drug mechanisms. While agents currently available for clinical use leave much to be desired, they do allow us to probe deeper into the various types of thromboembolic problems and to learn much more about the underlying pathological mechanisms.

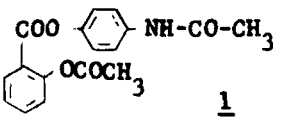
Aspirin and Other Non-Steroidal Anti-Inflammatory Agents. This class of compounds has been studied more extensively than any other with respect to the possible development of a clinically useful antithrombotic agent which acts through inhibition of PA. The current status of the clinical use of aspirin as well as several other compounds is nicely summarized in two recent reviews.[6,7] While encouraging clinical results have been obtained with aspirin, both authors indicate that further testing is necessary before its widespread use as an antithrombotic agent can be recommended. The results of two large hospital studies reported by the Boston Collaborative Drug Surveillance Group[8] and a sizable British study[9] both suggest that aspirin may be effective in preventing myocardial infarction.

While clinical evidence accumulates regarding the efficacy of aspirin

in preventing arterial thrombosis, additional supportive laboratory results continue to be reported. Vascular injury induced in rats by means of a laser[10] and in dogs by means of intimectomy[11] led to thrombosis which was effectively inhibited by aspirin pretreatment. Such evidence lends further support to the concept that aspirin may prove effective clinically in those conditions where arterial thrombosis is triggered by platelet adhesion to abnormal surfaces whether they be disrupted vascular endothelium or prosthetic devices.

Recent evidence indicates that the action of aspirin on platelets may not be totally dependent upon platelet acetylation. Various salicylate analogues were compared to aspirin for their ability to inhibit the second phase of ADP induced PA as well as PA induced by collagen.[12] Results indicate that, while the presence of the acyl group at the 2 (ortho) position of the benzene ring was necessary for activity, other structural features were also important. In addition, these same investigators demonstrated that relative degree of platelet acetylation and inhibition of PA were not similar throughout the series.[13] They, therefore, concluded that acetylation may be responsible for the long duration of the aspirin effect but the findings are not consistent with the hypothesis that acetylation of platelets by aspirin is solely responsible for its observed effects on platelet function.

The effects of two additional anti-inflammatory agents on platelet function have recently been described. Benorylate (1) and HP-129 (2) appear to be similar to aspirin with respect to profile of activity. Benorylate is a lipid-soluble ester which is equally as effective as aspirin in inhibiting collagen induced PA but appears to possess less potential for inducing gastrointestinal bleeding.[14] HP-129 was reported to be 2 - 5 times as active as aspirin in the laboratory and effective in ex vivo human aggregometry studies at oral doses in the range of 100 to 250 mg.[15]



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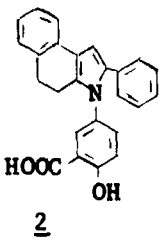

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Prostaglandins. The fact that the prostaglandins are naturally occurring compounds with extremely potent effects on platelet function continues to generate interest in them and in their possible role in thrombosis. PGE_1 has long been regarded as one of the most potent inhibitors of PA known while other prostaglandins and certain intermediates in the synthesis of prostaglandins are thought to play a role in inducing PA.

Willis has described a labile aggregation-stimulating substance (LASS) which is produced during the enzymatic conversion of arachidonic acid to prostaglandin.[16] Further studies have shown that LASS is most likely composed of endoperoxide intermediates which arise during prostaglandin biosynthesis from arachidonic acid in the presence of microsomal fractions.[17] Two such endoperoxides have been identified and were designated PGG_2 and PGH_2. These substances are potent inducers of PA and are released from platelets in response to thrombin, collagen and L-epinephrine in addition to arachidonic acid.[17,18] Aspirin cannot block PA induced by these substances but is able to block the formation of these

endoperoxides as well as the platelet release reaction. In addition, it has been shown that sodium arachidonate administered intravenously to rabbits causes death due to massive platelet thrombi which occlude the microvasculature of the lung.[19,20] Aspirin pretreatment was able to protect rabbits from death for a period of up to several days. Also, in six human cases of unexpected sudden death, postmortem microscopic examination revealed the presence of platelet aggregates in small pulmonary arteries and arterioles.[21] The similarities between these recently reported clinical cases and the rabbit model described above suggest that prostaglandin endoperoxides may play a role in human disease.

Smith et al. indicate that the endoperoxide intermediates seem to be important for irreversible aggregation but not primary aggregation.[18,22] These findings may require that we reevaluate the concept that ADP release is solely responsible for irreversible aggregation. Willis and Kuhn have reported that 5,8,11,14-eicosatetraynoic acid (TYA) (3) is another compound, like aspirin, which is able to inhibit the enzymatic conversion of arachidonate to LASS.[23] The potential antithrombotic properties of this compound are currently under study.

Smith et al. have also recently reported that PGD_2 (4) has been found to be more than twice as potent as PGE_1 as an inhibitor of both ADP and collagen induced PA in human platelet rich plasma.[24] In contrast, PGD_2 was less potent than PGE_1 in rabbit and rat platelet rich plasmas.

$CH_3(CH_2)_4C{\equiv}CCH_2C{\equiv}CCH_2C{\equiv}CCH_2C{\equiv}C(CH_2)_3COOH$

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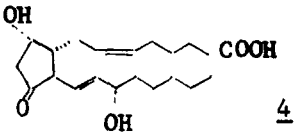

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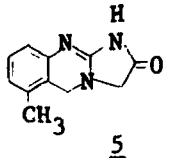
Miscellaneous Agents. While a wide variety of new structures have been reported to inhibit various forms of PA, the majority of in depth studies have delt with drugs which are on the market for other purposes. There have been relatively few new compounds introduced as potentially useful antithrombotic agents which have been supported by comprehensive pharmacological investigation.

BL-3459 (5) is one of the more interesting members of a series of 1-2,3,5-tetrahydroimidazo [2,1-b] quinazolin-2-ones recently reported to be potent inhibitors of PA and experimental thrombosis.[25] This compound was shown to inhibit PA induced by a variety of substances, including ADP, at concentrations in the range of 0.4 - 4 μM in platelet rich plasma obtained from various species.[26,27] Furthermore, the potency of BL-3459 carried over to the ex vivo situation and the compound demonstrated impressive activity in a variety of models of experimental thrombosis. In general, activity was observed following single oral doses in the range of 0.5 - 10 mg/kg.



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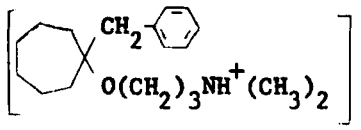
Bencyclane (6) has recently been evaluated for activity against platelet adhesion in human blood both in vitro and ex vivo.[28] The compound reduced platelet adhesion to glass beads by 50 % when added to blood



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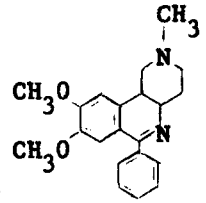
at a concentration of 100 μg/ml. In addition, significant reduction in platelet adhesiveness was also observed in human subjects following intravenous infusion of bencyclane at a dose of 75 mg/hr over a four hour period. Platelet adhesiveness returned to normal two hours after the infusion had been terminated. These results indicate that bencyclane may be effective clinically but its utility could be quite limited unless oral activity can be demonstrated.

AG 19,417 (7) was evaluated *ex vivo* and *in vivo* in rabbits and rats for activity against platelet adhesion, PA and thrombus formation.[29] This compound was active in all three models employed at doses ranging from 25 - 50 mg/kg po or at 10 mg/kg iv.



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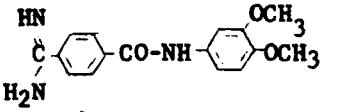
4-Amidinobenzoic acid 3',4'-dimethoxyanilide (8) was the most potent of a series of benzamidine derivatives reported to inhibit ADP, collagen and thrombin induced PA *in vitro* in the range of 10 - 20 μM in human platelet rich plasma.[30] However, the *in vivo* potential of these compounds as antithrombotic agents has not yet been reported.



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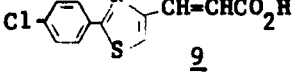
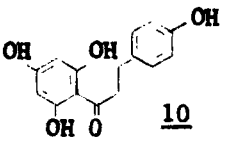
WY-23,049 (9) has been reported to be an inhibitor of the first phase of ADP induced PA and aggregation induced by epinephrine in human platelet rich plasma.[31] However, this agent appears to be relatively weak, having an EC_{50} of approximately 2.5 mM against the first phase of ADP induced PA. *Ex vivo* activity was demonstrated in guinea pigs and the compound partially antagonized ADP-induced platelet loss in rats at oral doses in the range of 10 - 50 mg/kg.



9

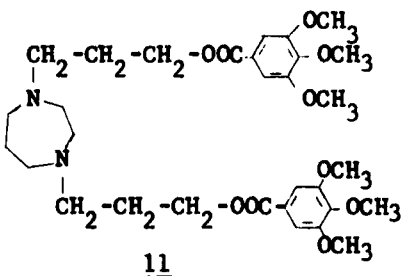
Polyphloretinphosphate is a polyester of phloretin (10) and phosphoric acid which has been reported to inhibit platelet adhesion to plastic surfaces as well as to inhibit hemodynamic and respiratory changes and the fall in platelet count induced by the infusion of thrombin or protamine to dogs.[32,33] It was concluded that this substance prevents increased pulmonary arterial pressure, decreased systemic pressure and increased tracheal insufflation pressure resulting from thrombin or protamine infusion by preventing platelet adhesion and the release of platelet contents.



10

The *in vitro* effect of dilazep (11) on PA has been reported.[34] This compound was compared to dipyridamole and adenosine for activity against both ADP and collagen induced PA. In general, dilazep was somewhat more

active than either dipyridamole or adenosine, possessing EC_{50}'s in the range of 0.1 - 1 mM. However, the efficacy of this compound on experimental thrombosis following in vivo dosing has not yet been established.



11

Further studies of the effects of barbituric acid derivatives[35] and antihistamines[36] have suggested that while these classes of drugs inhibit platelet function in vitro, blood levels sufficiently high to produce these effects are probably not obtained under normal clinical conditions. However, carbenicillin, like penicillin G, has been shown to produce definite defects in platelet function at doses which are within the therapeutic range.[37]

General Considerations. The role of platelets in rapidly developing, severe atherosclerosis represents an approach to plaque formation which may have important therapeutic implications. Immunologic endothelial injury, induced experimentally or clinically as a result of tissue transplantation, leads to rapid plaque formation.[38,39] Electron microscopy has revealed the early formation of platelet aggregates in association with denuded endothelium. Platelets were also found deep in arterial smooth muscle tissue indicating that they may be associated with delivering damaging substances to this region of the arterial wall. Early lesions were composed almost entirely of platelets adherent to damaged endothelium and basement membrane. Other experimental studies have confirmed that when endothelial abrasion is produced using a balloon catheter, microthrombi quickly form at injury sites.[40] These lesions then progressed with smooth muscle proliferation and infiltration of lipid when dietary cholesterol was elevated. Thus, it would appear that rapidly developing, severe atherosclerosis, which is a serious problem in cases of cardiac transplantation, may involve the following sequence of events: immunologic endothelial damage, platelet adhesion and aggregation to form microthrombi, release of platelet contents, further vessel wall damage, lipid infiltration and eventual plaque formation. An inhibitor of PA and release may prove to be an important addition to the post-transplant therapeutic regimen.

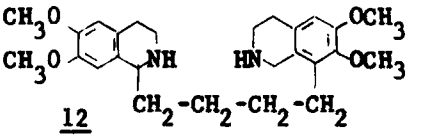
Anticoagulants

The state of anticoagulant therapy remains essentially as reported last year.[5] Few new anticoagulant agents have been introduced in recent years and only several of the defibrinating snake venoms have progressed to clinical trials. Nearly all clinical anticoagulation is currently managed either with heparin or the coumarin like compounds. Nossel and Wilner have recently published an excellent review of the subject.[41]

Fibrinolysis

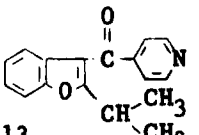
The recent literature contains several comprehensive reviews on fibrinolysis, thrombolysis and thrombolytic agents in general,[5,42,43,44] and the published proceedings of the First International Conference on Synthetic Fibrinolytic/Thrombolytic Agents held in Paris in 1972.[45] Although no promising clinically active, non-enzymatic fibrinolytic agents have been developed, the enzymatic and the non-enzymatic approaches to this therapeutic area both continue to receive attention. It has been advocated by von Kaulla[46] that it is not desirable to concentrate all efforts on the evaluation and development of enzymatic thrombolytic agents alone; questions have been raised with respect to the suitability of current methods used for the assessment of thrombolytic activity and with respect to whether or not investigative efforts are being concentrated on the right goal.

With respect to progress in the non-enzymatic fibrinolytic category, a series of monosubstituted bis (tetrahydroisoquinolines) related to bisobrin[47] (12) have been reported.[48,49] Many of these analogues were found to induce parenteral fibrinolytic activity comparable to bisobrin in rats as measured in the dilute whole blood clot-lysis assay but none possessed a useful level of oral activity. Pharmacological studies with this type of structure[50,51] strongly suggest that the fibrinolytic activity produced is the result of induction of histamine release from vascular walls and therefore subject to depletion of vascular histamine stores with consequential loss of fibrinolytic response.



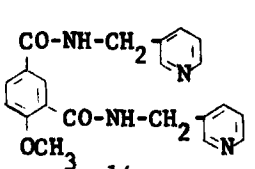
12

Inicarone (13), a benzarone analogue, has been reported to exert potent, long lasting fibrinolytic effects in plasma and in the vascular wall of experimental animals through activation of plasminogen without effect on coagulation steps.[52]



13

The drug G-137 (14), one of the most active of a mono- and bis- picolylamide series of 4-hydroxy-isophthalic acid and its methoxy derivatives, has been reported to exert a direct anticoagulant and fibrinolytic effect in rabbits at doses of 10 mg/kg i.p.[53] The mechanism of action is not known.



14

Lysine vasopressin has been demonstrated to release plasminogen activator in humans subsequent to intravenous infusion but it is associated with unpleasant side effects. The new analogue, 1-deamino-8-D-arginine vasopressin, caused a rapid and significant release of activator in healthy humans.[54] The response was similar to that observed after moderate exercise and intravenous epinephrine administration.

With respect to the enzymatic fibrinolytic category the results of ten major international clinical trials of streptokinase and urokinase in

acute myocardial infarction have been reviewed;[55] it is evident that the beneficial effects of thrombolytic agents have not been unequivocally demonstrated in treatment of myocardial infarction. Currently a National Heart and Lung Institute-sponsored study entitled the Streptokinase Myocardial Infarction Trial (SMIT) is underway and an associated pilot study has revealed observations on complications of streptokinase therapy and their relation to trial design.[56] It was emphasized that the ramifications of minor complications and the difficulty in blinding investigators must be clarified before a universally acceptable definitive trial can be completed.

The Urokinase-Streptokinase Embolism Trial, initiated in 1967 by the National Heart Lung Institute is considered to be the first controlled randomized comparison of urokinase and streptokinase in thromboembolic disease. Phase I established that urokinase increased the resolution rate of pulmonary thromboemboli, especially massive emboli, as judged by arteriography, hemodynamics and lung scanning.[57] The trial was not designed to demonstrate a difference in mortality and none was found. Phase II results have been recently reported[58] and serve to demonstrate that: regimens of either 24 hours of streptokinase, 24 hours of urokinase or 12 hours of urokinase are superior to heparin alone in accelerating the rate of resolution of acute pulmonary embolism; no benefits were derived from 24 hours of urokinase treatment as opposed to 12 hours; any difference between urokinase and streptokinase in resolution of pulmonary emboli is probably small; and side effects are not serious. Although there was an inability to detect any differences in mortality in the phase I study between patients treated with heparin and patients treated with thrombolytic therapy, Sherry[59] has concluded that thrombolytic therapy should be a useful adjunct to the management of massive embolism.

There is evidence in the literature to suggest that dose regimens of thrombolytic therapy other than those in current use may be worthy of clinical examination. The combination of heparin and low doses of brief courses of streptokinase appear to be synergistic and to produce resolution equivalent to a standard high dose of streptokinase alone as assessed in a newly developed model of experimental pulmonary embolism in dogs which permits quantitation of the degree of lysis of emboli.[60]

Potentiation of urokinase-activated lysis of human fibrin clots has been demonstrated with adenosine diphosphate and flavinadenine dinucleotide[61] as well as with other nucleotides, nucleosides, purines and pyrimidines. These agents are thought to be related to the fibrinolytic potentiating factor present in human red cell lysates. It has been reported that the sulfate esters of 2-aminoethanol including the corresponding C-alkyl and/or N-alkyl substituted derivatives have been found to potentiate blood clot lysis induced by urokinase when a combination of urokinase and one of these sulfate esters is brought into contact with clots of blood from vertebrate animals.[62] Potentiation of activity serves to reduce the amount of urokinase required for clot lysis.

The relationship between the use of fibrinolytic agents and anticoagulants versus the spread of cancer has been reviewed.[63,64] Many investigators have observed a hypercoagulable state in patients with ad-

vanced cancer. Two important considerations which have emerged to explain observed antimetastic effects are the facilitation of microcirculation within tumors and both the direct and indirect specific and selective actions that these agents may exert on cancer cells as well as on the host. Some of those agents such as fibrinolysin, warfarin and heparin appear to enhance the efficacy of chemotherapy with cytotoxic agents or radiotherapy in patients with various types of cancer including stage III and IV disease. The rationale for the use of these agents is based on the observation that fibrin is always found in and around a cancer, particularly at its growing edge and on the hypotheses that circulating cancer cells need a fibrin meshwork to support and initiate metastases. If this fibrin meshwork can be decreased or prevented, the natural mechanisms of cellular control of the host can produce cytotoxic antibodies and overcome the cancer cells.

A double-blind controlled trial designed to assess the value of combined treatment of phenformin and ethylestrenol against the development of deep-vein thrombosis in patients having gynecological surgery demonstrated the failure of this drug combination to lower the incidence of postoperative deep vein thrombosis despite preoperative stimulation of fibrinolysis.[65]

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Section III - Chemotherapeutic Agents

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

Chapter 12. Antibiotics

Gerald H. Wagman and Marvin J. Weinstein, Schering Corporation, Bloomfield, New Jersey

General. Collected papers from symposia on drug-inactivating enzymes and other antibiotic problems with resistant bacteria,[1] including Pseudomonas aeruginosa,[2] as well as recent advances in staphylococcal research,[3] were published. Proceedings of conferences on gentamicin,[4] clinical perspectives of amoxicillin,[5] and mode of action of antibiotics on microbial cell walls and membranes[6] appeared. Abstracts of an international microbiological congress[7] included sessions on infections, new antibiotics, non-specific resistance, and antibiotic biosynthesis. A symposium on laboratory evaluation of antimicrobial agents was held.[8] Abstracts of a conference consisting of symposia and sessions relating to structure and synthesis of antibiotics, combined therapy, new antimicrobial agents, clinical effectiveness, pharmacology, mechanisms of biosynthesis, and other related topics were published.[9] Reviews appeared on the chemistry and biosynthesis of actinomycins,[10] pathogenic anaerobic bacteria,[11] and newer cephalosporins and expanded-spectrum penicillins.[12] Published papers from a symposium on secondary metabolism in microorganisms[13] included fermentation and mutation studies with butirosin, and biosynthesis of β-lactam antibiotics, macrolides, novobiocins and related coumarin antibiotics. New books on antibiotic chemotherapy,[14] biosynthesis and enzymic hydrolysis of penicillins and cephalosporins,[15] and infrared spectra of antibiotic substances[16] were made available. A reference work on chromatography of antibiotics was published.[17]

Aminoglycosides. Tobramycin was evaluated as therapy in gram-negative infections in seriously ill patients with underlying diseases[18]. Gentamicin and tobramycin were compared in vitro and in single drug therapy in patients with serious gram-negative infections and the clinical effectiveness was similar.[19] The distribution of these antibiotics in body fluids of dogs was compared.[20] An acute intravenous toxicity study of 25 aminoglycoside antibiotics was carried out[21] and the effect of various molecular changes noted. Amikacin was found to be effective in the treatment of significant infections,[22] although the expected blood and urine levels for a given dosage were not always obtained. The clinical response to amikacin was satisfactory in patients with gram-negative infections resistant to other aminoglycosides,[23] and in another study cures were achieved in 10 of 12 patients with gram-negative infections.[24] The pharmacology of amikacin in humans was studied,[25] as was the activity against clinical isolates resistant to one or more aminoglycoside antibiotics.[26] In 12 cases of resistant Proteus rettgeri urinary infections which were controlled or eradicated with amikacin, relapse or re-infection was frequently encountered.[27] Ten of eleven patients treated with single dose gentamicin therapy were cured as compared with 8 of 10 patients treated with three injections daily.[28] Verdamicin (1b)[29] demonstrated activity similar to gentamicin[30] against Enterobacteriaceae and Pseudomonas aeruginosa; Proteus and Providencia were notably more susceptible to verdamicin. In a study of susceptibility of Pseudomonas aeruginosa

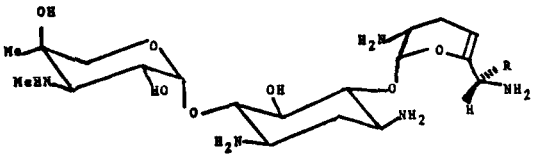
to three aminoglycosides and carbenicillin,[31] organisms with increased resistance to gentamicin also had increased resistance to tobramycin and amikacin and there was no correlation between carbenicillin and aminoglycoside resistance.

The structure of sisomicin, (1a) which contains a novel unsaturated sugar unit, was determined.[32] A resistant strain of Pseudomonas inactivates sisomicin by 6'-N-acetylation.[33] Sisomicin, gentamicin and tobramycin had about equal activity against 273 clinical bacterial isolates and all were more active than amikacin.[34] Gentamicin A, which is relatively weakly active, was converted to the 6'-amino-6'-deoxy derivative (2a) and its in vitro activity against some sensitive gram-positive strains was superior to the starting material, gentamicin C complex, and the structurally related kanamycin B.[35] A variety of selectively protected garamine derivatives have been prepared[36] that undergo selective glycosylation at the 4-position. The syntheses of 1-N-[(S)-4-amino-2-hydroxybutyryl] gentamicin C_1 and 1-N-[(S)-3-amino-2-hydroxypropionyl] gentamicin C_1 were reported; these are potent inhibitors of organisms that inactivate gentamicin C_1 by 2"-O-adenylylation and 3-N-acetylation, but they are not active against a strain of Providence which inactivates gentamicin by 2'-N-acetylation.[37] The synthesis of 2"-deoxy-gentamicin C_2 (2b) was reported[38] and, as predicted had good activity against gentamicin-resistant adenylylating strains of E. coli and K. pneumoniae.

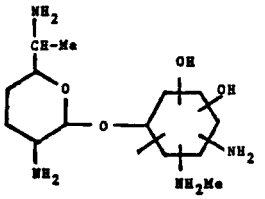
The synthesis of 4'-deoxy-kanamycin A was described,[39,40]; this compound is significantly more active than kanamycin against Pseudomonas strains and also inhibits the resistant organisms which produce neomycin-kanamycin phosphotransferase II. The synthesis and antibacterial activities of several derivatives of kanamycin, kanamycin B and 3',4'-dideoxy-kanamycin B acylated at the 1-amino group with isoserine instead of 4-amino-2-hydroxybutyric acid were reported,[41] as were twenty-three 1-N-acyl derivatives of kanamycin A.[42] The new aminoglycoside antibiotics 4-deoxybutirosins A and B, which contain the new deoxyamino sugar, 2,6-diamino-2,4,6-trideoxy-α-D-xylo-hexopyranose (4-deoxy-neosamine C), have been shown to have broader antibacterial activity than that of butirosin and increased antipseudomonal activity.[43,44] Cross-resistance between butirosin and gentamicin occurs in a variable manner.[45] The synthesis of 3',4'-dideoxybutirosin A was reported[46]; this agent as expected was found to have greater activity against butirosin-phosphorylating strains.

6'-Desamino-6'-hydroxyribostamycin[47] and 6-O-(β-D-ribofuranosyl) paromamine[48] have been prepared. The addition of analogs of 2-deoxystreptamine to a mutant of Micromonospora inyoensis, the sisomicin-producing organism, has resulted in the formation of new antibiotics called mutamicin 1, produced by the addition of streptamine, and mutamicin 2, produced from 2,5-dideoxystreptamine.[49] The latter has favorable activity against gentamicin/sisomicin-acetylating strains. Similarly, a mutant of S. rimosus forma paromomycinus converts streptamine to two new antibiotics, hybrimycins C_1 and C_2, which are analogs of paromomycins 1 and 2 respectively.[50] Selective hydrolysis yielded a third new antibiotic, hybrimycin 3, an analog of paromamine.

A new aminoglycoside, fortimycin B (3), has been isolated from cultures of Micromonospora olivoasterospora.[51] Antibiotic XK-62-2-(sagamicin), produced by Micromonospora sagamiensis[52] was identified as 6'-N-methyl gentamicin C_1a,[53] and is identical with gentamicin C_2b produced by Micromonospora purpurea mutant JI-33.[54] A new 6'-deoxy-6'-substituted aminolividomycin has been prepared from lividomycin.[55] Novel aminoglycosides 66-40B and 66-40D co-produced as minor components in the



1 1a R=H
1b R=Me



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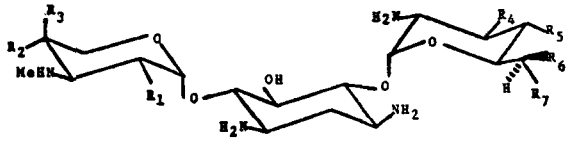


2 2a $R_1=R_2=R_4=R_5=R_7=OH$; $R_3=R_6=H$

2b $R_1=R_4=R_5=H$; $R_2=R_6=Me$; $R_3=OH$; $R_7=NH_2$

2c $R_1=R_3=R_4=R_5=R_7=OH$; $R_2=R_6=Me$

sisomicin fermentation, have been reported,[56,57] as has Sch 17726, a new broad spectrum antibiotic which contains a double bond in one of the amino sugar moieties.[58] Antibiotic G-418 (2c)[59] is a new broad spectrum aminoglycoside,[60,61] which is also highly active against protozoa, amoebae, tapeworm, and pinworm infestations in mice. When neamine, kanamycin, or gentamicin C_1 are mixed with aldehydo sugars and solutions incubated at 30°C or higher at pH 7, base-labile, acid-stable compounds which appear to be N-glycosides are formed.[62,63]

A number of rapid enzymatic gentamicin assays have been described,[64-68] as has a rapid specific assay for amikacin utilizing a strain of Providencia stuartii resistant to multiple antibiotics with the exception of amikacin[69], and a rapid microassay devised for gentamicin and clindamycin when present together.[70]

β-Lactams, Cephalosporins and Penicillins. New cephalosporin antibiotics continued to be studied. Cephapirin was reported to be comparable to cephalothin in vitro and is an effective agent in the treatment of infection due to S. aureus and Streptococcus pneumoniae, although its intramuscular injections were moderately painful and intravenous infusions caused phlebitis in three of nine patients treated with doses up to 18 g per day.[71] Pseudomonas species were resistant to cefamandole, but it was reported to be more active than cephalothin, cephaloridine or cephalexin against members of the Enterobacteriaceae.[72] Cephacetrile gave significantly higher peak serum levels than cephalothin; after 2 g intravenous doses the levels were 74.9 and 21.5 μg/ml, respectively. The MIC's for cephacetrile were superior to cephalothin for Escherichia coli and Klebsiella and inferior for staphylococci and Proteus mirabilis.[73] In vitro studies were carried out with cefazolin,[74] and it was compared with penicillin G alone and with probenecid in the treatment of uncomplicated gonorrhea.[75] Two grams of cefazolin resulted in 29% failures among 31 patients and the same dose with 1 g probenecid resulted in 19.3% failures. The control schedule of 4.8×10^6 units of aqueous procaine penicillin G plus 1 g probenecid resulted in only one failure among 30 patients. Cefoxitin, a semisynthetic derivative of cephamycin C, was the subject of numerous papers.[76-84] Against gram-positive organisms, cefoxitin was less

active than cephalothin, but activity was equal against gram-negative organisms. Cefoxitin was reported to be remarkably stable in the presence of organisms which produce β-lactamase. Three papers appeared on a new parenteral cephalosporin, SK and F 59962, which was reported to have in vitro activity superior to cefazolin and cephalothin against gram-negative organisms; however, it failed to demonstrate significant activity against enterococci, indole-positive Proteus species, and Pseudomonas.[85-87] In vitro studies with cephanone were reported[88] and comparative in vivo studies were performed with six cephalosporin antibiotics.[89] Three new cephalosporin antibiotics were reported, BL-S217,[90] BL-S640[91] and FR-10612,[92] an orally active agent.

A series of new derivatives of 7-aminocephalosporanic acid which were well absorbed following oral administration to mice were prepared; in these derivatives the acetoxymethyl function at C_3 was replaced with a heteroaromatic carbonylthiomethyl moiety and the 7-amino group acylated with D-phenylglycine.[93] A number of derivatives of 7-(α-sulfophenylacetamido) cephalosporanic acid were synthesized and their structure-activity relationships described.[94] 7(X)-Mandelamidocephalosporanic acid was synthesized, its derivatives and analogs were prepared, and their structure activity relationships were determined.[95] A variant of the one step methoxylation procedure has enabled the synthesis of 6-α-methoxycarbenicillin and the corresponding 7-α-methoxycarbenicillin analogs.[96] Differences in antibacterial activity as a result of 6-α-and 7-α substitution in penicillins and cephalosporins are paralleled by differences in chemical reactivity of their corresponding β-lactams , in both cases attributed to steric factors resulting from α-substitutions.[97] Novel syntheses reported include α- or β-alkoxy-β-lactams,[98,99] replacement of the carbamoyloxy group by cleavage of the C_{10}-O bond of Δ^2 cephems with hydrohalic acids[100,101] and total synthesis of (±)-3'-methylcephalothin.[102] The total synthesis of (±)-1-oxacephalothin was reported[103] which has all the features of cephalothin except that the sulfur atom has been replaced by oxygen and, similarly, the stereospecific total synthesis of (±)-1-carbacephalothin has been described,[104] in which the sulfur has been replaced with methylene.

Ticarcillin, a new semi-synthetic penicillin was found to be twice as active in vitro as carbenicillin against P. aeruginosa strains,[105] and further in vitro comparisons were made[106] as well as the clinical pharmacology studied.[107] A series of reports appeared on BL-P1654, a new ureido-penicillin.[105,106,108-112] It is reported to be active against many ampicillin-resistant and some carbenicillin-resistant bacteria, especially strains of Enterobacter and Serratia; however, the data on the activity against P. aeruginosa isolates are conflicting. The antibiotic is not bactericidal. Amoxicillin, a new orally administered penicillin, was shown to have greater gastrointestinal absorption than ampicillin, giving 2-3 times the peak serum levels.[113,114] In uncomplicated gonococcal infections, amoxicillin given at 3 g was approximately as effective as 3.5 g of ampicillin plus 1 g of probenecid.[115] Five new semisynthetic penicillins were tested with resultant varied claims for superior serum levels (BRL 8988)[116] (PC-183)[117]; more rapid absorption (cyclacillin)[118]; and greater activity than carbenicillin (BAY e 6905 and BAY f 1353).[119,120]

Aminoglycoside β-Lactam Synergy. The inhibitory activity of combined carbenicillin and gentamicin was better against 114 of 130 clinical isolates of Pseudomonas aeruginosa compared to the most effective antibiotic alone.[121] Similarly, enhanced activity resulted by combined use of sulbenicillin and gentamicin,[122] and combinations of tobramycin, gentamicin or amikacin with carbenicillin were synergistic.[123,124,125]. Various

combinations of gentamicin or amikacin combined with carbenicillin or BL-P1654 showed significant differences.[126] Penicillin plus gentamicin or streptomycin was active against S. viridans in 3-4 days in rabbits with catheter-induced aortic valve endocarditis[127]; nafcillin combined with gentamicin or tobramycin had significant activity against S. aureus from patients with endocarditis or septicemia.[128]

Macrolides. Recent studies on the chemistry and biochemistry of 16 membered macrolides indicated that the presence of one ketone or aldehyde group in the aglycone is essential for antibacterial activity.[129]

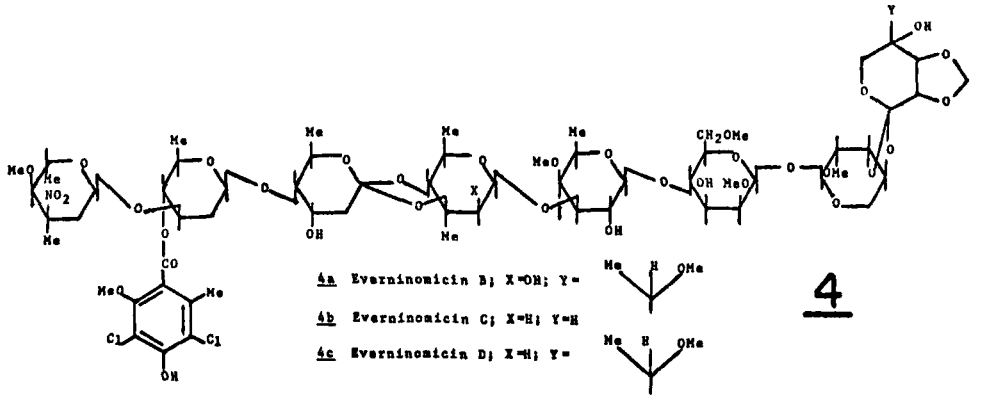
Rosamicin was reported to be more active than erythromycin against gram-negative organisms[130] and showed lack of complete cross-resistance. A new basic macrolide complex, designated YL-704, contains at least 13 components.[131-134] The structures of erythromycin A carbonate[135] and erythromycin D[136] were elucidated; and 4"-deoxy-4"-oxoerythromycin derivatives were described.[137] Two cyclic carbonates of 8-hydroxyerythromycin A were reported.[138] In vitro development of resistance[139] and microbial inactivation of erythromycin[140] were reported, as was the effect of erythromycin therapy on diphtheria carriers.[141] A number of studies were detailed on the effects of erythromycin on binding to E. coli ribosomes.[142-146]

Antibiotic XK-41-B_2 has been identified as 4"-O-propionyl-megalomicin A,[147] while co-produced components A_1, A_2, B_1 and C_1 were found to be identical to the megalomicins.[148] Microbial conversion of macrolides, including deacylation of maridomycin,[149,150] and reduction[151] and hydroxylation of maridomycin and josimycin,[152] were reported. Antimicrobial activities of leucomycin congeners were compared[153] as was the in vitro efficacy of kitasamycin (leucomycin A6).[154] The mode of action of kujimycins was determined.[155]

Clindamycin. A high level of resistance to clindamycin was reported for 16% of 49 strains of Clostridium ramosum. All isolates were resistant to rifampin and gentamicin and most were resistant to lincomycin.[156] The sensitivity of recent clinical isolates of anaerobes at the Mayo Clinic was investigated. All strains of Clostridium perfringens were inhibited by clindamycin at 3.1 μg/ml, but only 60% of other clostridial species were inhibited by this concentration, suggesting a somewhat decreased susceptibility since 1971.[157,158] A study in which clindamycin (35 patients) was compared with penicillin (49 patients) for treatment of anaerobic pulmonary infections showed no significant difference in response according to duration of fever, X-ray resolution, or outcome of treatment.[159] The combination of clindamycin and gentamicin is often used to treat mixed bacterial infections and is initial therapy for suspected sepsis.[160] Against anaerobic bacteria the results of one study[161] showed no antagonistic effects of the combination in vitro, and in some strains a synergistic effect may be present. With Enterobacteriaceae and Pseudomonas clindamycin and gentamicin demonstrated no significant interaction in vitro, although clindamycin, which was consistently inactive at some concentrations, occasionally altered the minimum inhibitory concentrations of gentamicin by several dilutions.[162,163] Clindamycin was found to inhibit the early in vitro killing of certain gram-negative bacilli by aminoglycosides.[164] Exposure to clindamycin did not significantly alter the 18-hour end-points of gentamicin cidal activity, but it was observed to interfere with the early bacterial killing activity of gentamicin.[165] This effect was variable in that it was not uniform for all species tested. Lincomycin and clindamycin are strongly implicated in the recent resurgence of pseudomembraneous enterocolitis.[166] Aminoglycosides interfere with the determination of clindamycin in standard

microbiological assays, and a new method was reported[167] involving addition of calcium chloride to agar, which blocks the aminoglycoside activity and does not affect clindamycin. The differential plasma binding of clindamycin and lincomycin requires consideration in assay designs.[168]

Miscellaneous Antibiotics. The novel structures for everninomicins B, C and D (4a-c) were elucidated[169,170,171] and microbiological characterizations of components B and D were reported.[172,173] The comparative



4a Everninomicin B; X=OH; Y=

4b Everninomicin C; X=H; Y=H

4c Everninomicin D; X=H; Y=

4

efficacy of tetracycline, doxycycline and minicycline was studied.[174,175] New, natural rifamycins were described,[176] and clarification of the biological effects of rifamycin and its derivatives was reported[177,178,179] as was the immunological responsiveness of tuberculosis patients.[180] The synthesis of the oximes of 3-formylrifamycin SV and the preparation of some of the O-substituted hydroxylamine intermediates were described,[181] and the effect of the rifamycin structure on RNA-instructed DNA-polymerase inhibition was studied.[182] The carbon 13 nmr spectrum of rifamycin S[183] has confirmed previous assignments at C-6 and C-8.

Field desorption mass spectra were shown to be useful indications of the composition of antibiotic complexes and useful for determination of molecular weights.[184] The absolute configuration of oryzoxymycin[185] and kidamycin,[186] and the revised structure of cerulenin[187] and magnesidin[188] were reported. A fast comparison of IR spectra for screening new antibiotics was devised,[189] and conformations of several transport antibiotics were studied using Raman spectroscopy.[190]

Resistance. Ampicillin resistance in clinical isolates of Shigella sonnei results from β-lactamase production under two types of control, one due to chromosomal mutation and the other to an R-factor, and one isolate was found to have both mechanisms.[191] Chromosomal uninducible resistance to tetracycline was studied in S. aureus[192] and appeared to be correlated with a chromosomal location for the resistance genes. Viomycin-resistant strains of Mycobacterium smegmatis demonstrated pleiotropic resistance to capreomycin, streptomycin and kanamycin as a result of mutational alteration of 30 S ribosomal subunits.[193] Gradually increasing concentrations of tobramycin produced resistance in clinically isolated strains of P. aeruginosa which resulted in cross-resistance to neomycin and gentamicin but not kanamycin. There was no loss of resistance after 20 transfers in antibiotic free medium.[194] Resistance to polymyxin in Salmonella was associated with altered structure of the outer cell membrane, which was shown to be caused by a point mutation.[195] The genetic basis of multiple antibiotic resistance of five clinical isolates of Neisseria gonorrhoeae

was investigated and found to be controlled by several gene loci.[196] Resistance to spectinomycin, streptomycin, rifampin and erythromycin was transferable during mixed-broth cultivation of sensitive and resistant strains of N. gonorrhoeae.[197] The process is DNase-sensitive and is presumably due to transformation.[196,197] A similar example of multiple antibiotic resistance with this organism was reported with six different antibiotics.[198] Gentamicin resistant strains of P. aeruginosa isolated at Parkland Memorial Hospital in Dallas were all found, by retrospective epidemiological investigation, to be exclusively from patients in burn intensive care units and geographically contiguous areas of the hospital.[199] A nine-year study in a urological ward showed resistant organisms decreased coincident to decreased consumption of antibiotics.[200] A strain of Providencia has been shown to inactivate aminoglycosides by 2'-N-acetylation.[201] Antibiotic-inactivating enzymes were studied by several investigators.[202-206]

R factor-mediated resistance to aminoglycosides continued to be reported, specifically to amikacin,[207] tobramycin,[208] gentamicin,[209] ribostamycin,[210] butirosin,[211] sisomicin,[211] and lividomycin A.[212] The enzymatic 3-N acetylation of gentamicin, tobramycin and kanamycin was found to be due to a new isoenzyme controlled by a new R factor obtained from a clinical isolate of Klebsiella.[213] A kanamycin-resistance plasmid was found in S. aureus which can be transduced into or can transform competent susceptible strains to kanamycin/neomycin resistance.[214] The induction of R factor mediated resistance to tetracycline in E. coli was found to be incomplete, suggesting that some chromosomally mediated function is necessary for full expression.[215] A Pseudomonas R factor inducing tetracycline resistance was found to be inactivated by low temperatures.[216] A plasmid determining resistance to erythromycin, lincomycin and vernamycin B_2 was isolated from a strain of Streptococcus pyogenes and is present to the extent of one to two copies per chromosomal genome equivalent.[217] Out of 117 gram-negative pathogenic bacteria examined, 46 were found to carry an R factor which decreased the resistance of a rifampin-resistant mutant of E. coli.[218] All 17 Salmonella typhi strains tested from an epidemic in Mexico carried R factors, as did 20 Shigella dysenteriae strains of epidemic origin.[219] R factors causing resistance to penicillins and cephalosporins were studied.[220-224]

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Chapter 13. Antifungal Agents

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Reviews - In a monograph dealing with the selection and use of antibiotics, the clinical aspects and chemotherapy of infections caused by such fungal pathogens as Candida albicans, Aspergillus species, Cryptococcus neoformans and the Phycomycetes as well as the history, toxicity and clinical use of amphotericin B and 5-fluorocytosine (5-FC, flucytosine) were reviewed.[1] In a singularly excellent account, aspergillosis was reviewed.[2] This article summarized essential aspects of the history, pathological diagnosis and physiology, mycological aspects, host response, serology, treatment, clinical manifestation and laboratory diagnosis of human aspergillosis as well as the more recent topic of aflatoxins. The superinfections most commonly seen in illicit drug users were reviewed and commonly seen fungal infections such as candidiasis and aspergillosis were discussed.[3] The role of the radiologist in the diagnosis of opportunistic pulmonary fungal infections and their symptoms was reviewed; specific fungal infections discussed included phycomycosis, aspergillosis and nocardiosis.[4] Chronic mucocutaneous candidiasis in immunosuppressed patients and patients with genetically determined diseases was reviewed; treatment with immunoreconstitution was discussed as well as treatment with 5-FC, clotrimazole or amphotericin B.[5] The combination of immunoreconstitution and drug therapy and the importance of iron deficiency and other nutritional factors also were discussed. Clinical aspects of the mycotoxicoses in man and animals was reviewed.[6]

Mycotoxin research in Japan, particularly in its relationship to the rice crop and specific organisms associated with the production of mycotoxins in foodstuffs, was reviewed together with the screening of organisms for mycotoxin production.[7] Pharmacokinetics and dosing with antimicrobics in patients with renal impairment were reviewed, and dosing considerations for antimicrobial drugs in such patients were given; the criteria for establishing the degree of renal impairment in these patients also were discussed, with particular reference to antifungal drugs.[8] Mechanisms of drug toxicity were reviewed, particular reference was given to the role of in vivo production of chemically reactive covalently bound metabolites.[9]

The more current developments in antimycotic chemotherapy were reviewed, with specific reference to 5-FC and the imidazole derivatives miconazole and clotrimazole.[10] The relative pharmacokinetics of antimicrobial drugs were reviewed, together with the means of prediction of blood levels as related to multiple doses, dosing in patients with renal impairment, and establishment of initial loading doses.[11] Without reference to specific antifungal agents, the bioavailability of drugs given orally was reviewed in relationship to their dosage forms.[12] The presence and biosynthesis of sterols in fungi and their importance in reproduction and growth were reviewed and a comparison was made with plant and animal sterols.[13] The synthesis, chemical reactions, specific reactions, and

spectroscopic properties of the benzimidazoles including those with fungicidal activities were reviewed.[14] A most excellent textbook on the medically important fungi, and the diseases they produce, together with diagnoses and treatments ,was presented.[15]

Methods - The problems and techniques of continuous sterilization of broth for use in fermenters in the pharmaceutical industry were examined and new types of equipment were described.[16] The conditions and kinetics for optimizing the operation of biochemical reactors for fermenters were examined.[17] Techniques for controlling aeration and agitation in antibiotic-producing fermentations were discussed and a method of choosing combinations of conditions for aeration and agitation was described.[18] Three methods for evaluation of topical antibacterial agents on human skin were described.[19] A new diluent for use in determining bacterial counts in anhydrous cosmetics was reported.[20] A novel technique, based on ultrafiltration, for the determination of free preservatives in oil-in-water emulsions such as those used in pharmaceuticals and cosmetic preparations was described.[21] A bilayer technique for determining antagonism between microorganisms was described; while its application was to wood-decaying fungi, it also should be applicable to human pathogenic agents.[22] Microbiological, photometric and densitometric methods for the determination of clotrimazole in biological materials were described.[23] The incorporation of radioactive precursors of RNA was used to measure the inhibitory effects of antifungal agents on slow growing fungi; there was excellent correlation between the results of regular tests for determination of minimal inhibitory concentration of antifungal drugs and those of the newly described technique.[24] A modification of the official method for the microbiological assay of nystatin in foodstuffs was described.[25] Concentrations of 5-FC and amphotericin B in bronchial secretions in a dog model were studied and it was shown that while 5-FC penetrated the blood-bronchus barrier well, amphotericin B did so only poorly.[26] The use of albumin microspheres as vehicles for achieving specific drug delivery in such infections of the reticuloendothelial system such as histoplasmosis was described.[27]

New Antifungal Agents - An extract from Batamote, a plant native to the southwestern United States and noted in native folklore as having therapeutic activity in the treatment of athlete's foot, was shown to be inhibitory for dermatophytic organisms *in vitro*.[28] Purpuromycin, a new antibacterial and antifungal agent, was shown to be structurally related to the rubromycins.[29] Materials with antibiotic activity effective against some gram-positive and gram-negative bacteria as well as against some yeasts were isolated from the plasmodial stage of the myxomycete *Physarum gyrosum*.[30] A9145, a new adenine-containing antifungal agent, was shown to be effective against *C. albicans* *in vitro* in a synthetic medium and also *in vivo* in mice, and there was evidence of synergism with amphotericin B.[31] Imidazoles having a variety of alkyl and aralkyl sulphur substitutions at the 2-position and their 5- and 4-nitro analogues were synthesized and tested for a broad spectrum of biological activities. Many of the nitroimidazoles were potent *in vitro* trichomonacides, and 5-nitrobenzyl sulfoxides and sulfones were found to be potent antifungal agents when tested against *Trichophyton mentagrophytes* and *Fusarium* sp., but not against *C.*

albicans.[32] New nitro-pyrrole derivatives were studied, those with chlorine atoms in the α position of the keto group and N-alkyl substitutions had optimal antifungal activity.[33]

Cycloeudesmol, an antibiotic strongly active against C. albicans in vitro was isolated from the marine alga Chondria oppositiclada, and characterized as a cyclopropane containing sesquiterpene alcohol.[34] The synthesis and antimicrobial activities of N-substituted 2-phenyl-4-amino-6-hydroxyquinazolines were described.[35] Hyalodendrin, a new antifungal antibiotic containing an epidithiadiketo piperazine ring system, while active against phytopathogenic fungi, was also shown to be active against some human pathogens.[36] The in vivo antifungal activities of a series of N-hydroxy pyridones against Microsporum canis were discussed in relationship to increases or decreases in the lipohydrophobic properties of the compounds.[37] SQ 18,506, a new nitrofurantoin (trans-5-amino-3[2-(5-nitro-2-furyl)vinyl]-Δ^2-1,2,4-oxadiazole), was found to be active in vitro against both bacteria and fungi and topically in vivo against experimental Candida vaginitis, but not parenterally against systemic Candida infections or topically against T. mentagrophytes.[38,39]

Three new actinomycins were isolated from a new species of Streptomyces, Streptomyces lisandri nov. sp.; their antimicrobial spectra included protozoa, fungi and helminths.[40] The mouse skin lesion model was used to determine the effectiveness of various antimicrobial compounds and also of a new compound, AHR-1911 (10-undecenyl-1-yl pseudothiourea hydroiodide), against various pathogenic organisms; AHR-1911 was less effective than amphotericin B against C. albicans in this model.[41] A series of derivatives of thiocarbamic acid were synthesized and screened for antibacterial and antifungal activity; three compounds characterized by methane and monocyclic aromatic ring (phenyl) substitutions were found effective against Aspergillus niger and Trichophyton asteroides.[42] A series of 3,4-dihydro-2H-1,3-benzoxazine-2,4-diones and 3,4-dihydro-2H-1,3-benzoxazine-4-ones were synthesized from several salicylamides and salicylanilides; activity against C. albicans was demonstrated.[43] The mode of action of haloprogin in Candida species was studied and was found to be related to inhibition of O_2 uptake and disruption of cell membranes; a comparison was made between the activity of haloprogin and amphotericin B and nystatin against C. albicans.[44] The isolation, purification and properties of the hexaene macrolids candihexin I and candihexin II were described; candihexin I but not candihexin II was active against a variety of yeasts and filamentous fungi.[45]

Clinical Experience - Considerable interest was shown in the use of transfer factor as an adjuvant in the treatment of the systemic mycoses. A case of cavitary pulmonary coccidioidomycosis was treated with immunotherapy; the patient was nonreactive to intradermal coccidioidin antigen and lymphocytes were nonresponsive to this antigen in vitro; following reconstitution with whole lymphocytes and transfer factor, the patient became skin test positive to spherulin and her disease stabilized.[46] Three patients with progressive coccidioidomycosis were treated with transfer

factor; two became reactive to intradermal coccidioidin and two had prolonged clinical remissions.[47] Ten patients with mucocutaneous candidiasis were treated with transfer factor, amphotericin B, or combinations of the two. Transfer factor alone caused conversion of delayed dermal responses but produced neither beneficial nor detrimental effects in four patients, while four responded favorably to amphotericin B alone and two responded to combined treatment but subsequently relapsed.[48] The use of transfer factor in the treatment of patients with infectious diseases was discussed, including its use in the treatment of systemic fungal infections.[49]

There was continued interest in the use of 5-FC in the treatment of mycotic infections. Two diabetic patients with urinary tract infections due to Torulopsis glabrata were successfully treated with 5-FC given over a two week period.[50] Eleven patients with disseminated candidiasis were treated with oral and intravenous 5-FC for an average of 31 days; therapy was successful in ten, while one patient died with cerebral Candida abscesses.[51] A singular case of Candida endocarditis caused by Candida parapsilosis was reported in which the organism became resistant to 5-FC during therapy; resistance was shown to be due to a lack of cytosine deaminase.[52] A catastrophic pancytopenia occurred in one patient following treatment with 5-FC.[53] The efficacy of 5-FC in the treatment of infections due to fungi other than the pathogenic yeasts was demonstrated. In one study, three patients with chromomycosis were successfully treated with oral 5-FC; in a second study, six of eleven patients with chromomycosis treated with 5-FC improved.[54,55]

Further studies with clotrimazole and miconazole were reported. In one study, over 600 patients with Candida vaginitis and over 300 patients with Trichomonas infections were treated with clotrimazole; 82 per cent of the patients with Candida infections were cured while only 52 per cent of those with trichomonal infections were cured by this treatment.[56] The pharmacokinetic effects, antifungal properties, clinical usage, and tolerability of clotrimazole were reviewed.[57] Miconazole and nystatin were compared topically in the treatment of Candida vulvovaginitis in 116 women; miconazole proved to have superior therapeutic activity.[58] In a study of the epidemiology of chronic fungal vaginal infections, infections due to C. albicans were found to respond better to clotrimazole than those due to Torulopsis glabrata.[59] Intravenous and intrathecal miconazole were shown to be of some value in treatment of human coccidioidomycosis.[60]

Several interesting studies with established antifungal agents were reported. Two patients with dermal leishmanoid were successfully treated with intravenous amphotericin B.[61] Various topical preparations were compared with griseofulvin in the treatment of 254 dermatophytic infections of glabrous skin; isolation of organisms was achieved from 130 pieces of clothing, and it was found necessary to disinfect all clothing material in order to prevent reinfection, while prolonged treatment with griseofulvin was required to treat hair and nail infections.[62] Studies on the absorption, metabolism and excretion of griseofulvin in man were reported; the major metabolite excreted in urine was found to be 6-desmethylgriseoful-

vin.[63] In a double blind study conducted in prison inmates, a one per cent tolnaftate powder was found to be more effective than a talc-cornstarch vehicle in prevention of tinea pedis.[64] A three year study of tinea pedis infections due to T. mentagrophytes conducted in a public swimming bath was reported; the use of foot powder containing tolnaftate decreased the rate of infection from 4.8 to 1.2 per cent.[65] A singular case of allergic contact dermatitis due to tolnaftate was reported.[66] Three patients with mycetoma were successfully treated with dapsone; Nocardia asteroides was cultured from two of the patients but not the third.[67]

There were several reports on newer agents or new applications of established agents. One article reported the successful treatment of ringworm infections with a 10 per cent solution of thiabendazole; it was found to be as effective as griseofulvin.[68] Boric acid was used in the treatment of 40 cases of Candida vulvovaginitis; 38 of the 40 patients were cured, with no recurrence of symptoms.[69] The use of hyperbaric solutions of amphotericin B in 10 per cent dextrose for intrathecal administration was reported.[70]

The incidence of epidemic T. mentagrophytes infections in U.S. military personnel in Viet Nam was studied; infections were found to be due to a unique zoophilic variant of T. mentagrophytes not normally isolated in the United States. This variant also was isolated from commensal rats in the area, and Americans appeared to be more susceptible to infections caused by it than were adult Vietnamese.[71] Spherulin, a new skin test reagent, was compared with coccidioidin for elliciting delayed dermal hypersensitivity responses in 298 humans; when tested in standard concentrations, spherulin detected 32 per cent more reactors than did coccidioidin.[72] The incidence of sepsis in debilitated patients receiving intravenous hyperalimentation therapy was studied; catheter contamination was noted in 7.3 per cent of patients receiving hyperalimentation therapy for a period of 10 days or more and C. albicans was the organism most commonly isolated.[73]

Biological and Chemical Studies - Cycloheximide is a proven inhibitor of protein synthesis, acting at the level of cytoplasmic ribosomes; as such it has found extensive use as a biochemical probe. In one study it was found to induce changes in the fine structure of bean chloroplasts and in their thylakoid systems.[74] Acting together with actinomycin D, cycloheximide was found to induce an inhibition in the rise of phenylalanine pyruvate aminotransferase produced by tetraiodoglucagon in rat liver.[75] An unidentified "messenger" involved in the reversible inhibition of ACTH-induced corticosteroid release by cycloheximide was detected.[76] Cycloheximide induced impairment of memory during prolonged training of mice and also induced amnesia and loss of spontaneous recovery in mice trained to a specific avoidance response.[77,78] Differential effects of analogues of cycloheximide on synthesis of RNA and protein were studied in Achlya, with variable results; activities of a lesser degree than that obtained with cycloheximide were reported.[79]

Continued interest was shown in potentially synergistic combinations of antimicrobial agents against pathogenic fungi. Ampicillin and erythromycin were shown to act synergistically in vitro against strains of N. asteroides; the synergistic effect of these two agents was influenced by duration of incubation, and antagonism was not observed.[80] The inhibition of RNA synthesis in Saccharomyces cerevisiae by rifampin following potentiation by low doses of amphotericin B was reported.[81] Derivatives of rifamycin in combination with amphotericin B were found to be synergistic when tested against Histoplasma capsulatum.[82] The treatment of C. albicans infections in mice with 5-FC and amphotericin B alone and in combination was reported; in combination the cure rate was increased from about 15 per cent for either drug alone to 79 per cent with combined therapy.[83] Effective treatment of experimental coccidioidomycosis in mice with combinations of amphotericin B and tetracycline was described; results showed that the combination of the two antibiotics was effective, with the dosage of amphotericin B being reduced 2.5 to 4 times from that required for effective chemotherapy alone.[84] Synergism between 5-FC and amphotericin B against sensitive strains of C. neoformans and partially resistant strains of C. albicans was described.[85] The synergism of combinations of amphotericin B and rifampin when tested against C. albicans was reported.[86]

Studies on the molecular basis of the mode of action of amphotericin B and other polyene antifungal agents continued. The molecular basis of the selectivity of amphotericin B for yeast cells and filipin for animal cells was investigated. Both the toxicity of filipin and the therapeutic value of amphotericin B could be explained at the cellular and molecular level from the following observations: first, these polyene antibiotics had different effects on yeast and red blood cells, with filipin being more active against human red blood cells while amphotericin B was more potent in inhibiting yeast cells; and second, filipin was more effectively inhibited by addition of cholesterol, the major membrane component of human red blood cells, while amphotericin B was most effectively inhibited by ergosterol, the major membrane sterol in yeast.[87] Other studies on the mode of action of polyene antibiotics on various natural membranes were reported. Results of one confirmed earlier studies made on liposomal membranes which demonstrated that the distribution of double bonds in the membrane sterol nucleus appears to be of major importance in conferring polyene susceptibility.[88] Prior studies on the selective toxicity of polyene antibiotics in liposome membrane models had demonstrated that polyene susceptibility is affected by the composition of membrane fatty acyl chains and the distribution of double bonds in the membranes.[89] Resistance to polyene antibiotics in Candida tropicalis was described; this effect was related to changes in the sterol composition of the cell membrane.[90] The effects of amphotericin B and nystatin on thin lipid membranes were described, and a model for a pore-containing membrane was postulated.[91] The in vitro effects of nystatin on S. cerevisiae were described, and ion release without lysis caused by this compound was reported.[92] A novel sterol in an ergosterol-deficient yeast mutant of S. cerevisiae was described, and the occurrence of this mutation was related to resistance to polyene antifungal agents.[93] The efficacy of certain

mutagens, together with polyene antibiotics, in the induction and isolation of sterol mutants in Neurospora crassa was reported; two genes affecting sterol synthesis and polyene resistance were defined.[94] Resistance to polyene antibiotics in mutants of Aspergillus fennelliae was reported; qualitative and quantitative differences in the sterol concentrations in the cell membrane of the resistant mutants were investigated, and the resistant mutants were found to be deficient in ergosterol.[95] Subsequently the mutants were shown to contain modified sterols rather than ergosterol.[96] Evidence that cholesterol is the common binding site for cerolysin, streptolysin O, and saponin was presented.[97] Death of nystatin-resistant strains of S. cerevisiae upon refrigeration was described; this apparently was due to alteration in cell membranes of these resistant organisms.[98] Inactivation of C. albicans by UV irradiation in reference to growth conditions following the irradiation period was reported, and resistance to amphotericin B was related to changes in composition of the cell membrane.[99] In a most excellent study, amphotericin B was found to elicit alterations in bilayer membranes; these alterations were associated with changes in cell membrane permeability. A model postulating the formation of membrane pores was presented.[100]

Studies into the basic biological and pharmacological aspects of the imidazole compounds continued. Oral miconazole was found to be well absorbed from the gastrointestinal tract, but not from either mucous membranes or skin.[101] The biochemical effects of miconazole in C. albicans were studied; miconazole was found to inhibit the transport of adenine, guanine, and hypoxanthine and to act primarily on yeast cell membranes, resulting in a selective inhibition of the uptake of precursors of RNA and DNA.[102] Miconazole also was shown to alter cell membrane permeability, thus causing leakage of 260-nm absorbing materials as well as inhibiting endogenous respiration.[103] The effect of clotrimazole on C. albicans was studied at the electron microscopic level, and changes were observed to occur primarily in the nuclear membrane.[104] Clotrimazole was found to be active in vitro against Naegleria fowleri.[105]

Various standard and new compounds were examined for their pharmacological and antifungal activity and for possible potentiation of antifungal activity. Mice injected with cyclophosphamide prior to infection with H. capsulatum showed a dramatic increase in the number of organisms isolated from various organs of the body; this increased pathogenicity was associated with suppresion of humoral antibody formation.[106] A crystalline β-glucanase capable of acting on cell walls of living yeasts degraded yeast glucan and laminarin and was identified as being an endo β-1,3-glucanase.[107] The molecular weight of this enzyme was reported to be 24,500.[108] Use of an oil-in-water emulsion as a vehicle resulted in a 2.5-fold increase in gastrointestinal bioavailability of griseofulvin in the rat.[109] Inhibition of Marek's disease herpesvirus plaque formation by nystatin and amphotericin B was studied, and nystatin was found to be the more effective.[110] 5-Fluorocytosine was found to be active both in vivo and in vitro against Acanthamoeba; there were differences in susceptibilities of virulent and avirulent strains to the drug, and virulent strains

appeared to be capable of developing a mechanism of resistance to 5-FC.[111] 6-Chlorouracils with alkyl or alkenyl group substitutions were found to be inhibitors of yeast-alcohol dehydogenase.[112] Bischelates of 5-, 7-, and 5,7-halogenated 8-quinolinols with copper were found to have antifungal activity; this activity was geometrically related to the size of the pores in the spore wall of the fungi tested, which included Aspergillus niger, Trichophyton viride, Aspergillus oryzae and T. mentagrophytes.[113] Not unexpectedly, tetracyclines were found to increase the virulence of Y and M forms of C. albicans in mice.[114] Griseofulvin was found to be inhibitory for the myxomycete Physarium polycephalum; effects included inhibition of mitosis, presumed induction of polyploidy, and changes in the organization of the plasmodial surface.[115] Tunicamycin, an antibiotic active against viruses, gram-positive bacteria, yeast and fungi, was found to act primarily as an inhibitor of glycoprotein synthesis.[116] 5-Fluorocytosine was found to have a significant effect on protein synthesis in susceptible yeasts; this effect was felt to contribute to the antifungal activity of the drug.[117] Daily intravenous administration of amphotericin B to rats was found to produce significant decreases in serum cholesterol levels.[118] Triclosan was reported to have a wide range of activity against bacteria and fungi and was more active than hexachlorophene and neomycin against Staphlococcus aureus and against some gram-negative organisms; it was also active against Candida species and dermatophytic organisms.[119] The mode of action of triclosan was found to be associated with changes in membrane function; there was interference with the uptake of amino acids and uracil from culture media at low concentrations, and at bactericidal concentrations there were lesions in membranes which led to leakage of the cellular content.[120] Interconversion between variotin and ketovariotin was described.[121]

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Chapter 14. Antineoplastic Agents

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There probably exists no better way of being made aware of the enigmatic nature of the cancer cell than is provided by a review of the antineoplastic agents developed during a given year. In the absence of definitive information on the molecular basis underlying the development and growth of the cancer cell and the resulting biochemical properties which differentiate it from normal cells, the identification of selective inhibitors becomes entirely a matter of trial and error. Thus, screening for potentially active agents from plant and animal sources, structural modification of natural metabolites, and evaluation of cytotoxic chemicals are still the primary means used for the development of clinically useful antitumor agents. Although intellectually not quite satisfying, this empirical approach has been rather successful. The dramatic advances made in the treatment of the childhood leukemias, choriocarcinoma, Wilms' tumor, Burkitt's lymphoma and other neoplasms[1] have been achieved despite the lack of exploitable information related to the molecular or biochemical makeup of these tumors. Much experimentation and endless trials involving a multitude of schedules and drug combinations were necessary but, in the balance, the result was well worth the effort. In addition to the therapeutic success, much new knowledge was gained concerning the pharmacokinetics of various classes of compounds, their effectiveness in relation to the cell cycle, and their use in conjunction with metabolites which modify their toxicity to the host. The emphasis now is on the treatment of solid tumors, which have been largely refractory to the action of existing agents. In moving forward, it becomes necessary to focus on those factors which have made the past progress possible and to explore in what manner they could possibly be improved upon.

Since complex molecules such as actinomycin D or vincristine or adriamycin could not possibly have been designed, on a rational basis, as active antitumor agents, which indeed they are, it is obvious that screening for cytotoxic agents from natural sources needs to be continued. And so does the synthesis of new agents. The fact that some groups of compounds, such as the alkylating agents or certain quinone derivatives, have proved useful in cancer therapy, does not exclude the therapeutic potential of classes of compounds not currently recognized as antitumor agents. Investigations in an area where the clinical utility of a type compound has been established, constitute essentially a search for derivatives with selectivity greater than that of the parent. This aim is not realized too often. However, relatively small structural changes <u>can</u> make a significant difference. For instance, a hydroxyl group present in adriamycin leads to a marked increase in therapeutic efficacy as compared to the companion antibiotic daunomycin which lacks that group. In preparing derivatives, the problem becomes one of ascertaining the structural modifications which can lead to improved activity. It is unfortunate that correlations between structure and activity become possible only after scores of derivatives of a given compound have been prepared and their biological activity has been evaluated. But once

established, such correlations can serve to partially remove the empirical component from further designs, improving the chances for success.

In a series of conferences, the structures of a relatively large number of quinones[2] and of actinomycins[3] were related to their antitumor activity, and the salient structural features associated with this activity were described. Similarly, the concepts and rationales underlying the combination chemotherapy of cancer were explored.[4] These evaluations emphasized the requirement for statistical methods suitable for establishing correlations, and a survey[5] focused on the currently available techniques which, among others, include multiple regression analysis, discriminant analysis, molecular orbital calculations, cluster analysis, pattern recognition,[6] and substructural analysis.[7] Equally important is the continued re-evaluation of the animal models used for predicting drug action against human tumors, and although leukemia L-1210 is currently considered the most useful *in vivo* model system,[8] increased attention will have to be paid to human tumor explants carried in suitable animals.

With these considerations in mind, it is of interest to review some of the advances made during 1974 with respect to antineoplastic agents.

The structural modification of alkylating agents is being based increasingly on information concerning their metabolic activation (bioactivation). Thus, because the antitumor effect of cyclophosphamide is dependent upon its enzymatic oxidation in the liver, a Fenton oxidation product, 4-peroxycyclophosphamide, was evaluated and found markedly active against H. Ep. #2 cells *in vitro* and leukemia L-1210 *in vivo*.[9] The oxidation product may offer some advantage over the parent compound in cases where impaired liver function is encountered. Bioactivation also is the concept underlying the design of various benzo- and naphthoquinones containing one or two side chains capable of alkylation following enzymatic reduction of the quinone nucleus *in vivo*.[10,11] Various of these compounds interfered with the growth of adenocarcinoma 755 in mice, with nucleic acid synthesis, and with the activity of some coenzyme Q mediated enzyme systems. A sufficient number of nitrosourea derivatives have now been synthesized to allow the quantitative evaluation of structure-activity relationships. A reasonable correlation was found between the octanol-water partition coefficient of 14 nitrosoureas and their ability to inhibit Lewis lung carcinoma in mice. The optimal log P value fell between -0.20 and 1.34.[12] Similarly, a computer analysis of biological, biochemical and physiochemical data relating to several 1-(2-haloethyl)-1-nitrosoureas with activity against leukemia L-1210 showed that carbamoylating and alkylating activity as well as solubility participate in determining the degree of antileukemic activity.[13] Solubility and carbamoylating activity are major factors in determining toxicity, whereas the antitumor effect depends to a large extent on the alkylating activity. It was suggested that for optimal effect, nitrosoureas should have low carbamoylating activity, relatively low chemical stability, high alkylating activity and marked lipophilicity. Structure-activity comparisons with antitumor-active 3-methyl-3-nitrosoureido derivatives of various amino sugars related to streptozotocin showed that the sugar moiety serves as a comparatively non-specific hydrophilic carrier for the N-methyl-N-

nitrosourea group.[14] However, the carbohydrate moiety must provide some additional properties to the derivatives, since their biological effects differ from those of N-methyl-N-nitrosourea itself.

Agents which interfere with the availability or cellular utilization of amino acids continue to hold interest in cancer chemotherapy. Thus because human lymphoblastic leukemia cells in culture demonstrate an absolute requirement for L-cysteine, whereas normal human lymphoid cells in culture do not, "cysteine scavenging" was suggested as a possible selective antitumor approach. Therefore, α-methylenebutyrolactone derivatives potentially capable of combining with intracellular or extracellular cysteine were synthesized.[15] The compounds prepared were, however, equally toxic to the two cell lines, probably due to non-specific S-alkylation. Establishing an arginine deficiency in the growth medium, through the introduction of rat or beef liver arginase caused the inhibition of leukemia L-5178 Y and L-1210 cell growth in vitro.[16] This approach parallels the well established observations with glutaminase-asparaginase, and is potentially applicable to many other amino acid metabolizing enzymes. To avoid the toxicity associated with systemically administered L-asparaginase, the enzyme was incorporated into an extracorporeal chemotherapy unit through which human and baboon blood was perfused. The resulting serum asparagine levels were reduced to almost zero.[17]

Because t-RNA methylase activity is increased in various tumors[18] and S-adenosyl-L-methionine (SAM) functions as a methyl donor for transmethylations, the use of analogs which can interfere with the activity of SAM constitutes one way by which a selective antitumor effect might be achieved. In carrying out the transformation reactions, SAM is converted to S-adenosyl-L-homocysteine (SAH) and 5'-deoxy-5'-(methylthio)adenosine, both of which inhibit the transmethylation reactions. Among various analogs, 2-fluoro-5'-(ethylthio)adenosine was more inhibitory to H.Ep.#2 cells in vitro than was the parent compound.[19] Numerous analogs of SAH with modifications in the amino acid or the base moieties were, in general, less effective than SAH itself in inhibiting selected enzymes involved in methyl transfer.[20-22] It is of interest that the replacement of methionine by homocysteine in the culture medium of some normal and malignant mammalian cell lines led to the selective inhibition of the growth of the tumor cells.[23]

In the search for more selective analogs of folic acid, numerous structural modifications have been introduced into the molecule. Whereas some of the newly prepared derivatives showed growth inhibitory activity equal to that of methotrexate (MTX) in various bacterial systems, they were generally less effective or inactive as inhibitors of tumor growth in vivo. For instance, 1-deaza-N^{10}-methylfolic acid was as inhibitory as MTX to Lactobacillus casei and Streptococcus faecium, but not to leukemia L-1210 cells in vivo, and the compound was a poor inhibitor of dihydrofolate reductase (DHFR) from pigeon liver.[24] Similarly, 8-deazafolic acid and its reduced derivatives were as potent as MTX against S. faecium and the compounds retained good activity against a MTX-resistant strain, but they were ineffective inhibitors of L. casei DHFR.[25] On the assumption that the interchange of the amino and methylene groups at positions 9 and 10 of the

folic acid molecule could effect an alteration in the enzyme binding characteristics of the compound, isofolic acid[26] as well as isoaminopterin[27] were prepared. Isofolic acid was markedly inhibitory against both S. faecium and L. casei, whereas isoaminopterin inhibited only the growth of L. casei. Isoaminopterin was approximately one-half as effective as MTX in inhibiting L. casei DHFR, whereas isofolate was only a weak inhibitor of the enzyme, reflecting the contribution which the 4-amino group makes to binding. When the N^{10} of aminopterin was replaced by carbon, inhibitory activity similar to that of MTX was seen in S. faecium and L. casei and in the L. casei DHFR assay.[28] Since 3',5'-dichloro MTX is detoxified in man and in experimental animals via 7-hydroxylation, the 7-methyl derivative was prepared;[29] this derivative, like 7-methyl MTX, was less active against experimental tumors and L. casei DHFR than was the parent compound. Another derivative, 3',5'-dichlorohomofolic acid, was inactive against L-1210 in the mouse and was a weak inhibitor of L-1210 DHFR.[30] Similarly, 3'-ethylfolic and 3'-isopropylfolic acid were inactive against L-1210 in vivo and against pigeon liver DHFR although 3'-ethylfolic acid was a rather pronounced inhibitor of the growth of S. faecium.[31] The antibacterial and DHFR inhibitory activity but not the antitumor effect of 1,3-diamino-7,8,9,10-tetrahydropyrimido[4,5-c]isoquinoline was effectively increased by the introduction of lipophilic alkyl, aryl or aralkyl substituents.[32] In contrast, the presence of long chain alkyl groups in positions 5 or 6 of 1,3-diaminobenzo[f]quinazoline led to some loss of inhibitory activity against S. faecium, whereas a 6-chloro substituent enhanced the activity quite markedly.[33] None of the longchain alkyl compounds was as active against KB cells in culture than was the parent member of the series. Among a large series of quinazolines, pyrido-[2,3-d]pyrimidines and pteridines evaluated for their capacity to inhibit DHFR from various sources, activity comparable to MTX was exhibited by some of the compounds.[34-36]

To improve the transport of MTX across biological membranes, particularly the blood brain barrier, various modifications of the glutamic acid portion of the molecule were undertaken to effect an increase in lipophilicity. Among MTX analogs containing modifications of the carboxyl groups, the diethyl ester showed activity against L-1210 in vivo comparable to that of MTX itself, due most likely to hydrolysis to free MTX.[37] Replacement of the L-glutamic acid moiety with D-glutamic acid caused a marked loss of activity against L-1210 cells in vivo, whereas its replacement with glutaric acid or l-lysine resulted in inactivity.[30] A study on the stereochemical characteristics of the folate-antifolate transport mechanism in L-1210 cells revealed that the influx mechanism exhibits the greatest affinity for aminopterin, somewhat less for MTX and poor affinity for folic acid.[38] The affinity for quinazoline derivatives is somewhat less than that for aminopterin, but is severely reduced for the corresponding pyrimidine analogs. The efflux mechanism is also most efficient for the pteridine analogs, but the pyrimidine analogs efflux almost as rapidly. The quinazolines have the lowest affinity for the efflux mechanism. Thus, both influx and efflux determine the internal level of free drug achievable at any given extracellular concentration.

A symposium on the "Chemistry, Biology and Clinical Uses of Nucleoside

Analogs" held under the auspices of the New York Academy of Sciences[39] demonstrated the prominent position among the antineoplastic agents which this group of compounds still holds. Arabinofuranosyl cytosine (ara-C), 7-deaza-adenosine, and 2,2'-anhydro-arabinofuranosyl-5-fluorocytosine represent but some of the clinically useful nucleosides discussed. Analogs whose synthesis was reported during the past year include 2'-deoxy-6-selenoguanosine, which was less active against leukemia L-1210 *in vivo* than was the corresponding ribofuranosyl derivative[40] and a group of 4'-oxo-nucleosides, consisting of some 7- and 9-[6-deoxy-α-L-lyxohexopyranos-2(and 4)-ulosyl]purines which showed some inhibitory effect against KB cells; replacement of the carbonyl group in the carbohydrate moiety led to inactivity.[41,42] From the point of view of structure *vs.* activity it is noteworthy that the α and β anomers of 1-(2-deoxy-D-ribofuranosyl)-4-pyridone were inactive when tested in the *Escherichia coli* system,[43] whereas 3-deazauridine was an effective inhibitor of these bacteria as well as of leukemia L-1210 cells.[44] Activity against some tumor cell lines *in vitro* was also shown by 2'-amino-2'deoxy-5-fluorouridine[45] and by 5-thiocyanato-2'-deoxyuridine,[46] the latter exerting its effect most likely following reduction to the 5-mercapto derivative. Because of the pronounced antitumor activity of 2,2'-O-anhydro ara-C, the 4'-thio analog was prepared,[47] with activity against KB cells equivalent to that of the 4'-oxygen compound.

Among cyclic nucleotides, 9-(β-D-arabinofuranosyl)guanine cyclic 3', 5'-phosphate proved to be markedly cytotoxic to various tumor cell lines *in vitro*,[48] whereas some 8-seleno and alkylseleno substituted derivatives of cyclic AMP inhibited the growth of L-5178 Y cells *in vitro* only slightly.[49] To facilitate the passage of nucleotides through the cell membrane, some trinucleoside monophosphates containing thymidine and 5-fluoro or 5-bromo-2'-deoxyuridine residues were prepared.[50] The moderate inhibition of cell growth they produced was attributed to the slow initial hydrolysis of the triester.

In view of the encouraging antitumor activity of the thiosemicarbazone derivatives of 5-hydroxy-2-formylpyridine (5-HP) and 1-formylisoquinoline (IQ-1), a number of additional derivatives were prepared. Among these, 2-formyl-4-(m-aminophenyl)pyridine thiosemicarbazone[51] and 5-methylamino-1-formyl-isoquinoline thiosemicarbazone[52] were the most potent inhibitors of the growth of Sarcoma 180 cells in mice. The isosteric replacement of sulfur by selenium in -NH or O abolished the inhibitory effect.[53] Significant activity against Sarcoma 180 was also shown by some 4'-substituted derivatives of 5-HP,[54] and by some carboxaldehyde derivatives of IQ-1.[55] An evaluation of 97 α-(N)-formyl heteroaromatic thiosemicarbazones as inhibitors of three murine tumors and of the enzyme ribonucleoside diphosphate reductase from H.Ep#2 cells demonstrated a significant correlation between the *in vitro* enzyme inhibition and the antitumor activity in mice.[56] From the design point of view it was concluded that to produce activity, highly ionic or readily metabolizable groups, such as phenolic hydroxyls, need to be avoided, whereas suitable complex ring substituents that are C- or N- linked and can contribute to drug-enzyme interaction are desirable.

Combinations or complexes of drugs and tumor-specific antibodies are being explored with increasing frequency for their antineoplastic properties.

The role of the antibodies is to lend selectivity to the cytotoxic agents.[57] For instance, by coupling the alkylating agent Trenimon (2,3,5-tris-ethylenimino-1,4-benzoquinone) to immunoglobulins, both alkylating activity and immunological specificity were retained.[58] However, antibodies do not require complexing with a drug to exert enhanced effects. Antibodies to polyoma-transformed cells augmented the cytotoxicity to chlorambucil, although they did not bind the drug preferentially.[59] Similarly, the administration of antitumor antibody to mice one hour after injection of nitrogen mustard or arabinofuranosyl cytosine effected a synergistic inhibition of the growth of lymphoma cells.[60] A variation of the combination approach envisions the specific attachment of haptens to the tumor cell surface, followed by the generation of antibodies against the haptens.[61] The problem, of course, is one of obtaining haptens with tumor cell specificity. Unique differences in tumor cell surfaces do exist. For instance, as compared to normal leukocytes, the surface membranes of human and murine leukemic cells display a marked deficiency of unesterified cholesterol.[62] This low level can be increased by treatment of the cells with a lecithin-cholesterol mixture[63] and analogs of cholesterol can, probably, be bound as well. Another possibility is suggested by the observation that phenylglyoxal, which reacts specifically with arginine moieties in protein, does not enter HeLa cells, but, by masking arginine residues in the cell surface proteins, inhibits their growth.[64] The observation that in vivo treatment of L-1210 cells with 5-(3,3-dimethyl-1-triazine)imidazole-4-carboxamide appeared to induce new antigens in their cell surface suggests that such an increase in immunogenicity may lend itself to "adoptive immunotherapy".[65] Tumor specific cell antigens have also been considered potential targets for ^{131}I or ^{133}I-radioantibodies, which were calculated to deliver a sufficiently high dose of radiation to be of therapeutic value.[66] Since the boron (B) nucleus has the capacity to absorb thermal neutrons and subsequently to undergo fission with the release of α-particles which dissipate their ionizing energy within a few cell diameters, the selective accumulation of ^{10}B in a tumor has been considered potentially useful for selective therapy. Antitumor antibodies are envisioned as the specific localizing carriers. Various ionically solubilized boron hydrides were used to attach B to human α-globulin and to bovine serum albumin, but only small amounts (0.6-1.7% by weight of B) could be bound without significant loss of protein solubility.[67,68] To avoid the exchange of ionically bound B with other proteins, neutral polyhedral borones containing protein incorporating functions were prepared,[69] but their incorporation levels into proteins were relatively low.

In evaluating these approaches it is essential to keep in mind that the antigenicity of spontaneous tumors varies markedly from that of transplanted ones, and unless such antigenicity can be enhanced, it may not be sufficiently pronounced to permit its exploitation for therapy.

Numerous new antibiotics with activity against various experimental tumors have been isolated. Among these are vermiculine, a nine-membered lactone, produced by P. vermiculatum,[70] two antibiotics produced by P. stipitatum[71] and an azamino acid found in cultures of S. candidus.[72] The search for more selective analogs of adriamycin led to the preparation of

various O-acyl derivatives (acetyl, propionyl, benzoyl, etc.), none of which was significantly more active than the parent compound.[73] Of interest in relation to structure vs. activity is the suggestion[74] that the cardiac toxicity of daunomycin and adriamycin may reside in the daunosamine portion, which allows the molecules to be taken up by the heart muscle, whereas the antitumor activity resides in the chromophore. Various actinomycin D derivatives have also been prepared, and among these 7-nitro and 7-amino-actinomycin D were comparable to the parent in inhibiting the growth of some experimental tumors.[75] Among materials extracted from plants, cordacin, obtained from Drymaria cordata, is reported to exert significant activity against leukemia L-1210 in vivo, with low toxicity to the host,[76] and extracts from Zaluzania robinsonii[77] and Sarracenia flava[78] show some antitumor effects. Polysaccharides and lipopolysaccarides isolated from bacteria and fungi continue to receive attention for their tumor growth inhibitory properties,[79-81] their effect being mediated most likely through stimulation of the primary immune response.[82-85] Continuing attention is also being paid to the immunoprophylactic and immunotherapeutic properties of killed Corynebacteriae and Bordetellae, which cause the inhibition of the growth of various experimental tumors in vivo.[86-90] In view of the fact that interferon is of value in the treatment of osteosarcoma in vivo and in vitro, it deserves close evaluation.[91] Although reports on the antitumor activity of animal extracts appear with great regularity, a survey of the antineoplastic activity of materials extracted from mollusks, echinoderms, porifera, and a variety of marine invertebrates showed that such extracts are frequently quite impure, and that their utility is compromised by the opposing biological effects of such impurities. The report also indicated that whereas some active antitumor compounds were encountered, no clinically useful agents have, to date, been found in materials obtained from the sea.[92]

Polymers of various types, including pyran copolymer[93] and poly I-C[94] continue to be evaluated as antitumor agents. Significant activity was obtained against solid L-5178Y and against Lewis lung carcinoma by means of a soluble double stranded RNA-polyquaternary ammonium complex,[95] and some inhibition of Walker carcinosarcoma 256 was effected by poly (sodium acrylate) and poly (ammonium acrylate).[96] Of interest is the observation that, as evaluated against leukemia L-1210 in DBA_2 mice, an adriamycin-DNA complex had a better therapeutic index than did free adriamycin. The increased chemotherapeutic efficiency is thought to result from the relatively high endocytic activity of the tumor cells, which is responsible for the uptake of the complex into the cells. After degradation of the DNA inside the lysosomes, adriamycin is liberated and exerts its inhibitory effects.[97] Significant activity against Ehrlich ascites carcinoma was also produced by polypeptides consisting of 5-30 α-amino acid residues, wherein the terminal carboxy group was bound to bis(2-chloroethyl)amine or N,N-bis(2-chloroethyl) hydrazine.[98]

Certain dyes such as hematoporphyrin, fluorescein and acridine orange, which accumulate selectively in neoplastic tissues, have potential therapeutic utility. They can be activated by irradiation with visible light[99] or with a laser beam[100] at energy levels that do not cause heat damage, and

can subsequently effect partial or complete regression of various experimental tumors.[101]

Because of the demonstrated antitumor activity of several platinum complexes, other metal derivatives such as rhodium (II)-acetate or propionate were tested, and were shown to be effective inhibitors of Ehrlich ascites carcinoma in mice.[102] A heteropolyanion 5-tungsto-2-antimonate protected mice against Friend and plasma variant-induced leukemias.[103]

Miscellaneous agents which have shown activity against experimental tumors include prostaglandin $F_{2\alpha}$, active against an estrogen-dependent DMBA-induced mammary tumor of the rat;[104] a number of derivatives of 4'-(9-acridinylamino)-methanesulfonanilide[105,106] and various amine-substituted analogs of bisquaternary salts of 4-[p-(p-[4-pyridylamino]-phenylcarbamoyl)anilino]quinoline,[107] some with potent activity against L-1210 *in vivo*. Significant activity against various experimental tumors was produced by derivatives of 1-acetyl-2-picolinoylhydrazine,[108] by a number of acetylenic Mannich bases containing the N-bis(2-chloroethyl)amino moiety,[109] by homocoralyn,[110] 2,2'-(methylenediimino)bis-1,3,4-thiadiazole,[111] and by berberinol thiophosphamide.[112]

It is evident that this brief review concerned itself only with the experimental aspects related to the development of new antineoplastic agents. It is to be hoped that the therapeutic utility of one or another of the agents or approaches discussed will be such as to merit their inclusion in future clinical reviews.

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Chapter 15. New Concepts in the Chemotherapy of Neoplasia

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New observations have been made which are leading to, or will lead to, rapid clinical development. These can be divided into the following significant areas:

1) The role of cyclic nucleotides in producing cellular differentiation in malignant cells, and the role of phosphodiesterase inhibition, in maintaining cyclic nucleotide effects, and in controlling lymphocyte immunologic responsiveness and cytotoxicity.

2) The role of the cyclic nucleotides (cyclic CMP) in controlling the mitotic cycle.

3) Synergistic antitumor activity between alkylating agents and platinum derivatives and the synergy between a wide ranging group of antitumor drugs and the piperazinyl propane chelator, ICRF-159. Clinical evidence for enhanced effectiveness of 5-fluorouracil with the nitrosoureas and adriamycin with cyclophosphamide.

4) The development of relatively non-marrow depressing water soluble glucose-containing nitrosourea derivatives with increased therapeutic activity against the L1210 leukemia.

5) The utilization of DNA physical-chemical complexes with adriamycin, daunorubicin and actinomycin-D which are reputed to show improved therapeutic index.

6) The role of ribonucleotide reductase as a factor in tumor growth.

7) The effects on methotrexate (MTX) cellular concentration by vincristine and corticosteroids, and the possibility that MTX action may also exert effects via thymidylate synthetase as well as inhibition of tetrahydrofolate reductase.

8) The evidence that nitrogen mustard (HN_2) can exert its cytocidal activity independent of cell cycle kinetics but dependent on protein synthesis and that its entry into cells can be stimulated by agents which affect choline transport.

9) Evidence for increased gluconeogenesis stimulated by the presence of tumor in the host.

10) The application of sequential combination chemotherapy and adjuvant chemotherapy and immunotherapy to the clinical treatment of solid tumors with evidence of successful clinical application of adjuvant chemotherapy (phenylalanine mustard (L-PAM) in breast cancer and the reports of the usefulness of BCG, methanol extract residual (MER), C. parvum, and tilorone in melanoma, leukemia, bowel and other tumors.

11) The development of agents which produce "chemical" adrenalectomy, or block pituitary trophic hormones, and estrogen trophic action which are now in clinical trial against breast and prostate cancer.

12) The systematic study of inhibitors of RNA and DNA polymerase for antiviral (oncorna viruses) and antitumor activity which may lead to effective inhibitors of latent tumor viruses as well as effective antitumor agents.

13) The relationship of tumor fibrinolysin activity to malignancy and the presence of acid proteases in tumors. Attempts at modulation have included protease inhibition (pepstatin) and the use of warfarin and streptokinase clinically.

14) The evidence that inhibition of metastatic disease may involve alternative forms of chemotherapy that inhibit tumor vascularization (ICRF-159) and nidation.

15) The evidence that complement dependent immune cytotoxicity, and cellular immune responses, can be mediated by diverse pharmacologic agents (i.e., tilorone, levamisole, diethyl dithiocarbamate, cholinergic agents, carbodiimide, insulin) and that chemotherapeutic tumor cell injury can predispose to immune lysis.

16) The demonstration that radioprotective thiols have antitumor activity.

Clinically, the concept of combination chemotherapy, based upon successes in the treatment of acute leukemias, has developed into a major clinical activity in the solid tumor area. In most cases, these clinical studies are based upon empirical trial with the rationale related to combinations between cell cycle specific and non-specific antitumor agents applied in a simultaneous or sequential manner. In some cases, the approach is designed to develop tumor synchrony so that tumor cells will become more vulnerable to the effects of cycle specific agents for enhanced antitumor activity. In this regard, asparaginase in the treatment of leukemia has shown increased success when methotrexate is given at 5-10 days, following the administration of asparaginase.[1] This is related to the effect of asparaginase in synchronizing the leukemic cell population so that S phase vulnerability coincides with methotrexate administration. If methotrexate is given simultaneously with asparaginase, the synergistic effect is lost and methotrexate action is impeded. More importantly, evidence that nitrosoureas cause delays at S and G2 of the cell cycle are supported by apparent improvement in therapeutic response when they are administered in combination with a drug that is active in S phase (i.e., 5-fluorouracil).[2,3] However, we should not forget previous experience with antibiotic combinations where cytostatic drugs could interfere with the effective action of cytocidal agents. Utilization of the L1210 tumor model has shown that dose timing is critical to the success of drug combinations and that sequential chemotherapy may be better than simultaneous use of several drugs.[4]

Clinical chemotherapy is now sophisticated enough to realize that solid tumor growth is not exponential but can become so on removal of the bulk of the tumor.[5] Repetitive chemotherapy can be most effective by increasing tumor vulnerability through development of mitotic synchrony or, alternatively, utilizing drugs whose metabolic pathways impinge sequentially on cellular events. Dose-kill concepts that require massive marrow destruction and supportive therapy with autologous marrow transplants, platelet and granulocyte transfusions, although currently in vogue, will hopefully not be necessary.

In another area, the concept has now been established that the smaller the tumor burden, the greater the possibility of chemotherapeutic success

through interreaction with endogenous host resistance. This has led to adjuvant chemotherapy where treatment is given in the absence of clinical disease (in association with surgical resection or curative radiotherapy). Prolongation of five-year survival has been seen in breast cancer with repetitive 5-FU treatment in patients with residual but asymptomatic disease,[6] and similar projected five-year results have recently been reported for the use of phenylalanine mustard (L-PAM).[7] Excellent prolongation of survival (inhibition of pulmonary metastases) has been seen with the administration of adriamycin and/or high dose methotrexate with citrovorum factor rescue in osteogenic sarcoma following tumor amputation[8,9] and similar results have been seen in Wilms tumor and pediatric rhabdomyosarcoma and neuroblastoma with adriamycin and other agents. However, in bowel cancer, recent 5-FU post-operative treatment of patients with residual disease at the time of curative surgery has been disappointing although BCG administration with 5-FU is promising in early data. Other studies are still in progress, and chemotherapy in the absence of symptomatic disease but with predictable symptomatic recurrence is undergoing wider testing.[10]

In the laboratory, utilizing the L1210 model, the most promising combinations are those that show true synergy. This is true for ICRF-159, -1,2-di(3,5-dioxopiperazinyl-yl)propane, which synergizes with 5-FU, Cytoxan, DTIC, vincristine, adriamycin and hexamethylmelamine (HXM).[11] Combinations of ICRF-159 with adriamycin and hexamethylmelamine are of particular clinical interest because adriamycin dosage can be reduced, thus decreasing clinical costs, and avoiding its myocardial toxicity. Reducing clinical dosage of HXM to avoid nausea and vomiting would have significant clinical benefit. ICRF-159 inhibits tumor vascularization and may exert its anti-metastatic effect through this mechanism,[12,13] and evidence is developing that indicates alternative therapies for primary tumors in contrast to metastatic disease. These include ICRF-159, triton WR 1339 (polyoxyethylene ethers), tetramisole and methyl-CCNU[12] as well as streptokinase.[14]

Hexamethylmelamine and ICRF-159, given singly, are relatively inactive against the L1210, but true synergy is seen with the combination producing increased life spans up to 175%.[11] Hexamethylmelamine shows a spectrum of activity against lung and lymphomas, and is the best single agent in ovarian carcinoma. Its activity in man and the mouse is characterized by demethylation to melamine, and it does not inhibit dihydrofolate reductase as is true for other triazines.[15]

Synergy has also been seen for cytoxan in combination with the organometallic platinum compound, cis-dichloro (dipyridine) Pt II (cis-PPC), and *in vitro* synergy has been reported for this compound with adriamycin, BCNU and camptothecin.[16] Another interesting synergy has been reported for amphotericin-B followed 24 hours later by BCNU[16] and amphotericin-B, which stimulates phagocytosis, shows enhanced toxicity to cells in tissue culture in combination with rifamycin derivatives.[17]

A variety of clinical combinations are now in use and these include: adriamycin, 5-FU, DTIC, vincristine, and Cytoxan or nitrosourea alkylating derivatives. Hydroxyurea in combination with 5-FU has reported clinical usefulness in bowel and gastric cancer.[18] However, the best drug combinations we have against gastro-intestinal cancer appears to be the combination of methyl-CCNU with 5-FU.[3] As mentioned previously, this is not surprising because of recent in vitro data indicating that nitrosoureas prolong S&G2 phases of the cell cycle making cells theoretically vulnerable to drugs acting on DNA synthesis or metaphase following nitrosourea administration.[2]

Streptozotocin, bleomycin and emetine are also used in combination studies with particular value because they do not have bone marrow depressing characteristics. Streptozotocin is a nitrosourea containing compound[19] while bleomycin is a glycopeptide with effects on DNA synethesis[20] as well as nucleolar activity.[21]

In relation to the newer agents, the nitrosoureas provide a most exciting compound in chlorozotocin, 2-3-(2-chloroethyl(3-nitrosoureido)2-deoxy-D-glucopyranose, (DCNU, NSC 178248).[22] This compound is a water soluble nitrosourea that shows minimal bone marrow toxicity and has produced an increased life span (ILS) of 701% when given on day 2 following L1210 inoculation, and when given to the established tumor on day 6, at an LD10, it has given a greater than 310% ILS. It is much more effective than BCNU and, unlike BCNU, is effective in the mouse at therapeutic levels that do not produce marrow depression. 1,3,4,6-tetra-O-acetyl (di-2-chloroethyl) amino 2-deoxy-d-glucopyranose (TGM) has related activity and is most effective when given in multiple dose schedules which suggests that aminoglucose modification of nitrosoureas changes schedule dependencies.[23] The entry of these drugs or related structures into the clinic are awaited with great interest.

The available nitrosoureas (BCNU, CCNU, methyl-CCNU) are lipophilic and enter the central nervous system, and are clinically active against glioblastomas. However, a water soluble nitrosourea in a rat brain tumor model has been found (PCNU, NSC 95466) which had superior antitumor activity to the more lipid soluble compounds.[24] This may correlate with greater alkylating activity versus low carbamoylating effects.[25,26] Study of the action of nitrosoureas in relation to effects on DNA polymerases is underway.[26]

A new triazine antifolate is ready for clinical trial, triazinate (TZT), (NSC 139105) which is a potent inhibitor of tetrahydrofolate reductase. This drug enters the brain with significant levels in the CSF up to 8 hours in contrast to methotrexate.[27] This is of interest in that alternative mechanisms for methotrexate activity now appear to involve direct inhibition of thymidylate synthetase.[28]

The anthracyclines are of major importance, and adriamycin has had extensive successful clinical trial in Ewing's, osteogenic and soft tissue

sarcomas as well as lung and breast carcinoma. The demonstration of enhanced in vitro activity for adriamycin-DNA complexes has resulted in development of actinomycin-D-DNA complexes which also have shown improved therapeutic index in animal model systems.[29] The concept behind DNA complexing was developed from the work of Trouet and DeDuve, who felt that producing a neutral adriamycin-DNA complex could permit endocytic activity to more readily pick up these agents, thus providing therapeutic advantages as endocytic activity is higher in tumor cells. Once in the tumor cell, lysozomal or phagasomal deoxyribonuclease splits off the DNA from the complex, releasing the active compound. These concepts most assuredly will be extended to other basic chemotherapeutic agents, but one of the major problems of this work is the variation in the DNA complexes formed, thus producing results that vary from laboratory to laboratory depending on the DNA polymers used.[30] The therapeutic effectiveness of the anthracycline DNA complexes could be due to a slow release phenomena changing the pharmacokinetic action of these agents. Other adriamycin compounds such as 14-O-acyl derivatives may work through slow release by cellular esterases with adriamycin as the active compound.[31]

In another area, the vinca alkaloids, vincristine and vinblastine, bind to microtubular protein with resultant depolymerization of this protein in the mitotic spindle and/or cellular cytoskeleton, explaining stathmokinetic effects and/or cytocidal action. Of interest to the latter are the recent observations[32,33] that methotrexate turnover in cells in vitro is blocked by vinca action and that elimination of methotrexate from cells can be inhibited. One should determine whether cytoskeletal (microtubular) integrity is necessary for the elimination of certain drugs within the cell. The interaction of drugs on membrane transport in cells is of importance with recent data indicating that corticosteroids can interfere with methotrexate action[34] while quinacrine, morphine and cocaine stimulate HN_2 uptake in in vitro tumor cell models.[35]

Of interest, new vinca alkaloids have been developed with different tumor spectrums from those of vincristine and vinblastine. These vinblastine amides, unlike the parent vincristine and vinblastine, have major bone marrow depressing activity, and it is hoped that these new vinca derivatives may not be as neurotoxic and thus may give us a whole new series of vinca derived compounds of differing clinical usefulness.[36] The podophyllotoxin derivatives are back in clinical trial, and newer derivatives are not metaphase inhibitors and may work by blocking DNA synthesis late in S phase.[37]

A variety of immunoadjuvants apart from BCG are now in clinical trial. These include fixed vaccines like methanol extract residual of BCG (MER) and Corynebacterium parvum. MER has resulted in regression of advanced bowel cancer in several patients and C. parvum has been reported to increase clinical responsiveness in a variety of cancer patients with concomitant chemotherapy.[38]

Of interest to medicinal chemists, synthetic compounds are available with similar action. Pyran copolymer, the divinyl ether maleic anhydride

copolymer, if provided as pure lower molecular weight fractions (<15,000 MW) should become clinically useful because of better therapeutic index.[39] Pyran has anti-coagulant activity and like polymethacrylic acid can decrease pulmonary metastases from Lewis lung tumor. Pyran, tetramisole and tilorone, given as immunoadjuvants, can prevent tumor growth of the LSTRA sarcoma following BCNU.[40] However, immunoadjuvants differ in mechanism of action as 2,3,5,6-tetrahydro-6-phenyl-imidazo thiazole "tetramisole" and its l-isomer, "levamisole" are potent T cell stimulators,[41] while pyran and tilorone show evidence of T cell inhibition.[42]

Tilorone (DEAE fluorenone) and MER 11,002 (DEA fluorene), which are interferon inducing antiviral agents, are in clinical trial for antitumor action. Tilorone and its analogs inhibit tumor virus DNA polymerases.[43] Tilorone has proven disappointing clinically despite transient melanoma regression in advanced cases, but is currently under study as an adjuvant in early disease.

Levamisole, as a potent T cell stimulator,[41] is currently in early trial as a post-surgical adjuvant. In regard to its mechanism of action, it may work through effects on the cyclic nucleotides of the lymphocyte.[44] Renoux[45] has found that diethyl dithiocarbamate (DDC) has similar action to levamisole as a T cell stimulator. This is of interest in that DDC was shown to have antitumor action against a wide range of tumors and was given a Phase I intravenous clinical trial. Although striking pain in tumors was seen, a Phase II clinical study was never conducted. DDC is currently used orally as a chelating agent to treat nickel poisoning and is the active agent in antabuse as an inhibitor of alcohol dehydrogenase. Of parallel interest, tetramisole has been reported to sensitize to alcohol and to possess clinical anti-depressant activity. DDC inhibits β-DOPA decarboxylase and blocks noradrenaline and protein synthesis in rat brain with destruction of latent memory. The antitumor action of DDC should not be surprising in that striking tumor necrotization of MC sarcoma and the KREBS-2 tumor has now been reported for radioprotective thiols. These include compounds which possess a free amino group and a free sulphydryl group, such as: mercaptoalkylamines, mercaptoalkylguanidine, and thiophosphates.[46] This is apparently the re-appraisal of a whole new area, which must be looked at for effects on host resistance.

The action of antitumor agents like DDC or tilorone (bizarre dreams) on CNS activity as well as immunologic response should not be surprising in view of antitumor activity for agents as diverse as the anti-serotonin, cinanserin, the muscle relaxant, chlorphenesin[47] and alkylating agents which possess cholinergic and/or barbiturate stimulating activity. Cholinesterase inhibition stimulates lymphocyte function,[48,49] and amidoximes have been synthesized whose potent L1210 antitumor activity in mice is limited by seizure activity of unexplained etiology.[50] Δ9-Tetrahydrocannabinol (Δ9THC) shows both immunosuppressive and antitumor activity at high, clinically inappropriate doses in mice, but further work is warranted because of selective effects in tissue culture on DNA synthesis and tumor cell replication.[51] The thioxanthone, hycanthone, has hallucinatory activity, and there

are structural similarities between Δ9-THC, tilorone, hycanthone and lucanthone (miracil D). Both hycanthone and lucanthone are in clinical trial.[52] Lucanthone is an oral schistasomide which also resembles actinomycin-D structurally, and has been shown to increase clinical regression in pulmonary metastases following radiation.[53]

In relation to immunologic response, there is an important observation indicating that idoxuridine (IUDR) (5-Iodo-2-deoxyuridine) stimulates hemolysin plaque formation up to 15 days after drug administration.[54] This thymidine analog, which has anti-DNA viral effects, is a potent 19S antibody stimulator. Of interest, IUDR stimulates virus C particle formation and may be similar to immunoadjuvant agents like pyran, poly I:C or tilorone in some experimental tumor systems.

The evidence for neuraminidase enhancement of tumor cell antigenecity has suggested that highly charged compounds that would react with negatively charged glycoproteins at the tumor cell surface might be equally useful in sensitizing tumors to immune response and this has led to the study of carbodiimide as an enhancer of complement dependent antibody lysis of tumor cells.[55] This may explain the antitumor action of polyethylene imines and polyionines.[47] Of importance to this, contact between sensitized lymphocytes and activated macrophages and target cells is a prerequisite for lymphocyte mediated cytotoxicity. Changes in surface charge could affect "killer" cell approximation to the tumor cell target. The observations that vitamin A stimulates immune response and that exposure of tumor cells to vitamin A alcohol produces loss of cell surface coat has potential clinical usefulness.[56]

Decreases in cyclic AMP, increases in 8-bromo guanosine 3', 5'-monophosphate, cholinergic stimulation[48] or insulin[49] elevate cyclic GMP and increase the target killing capacity of sensitized lymphocytes. In another area, streptokinase[57] induced fibrinolysin has been reported to activate cell mediated immunity and is in clinical trial as a post-surgical adjuvant. Chemotherapeutically injured tumor cells are more sensitive to the lethal action of complement mediated antibody effects.[58]

The role of adriamycin and newer anthracyclines as biologically useful agents relates to the level of reverse transcriptase (RT), and its presence in oncogenic RNA viruses may be controlling factors in a variety of animal tumors and the presence of this enzyme may be a clue to malignant transformation. Apple[59] has screened anthracyclines which block *in vitro* viral RT for *in vitro* and *in vivo* prophylaxis against Rous sarcoma virus (RSV) in chicken tissue. These agents block RSV focus formation and *in vivo* sarcoma development is blocked when given in close conjunction with virus inoculation. The most potent inhibitors, both *in vitro* and *in vivo*, were cinerubins and 14-O-alkyl-adriamycins. In other models, O'Connor et al.[60] have studied the inhibition of transformation of mammalian cell cultures by sarcoma-inducing oncorna viruses following administration of rifamycin antibiotics. Inhibition of virus yield and cell transformation was seen with rifamycin antibiotics that were independent of 3' substitution

and the ability of the rifamycin to inhibit viral DNA polymerase. O'Connor et al.'s screening program is testing a wide variety of chemotherapeutic agents for simultaneous inhibition of DNA and RNA polymerases and correlating this with effects on oncornavirus yield and cellular transformation. Of interest to the above, most recently, purified liver DNA polymerase II has been found to be significantly inhibited by nitrosoureas and isocyanates.[26] Jasmin[61] has found that phosphotungstates can affect tumor growth with effects on nuclear polymerases. It is hoped that from this work, agents will be found that can be used prophylactically or therapeutically against viral-induced or established tumors.

Ethidium bromide and related structures have potent antitumor activity and are inhibitors of RNA dependent DNA polymerases.[62] Ethidium chloride and isometamidium have been in Phase I clinical study with cardiotoxicity as a dose limiting problem for ethidium, but one should remember that ethidium synergizes with aza-serine as phenanthridinium compounds block the exogenous incorporation of purines into DNA.[63] Nine alkyl substituted purines, which had clinical activity, behave similarly,[64] but apparently they have not been looked at as inhibitors of the reverse transcriptase.

Cyclic AMP and GMP are becoming increasingly important to concepts of tumor growth and the differentiation of tumor cells toward normal morphology. In hepatomas, in parallel with increasing growth rates, the activity of adenylate cyclase decreased and that of phosphodiesterase increased.[65] The ratio of phosphodiesterase to adenylate cyclase correlated positively with increasing growth rate.[66] Similar relationships have been seen for metastasizing versus non-metastasizing tumors.[67]

Growth inhibition of a DMBA transplantable mammary tumor and the Walker 256 tumor was produced by N-6,O-2-dibutryl cyclic adenosine 3', 5' monophosphate.[68] In addition, a series of nucleoside 3', 5' cyclic phosphates has been developed as antitumor agents. These have been coupled to 6-mercapto purine riboside, arabinosyl cytosine, 5-fluorodeoxyuridine, and arabinosyladenine.[69] While their effects against the L1210 system were not that much greater than with the parent compounds, they inhibited intracerebrally inoculated leukemia L1210 cells. Similarly, prostaglandin E_1 increased the survival of mice with B16 melanoma by up to 60%.[70] This mechanism of action is thought to involve cyclic AMP which has been shown to control the differentiation of B16 melanoma cells.

Papaverine, a potent phosphodiesterase inhibitor, has produced reversion of neuroblastoma cells to normal and apparently inhibits lymphocyte response to phytohemaglutinin.[71] Neuroblastoma cells also revert toward normal morphology on exposure to dopamine and epinephrine as well as sodium butyrate which increases cyclic AMP levels.[72] Prostaglandins behave similarly. Neuroblastoma differentiated cells are distinct from malignant neuroblastoma cells in relation to interaction with guanosine triphosphate and divalent cations.[73]

Cyclic AMP is also important to modulation of viral replication as it has been shown that dexamethasone increases mouse mammary tumor virus

replication, and dexamethasone given with dibutyrul cyclic AMP produces higher yields of the mouse mammary tumor virus.[74] The relationship of phosphodiesterase inhibitors is particularly of interest in view of apparent reports of caffeine-BCNU synergy which is now undergoing clinical test. Of tremendous chemotherapeutic potential is this year's observation by Bloch[75] that cyclic CMP controls the mitotic cycle of L1210 leukemia cells.

In relation to hormonally dependent tumors, prolactin as a controlling factor in promoting mammary cancer has given rise to L-dopa and ergotamine related compounds which prevent pituitary release of this mammotrophic hormone.[76,77] L-Dopa has produced clinical regression of mammary tumors and 2-bromo-alpha-ergocryptine (CB-154) has virtually prevented mammary tumors in mice with and without estrogen secretion.[78] This is of interest to the anti-estrogen action of diethyl dithiocarbamate which blocks β-dopa-decarboxylase. In another area, "medical" or "chemical adrenalectomy" utilizing the anti-convulsant, amino-glutethimide, has produced symptomatic relief in human breast cancer[34] and is in trial against prostatic cancer as well. Anti-estrogens such as tamoxifen (ICI 46,474), the trans-isomer of 1(4-(2)-dimethylaminoethoxy) phenyl) 1,2-diphenyl but-1-ene citrate is a powerful anti-estrogen with clinical effectiveness in breast cancer.[79] Spironolactone has reported activity in pancreatic cancer[80] which is not surprising because of previous reports of aldosterone dependency of transplanted tumors.

It has also been found that nitrogen mustard (HN_2) exerts cytocidal action independent of cell cycle[81] and related to synthesis in that cyclohexamide can protect intestinal mucosa from *in vivo* injury produced by HN_2, radiation and cytosine arabinoside.[82] In other areas, the action of alkylating agents has been suggested to possibly relate in some cases to acrolein formation. Cytoxan, isophosamid, trilophosphamid, bis(3-mesloxypropyl) amine, propanediamine mustard and the phenothiazine, chlorpromazine, have all been shown to release acrolein.[83,84] Nitrosoureas are activated by microsomal enzymes to give rise to isocyanates which have carbamoylating activity as well as alkylating effects.[25]

The combination of antitumor with anti-viral agents may have clinical usefulness. In the AKR leukemia, imidazole carboxamide riboside (virazol) prolonged survival in mice which received systemic chemotherapy.[85] Whether this is indicative of a direct additive tumorocidal action for virazol to other chemotherapeutic agents, or that an anti-viral agent like virazol might be useful to prevent leukemic viral reinduction in mice whose tumor burden is decreased by chemotherapy, awaits further trial.

Of major clinical potential, the role of adenosine in inhibition of lymphocyte response[86] becomes pharmacologically controllable with the development of adenosine deaminase inhibitors,[87] and we may be in a position to develop a new group of immunosuppressants and/or antitumor agents. In recent work, the lymphokine Migration Inhibitory Factor (MIF) has shown macrophage activating activity and on pre-treatment of macrophages with di-isopropyl-fluorophosphate or with soybean trypsin inhibitor increases are seen in macrophage phagocytic function.[88] The fact that esterase

inhibitors have modulating function of this kind is of increasing importance with recent evidence that activated macrophages may be a prime killing system for tumor control.[39]

Numerous attempts are underway to develop slow release forms of ara-C to decrease bone marrow and intestinal epithelial toxicity and the need for frequent drug administration. Benzoate-ara-C (BARA-C) has been developed which, in animal studies, shows that selective toxicity can be obtained with normal tissue recovering more rapidly than tumor tissue despite prolonged exposure.[89] The combined use of tetrahydrouridine, a cytidine deaminase inhibitor, with ara-C, is finally entering clinical trial.[90]

The evidence that gluconeogenesis is stimulated in animals with tumors and relates to glucose utilization and steroid effects has been presented.[91] Clinical application of hydrazine SO_4[92] in an attempt to therapeutically moderate this effect for host benefit has been disappointing but deserves further laboratory effort.

Two other areas of fundamental importance to tumor growth control capable of pharmacologic approach have been based on the evidence that tumor asites accumulation can be altered *in vivo* by pepstatin, the acid protease inhibitor.[93] The pH optima for proteases inhibited by pepstatin is similar to that of acid proteases at a pH 6 implying that there may be micro-pH environments at the cell surface or within the cell affected by acid proteases and their inhibitors. This is of interest as protease inhibitors have been reported to decrease the incidence of skin cancers and colon carcinogenesis in animal models.[94]

The author urges that more activity be directed toward the study of ribonucleotide reductase inhibitors. The reduction of ribonucleotides is a critical rate limiting step[95] to the formation of deoxyribonucleic acid. Data indicates that correlates are found between increases in this enzyme and malignant transformation of liver cells.[95] Hydroxyurea is an inhibitor of this enzyme,[96] and pyridoxal phosphate and the dialdehyde derivates of adenosine and adenosine 5' mono and triphosphate inhibit *in vitro* ribonucleotide reductase.[97]

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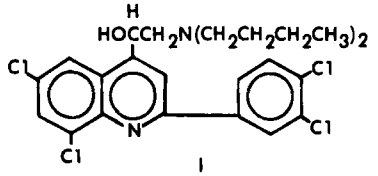
Chapter 16. Antiparasitic Agents

Edgar J. Martin, Food and Drug Administration, Rockville, Md.

Introduction - An encyclopedic work on chemotherapy of protozoan diseases was compiled.[1] Reviews appeared on the chemical control of mosquito larvae[2] and the important problem of drug-resistant malaria.[3,4]

Antimalarials - A monograph appeared on antimalarial agents.[5] The DNA binding and effect on RNA synthesis by chloroquine analogs were found to be functions of length and number of basic groups in the side chain.[6] Acid protease of Plasmodium falciparum and P. knowlesi differed from those of host-erythrocyte ghosts; pepstatin and chymostatin were their most potent inhibitors.[7] An enzyme of folic acid biosynthesis in P. chabaudi was purified.[8] Dihydropteroate synthetase was isolated from P. berghei.[9] The thymidylate synthetase of mouse erythrocytes was found to increase about seven fold during P. berghei infection.[10] The half-life of pyrimethamine in 6 healthy humans averaged 92 hr.[11]

A common triangular structure feature has been proposed for several classes of antimalarials.[12] Epidihydroquinidine and some of its analogs were found active against P. berghei.[13] Trials with some quinolones were not encouraging.[14] Sixty-six quinoline methanols varied in activity against P. berghei and P. gallinaceum.[15] Compound WR-30090, I, was active against certain strains of P. falciparum and P. vivax.[16] Various 8-aminoquinolines were laboratory tested with various results.[17] Some methoxyacridine analogs were found curative against P. berghei at high dose levels.[18] Threo-compounds of pyridine-methanols were generally more active than the erythro compounds.[19] Quinazolinemethanol analogs were found highly photosensitizing.[20] Certain thiadiazoles were active against chloroquine resistant P. berghei, and a phenothiazine was twice as effective as quinine against P. berghei.[21,22] The phenanthrene-methanol WR 33063, II, was active against the Chesson strain of P. vivax and seven P. falciparum strains, including the chloroquine and pyrimethamine resistant Camp strain.[23,24] Phenanthrene amino alcohols, whose triangular pharmacophore contained nitrogen and oxygen atoms in close proximity, were active against P. berghei.[25] In a series of anthracene aminoalcohols the analog III was the most active against P. berghei.[26] It has been proposed that in naphthothiophenes the analogs whose side chains



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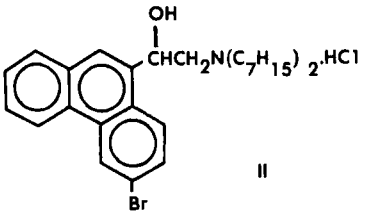

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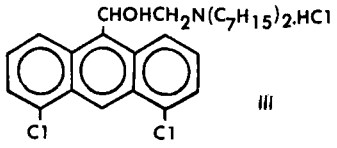
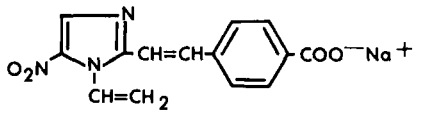


III

enhance DNA binding afford the greatest antimalarial activity.[27] But in naphthoquinone analogs the relationship between physical properties and selective toxicity were unclear.[28] From a series of guanidinepyrimidines and amidobenzimidazoles analogs were selected for clinical trials.[29,30] A P-nitrophenylsulfonyl acetanilide derivative was found less toxic to mice and more effective against P. berghei than chloroquine.[31] The compound 4-bis(N,O-diacetylhydroxylamino)diphenylsulfone (TAHDS) was found more active than DDS against P. berghei.[32] Some fluorene derivatives were found active against P. berghei.[33] The potency of lincomycin was similar to that of its derivatives.[34] Combinations of specific drugs are sometimes beneficial, particularly where single or multi-drug resistant malaria strains have become a problem.[35]

Antitrypanosomals and Antileishmanials - Reviews appeared on these agents and their problems of drug resistance.[36,37] Carbazole and metronidazole derivatives have been synthesized and tested for antitrypanosomal[38] and antitrichomonal activities.[39] In styryl-nitroimidazole derivatives, antitrypanosomal activity was found when the styryl function was placed in the 2 position of the imidazole nucleus, but not in the 4 or 5 position.[40] The nitrovinylfuran SQ 18,506 was found active against Trypanosoma brucei in mice.[41] The antibiotic dactylarin was active against Leishmania brasiliensis at an MIC of 0.5mcg/ml.[42] Cleavage of diminazene aceturate occurs in acid solution; the diazonium fragment is trypanocidal but has only short term stability.[43]

Antitrichomonal Agents - In vitro and in mice testing showed activity of analogs of nitrothiazole,[44,45] metronidazole,[37,46,47] nitroimidazole sulfides and sulfoxides,[48] benzofuran,[49] and quinoloncarboxylate.[50] The nitrovinylimidazole IV was found to be strongly protein bound in mammals, including humans. Trichomonas vaginalis takes up IV only in the unbound form, and degrades it to some extent. IV inactivates certain energy-producing enzymes and blocks ATP synthesis in T. vaginalis and Trypanosoma rhodesiense. It does not inactivate glycolytic enzymes in the mouse.[51] Antitrichomonal activity was reported for the antibiotics SC-28763 (isolated from Spicaria divaricata),[52] EM 49 (a peptide from a Bacillus circulans strain),[53] SPA-S-132 (patricin, isolated from a Streptomyces aureofaciens strain),[54,55] X-5108 (isolated from Streptomyces goldiensis),[56] and actinotiocin (a sulfur containing peptide isolated from Actinomadura pusilla).[57]

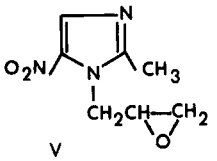


IV

Anticoccidia and Antitoxoplasma Agents - A review on chemotherapy of chicken coccidiosis appeared.[58] An aryltriazine[59] and some derivatives of azouracil,[60] 6-hydroxypyranone,[61] pyridoxal,[62] 4-hydroxyquinoline-3-carboxylate,[63] and the antibiotic rosamicin (Sch 14947)[64] were found active against Eimeria species. Clindamycin and its N demethyl-4'-pentyl derivative were found active against Toxoplasma gondii in mice.[65]

Anaplasmosis, Babesiasis, and Theileriasis - A review of these infections was published.[66]

Amebicides - A review appeared on the chemotherapy of amebiasis.[67] Amebicidal activity was reported from quassin and some of its analogs extracted from Simarubaceae.[68] New amebicidal benzofuran analogs were synthesized.[69] The nitroimidazoles V and VI were effective against Endamoeba histolytica and T. vaginalis.[47] The proton magnetic resonance spectra of antiamebic and antitrichomonal nitroimidazoles were studied.[70]



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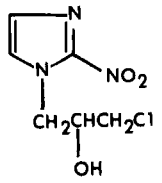

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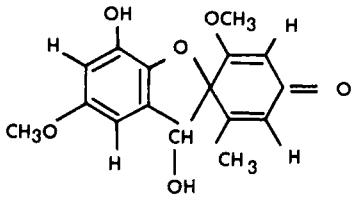
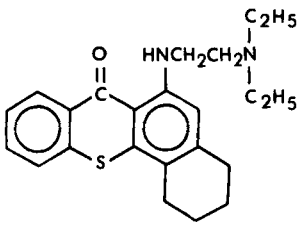


VII

Some substituted triazines were active against E. histolytica and T. vaginalis.[71] Anisomycin analogs were found active against E. histolytica,[72] dactylarin VII against E. invadens,[42] and clotrimazole (BAY5097) against Naegleria fowleri.[73] The prolonged use of diiodohydroxyquinoline caused optic nerve atrophy in a patient;[74] related drug injuries were previously described as subacute myelo-optico-neuropathy (SMON).[75]

Antischistosomal Agents - A review appeared on antischistosomal drugs.[76] Certain modifications of the hycanthone molecule reduced its mutagenicity as measured by the Ames technique.[77] Nitrofuryl-vinyl compounds were active against Schistosoma mansoni.[78] Replacement of the 5-nitro group with a cyano, carboxy or carbomethyl group caused loss of activity.[79] Tubercidin was found active against the females of S. mansoni and S. japonicum.[80] A nitrothiazolylurea analog was found active against schistosomes and T. vaginalis,[81] and the thioxanthene VIII was found active against S. mansoni in mice.[82] A preliminary report indicated that certain thiophene analogs have antischistosomal activity.[83] The tetrahydroquinoline oxamniquine (UK4271) IX was effective against S. mansoni in



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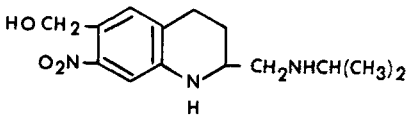
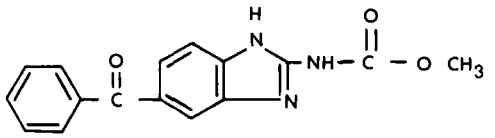


IX

humans.[84] Some analogs of tetrahydroquinoline and of pyrazinoquinoline were found active against S. mansoni in mice and Cepus apella monkeys.[85] The naphthoquinoline, Lapachol, given by gavage to mice, caused reduction of S. mansoni cercaria penetration.[86] Niridazole was found to be a frameshift mutagen.[87]

Other Anthelmintics - A monograph appeared on anthelmintics [88] as well as reviews on chemotherapy of Trichinella spiralis[89] and of trematodes and cestodes.[90] Reviews appeared on the chemistry[91] and chromatography[92] of benzimidazoles. Hansch analysis determined structure-activity relations between dihydrobenzanilides and inhibition of succinate dehydrogenase.[93] The benzimidazole-carbamate analogs mebendazole X and fenbendazole XI



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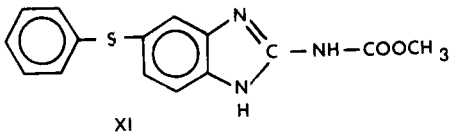

XI

were found to have broad spectrum anthelmintic activity.[94] In infected humans, mebendazole treatment causes trichuris eggs to develop abnormally.[95] Certain benzanilids were found active against Hymenolepis diminuta, Aspicularis tetraptera, and liver flukes and certain aminoketones against Ascaris suum.[96,97] Among antibiotics, aspiculamycin (Sankyo) was found active against oxyuris and ascaris[98] and some fractions of axenomycin (Farmitalia) against cestodes.[99] In the filarial nematodes Litomosoides carinii, Dipetalonema vitae, and Brugia pahangi the primary effect of l-tetramisole is on the neuromuscular system.[100]

Immunologic Mechanisms - A textbook was published dealing with immunology and its techniques in parasitic infections,[101] and a review appeared on immunology research in protozoan diseases.[102] The possible effect of certain antiparasitic drugs on the immune system deserves attention. Levamisole was reported to stimulate immune responsiveness under certain conditions.[103,104] Niridazole was found to suppress granuloma formation around S. mansoni eggs.[105] Chloroquine was reported to be an immune suppressor.[106]

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Chapter 17. Antiviral Agents

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During the period covered by this review the pace of development of antivirals has accelerated. This chapter highlights some of these activities.

Exogenous Interferon - Until recently, the technology needed to produce sufficient amounts of exogenous interferon for significant clinical trials did not exist. However clinical as well as animal trials are now possible due to improved production techniques for both human and mouse interferons.[1-3] The efficiency of production has increased an average of two fold per year for the last several years. Human interferon has been produced on a large scale from primary human leukocyte cultures obtained from blood donors in Finland.[2] Although currently available for clinical trials, this material remains quite expensive. It has also been obtained by the so-called "superinduction" method in human foreskin fibroblast cells.[1]

determine its molecular structure and possible method of synthesis. Interferon is a glycoprotein of about 30,000 molecular weight. Recent studies reveal that the antiviral activity probably resides mainly with the protein part of the molecule since removal of most of the carbohydrate did not destroy its activity (C. Anfinsen, S. Bose, D. Rotman, U. Ruegg, L. Corley, personal communication) while proteolytic action did destroy its activity.

Attempts at interferon-prophylaxis of human respiratory virus infections in volunteers were unsuccessful when smaller amounts of interferon were available.[4] More recently large doses were shown to be prophylactic against respiratory infection when the interferon was administered intranasally.[5] This was a double blind placebo-controlled study first against challenge with influenza B virus and then against rhinovirus 4 in separate volunteer groups. In the influenza portion of the study, a total of 800,000 units of human interferon did not alter the severity of the disease but did delay disease onset. In the second study, which used a rhinovirus challenge, a total of 14,000,000 units of interferon decreased the severity of symptoms as well as the frequency and amount of virus-shedding after infection. Safety, and lack of toxicity of interferon also were indicated. Thus, the effective intranasal dose was in the range of 800,000 to 14,000,000 units. An important factor governing the unexpected requirement for large quantities of interferon may be the mucosal barrier in the nose.

clinical trial employing systemic administration of interferon during varicella-zoster in immunologically suppressed patients.[6] This study is still in progress but it has confirmed the safety of interferon,[7] and the maintenance of significant serum levels.

Early studies in cancer patients indicate that there may be some protective action against osteogenetic sarcoma metastases in man (H. Strander, K. Cantell, S. Iugimarsson, P. A. Jakobsson, U. Nilsonne and G. Soderberg, International Workshop on Interferon in the Treatment of Cancer, Memorial Sloan-Kettering Cancer Center, N. Y., March 31-April 2, 1975). Suggestive findings like these must be confirmed in controlled studies.

An attempt can be made to estimate the doses of exogenous interferon required in man from studies in tissue culture (F. Dianzani and S. Baron submitted, 1975), mice[8-10] and man.[11] Parenteral administration of 1 million to 1 billion units of interferon per dose may be required depending on viral sensitivity, time of initiation of treatment, and site of infection.

Interferon Inducers, Polynucleotides - In the past the polynucleotide inducers of interferon were shown to be highly active in the mouse and rabbit[12] but were poorly active in man and subhuman primates.[13] Despite its low interferon inducing capacity for man, poly I·poly C provided significant protection against respiratory infections and herpes eye infections.[13,14] The low interferon response in man to polynucleotide inducers does not seem to be caused by a poorly functioning interferon system since (a) polynucleotides are good inducers of human cells in culture,[1] (b) man produces interferon in response to a broad range of virus infections,[15] and (c) high concentrations of interferon have been reported during infections with herpes simplex virus (HVH),[13] varicella-zoster virus [16] and influenza virus.[17] A possible explanation of the low interferon response of man to polynucleotides is the occurrence of high levels of nucleases in the serum.[18] With this possibility in mind, a nuclease resistant complex of poly I·poly C, poly-l-lysine and carboxymethylcellulose has been developed.[19] When injected intravenously into monkeys and chimpanzees peak serum levels of interferon ranging from 125-6000 units/ml were obtained. This inducer provided prophylactic and early therapeutic protection against Simian hemorrhagic fever virus[19] yellow fever virus[20,21] and rabies virus (G. Baer, S. A. Moore, J. H. Shaddock, H. B. Levy, S. Baron, personal communication) infections of monkeys. These studies demonstrate that primates can make good quantities of interferon with resulting protection against certain interferon-sensitive viruses. Studies in man are planned.

Other active polynucleotide inducers of interferon have been developed by altering the structure of the nucleotide used to make up the polynucleotides.[22] These studies have also further defined the structural requirements for induction of interferon.

In the past, toxicity studies of poly I.poly C in animals raised the possibility of several serious toxicities including (a) teratogenetic effects,[23] (b) induction of autoimmune disease,[24] (c) enhancement of infection by certain fungi[25] and (d) transformation of rat cells to the malignant state.[26] When poly I.poly C was tested in a limited number of

patients, the only observed toxicities were a short-lived febrile response[13] and 2 allergic reactions involving bronchospasm and edema (R. Robinson, V. DeVita, A. Levine, H. B. Levy, and S. Baron, personal communication). Possible side effects might be avoided by the topical application of interferon inducers to the nasal tract or eye.

Virus Inducers of Interferon - In the past induction of interferon and concomitant protection against severe virus infections of animals have been shown to occur following the injection of interferon inducing viruses.[27] It has been reported that avirulent viral infections of man could lead to the appearance of interferon in the serum and nasal fluids of man.[28] Associated protection against human respiratory viral infections was also reported. However attempted confirmation with one of these viruses was not successful (S. Reed, and D. A. J. Tyrrell, presented at NIAID Influenza Workshop, Bethesda, Md., 1974).

In a recent related study attenuated influenza virus was administered to volunteers who then received wild type parainfluenza virus 7 days later.[29] Interferon production following inoculation of the attenuated virus was low or undetectable and so it was not surprising that no protection was observed even though the challenge parainfluenza virus was highly sensitive to interferon. This study also demonstrated a late production of nasal interferon in volunteers infected with parainfluenza virus only. This suggests that treatment of this interferon-sensitive parainfluenza virus infection may be feasible up to several days after infection in future studies.

Propanediamine Derivatives as Inducers - These inducers were initially found to be moderately active in the mouse.[30] There have been two studies of the topical application of such an inducer to the nasal epithelium of man as prophylaxis against respiratory virus infection. In one study the inducer stimulated moderate levels of interferon, good protection against rhinovirus induced-disease and decreased virus shedding.[31] In the other study, similar results were obtained however, no decrease of virus shedding was observed.[32] These findings are similar to earlier studies performed with poly I·poly C as the inducer of interferon in the nasal tract.[33] Here also symptomatic protection was observed against both rhinovirus 13 and influenza type A2 infection in volunteers but decreased virus shedding was observed in only one of the two studies with rhinovirus 13.

Other Inducers of Interferon - It has been reported that small molecular weight radioprotective chemicals also induce interferon and provide protection against viral infections.[34] Another newly recognized interferon inducer is 9-methylstreptimidone, a glutarimide antibiotic from Streptomyces.[35]

Biological Inducers of Interferon - These include the lipid fraction of the *E. coli* lipopolysaccharide,[36] the cell wall fraction of brucella,[37] a protein derived from *E. coli*,[38] and a group of polysaccharides.[39] It is

possible that chemical identification of the inducing moiety could identify new classes of inducers.

Idoxuridine - The topical application of 5-iodo-2'-deoxyuridine (idoxuridine, IDU) has been shown to be efficacious in the treatment of herpetic keratoconjunctivitis[40-41] and is the current treatment of choice. Because of this clinical effectiveness and *in vitro* effectiveness against other DNA viruses such as cytomegalo, vaccinia and varicella-zoster viruses, several clinicians were encouraged to test this compound clinically against the highly lethal herpes encephalitis. This resulted in a series of articles attesting to its efficacy.[42-45] However, there continued to be some evidence of toxicity following the systemic administration of the drug.[46]

Because of the uncontrolled nature of the early investigations double blind studies were initiated independently by two groups.[47] Both of these studies were terminated prior to completion because of the appearance of intolerable toxicity with no indication of amelioration of the disease. As a result of these carefully done studies, the use of IDU against herpes encephalitis is contraindicated and should be discontinued unless a nontoxic and protective dose schedule can be found. The discrepancy between the *in vitro* effectiveness and *in vivo* failure is partially explained by the finding that HVH was not inhibited by IDU in the brain tissue of mice.[48] Also no nontoxic level of IDU could be found which protected mice against disseminated HVH infection (E. Lefkowitz, M. Worthington and S. Baron, submitted, 1975).

A possible side effect of the use of IDU in the therapy of herpetic infections is the ability of this drug to induce or enhance the replication of a broad range of both DNA and RNA viruses under conditions of incorporation of IDU into cellular DNA *in vitro*[49-51] (J. Green and S. Baron, submitted, 1975). These virus inducing and enhancing effects of IDU must be considered as potentially important side effects of treatment with the drug.

Cytosine Arabinoside (Ara-C or 1-β-D-arabinofuranosyl cytosine) - Ara-C has been found to have *in vitro* activity against herpes viruses, poxviruses and rhabdoviruses.[52] Recent studies in mice infected with HVH failed to demonstrate protection against systemic infection by doses which were nontoxic (E. Lefkowitz, M. Worthington and S. Baron, submitted, 1975).

Because of the severity and potential lethality of herpes zoster infection in patients with a compromised immune system, treatment with Ara-C was attempted. However, evaluation of the encouraging reports[53-56] was difficult due to the different regimens used, lack of quantitative evaluation of indices of disease and most important the lack of randomized placebo-controlled studies. In a carefully controlled double blind study Ara-C was administered within 48 hours of the onset of dissemination by continuous IV infusion until dissemination ceased or for a maximum of 72

hours. The duration of dissemination was greater in the treated than the placebo group and was even more prolonged for Ara-C treated patients with stage III or IV lymphoma.[57] Also, the drug gave evidence of hematologic toxicity and depression of antibody formation. This and other studies[58,59] demonstrated that Ara-C had no value against varicella-zoster. The discrepancy between *in vitro* and *in vivo* effectiveness might be that in man intravenous Ara-C is rapidly deaminated to 1-β-D arabinofuranosyl uracil (Ara-U) which lacks antiviral activity.

Adenine Arabinoside (Ara-A) - A highly promising chemotherapeutic agent under clinical study is 1-β-D-arabinofuranosyl adenine, which differs from Ara-C in containing adenine instead of cytosine. More importantly in man, Ara-A is deaminated to 1-β-D arabinofuranosyl hypoxanthine which is antiviral. Therefore, the duration of antiviral effect in man is longer than that of Ara-C.

Adenine Arabinoside - A Symposium on Current Status (Virology) was held in September, 1974, jointly sponsored by the Parke, Davis Company and the NIAID (In press, Raven Press). It was reported that Ara-A was highly active in model systems against herpes and pox DNA viruses, partially active against adeno and papova DNA viruses as well as rhabdo and oncorna RNA viruses.[60-66]

Most recent studies of disseminated HVH infection in mice have shown the drug to be well tolerated at doses which are protective when begun as late as 3 days after infection. However, using combination therapy synergistic toxicity with poly I·poly C was observed (E. Lefkowitz, M. Worthington, and S. Baron, submitted, 1975).

Well controlled studies against HVH epithelial keratitis of man[67] showed equal efficacy of trifluorothymidine, IDU and Ara-A. However, Ara-A is preferable because of low toxicity, low allergenicity and more rapid action. In a small study, deep stromal disease and iritis could be treated with systemic Ara-A utilizing 20 mg/kg/day given intravenously.[68]

Following pilot studies,[69] a double blind placebo-controlled clinical study was initiated to evaluate Ara-A in biopsy proven herpes encephalitis, neonatal herpes, severe localized herpes and varicella-zoster particularly in immunocompromised patients. Antitumor activity is also undergoing efficacy study.[70]

Amantadine - Although 1-adamantanamine hydrochloride (amantadine) has been proven efficacious against influenza type A, it has not received wide acceptance as a result of mild gastrointestinal irritation and transitory central nervous system reactions noted in 1-2% of patients.[71,72] The drug has been used extensively for the treatment of Parkinson's disease with only livedo reticularis and mild ankle edema seen as additional adverse effects.[73] Efficacy studies in man have shown more than 50% prevention of disease.[74-79] It is also effective in the reduction of fever during natural outbreaks of either the Asian or Hong Kong strains of influenza A2.[80-83] Recent studies with the

related rimantadine, indicate protection of mice against influenza virus infection when therapy was initiated as late as three days after infection. Crucial to the late protection was the local administration by small particle aerosol rather than by parenteral administration. (J. S. Walter, E. L. Stephen, J. B. Moe, R. O. Spertzel, J. W. Dominik, and H. W. Young, personal communication, 1974). However, neither virus titer nor lung lesions were decreased in the treated group indicating that the mechanism of protection is not understood. Amantadine derivatives such as rimantadine are deserving of further testing. The use of these compounds is not advocated as a replacement for the vaccine, but as an adjunct or for intermediate chemoprophylaxis when an appropriate vaccine is not available.

Ribavirin - Another broad spectrum antiviral with clinical potential is 1-β-D-ribofuranoxyl-1, 2, 4-triazole-3-carboxamide (ribavirin, Virazole). It is effective in vitro and in vivo against influenza A and B, parainfluenza (Sendai), herpes, vaccinia and some strains of mouse hepatitis viruses.[84,85] Ribavirin appears to inhibit an early step in viral replication that leads to the synthesis of viral nucleic acids--RNA or DNA. It is believed that the compound is phosphorylated in vivo and its antiviral action is due to the inhibition of the biosynthesis of guanosine 5' monophosphate.[86]

Recently ribavirin has been found to protect mice against influenza virus infection when therapy was initiated as late as three days after infection. A critical factor in the protection was the local administration of ribavirin by small particle aerosol rather than by parenteral administration (E. L. Stephen, J. W. Dominik, J. B. Moe, R. O. Spertzel and J. S. Walker, submitted, 1975). Although ribavirin is not free of toxic reactions, it is sufficiently safe to warrant limited clinical studies for the prevention and/or treatment of influenza, parainfluenza and hepatitis. At the 14th Interscience Conference on Antimicrobial Agents and Chemotherapy, Dr. F. Salido-Tenegell, reported effective therapy against an outbreak of Influenza A2 in a double blind, placebo-controlled study.

Disodium Phosphonoacetate (PAA) - This highly promising antiviral compound was found to be effective against HVH types 1 and 2 in tissue culture and subsequently confirmed in vivo with herpes dermatitis in mice and herpes keratitis in rabbits (NIAID sponsored Cooperative Antiviral Testing Group, personal communication, 1975). It is also effective against other viruses of this group such as cytomegalovirus and pseudorabies virus.[87] In vitro studies using WI-38 cells and HVH demonstrated that the mode of action was to inhibit selectively the DNA-dependent DNA polymerase from HVH.[88,89] Efforts are currently underway to initiate limited controlled clinical trials, initially against cutaneous herpes.

2-Deoxy-D glucose (2-D-G) - This sugar analog was found to be effective in preventing or reducing the severity of herpetic keratitis in rabbits.[90] The mode of action of this compound is believed to be interference with the synthesis or elongation of the oligosaccharides which are incorporated

as part of viral specific glycoproteins. The glycoproteins are essential for formation of the envelopes of orthomyxoviruses, paramyxoviruses and probably herpesviruses. Although the protection obtained with this compound is not dramatic, it does suggest that sugar analogs may be useful antiviral compounds.

Oxoline, Bonaphton, Tebrophen and Florenal - Soviet scientists have developed two compounds which they find effective against influenza virus as well as other viruses.[91] In volunteer studies the Soviet scientists have stated that morbidity was decreased 39% by oxoline ointment which is currently available to their public. In controlled challenge studies bonaphton (6 bromonaphthoquinone) given orally, to patients infected with partially attenuated influenza virus (H3N2), decreased morbidity from 78.6% in the placebo group to 38.4% in the volunteer group receiving bonaphton. This compound was also found to be non-toxic and is currently available for general use. Unfortunately, the NIAID Cooperative Antiviral Testing Group was unsuccessful in detecting significant antiviral effect by these 2 compounds in its animal studies (personal communication). This was also true for tebrophen, a substituted biphenyl compound and florenal, a benzyl fural benezene compound. These latter compounds had previously been reported to be effective against herpetic keratitis in man.[92]

Photodynamic Inactivation - In recent years photodynamic inactivation[93,94] of HVH during infections of the eye, lip, skin, or genital area has been reevaluated. The method utilizes heterotricyclic dyes such as neutral red, acridine orange or proflavine. The dyes act by binding to viral nucleic acid, absorbing visible light and inactivating the virus via oxidation. Photosensitization appears to be particularly effective against the large DNA viruses such as herpes but it also inactivates many other viruses.

Fifty percent effectiveness against recurrent facial or genital HVH infections of man has been reported in a controlled study using photodynamic inactivation.[95] In an uncontrolled study of herpes genitalis (HVH-2) in women[96] a favorable response was also reported. More recently[97] proflavine photoinactivation was as effective as IDU in rabbits whose eyes had been infected with HVH. However, confirmation of the therapeutic action against human cutaneous herpes was not obtained in the most recent trial (M. G. Myers, M. N. Oxman, J. Clark, and K. Arndt, manuscript in preparation). Careful study of the variables which influence activity[93] must be done in patients to achieve reproducible effects.

A warning has appeared in the literature[98] and in the FDA drug bulletin[99] stating that photoinactivation of herpesvirus may be clinically hazardous because the inactivated virus was capable of transforming normal mammalian cells to malignancy. Other possible complications of such treatment include damage to cells and their genetic apparatus due to the strong affinity of the dyes for the nuclei of cells.[100-103]

Streptovaricins - Streptovaricins and rifamycins represent a group of structurally related antibiotics and their derivatives which are active against bacteria and certain viruses. Streptovaricins were found to inhibit the virus specific reverse transcriptase enzyme of the RNA tumor viruses.[104,105] The streptovaricins were also reported to inhibit moderately murine sarcoma virus induced transformation in vitro[106] and in vivo.[107-109]

Rifamycin Derivatives - Rifamycins, in addition to being important antibacterial agents, have been shown to inhibit the growth of poxviruses.[110,111] More recently a number of the rifamycin derivatives have been found to inhibit the reverse transcriptase enzyme of the RNA tumor viruses[112-114] and transformation in tissue culture.[115] The inhibitory effect is relatively specific in that rifamycins do not inhibit a DNA tumor virus or normal cell growth[116] but do inhibit the growth of virus-transformed chick embryo cells.[117,118] In the mouse rifamycins inhibit several parameters of oncornavirus-induced leukemia.[115-119] A problem associated with many rifamycin derivatives is that they are toxic for mice.[120]

Narcissus alkaloids - These alkaloids are being studied for their antiviral properties, especially anti RNA tumor virus properties.[121,122] Recently inhibition of leukemia virus replication by these alkaloids was confirmed in tissue culture studies.[123,124] In leukemia virus infected mice the alkaloids were also more effective in increasing survival than were rifamycin or adriamycin and the protective action of the alkaloids was further enhanced when they were used in combination with other chemotherapeutic agents.[124]

Acknowledgement: The authors wish to thank Mrs. C. P. Uhlendorf and Mrs. C. Hawley for their assistance in the preparation of this review.

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Section IV - Metabolic Diseases and Endocrine Function

Editor: Walter T. Moreland, Pfizer Inc., Groton, Connecticut

Chapter 18. Non-steroidal Anti-inflammatory Agents

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Introduction - The intensity of research in the field of inflammation during recent years can be most readily gauged by the large number of candidates on which clinical testing was reported in 1974. Since the etiology of the major chronic inflammatory diseases remains unknown, current therapeutic approaches to their treatment[1-4] still rely heavily on non-steroidal antiinflammatory agents (NAA).[3,5] With the suffering and disabling effects caused by the rheumatic diseases afflicting some 20 million patients in the U.S. alone and with approximately a quarter of a million new victims each year, the economic cost to the nation in terms of lost wages and other medical expenses is formidable. There is great need for more effective antirheumatic drugs. Monographs,[5,6] reviews,[1,3,7] symposia[8,9] and stimulating articles[10-12] reveal the trend of current research[10,13] towards the discovery of new drugs for inflammatory diseases.

Pathogenesis - Researchers (veteran or newcomer) and physicians alike are greatly hampered by a lack of clear understanding of the etiology and pathogenesis of the inflammatory disease(s) that they study and treat.[13] Textbooks and monographs[14-18] and a few relevant articles[19-26] on the pathogenesis of rheumatoid arthritis (RA), reveal some of the complexities involved. Further confusion is introduced by the fact that osteoarthritis (OA) and other degenerative joint diseases exhibit similar symptoms, but respond differently to treatment and presumably have different etiologies.[27-31] A recent review of Reiter's disease (RD)[32] contains descriptions of several disease syndromes with special attention given to cardiovascular and neurologic sequellae. Evidence that psoriatic arthritis may be one specific disease, instead of the coincidental occurrence of two common diseases, was presented by Moll and Wright.[33]

A detailed discussion of the various components of any or all these inflammatory diseases is obviously far beyond the scope of this review. While pathogenesis of some of these diseases has been reasonably well described, the etiology of none has been proven.[34]

Mechanism of Action - The complex nature of the inflammatory diseases accentuates the difficulty of studying the mechanism of action of antiinflammatory agents. It has been speculated that NAA's may exert their effects by inhibiting leucocyte migration,[35] inhibiting prostaglandin synthesis,[2,36] interfering with phagocytosis,[37,38] modifying lymphocyte activity,[39,40] decreasing the adherance of platelets,[41] or by stabilizing lysosomal membranes and thereby preventing the release of degradative enzymes.[22,42-45] However, these postulated mechanisms remain speculative because, at

present, there is no way to evaluate the relative importance of any one of them in the various disease states. Perhaps, when the etiology of the disease(s) becomes known and the pathogenesis sequence has been more clearly defined, the full implication of some or all these mechanisms will be appreciated.

Methodology - Models of inflammation were reviewed by Swingle[16] who organized screening procedures into categories according to criteria for detection of activity. Under "Antirheumatics", there was no experimental model cited. However, a list of test systems was presented in which activity has been demonstrated for chloroquine, for which arrest or remission of RA has been claimed.

Researchers are increasingly turning to experimental models that have an immunological basis.[5] According to Swingle,[46] immunosuppressive screens do not appear to be much more relevant to the problem than the antiinflammatory screens currently in use. There is disagreement whether immunosuppressives[47] or stimulants[51-54] should be used in RA therapy. Even those who believe immunologic agents should be employed disagree[46] whether humoral[47] or cell-mediated immunity[21] or both[55] should be influenced. Such controversy vividly points out the lack of knowledge of the primary etiology of RA. Perhaps when the properties and functions of B and T lymphocytes, which are responsible respectively for humoral and cell-mediated immunity, become more clearly defined and applied in the laboratory, a more meaningful search for immunologic agents specific for RA may be possible.[47]

The potential advantages of "quick" in vitro assays or "simple" in vivo procedures seem obvious, but until a better understanding of the human disease state is acquired, we must settle for a cruder whole-animal model.[46] Adjuvant-induced arthritis is a delayed hypersensitivity response to mycobacterial antigen in rats. It appears to be the most relevant model of RA and RD[46,47,56,57] that is presently available and has become the most widely used model for screening potential antiinflammatory drugs.[46] Hopefully, basic research with this polyarthritis model, particularly those studies delving into the chronic degenerative changes[57] in the bone, articular and periarticular structures, will increase our insight into the etiology as well as the pathogenesis of the human rheumatoid disease(s).

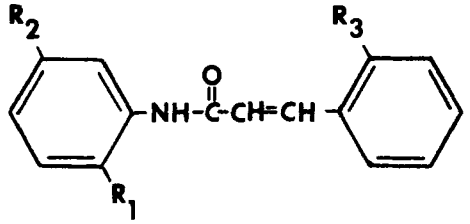
Compounds Under Investigation

Immunological Agents - Emphasis in the past has been primarily on immunosupression, but more recent reports suggest the possibility of treating RA by immunostimulation.[21,51-54] Schuerman[58] reported striking improvements in RA patients receiving levamisole, 150 mg/day. A non-specific activation of the immune response by BCG (bacille Calmette Guerin - vaccine for inoculation against tuberculosis) seems to have a beneficial effect in RA, which is accompanied by decreases in both erythrocyte sedimentation rate (ESR) and rheumatoid factor (RF).[59] Tuberculin was reported to permantly arrest disease symptoms in RA patients.[53] Since these agents stim-

ulate the immune system, it is difficult to reconcile such results with reports that the immunosuppressants, azathioprine (AZP) and cyclophosphamide (CYC), also have definite beneficial effects in RA.[48,60,61]

CYC is an alkylating agent[60] that has a cytotoxic effect, which is reflected in the decreased number of circulating small lymphocytes.[62] Decrease in blood monocytes was also observed.[60,62-64] Adverse side effects constitute a major limitation to the use of CYC in the clinic.[65] With long term usage there is an additional hazard of malignancy and mutagenesis.[61,65] RA patients treated with AZP at either 2 - 2.5 mg/kg/day or 1 - 1.25 mg/kg/day showed equal improvement in symptoms without a corresponding change in ESR or RF titer.[66,67] No increase in mean lymphocyte diameter was associated with AZP therapy[63] as compared with CYC treatment. Though a meaningful interpretation of this observation has not been presented, lymphopenia occurring in RA patients treated with AZP[67] was probably induced by decreasing the absolute numbers of lymphocytes rather than a selective effect on B or T cells. Occasionally, the administration of large doses of AZP (5-6 mg/kg/day) may be responsible for the complete remission of RA symptoms in cases where smaller doses had failed.[68] Both CYC and AZP act slowly and must be continued over a period of months to be effective.[60,61] Swingle[46] emphasized that most immunosuppressive drugs have antiinflammatory as well as immunological activity, and thus caution may be needed for proper interpretation of results.

Other immunosuppressives are in earlier phases of investigation. Cinanserin (I) is a potent antagonist of serotonin with analgesic activity.

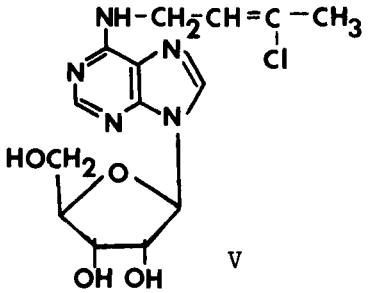


	R_1	R_2	R_3
I	$-S-(CH_2)_2-N(CH_3)_2$	$-CF_3$	H
II	$-O-(CH_2)_3-N(CH_3)_2$	$-CH_3$	H
III	$-O-(CH_2)_3-N(CH_3)_2$	$-C(CH_3)_3$	H
IV	$-S-(CH_2)_3-N(CH_3)_2$	$-CF_3$	Cl

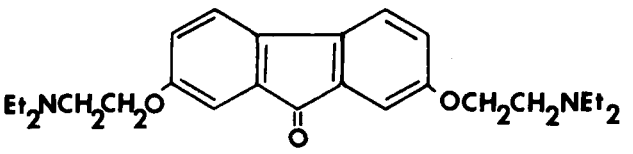
The immunosuppressive activity of I is comparable to AZP.[69,70] Two analogs II and III also have immunosuppressive but low antiserotonin activity. A third analog IV had antiserotonin activity but little immunosuppressive activity. The analogs II, III and IV and AZP were effective in the suppression of both local and systemically induced lesions in adjuvant arthritic rats. Another potential immunosuppresive,[71] N^6[3-chloro-2-butenyl] adenosine (V) has antiinflammatory activity (carrageenan edema and adjuvant arthritis tests) comparable to phenylbutazone. V was found to suppress the PFC-response to sheep red blood cells in Jerne's plaque test.

Tilorone (VI)[72] is an active antiviral compound. It is claimed that VI will stimulate the humoral, but inhibit the cell-mediated responses. Activity has also been demonstrated in the rat adjuvant arthritis test. The unique immunologic properties of this compound suggest potential for

the control of inflammatory diseases.

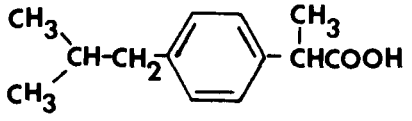


V

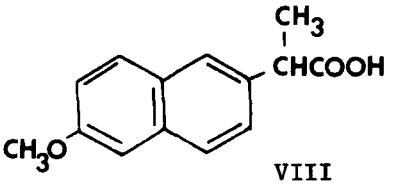


VI

Arylalkanoic Acids and Related Compounds - The phenylalkanoic acids are, by far, the most prevalent among the candidates being tested in the clinic at the present time.[3,73-76] A number of these are already widely used overseas. Motrin (ibuprofen, VII) recently became available for treatment of arthritis in the United States and has had extensive clinical evaluations.[73,77-80] In the treatment of RA, VII appears to be comparable to aspirin,[76] with fewer side effects.

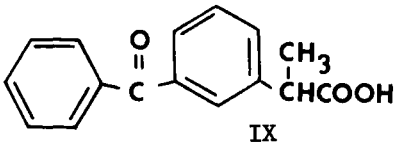


VII

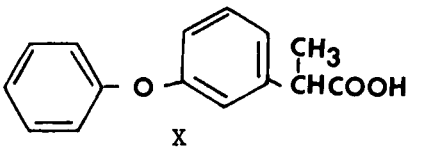


VIII

In two clinical trials, naproxen (VIII) 500 mg/day was found to be equal to aspirin 4 gm/day for the treatment of RA. VIII was better tolerated with less dyspepsia or G.I. bleeding. No serious side effects were observed in the four week studies.[81,82] In another study several cases of G.I. bleeding were reported.[83]



IX



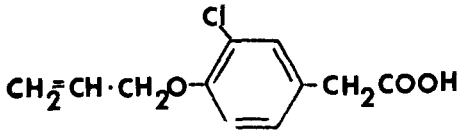
X

Ketoprofen (Orudis, IX) has been studied in various rheumatic diseases.[84] The antiinflammatory activity of IX(150 mg/day) was equal to that of indomethacin at 100 mg/day.[85] In RA, IX was effective and well tolerated with less CNS side effects than indomethacin but its overall effects and analgesic properties were not marked.[84,86,87] Antiinflammatory, antibradykinin and analgesic tests in experimental animals demonstrated that the activity of IX was about equal to that of indomethacin.

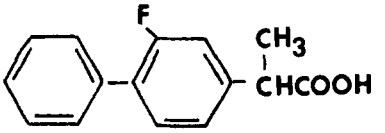
Fenoprofen (X) which has analgesic, antiinflammatory and antipyretic properties, was evaluated in 60 RA patients. Good benefits were obtained with few side effects.[88] X was reported to be equal to phenylbutazone in the treatment of osteoarthritis of the hip.[89] In a double-blind cross-over trial, X and aspirin were about equal in patients with classical RA.[73] The incidence of side effects was found to be greater with aspirin than with X.

Alclofenac(XI), clinically tested at 3 gm/day, was reported to be slightly better than aspirin at 4.8 gm/day for the treatment of RA.[90] After 17 weeks of treatment, no significant change in digital joint size was noted, though ESR decreased and serum IgM and IgG levels were lowered. Gastro-intestinal side effects were less with XI than with aspirin in this clinical evaluation.

Flurbiprofen(XII) was compared to indomethacin in ankylosing spondylitis and found to be equally potent in improving spinal mobility.[91] The analgesic properties of XII were greater than those of indomethacin.



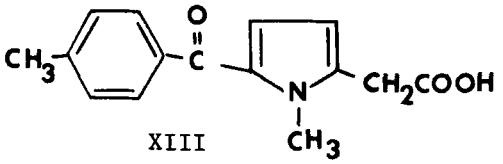
XI



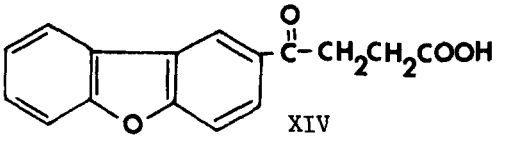
XII

Tolmetin(XIII) was significantly better than placebo[73,92] in the treatment of RA. In another study, tolmetin was compared to aspirin. Fifty patients with active RA were treated for six months and tolmetin was significantly superior to aspirin in reducing the symptoms of RA.[73] No severe side effects were observed.

Furobufen(XIV) is also undergoing clinical testing. Preclinical results[93] showed XIV equal to phenylbutazone in potency using the carrageenan paw edema test. XIV also inhibited the development of adjuvant arthritis in rats.

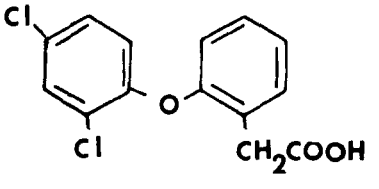


XIII

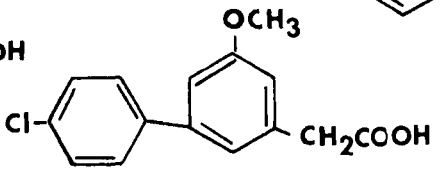


XIV

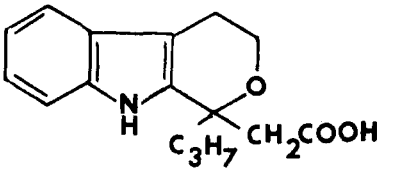
Other arylalkanoic acids are reported to be in various phases of preclinical evaluation. Fenclofenac(XV), which has been shown to have eight times the potency of ibuprofen (VII) in the adjuvant arthritis test,[94] is much less potent in the acute antiinflammatory tests. DKA-9(Nippon Shinyaku,XVI)[95] is claimed to be more potent than phenylbutazone, oxyphenbutazone, flufenamic acid, and ibuprofen using *in vivo* and *in vitro* tests.



XV

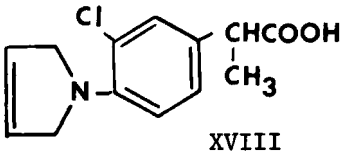


XVI

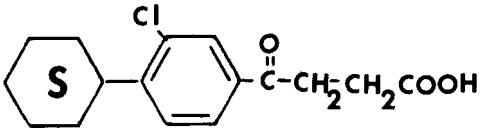


XVII

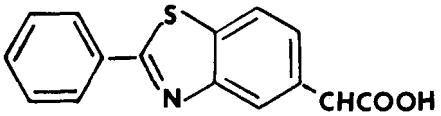
Prodolic acid (XVII) has antiinflammatory, analgesic and antipyretic properties[96] approximately equal in potency to phenylbutazone. XVII was active in the adjuvant arthritis test model. Pirprofen (XVIII) has shown antiinflammatory activity in the paw edema, cotton pellet granuloma and adjuvant arthritis tests.[97] Normal humans will tolerate XVIII up to 600 mg/day with occasional fecal blood loss. Some early clinical results indicate that 400 mg/day of XVIII is equal to 100 mg/day of indomethacin. Bucloxic acid (XIX), the best candidate from a series of analogs,[98] was reported to be active in the clinic. In rat tests, the XX compounds are similar to ibuprofen.[99,100] Some human metabolic studies[101] and also studies in rhesus monkeys[102] have been reported. The antiinflammatory activity of C4632 (XXI) was reported to be equal to that of indomethacin[103] with less toxicity.



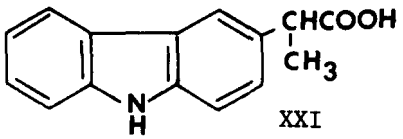
XVIII



XIX

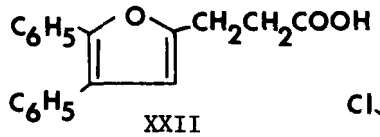


K308, R=H; K309, R=CHCOOH XX

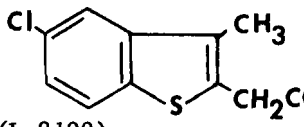


XXI

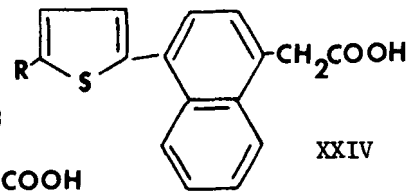
Oxoprozin (XXII) was reported to be less potent than phenylbutazone and equal to aspirin in the carrageenan edema test.[104] XXIII was reported to have ulcerogenic properties but a wide margin was found between antiinflammatory and ulcerogenic doses.[105]



XXII



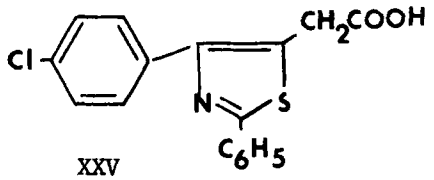
XXIII (L-8109)



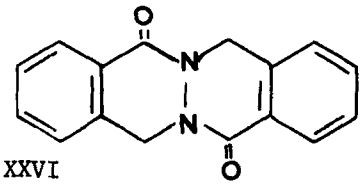
XXIV

The potency of XXIV where R = Br, H and Cl was 6.0, 6.8 and 15.0 respectively relative to phenylbutazone(1.0).[106] In the carrageenan paw edema test XXV was as potent as indomethacin and five times phenylbutazone in the adjuvant arthritis test.[107] Other related compounds have appeared in the USAN Council List of New Names: cliprofen,[108] suprofen,[109] cicloprofen[110] and piroxicam.[110]

Miscellaneous Agents - Extensive preclinical pharmacology of diftalone (XXVI) was presented.[111] XXVI was shown to be in the phenylbutazone and aspirin range of potency in acute antiphlogistic and chronic adjuvant arthritis tests. It also has analgesic and antipyretic activity. A controlled double-blind clinical trial with 32 RA patients showed activity and safety at least comparable to that of indomethacin.[112]

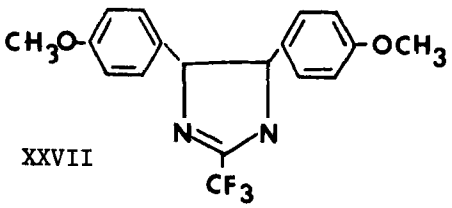


XXV

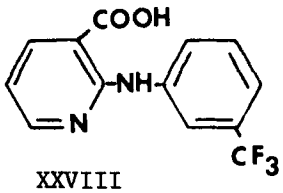


XXVI

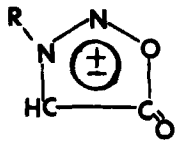
Flumizole (XXVII) is a trisubstituted imidazol found to be more potent than indomethacin in preclinical animal experiments.[113] Niflumic acid (XXVIII) was evaluated in a two-week clinical trial at 800 mg/day.[114] Therapeutic effects were observed in chronic polyarthritis.



XXVII



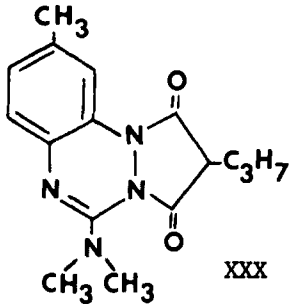
XXVIII



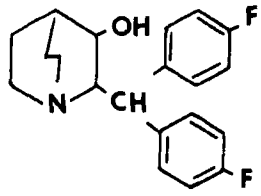
XXIX

R = C_6H_5-S-CH_2-CH_2- or biphenyl

Several sydnones (mesoionic compounds) related to XXIX have been discovered with antiinflammatory activity.[115] Apazone (AHR3018,XXX) has been evaluated in a controlled clinical trial for ankylosing spondylitis.[116] In a series of quinuclidines, XXXI was reported active against granuloma pouch exudate formation[117] with the cis-form one-fourth as potent as indomethacin. No activity against adjuvant arthritis was observed.



XXX



XXXI

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Chapter 19. Disorders of Lipid Metabolism

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Introduction – Coronary heart disease remains the leading cause of death in the United States although the rising mortality rate observed since World War II appears to have leveled off.[1] Stimulated by the established relationship between hyperlipidemia and the risk of coronary heart disease, research activity in the field of lipid metabolism continued with undiminished intensity. Particularly important advances in understanding have been made in the areas of metabolism of plasma lipoproteins and their role in the atherogenic process, the cellular basis of familial hypercholesterolemia, and the clinical effects and mechanisms of action of clofibrate, and these have been selected for emphasis in this report.

Etiology of Atherosclerosis – Goldstein and Brown, using cultured skin fibroblasts from high risk patients with familial hypercholesterolemia (FH), have gained new insight into genetic factors leading to this disease.[2] Normal human fibroblasts have cell surface receptors that bind low density lipoproteins (LDL) with high affinity and specificity. This binding of LDL regulates cholesterol metabolism by suppressing the activity of the rate-controlling enzyme in cholesterogenesis, β-hydroxy-β-methylglutaryl coenzyme A reductase (HMG-CoA reductase), and by increasing the rate at which fibroblasts degrade LDL. Cells from homozygotic individuals show a severe deficiency in the number of functional LDL receptors, which leads to overproduction of cholesterol and reduced ability to catabolize LDL, resulting in abnormally high levels of cholesterol and LDL. Cells from heterozygotes display normal affinity for LDL but the number of receptors is reduced by about 50 percent, and require 2-3 fold higher concentrations of LDL to maintain normal rates of cholesterogenesis and LDL degradation. In addition to defining a genetic deficiency in FH, these findings suggest that dietary and drug therapy aimed at depressing cholesterol levels by inhibiting intestinal absorption or accelerating its removal as bile acids may be disappointing in FH patients because the defect is in the feedback control of cholesterogenesis. This work suggests that an agent that specifically inhibits HMG-CoA reductase would be effective in FH. 7-Ketocholesterol, and other oxygenated cholesterol derivatives, are more effective than LDL in suppressing HMG-CoA reductase in normal cells and those derived from hypercholesterolemic homozygotes.[3] Their potential clinical application is being explored.

The finding of intact LDL in the human aortic intima and the fact that aortic LDL levels are related to plasma lipid levels indicate that lipoproteins play an important role in the uptake and deposition of lipids in the aorta.[4] Miller and Miller,[5] reviewing the work of several investigators, described an inverse relationship between plasma concentrations of LDL cholesterol and HDL cholesterol and suggested that the reduced level of HDL, rather than elevated LDL or VLDL, may be a causal factor in ischemic heart disease. Also, the removal of cholesterol from ascites cells and aortic smooth muscle cells *in vitro* was enhanced by the presence of human high density apolipoprotein.[6] Fractions derived from HDL apoprotein had reduced capacity for cholesterol removal but activity could be restored by the addition of phospholipids. The formation of linear arrays of stacked discs when sphingomyelin was added to the apoprotein fractions, is suggestive of a nascent HDL devoid of neutral lipid. This "unburdened" lipoprotein has high capacity for promoting the removal of cholesterol from these cells *in vitro*. Intact HDL and LDL share the ability to accept cholesterol from ascites cells. A reciprocal relationship between VLDL and LDL or HDL concentrations has been reported during weight reduction, carbohydrate induction and clofibrate treatment.[7] Earlier findings[8] indicate a strong inverse relationship between VLDL and HDL concentrations during clofibrate treatment.

Lipoprotein lipase (LL) has been implicated in a newly proposed mechanism[9] which attributes key roles, not only to LDL, but also to VLDL and chylomicrons. LL presence in the arterial wall suggests that the concentration of LDL at the blood-artery interface may greatly exceed concentrations in circulating blood. Focal cholesterol is deposited as follows: VLDL and chylomicrons are absorbed at arterial foci in proportion to the local concentration of heparin or heparin-like material. Arterial LL degrades them to cholesterol-rich LDL and chylomicron remnants. Much of the LDL produced is released into the blood, as

the cross-linking effect of heparin is weaker for LDL than for VLDL and chylomicrons, but some of the LDL and chylomicron remnants stay bound to the intimal surfaces long enough to be incorporated into the arterial intima. The lipoprotein lipase mechanism does not necessarily replace current views of the importance of LDL in atherosclerosis but does raise the possibility that in some patients high concentrations of LDL may be a consequence of an atherogenic lipolytic process rather than a primary cause of atherosclerosis.

The understanding of the physical-chemical state of lipids helps to explain lipid deposition and lesion reversal in atherosclerosis.[10] Phospholipids mixed with water form a sandwich consisting of layers of water interspersed with layers of phospholipid, which can absorb a considerable amount of cholesterol but not cholesterol esters. The latter tend to form a separate oily layer. When all the components are present together, cholesterol dissolves in both the cholesterol ester layer and in the phospholipid-water sandwich. Solid cholesterol crystals form when both these layers become saturated with cholesterol. By collecting data on arterial wall composition at various stages of disease, it was shown that, for children, cholesterol is readily absorbed by the phospholipid-containing membrane of the arteries. After puberty, the membrane becomes saturated with cholesterol, and excess cholesterol is converted to cholesterol esters which form small intracellular fatty streak globules. In advanced stages of arterial disease, crystals of cholesterol are found in the fibrous and fatty plaques. Once deposited, these crystals are difficult to remove, but the success in dissolving gallstones (cholesterol crystals) by altering bile composition suggests the therapeutic possibility of promoting the removal of such crystalline cholesterol deposits from the arterial wall with an agent capable of making the surrounding oily phase unsaturated with respect to cholesterol.

A primary role for circulating immune complexes in development of lesions is suggested from experiments in immunized rabbits fed a high-cholesterol diet.[11-13] The pathogenic immune complexes are presumed to form in response to antigens that are released by cell death and turnover. The sequence of events postulated is that immune complexes become localized in the vascular endothelium which proliferates in response to complement-mediated cytolytic attack. Factors released, such as histamine, lead to increased permeability which allows deposition of lipids, including cholesterol, in the vascular wall. This immunological model does not conflict with the lipid hypothesis, since any increase in vascular permeability would lead to an increased deposition of lipid. If immunological mechanisms are involved in atherogenesis, suitable inhibitors of this immune response could prove to be of therapeutic value. It is of interest that certain antiproliferative and anti-inflammatory agents have been shown in rabbits to have specific inhibitory effects on the development of atherosclerosis.[14]

Lipoprotein Formation and Secretion – Factors influencing the synthesis and assembly of hepatic lipoprotein components and the secretion of the completed lipoprotein have been examined and an improved understanding of these highly integrated processes is emerging. The components of newly secreted VLDL occur in relatively fixed proportions,[15-17] suggesting the presence of a mechanism providing appropriate amounts of the components for the assembly process. Early studies demonstrated a direct relationship between the plasma FFA concentration and hepatic triglyceride synthesis.[18-20] In addition, it was demonstrated that the incorporation of amino acids into VLDL apoprotein was also dependent upon the concentration of fatty acids entering the liver.[21,22] This increased apoprotein synthesis was accompanied by increased VLDL triglyceride synthesis and secretion. Norepinephrine administration to rats resulted in a delayed increase in cholesterol synthesis *in vitro* which could be abolished by puromycin administration.[23] The suggestion that the increased cholesterol synthesis was mediated by elevated plasma FFA levels is supported by the finding that oleic acid is a potent stimulus of cholesterol synthesis and secretion[24] and that the diurnal fluctuations of hepatic cholesterol and fatty acid synthesis are synchronized with the plasma FFA level.[25] The authors concluded that the tidal increases in plasma FFA levels may be the signal stimulating the synthesis of β-hydroxy-β-methylglutaryl CoA reductase. Recent evidence[26] indicates that, in a rat liver microsomal preparation, high concentrations of fatty acids inhibit cholesterol esterification. Since VLDL cholesterol is largely in the free form, this

inhibition of cholesterol esterification may channel fatty acids into triglyceride formation and provide cholesterol in a form suitable for assembly into the developing lipoprotein. The question of whether concomitant apoprotein synthesis is required or that a pool of existing apoprotein is available for lipoprotein assembly and secretion has been examined by Buckley *et al.*[27] Palmitate was incorporated maximally into lipoprotein after approximately 40 minutes whereas peak apoprotein synthesis and secretion did not occur until 90-120 minutes after administration of labeled precursors.[28] These findings indicate that most of the lipid secreted early was probably linked to apoprotein derived from a pre-existing pool. These conclusions have been confirmed by the demonstration that VLDL secretion continues for 50-60 minutes following inhibition of protein synthesis by cycloheximide.[29] Together, these findings indicate that, although a pre-existing pool of apoprotein is available, it is probably very limited and, furthermore, that continuing, long-term secretion of VLDL (more than one hour) requires concomitant apoprotein synthesis. In these studies, altered patterns of electrophoretic mobility of VLDL and a reduced ratio of triglyceride to protein indicate the secretion of abnormally proportioned lipoproteins following cycloheximide administration. Reduced VLDL triglyceride to protein ratios are also observed in short term studies with the hypolipidemic agent, clofibrate.[30,31] Reduced synthesis of VLDL protein with more prolonged clofibrate administration suggests a compensatory response to a diminished need to transport triglyceride from the liver.[30] In hypercholesterolemic subjects with and without hypertriglyceridemia,[32] a close relationship has been demonstrated between the plasma triglyceride concentration and the rate of cholesterol synthesis. If one assumes that the hypertriglyceridemia is due to increased triglyceride synthesis, then it appears likely that, in man as well as laboratory animals, the rate of cholesterol synthesis is determined by the rate of FFA esterification in liver.

The weight of the combined evidence indicates that the synthesis of the lipoprotein components is delicately attuned to the FFA flux to the liver and, therefore, to the rate of triglyceride synthesis suggesting that triglyceride transport is the *raison d'etre* of the plasma lipoproteins. Although several possible mechanisms suggest themselves, e.g., derepression of DNA or increased synthesis of mRNA, the molecular basis by which the rate of triglyceride synthesis determines the rate of synthesis of the other lipoprotein components has not been defined.

Although it is well established that the endoplasmic reticulum (ER) is the main site of lipid synthesis in the liver cell, the site of the assembly process is not known. Study of lipoprotein assembly was made possible by the observations of electron microscopists who described the presence of 300-1000 A electron-dense granules in the liver cell. These granules became more numerous under conditions of increased lipoprotein secretion and were believed to be lipoproteins.[33] Studies with the isolated, perfused rat liver described the presence of osmiophilic granules, presumably lipoproteins, in the smooth ER, the Golgi apparatus and, after secretion, in the space of Disse.[34] Stein and Stein[35] followed the time course of the appearance of lipid in the liver and concluded that lipoprotein particles were formed in the ER, "processed" in the Golgi apparatus and transported to the periphery of the cell prior to discharge into the space of Disse. "Processing" in the Golgi apparatus appears to include the attachment of carbohydrate to the otherwise completed lipoprotein. Electron-opaque granules are rarely observed in the rough ER but are first detected on the smooth side of the rough-smooth ER junction. Glaumann *et al*[36] demonstrated that rough ER lipoprotein particles were rich in protein and had lesser amounts of phospholipid and triglycerides. The lipoprotein particles isolated in the Golgi apparatus were rich in triglycerides and phospholipids and resembled serum lipoproteins. Pulse labeling experiments revealed a sequence beginning in the rough and smooth microsomal fraction, proceeding to the Golgi apparatus and terminating in serum VLDL. These results indicate that the assembly of the apoprotein and lipid moieties of VLDL begins in the rough ER and progresses to the smooth ER and Golgi apparatus where terminal remodeling of the lipoprotein particles takes place prior to secretion.

Reconstitution experiments have demonstrated that phospholipid is required for the incorporation of neutral lipids into aggregates resembling lipoproteins.[37,38] Reconstitution of several HDL apoproteins with phospholipid and cholesterol ester restores the physical characteristics of the lipoprotein.[39] Morrisett *et al*[38] have shown that a VLDL peptide, apoLP-alanine, interacts with phosphatidylcholine and, in the process, the ordered, α-helical content of the apoprotein is distinctly increased. Electron microscopy of the complexes formed from apoLP-alanine and phosphatidylcholine revealed that the addition of the apoprotein transformed the spherical phospholipid vesicles to a linear arrangement of stacked discs (rouleaux formation).[40] Forte and Nichols[41] have made a similar observation with both apoLP-glutamine I and apoLP-glutamine II and phospholipid. Rouleaux structures have also been reported in the plasma lipoproteins of patients with lecithin:cholesterol acyl transferase deficiency.[42] If cholesterol esters are added to the apoprotein and phospholipid components from normal or LCAT-deficient plasma, the array of stacked discs is converted to spherical shapes with diameters approximating those of HDL.[41] These findings indicate that cholesterol esters and other neutral lipids may play an important role in determining the conformation of plasma lipoproteins.

Studies with apoLP-glutamine II indicate that phospholipid binds selectively to sites in the apoprotein structure.[43] Selective binding of phosphatidylcholine to synthetic fragments of apoLP-alanine further illustrates the existence of specific phospholipid binding sites on the apoprotein.[44] These and other findings have led Jackson *et al*[45] to suggest that phospholipids bind preferentially to amphipathic helical regions of the apoproteins containing oppositely charged amino acid side chains spatially arranged to accommodate the phospholipid structure.

Although there is evidence that the secretion of completed lipoproteins involves the migration of secretory vesicles to the plasma membrane, little is known of the mechanisms controlling this transfer. Colchicine, an agent which binds to microtubular protein subunits, inhibited the release of triglyceride into rat serum. The accumulation of lipoprotein granules in secretory vesicles indicates that colchicine blocks a late, perhaps final, step in lipoprotein secretion.[46] Further support for the involvement of microtubules in hepatic VLDL secretion is found in the observation[47] that both colchicine and vincristine, another inhibitor of microtubule function, block the secretion of lipoprotein particles from the liver. Elucidation of the colchicine effect by Stein *et al*[48] demonstrates that colchicine inhibited the secretion of lipoproteins and protein but had no effect on protein synthesis. The secretion of biliary phospholipids and cholesterol, processes apparently not requiring microtubule participation, were not affected by colchicine administration to rats.

Hypolipidemic Drugs — The Coronary Drug Project study, designed to examine the long-term effects of several hypolipidemic drugs in male subjects who had already experienced at least one myocardial infarction, has now reached its scheduled conclusion.[49] The earlier discontinuation of estrogen[50,51] and D-thyroxin[52] left only the clofibrate (1.8 g/day) and nicotinic acid (3.0 g/day) treatment groups. During a followup period ranging up to 8.5 years, neither clofibrate nor nicotinic acid caused significant reductions in total or cause-specific mortality. The somewhat lower rates of coronary death and nonfatal myocardial infarction in the clofibrate group did not attain statistical significance. In the nicotinic acid group, there was a statistically significant lower incidence of nonfatal myocardial infarction. Both drugs caused reductions in plasma cholesterol (clofibrate, 6.5%; nicotinic acid, 9.9%) and triglyceride levels (clofibrate, 22.3%; nicotinic acid, 26.1%) in this moderately hyperlipidemic population (mean baseline plasma cholesterol level, 251 mg%; plasma triglyceride level, 165-170 mg%). In view of these findings and those reviewed earlier,[53-55] it appears that hypolipidemic therapy, if it is to be effective in the treatment of coronary heart disease, should be initiated during the pre-infarction stage and, probably, in a younger population in which elevated plasma lipid levels are a more prominent risk factor.[56] Primary prevention studies now in progress with clofibrate[57] and cholestyramine[58] are expected to shed light on this question.

The clinical prominence of clofibrate as a hypolipidemic agent has provided the impetus for numerous studies of its mechanism of action. Because clofibrate is primarily a hypotriglyceridemic agent, any proposed mechanism of action should attempt to explain that relative specificity of action. Such proposals have been reviewed in the past[59] and recent findings continue to support the view that the primary effect of clofibrate is the reduction of hepatic triglyceride output by decreasing the rate of synthesis. In addition, it may enhance the rate of triglyceride removal.

In a population of hyperlipoproteinemic subjects characterized by elevated rates of triglyceride production, Kissebah *et al*[60] demonstrated a normalized triglyceride production rate during clofibrate administration. In addition to this effect, improved triglyceride clearance was observed. Nikkila and Kekki,[61] studying the clofibrate analog, SU-13,437, in hypertriglyceridemic patients, concluded that the primary defect in most cases of type IV hyperlipoproteinemia is an increased production rate of plasma triglycerides and that the drug reduces the plasma triglyceride concentration by decreasing their production in the liver. Wolfe *et al*[62] did not observe decreased production of triglyceride during clofibrate treatment and concluded that the drug improved disposal of triglycerides in extrasplanchnic tissues. However, an increased conversion of fatty acids to acetoacetic acid and CO_2 indicates increased rates of fatty acid oxidation. Since net splanchnic fatty acid uptake was not affected by clofibrate, the increased rate of fatty acid oxidation is suggestive of decreased triglyceride formation.[63] The decreased rate of triglyceride production observed in human subjects may be related to the decreased availability of α-glycerophosphate (α-GP) observed in studies in rats.[31] Single oral doses of ethanol, which increased the levels of α-GP, caused a shift toward a more reduced intracellular redox state and increased the liver triglyceride concentration. Pre-treatment with clofibrate suppressed these effects of ethanol and caused a marked reduction in the plasma triglyceride concentration, indicating that clofibrate interfered with hepatic triglyceride formation.[64] Under similar circumstances Hawkins *et al*[65] concluded that the clofibrate-enhanced rate of ethanol removal could be explained simply on the basis of increased liver size. A contributing factor may have been in the increased regeneration of NAD, a co-factor requirement for ethanol oxidation.

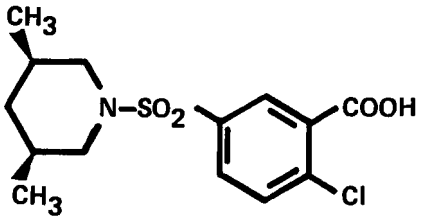
Clofibrate and structurally related agents prevented fatty livers produced by orotic acid.[66,67] Since orotic acid interferes with the secretion of triglycerides, these findings suggest that clofibrate suppressed triglyceride synthesis. Several mechanisms have been proposed to explain this effect of clofibrate on triglyceride synthesis. Besides the α-GP hypothesis reviewed earlier, Solberg *et al*[68] have described increased liver carnitine acyltransferase activity in rats fed clofibrate. This group of enzymes mediates the transport of activated fatty acids through the mitochondrial membrane and, therefore, promotes fatty acid oxidation. If the increased rate of fatty acid oxidation reduces the availability of activated fatty acids for triglyceride formation, then such an effect may, at least partially, account for the decreased triglyceride production during clofibrate administration. However, the significance of the increases in acyltransferase activity must be questioned since the most prominent effect of clofibrate is on the short- and medium chain acyltransferases,[69] the functions of which are uncertain.

Studies with isolated rat hepatocytes indicate that clofibrate inhibits the incorporation of acetate into fatty acids and cholesterol and stimulates palmitate oxidation and esterification.[70] Using rat and monkey hepatocytes, Capuzzi *et al*[71] reported similar findings and concluded that clofibrate does not interfere with triglyceride production or secretion. These findings are at variance with results derived from studies *in vivo* and may be due to the use of clofibrate concentrations much higher than those encountered during clinical use.[72] The reported inhibition of mitochondrial respiration *in vitro* by clofibrate appears to be subject to the same criticism.[73,74]

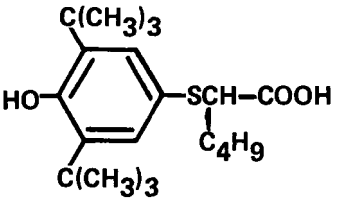
Gear *et al*[75] have demonstrated an inhibitory effect of clofibrate on hepatic mitochondrial protease activity *in vivo*. They propose that the increased mitochondrial protein mass observed in the livers of rats given clofibrate may be due to the decreased degradation of newly synthesized mitochondrial proteins. Such a mechanism appears unlikely in view of the distinctly increased specific radioactivity of mitochondrial

protein and the increased rate of hepatic RNA synthesis observed within two hours of clofibrate administration.[76] Furthermore, the decreased degradation of mitochondrial protein, which has a half-life of 5.8 days, would not be expected to produce the observed rapid increase in the mass of mitochondrial protein.

Several reports describing the effects of tibric acid in plasma lipid levels in man[77-82] and animals[83-85] have appeared. This structurally distinct agent is the most potent member from a series of sulfamylbenzoic acids[86] and owes its hypolipidemic effect to a reduction in the hepatic level of α-glycerophosphate,[83] an essential triglyceride precursor.



TIBRIC ACID



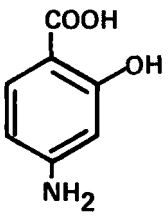
DH-990

Additional studies[87-89] with probucol confirm the 13-20% reduction of plasma cholesterol reported earlier. Although the triglyceride response is generally erratic and inconsistent, several studies show significant reductions.[90,91] DH-990, an analog of probucol, differs from it in significantly lowering triglyceride levels in rats and appears to be different mechanistically.[92]

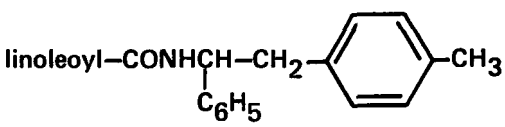
A study of almost three years duration with halofenate confirms its value in lowering triglyceride and uric acid levels in man.[93] Halofenate does not seriously affect the exercise performance in anginal patients or the resting, exercise or postexercise electrocardiograms in anginal patients or in asymptomatic patients with or without coronary heart disease.[94]

Long-term studies show colestipol to be safe and effective in lowering cholesterol levels in hypercholesterolemic individuals.[95,96] Decreases in plasma LDL and cholesterol have been accompanied by reciprocal increases in VLDL levels;[97,98] similar effects have also been reported for cholestyramine. The elevation of VLDL probably accounts for the consistent increases in triglycerides that have been reported for both resins.

Studies show that para-aminosalicylic acid (PAS) effectively reduces serum cholesterol and triglycerides in Type II patients.[99,100] In a one year study PAS was effective, well tolerated and showed no decrease in effectiveness with time.[99]



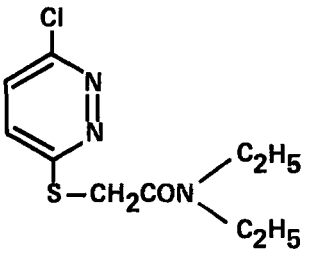
PAS



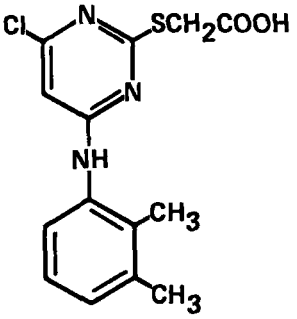
moctamide

Daily administration of moctamide to man inhibits the elevation of serum cholesterol due to egg yolk feeding. In only a few patients were serum cholesterol levels lower than initial levels indicating that in man, like animals, moctamide may have its main effect on cholesterol absorption.[101]

The choleretic agent ST9067, the antilipolytic activity of which is comparable to that of nicotinic acid, causes significant reductions in plasma cholesterol and triglyceride levels.[102] WY-14643, a heterocyclic thioacetic acid which appears to be mechanistically related to and more potent than clofibrate, is the most potent member of the series.[103]

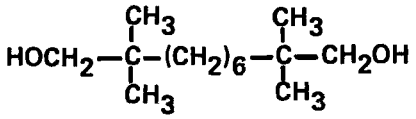


ST 9067

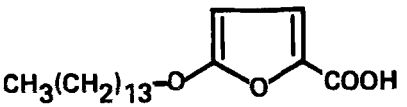


WY-14643

CI-720, a new hypolipidemic agent, depresses plasma cholesterol and triglyceride levels in patients at daily doses of 1.2-2 g. The drug appears to be well tolerated.[104]



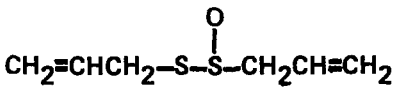
CI-720



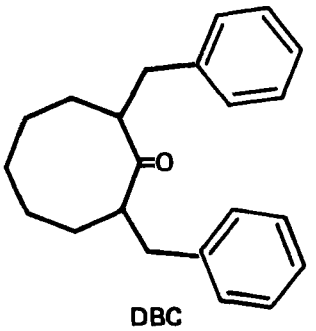
RMI 14,514

RMI 14,514 inhibits hepatic biosynthesis of fatty acids, as well as reduces plasma cholesterol and triglyceride levels in rats. The mechanism of action of RMI 14,514 on hepatic lipogenesis appears to be distinct from that of clofibrate.[105]

Allicin, a constituent of garlic, reduces rat serum and liver lipid levels,[106] with its most pronounced effect



allicin



DBC

on liver triglycerides. Freshly-extracted garlic juice was found to protect human subjects from fat-induced

increases in serum cholesterol.[107]

The most potent cholesterol-lowering agent in rats from among a series of cycloalkanones is 2,8-dibenzylcyclooctanone (DBC).[108] Estrogenic activity of this agent and other members of the series limits their usefulness.

The antilipolytic agent, 5-fluoro-3-pyridinemethanol, reduces plasma FFA levels and, when administered to patients within five hours of the onset of symptoms of myocardial infarction, reduces the incidence of serious ventricular arrhythmias.[109] This beneficial effect was related to the degree of reduction of plasma FFA concentrations. Another antilipolytic treatment, infusion of high levels of glucose-insulin-potassium, which reduces and maintains FFA levels below the myocardial threshold for FFA uptake, may also be of benefit during the postinfarction period.[110]

Propranolol in combination with nicotinic acid was reported to be extremely effective in reducing triglyceride levels in Type IV patients.[111] Although subsequently withdrawn for reasons unrelated to efficacy, a combination of practolol and clofibrate was introduced during 1974 in the United Kingdom. By themselves, however, β-blockers such as propranolol and practolol do not appear to appreciably affect plasma lipid levels.[112]

Miscellaneous Agents — There are conflicting reports on the value of dietary fiber in reducing lipid levels.[113] Differences in diet, type and amounts of fiber used and lipid types studied probably account for the confusion. Carefully controlled clinical studies are needed to determine the usefulness of fiber. The polyene antibiotics, candicidin[114] and amphotericin B,[115] decrease plasma cholesterol levels in experimental animals. Candicidin has been shown to significantly reduce cholesterol absorption which could account for its hypocholesterolemic properties.[116] Secholex, a diethylaminoethyl derivative of a cross-linked dextran, reduces LDL cholesterol of Type IIa patients.[117] VLDL was moderately reduced and HDL unchanged in one study,[117] while triglycerides were increased slightly in another.[118] Ascorbic acid does not appear to have a consistent effect on plasma cholesterol concentrations.[119,120] It depresses plasma cholesterol in young subjects but elevates it in older individuals.[121] Differences in the hypothyroid rat (depression)[122] and baboon (no effect)[123] have been reported. Chenodeoxycholic acid[124] reduces plasma triglyceride levels in hyperlipidemic patients, probably due to a decreased secretion of VLDL into plasma. It appears to have much less effect on plasma cholesterol levels.

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Chapter 20: Cyclic Nucleotides as Mediators of Drug Action

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Cyclic nucleotides now appear to be involved in the mechanisms of action of a large variety of drugs, both old and new, satisfying earlier predictions.[1] In fact it is exceptional to find a drug that does not invoke, in responsive tissues, a change in intracellular cyclic nucleotide levels, and they thus appear to mediate at least part of the activity of almost all classes of drugs.[2] This is not unexpected since the cyclic nucleotides appear to function primarily as universal messengers that translate changes in the extracellular milieu into intracellular biochemical, and hence functional, responses. For this reason, in many instances the relationship between the cyclic nucleotide changes and the pharmacologic effects of drugs may only be incidental and not causally related and care must be exercised in drawing the conclusion that cyclic nucleotides mediate the pharmacologic effects of any particular drug.

That a drug may influence the intracellular metabolism of the cyclic nucleotides in a particular *in vitro* system, is not by itself sufficient to implicate these messengers in mediating its effects on that system under normal therapeutic circumstances. Other criteria similar to those established for the hormones[3,4] must be satisfied before it can be concluded that the drug in question acts *via* the cyclic nucleotide system. Such criteria may require that the effects on the cyclic nucleotide system a) precede its pharmacological actions, b) be shown to occur in the appropriate intact organ system in a dose related manner, c) occur at *in vivo* therapeutic concentrations of the drug, d) be modified in a predictable way by other compounds with known activity on the cyclic nucleotide system, e) be blocked under the same conditions that result in blockade of the drug-induced pharmacologic effects and f) be mimicked by the appropriate cyclic nucleotides or their derivatives.

Drugs may produce their effects on the cyclic nucleotide system by direct action at any one of a large number of sites.[5,6] In addition they may also act indirectly by altering the sensitivity of the system to certain hormones or by interfering with the storage or action of information transferring molecules or other factors.[5,6] These indirect effects should still be reflected in changes in cyclic nucleotide metabolism that may not be immediate or dose related in the responding tissue. These complexities are further compounded by the fact that altering the rate of degradation or synthesis of one of the two main naturally-occurring cyclic nucleotides cyclic AMP and cyclic GMP may also result in changes in the intracellular concentrations of the other, since each appears able to either stimulate or inhibit, depending on its concentration, the rate of hydrolysis of the other.[2,5,6] The pharmacologic effects of some drugs, therefore, may depend not only on their primary effects on one nucleotide

but also on the secondary effects on the other, since both cyclic nucleotides generally take part in mediating the final tissue response, sometimes promoting antagonistic actions. Although cyclic cytidine monophosphate has been isolated from extracts of leukemia L-1210 cells,[7] there is presently no evidence for the occurrence of other cyclic nucleotides in nature and thus no evidence for their involvement in drug action.

It should also be recognized that actions on the cyclic nucleotide system may represent only one of several effects a particular drug may produce and hence may not account for all the activities of a compound in a particular system. This has been shown to be the case for a number of PDE inhibitors.[2] In addition, the cyclic nucleotides not only mediate the activities of a large variety of drugs but they also appear to mediate some of their well-known side effects; *e.g.* salivary gland enlargement following isoproterenol therapy appears to be mediated by cyclic AMP.[8]

A final complication in understanding the role that cyclic nucleotides may play in the mechanism of action of drugs is the present sparsity of data on the involvement of cyclic GMP. Most of the reported studies deal primarily with cyclic AMP which represents only part of the total picture. It is hoped that the availability of better and simpler methods for the study of cyclic GMP will expedite fuller characterization of its role in the actions of drugs. This is expected to result in better correlations between cyclic nucleotide metabolism and drug action.

We propose that it is possible to classify drugs on the basis of their effects on the cyclic nucleotide system. For example, using the drug-induced effects on cyclic AMP in a particular system drugs may be classified as a) c-adenylogenic *i.e.* to stimulate the synthesis of cyclic AMP *e.g.* isoproterenol b) anti-c-adenylolytic *i.e.* to inhibit the hydrolysis of cyclic AMP *e.g.* theophylline or papaverine, c) anti-c-adenylogenic *i.e.* to inhibit the synthesis of cyclic AMP *e.g.* lithium[9] d) c-adenylolytic *i.e.* to stimulate PDE *e.g.* insulin,[10] e) c-adenylomimetic *i.e.* to mimic the effects of cyclic AMP *e.g.* dibutyryl cyclic AMP; and f) c-adenyloantagonist *i.e.* to antagonize the effects of cyclic AMP on protein kinase *e.g.* tolbutamide.[11] By substituting "gua" for "ade" in the above terms similar ones can be coined to describe the effects of drugs on cyclic GMP. The classification of drugs according to their effects on cyclic nucleotide metabolism can bring together varying classes of drugs that have similar effects on some system; *e.g.* isoproterenol and glucagon are both c-adenylogenic in the heart and both produce similar effects on cardiac function.

The mediator role of cyclic nucleotides in the mechanism of action of drugs will be discussed according to the organ system on which the drugs exert their actions. Some cross-over between the different organ systems will occur to facilitate the discussion and to cover similar aspects of drug action in different tissues. The actions of various classes of drug on phosphodiesterase (PDE) have been reviewed recently.[2] Where possible, late references that will lead the reader to the bulk of the pertinent literature were selected.

I. DRUGS ACTING ON THE NERVOUS SYSTEM

A. General - The metabolism and functions of cyclic nucleotides in nerve function have been adequately reviewed.[12] The cyclic nucleotides appear to be involved in nearly all facets of nervous activity[13] and thus teleologically well suited to mediate the effects of most centrally acting drugs. This is supported by the fact that the central nervous system is one of the richest tissues in cyclic nucleotides and related enzymes.

It has been infrequent that agents injected peripherally have been shown to change the intracellular concentrations of cyclic nucleotides or their metabolism in the brain. Reports are now beginning to appear, however, that show area-specific changes in the metabolism of specific cyclic nucleotides in the brain and that correlate those changes with behavioral or functional effects. Most of the information on the possible involvement of cyclic nucleotides in the activity or action of drugs on the central nervous system has been accumulated in studies conducted *in vitro*.[14-16] Most of these effects have been on PDE.[2] Other sites were also studied.[17-19] The effects of the drugs tested on the *in vitro* activity of enzymes related to the cyclic nucleotide system appear to correlate well with their pharmacologic effects. This was shown particularly well to be the case in a series of antianxiety/antidepressant drugs.[20-22] Other investigators observed a lack of correlation between activity on certain cyclic nucleotide-related enzymes and pharmacologic effects.[23] The inability to demonstrate changes in brain concentrations of the cyclic nucleotides in response to peripherally administered, but centrally active, drugs could be due, at least in part, to the inappropriate methods used for brain fixation. The introduction of focused microwave radiation[24] to minimize changes in cyclic nucleotide levels prior to determination may help immeasurably in this regard. Ether anesthesia increased cyclic GMP and cyclic AMP levels in the cerebellum, whereas pentobarbital anesthesia decreased cyclic GMP and increased cyclic AMP in the cerebellum and increased the latter nucleotide in the cerebral cortex of rats.[25] Oxotremorine also raised cyclic GMP levels in the cerebral cortex and cerebellum.[26] D-Amphetamine elevated, while chlorpromazine and reserpine reduced, cyclic GMP levels in the mouse cerebellum *in vivo*.[27] Low doses of tetrahydrocannabinol elevated and high doses depressed cyclic AMP levels in mouse brain.[28] Cyclic AMP levels in the urine also appear to reflect the depressed state of the nervous system and exhibit marked changes that seem to correlate well with the changes in the clinical picture produced by various drugs[29] and electroshock therapy.[30]

B. Morphine - That the analgesic activity of morphine or similarly acting drugs may be mediated *via* the elevation of intracellular cyclic AMP levels in the midbrain-thalamus has been demonstrated.[31] The effect appears to correlate well with the onset of the analgesic action. The rise in cyclic AMP appears to be mediated primarily *via* increased adenylyl cyclase activity possibly due to elevated prostaglandin levels.[32] Withdrawal of morphine from addicted rats was accompained by a decrease

in adenylyl cyclase activity in the cerebral cortex, an effect that was antagonized by methadone treatment.[33] These effects contrast with the reported inhibitory effects of morphine on the stimulation of cyclic AMP formation in rat brain homogenates[34] and hemoblastoma x glioma hybrid cells *in vitro*[35,36] and the antagonism of morphine analgesia by cyclic AMP in mice, an effect that may be secondary to the effects of morphine on biogenic amines in the brain.[37,38] Also, morphine was reported to inhibit the stimulation of cyclic AMP synthesis by prostaglandin E_1 and E_2 in rat brain homogenates.[34] In this latter system heroin was more active and methadone less active. The action of morphine in this system also appears to be sterospecific and is antagonized by naloxone.[39]

C. Apomorphine - The beneficial effects of DOPA and apomorphine in parkinsonism may be mediated by cyclic AMP *via* stimulation of the dopamine receptors in the nigro-striatal pathway which are coupled with adenylyl cyclase[40] possibly *via* a primary effect on prostaglandin biosynthesis.[31]

D. Ethanol - The effects of alcohol in the brain may also be, at least in part, mediated *via* changes in cyclic AMP.[41] Although acute administration of ethanol did not change adenylyl cyclase activity in the brain cortex of mice, significant increases were evident in the activity of the enzyme following the chronic administration of alcohol.[42] This was also coupled with a significant increase in the concentration of cyclic AMP.[43] Whether those effects are mediated *via* increases in the concentration of brain catecholamines or due to the direct prolonged effects of alcohol itself, or its metabolites is not known. Chronic ethanol ingestion did not significantly change the brain concentration of norepinephrine in one study.[44] On the withdrawal of ethanol, the response of cyclic AMP to norepinephrine however, is increased in the cerebral cortex.[45] Acute administration of ethanol was reported to selectively lower the cyclic AMP level in the cerebellum.[46]

E. Local Anesthetics - There is also a suggestion that local anesthetics may act *via* increasing the intracellular concentrations of cyclic AMP.[47] The effect seems to be tied closely to the effects on Ca^{++} movement, and agrees with the well demonstrated effects of both local anesthetics and cyclic AMP in stimulating the sodium pump and stabilizing cellular membranes.[12]

II. DRUGS ACTING ON THE CARDIOVASCULAR SYSTEM

A. Drugs that affect cardiac contractility - A large effort has been devoted to understanding the role that cyclic nucleotides play in cardiac function. Although cyclic AMP is believed to mediate the ino- and possibly chronotropic effects of the catecholamines, the question is far from settled.[48] The possible role of cyclic GMP in the process is beginning to be understood.[49,50] As far as drugs are concerned, those catecholamine derivatives that stimulate the β-adrenergic receptors, *e.g.* isoproterenol, appear to act *via* adenylyl cyclase stimulation and the consequent accumulation of cyclic AMP. This is apparently also the site at which β-adrenergic blocking drugs exert their important effects on

the heart by blocking adenylyl cyclase stimulation by the catecholamines[51] or by direct effects on the enzyme.[52,53] Compounds that elevate cardiac contractility may also elevate cardiac cyclic AMP levels *via* other mechanisms *e.g.* inhibition of PDE, by theophylline and quazodine.[54] Elevation of the intracellular levels of cyclic AMP in the heart may also account for the side-effects of a number of drugs *e.g.* arrhythmias associated with diuretic therapy.[55]

B. Antihypertensive agents - The cyclic nucleotides also appear to mediate the antihypertensive activity of many drugs at the level of the vascular smooth muscle irrespective of their mechanism of action.[56] Chronic forms of hypertension in rats were shown to be characterized by an elevated cyclic GMP/cyclic AMP ratio in the vasculature.[57,58] Antihypertensive agents appear to change this ratio and bring it closer to that in the normotensive state, an action that appears to correlate well with their reduction of blood pressure.[56] Some antihypertensive agents achieve their effects by a direct action on cyclic nucleotide metabolism, *e.g.* diazoxide[59] and minoxidil that appear to inhibit PDE with greater activity on the cyclic AMP rather than on the cyclic GMP enzyme.[56] This would tend to produce greater elevations of cyclic AMP relative to cyclic GMP in the vascular smooth muscle and thus redress the imbalanced cyclic GMP/cyclic AMP ratio characteristic of the hypertensive state. Drugs that lower blood pressure *via* interference with sympathetic tone either at central (*e.g.* clonidine) or peripheral sites (*e.g.* guanethidine and reserpine) also result in elevation of the cyclic AMP/cyclic GMP ratio in the vascular smooth muscle. Sympathetic tone at the level of the vascular smooth muscle appears to be translated primarily into rises in intracellular levels of cyclic GMP. Sympathetic neuronal blockade results in lower cyclic GMP levels with little or no action on cyclic AMP, producing a more normal cyclic GMP/cyclic AMP ratio.

Sites other than the vascular smooth muscle involved in the antihypertensive effects of a variety of drugs also appear to involve changes in cyclic nucleotides. An example is the inhibition of renin-release which appears to underlie the antihypertensive activity of β-adrenoceptor blocking drugs. Renin release appears to be under the control of the cyclic AMP generated by the β-adrenergic receptor, located in the juxtaglomerular cells, that is susceptible to blockade by β-adrenoceptor blocking agents.[60]

C. Drugs that lower intraocular pressure - The possible mediator role of cyclic AMP in the action of drugs used to lower the elevated intraocular pressure in glaucoma is presently being examined. It appears that lowering intraoclar pressure is accompanied by a rise in the cyclic AMP content of the aqueous humor[61,62] although the site of cyclic AMP production is not clearly defined.[63] It must be noted however that the cyclic AMP rises elicited by norepinephrine in the aqueous humor are not blocked by propranolol[64] which by itself lowers intraocular pressure.[65] *d*-Isoproterenol, a compound with weak β-adrenergic receptor stimulant properties, effectively lowers intraocular pressure[66] accompanied by a rise in cyclic GMP levels in the aqueous humor.[67] Thus, it may be cyclic GMP

rather than cyclic AMP that mediates the intraocular pressure lowering effects of the catecholamines.

D. Diuretics - A number of diuretics appears to act *via* their selective activity on the cyclic nucleotide system. Furosemide was shown to antagonize the effects of cyclic AMP in the toad bladder by inhibiting the binding of the cyclic nucleotide to protein kinase,[68] a unique mechanism, in addition to its direct inhibition of the cyclase.[69] Ethacrynic acid also inhibits renal adenylyl cyclase although apparently at a different site.[69] Several diuretics also appear to directly inhibit cyclic AMP-dependent protein kinase.[70] This does not necessarily mean that diuresis is mediated by lower cyclic AMP levels or activity since cyclic AMP apparently also mediates the effects of antidiuretic hormone.[71] Elevation of cyclic AMP levels also appears to play an important role in the diuretic activity of hydrochlorothiazide[72] and other diuretics.[55] The apparent inconsistency probably results from a) the paucity of data on cyclic GMP and its role in kidney function and b) the lack of realization that the kidney is a complex tissue[73] and that determination of the levels of one cyclic nucleotide or the activity of related enzymes in the whole kidney is of little value. This was clearly emphasized by the work on prostaglandin metabolism[74] where the synthetic and degradative enzymes are located in different parts of the kidney.

III. DRUGS AFFECTING THE GASTROINTESTINAL TRACT

The cyclic nucleotide system appears to mediate the actions of a large variety of drugs on gastrointestinal function.[75] Apart from its well known involvement in the control of smooth muscle contraction, it appears to be involved in the control of gastric acid secretion,[76,77] exocrine pancreatic secretion[78,79] and intestinal secretion as well.[80] Cyclic AMP also appears to mediate the effects of several agents that induce diarrhea,[81,82] some gastrointestinal hormones,[83-85] and cholera and other enterotoxins[86-89] on water and electrolyte secretion. The role of cyclic GMP in these processes is now beginning to be investigated. It appears that cyclic GMP elevation may precede the well known cyclic AMP increases that characterize the action of cholera and enterotoxin.[90] Cyclic AMP also appears to mediate the gastric secretory inhibition produced by glycyrrhetinic acid.[91]

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the possible role of the cyclic nucleotides is that of smooth muscle. It is the general concensus that cyclic AMP mediates smooth muscle relaxation while cyclic GMP mediates contraction.[92,96] This also includes the coronary vessels[97,98] and vascular[99] and bronchial smooth muscle. As a rule, drugs affecting smooth muscle function produce the anticipated changes in the levels of the cyclic nucleotides or in the activity of the related enzymes. Bronchodilation is always accompanied by elevation of cyclic AMP or inhibition of PDE[100-103] while bronchoconstriction may be accompanied by rises in cyclic GMP.[104,105] The improve

ment of the response to adrenergic agents by asthmatics brought about by steroid therapy also appears to be related to the ability of the latter agents to improve the cyclic AMP response.[106,107] Asthma is generally characterized by decreased sensitivity of adenylyl cyclase to stimulation[108] which appears to be the biochemical translation of the β-adrenergic receptor blockade theory of the disease.[109]

V. DRUGS THAT AFFECT THE IMMUNE RESPONSE

A. General - The importance of the cyclic nucleotides in mediating various components of the immune response is widely recognized.[110-113] Although the exact mode of participation of either cyclic AMP or cyclic GMP in the various phases of this complex process is not entirely clear, several drugs that interfere with immune responses at different loci are thought to act *via* the cyclic nucleotide system.[110,114] An important example is disodium cromoglycate (Intal) and similarly acting antiasthmatic drugs [115,116] which appear to act primarily *via* the inhibition of the release of histamine and other mediators from mast cells.[117,118] Disodium cromoglycate was shown to inhibit cyclic AMP-PDE from a number of tissues[119] and to interact predictably with other drugs that are known to affect the cyclic nucleotide system.[120,121]

Pertussis-induced hypersensitivity to histamine appears to be mediated by cyclic GMP in mouse lung[122] and can be blocked by agents causing cyclic AMP accumulation.[123] This may be similar to the situation in asthma which is characterized by a decreased ability to synthesize cyclic AMP.[124]

B. Anticancer and antiproliferation drugs - The role that cyclic nucleotides may play in tumorigenesis and cellular proliferation is the subject of great interest.[125,126] A number of anticancer and antiproliferation drugs (primarily antipsoriatic) appear to act *via* altering the intracellular levels of the cyclic nucleotides in the target tissues. Cyclic AMP itself and its dibutyryl derivative inhibit the growth of a variety of tumors[125] and have beneficial effects in psoriasis. Present emphasis appears to be on cyclic nucleotide derivatives that mimic cyclic AMP action or antagonize that of cyclic GMP.

C. Non-steroidal anti-inflammatory agents - Although these agents appear to affect cyclic nucleotide metabolism in the target organs primarily *via* their effects on prostaglandin synthesis, they also seem to have some direct effects on the system.[127-130] In any case cyclic nucleotide changes in the responsive tissues appear to be involved in the final translation of the activity of these compounds and may even play a part in their effects on prostaglandin production. These compounds may also act at other sites in the cyclic nucleotide system.[131]

VI. MISCELLANEOUS DRUGS

A. Oral antidiabetic agents - Cyclic AMP is assumed to control the release of insulin from the pancreatic β-islet cells.[132] The only problem

in fully accepting this assumption has been the lack of a demonstrable change in cyclic AMP contents of the islets exposed to glucose which is presumably the most important stimulus for insulin release. Recently, however, reports are beginning to appear supporting the possible mediation by cyclic AMP of the insulinogenic effects of glucose.[133-135] Oral antidiabetic agents in general appear to act by elevating the intracellular levels of cyclic AMP primarily *via* PDE inhibition[2,136-138] although they may act at other sites as well, *e.g.* stimulation of adenylyl cyclase.[139-141] The antilipolytic effects of these compounds, however, appear to result from their inhibitory effects on the cyclic AMP-activated protein kinase in fat cells.[10,137,142]

B. Muscle Relaxants - Drugs that increase skeletal muscle contractility (antagonize tubocurarine toxicity) may facilitate the release of acetyl choline from motor nerve endings *via* the accumulation of cyclic AMP brought about primarily *via* PDE inhibition.[143] Both cyclic AMP and cyclic GMP are effective in antagonizing tubocurarine toxicity.[144] Apparently the inhibitory effects of tubocurarine, in relatively high concentrations, on PDE are of toxicologic rather than pharmacologic importance.[145] This agrees well with the utility of theophylline and papaverine in myasthenia gravis.[146] This mechanism is different however from the direct inhibitory effects of these same agents on skeletal muscle contraction.[147]

C. Carcinogenic agents - The lowering of intracellular cyclic AMP levels possibly coupled with elevated cyclic GMP levels appears to mediate the tumorigenic effects of phorbol-myristate-acetate.[148] Prevention of the decrease in cyclic AMP levels significantly modulates the tumorigenicity of the compound. The decrease in cyclic AMP levels is probably mediated by decreased sensitivity of adenylyl cyclase to stimulation which appears to characterize a number of tumors.[149-153] This is supported by the ability of agents that can raise the intracellular levels of cyclic AMP to inhibit the growth of tumors.[154,155]

D. Contraceptive Drugs - Although steroids are generally thought to act on the cell nucleus *via* mechanisms more or less independent of the cyclic nucleotide system, many of these agents do affect the cyclic nucleotides in ways that appear to be directly coupled to their sex-related effects.[156] Cyclic AMP itself has strong contraceptive activity in mice[157] primarily by inhibiting ovulation.[158]

E. Others - Cyclic AMP not only appears to mediate the actions of a number of antibiotics with widely varying spectra of activity,[159,160] but also mediates the induction of antibiotic inactivating enzymes.[161] It also appears to play an important role in the effects of radioprotective drugs,[162,163] the effects of interferon, poly A or poly U on L cells[164] and the metabolic effects of DDT.[165]

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Chapter 21. Peptide Hormones of the Hypothalamus and Pituitary

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The most interesting developments since the last review[1] have occurred in studies on: (a) somatostatin, the newcomer in hypothalamic hormones, and its surprising influence on pancreatic hormones; (b) superactive and inhibitory analogs of GnRH*; (c) the concept of enzymatic cleavage of hormones to form releasing factors, e.g. MRF and MIF from oxytocin; (d) critical evaluation of CNS and behavioral effects elicited by TRH; (e) highly active cores of hGH; and (f) detailed conformations of neurohypophyseal hormones, allowing rational analog design. Readers are referred to recent books and reviews for excellent summaries of the rapidly expanding work in the field[2-5] and especially in radioimmunoassay development[2,6].

I. Hypothalamic Hormones

A. General — One of the most important realizations is the lower than originally expected specificity of hypothalamic hormone control of pituitary secretion. All three hormones[7] of known structure have at least two targets, i.e. gonadotropin releasing hormone stimulates release of LH and FSH, thyrotropin releasing hormone controls TSH and PRL release, and somatostatin inhibits secretion of GH and TSH, as well as the pancreatic hormones insulin and glucagon. Other proposed releasing factors (e.g. for GH or ACTH) have not yet been isolated and physicochemically characterized and will not be reviewed here. The conclusion[8] that biosynthesis of TRH and, perhaps, other releasing hormones proceeds through nonribosomal enzyme systems (synthetases) may be premature in view of the absence of unusual structural features (ω-peptide bonds, D-amino acids, N-methylamino acids) in the known hormones. — Numerous comprehensive reviews and books have appeared[7,9-16], and studies on neuroendocrine control of anterior pituitary function through catecholaminergic and serotonergic transmitter input into hypothalamic releasing hormone-secreting neurons have been critically evaluated[17].

In addition to releasing hormones, the hypothalamus contains other peptides of high biological activity. The recent isolation of substance P [I][18] and neurotensin [II][19] may encourage searches for other peptides. I and II

H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 [I]

<Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH [II]

*Abbreviations: ACTH, Adrenocorticotropin; Asu, α-Aminosuberic acid; AVP, Arginine-vasopressin; AVT, Arginine-vasotocin; CNS, Central nervous system; FSH, Follicle stimulating hormone; GH, Growth hormone; <Glu, Pyroglutamic acid; GnRH, Gonadotropin releasing hormone; hGH, Human growth hormone; LH, Luteinizing hormone; LVP, Lysine-vasopressin; MIF, Melanotropin release-inhibiting factor; MRF, Melanotropin releasing factor; MSH, melanotropin; PRL, Prolactin; TRH, Thyrotropin releasing hormone; TSH, Thyrotropin.

have been synthesized[18,20]. Substance P elicits central depressant effects[21] and several potent peripheral and kinin-like activities[22,23]; neurotensin may be involved in regulation of liver glycogen metabolism[24] in addition to its kinin-like effects.

B. Gonadotropin Releasing Hormone or Luteinizing Hormone/Follicle-stimulating Hormone Releasing Hormone (GnRH) — A consensus between the schools of Guillemin and Schally seems to appear now in the literature, that (a) the decapeptide III controls physiologically the release of both luteiniz-

<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 [III]
1 2 3 4 5 6 7 8 9 10

ing hormone and follicle-stimulating hormone in man and in animals, and (b) that possibly another hormone might be present in hypothalamic tissue which releases only or predominantly FSH[10,11]. GnRH does not stimulate secretion of GH, TSH or ACTH. Species specificity might not exist. Several reviews and books on chemistry, biology, and clinical studies have appeared[9,11,25-30].

Intensive synthetic efforts have been aimed at three main goals: (1) preparation of large amounts of GnRH for biological studies, (2) analog synthesis for structure-activity studies, and (3) search for analogs with inhibitory properties. Of ca. 15 GnRH syntheses published to date (lit. survey[31]) several carefully executed conventional syntheses in solution provide for pure products in gram quantities[31-34]. Synthesis of ca. 150 analogs allows several structure-activity correlations*. Synthetic modifications at the Gly^{10}, Gly^{6} and His^{2} positions of III have remarkable influence on biological activity. Omitting Gly-$NH_2{}^{10}$, several (1-9)-nonapeptide alkylamides with increased potency were obtained[35]. Des(Gly-$NH_2{}^{10}$)-GnRH ethylamide exhibited 3-fold higher activity than GnRH. Replacement of Gly^{6} by D-alanine[36] or by D-leucine[37] resulted in 3- to 7-fold increase of in vivo and in vitro activities. All other position diastereoisomers showed strongly reduced activities[38]. Omission of His^{2} caused loss of all agonist activity[39]. Des-His^{2}-GnRH inhibited in vitro LH release by GnRH to 50%, at an agonist to inhibitor ratio of ca. 1:4000. Interestingly, these activity shifts appear to be retained and are sometimes additive or even synergistic in multiple substitutions. Increased potencies along with prolonged in vivo activities were observed for [D-Ala^{6}]- and [D-Leu^{6}]-des(Gly-$NH_2{}^{10}$)-GnRH ethylamide which showed, respectively, 16- and 54-fold greater effects than GnRH when integrated levels of LH or FSH, released over a 6 hr period after injection, were compared[37,40]. The octapeptide des-(His^{2},Gly-$NH_2{}^{10}$)-GnRH ethylamide[41] turned out to be a more potent inhibitor than des-His^{2}-GnRH and effective in vivo. Since des-His^{2}-[D-Ala^{6}]-GnRH was also a 3 times more potent inhibitor than des-His^{2}-GnRH[36],

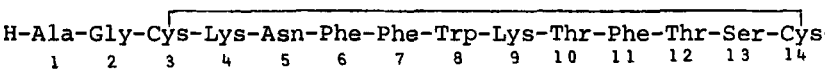
*The comparative activity ratios given below are approximations since assay methods vary between laboratories.

one looks forward with interest to a synthesis of des-(His^2,Gly-$NH_2{}^{10}$)-[D-Ala^6]- or [D-Leu^6]-GnRH ethylamide. Highly active analogs smaller than nonapeptides or inhibitors smaller than octapeptides have not yet been found[11] indicating that many parts of the GnRH molecule are required for high potency. — NMR and CD studies indicate[42,43] that GnRH exists in aqueous solution in random coil conformation with no appreciable stacking of the aromatic rings and no electrostatic intramolecular interactions involving the charged imidazole ring.

GnRH might be expected to find diagnostic, prophylactic, and therapeutic applications in human and animal fertility[10]. One problem facing in vivo applications is the short duration of action of GnRH. Its biological half-life was found to be 6-7 minutes in rats. Synthesis of a long-acting polymer-bound GnRH analog[44] represents one possible approach; alternatively, slow-release formulations may be developed.

C. Melanotropin Release-inhibiting Factor (MIF) and Melanotropin Releasing Factor (MRF) — The significance and physiological roles of peptides which exhibit MIF or MRF activities[7] are still subjects of debate, due in part to great experimental difficulties with bioassays. Concerning biosynthetic aspects, however, the enzymatic formation of H-Pro-Leu-Gly-NH_2 (MIF) from oxytocin[45] was the first example, on a molecular basis, of a well defined peptide hormone serving as a precursor or "second order prohormone" for a smaller peptide with entirely different hormonal activities[46]. This concept has since been broadened to include ACTH-releasing activity, ACTH, vasopressin, gastrin, and others[46].

D. Somatostatin (Growth Hormone Release-inhibiting Hormone) — Guillemin and collaborators isolated a peptide [IV] from ovine hypothalamic extracts



which consistently inhibited in vitro the release of radioimmunoassayable growth hormone in a dose-dependent manner[47]. Structure IV, a heterodetic tetradecapeptide with a 3-14 disulfide bond forming a 38-membered ring, has been confirmed by synthesis[48] (lit. survey[49]). Oxidative cyclization of bis-sulfhydryl intermediates ("reduced somatostatin") gives low yields (25-35%) of IV, due to polymerization. However, reduced somatostatin is equipotent with IV[50] in suppressing GH release in vitro (but not always in vivo), probably due to spontaneous oxidation.

Biological activities: Somatostatin inhibits secretion of growth hormone, thyrotropin, insulin and glucagon. It does not affect secretion of gonadotropins or ACTH. Both basal and stimulated GH secretion in vitro in dispersed rat pituitary cell culture[51], is suppressed by 1 nM levels of somatostatin[47,52]. Acutely, somatostatin appears to inhibit release but not synthesis or storage of GH. Somatostatin inhibits stimulated GH se-

cretion in vivo in man and animals without apparent species specificity. Its biologic half-life is short (<4 min). Constant intravenous infusion (e.g. 0.5-1 mg per adult human over 30-60 min) is required to obtain measurable effects. Rapid rebound of suppressed GH occurs when infusions are stopped[53,54].

Somatostatin inhibits TSH secretion in vitro and in vivo, induced by TRH or other stimuli in a dose-dependent manner[55,56] indicating a potential physiological role in thyroid regulation. Insulin and glucagon secretion in animals and in man are reversibly suppressed by somatostatin in a dose-dependent way. Basal and stimulated secretion of both pancreatic hormones is affected by direct action of low doses of somatostatin on pancreatic α and β cells, indicating a physiological role in glucoregulation[57-60].

Few analogs have thus far been synthesized. However, it has already been shown that the NH_2-terminal part of the molecule is not required for high activity. Des-(Ala^1-Gly^2)-somatostatin, its reduced form, the 3-N-acetyl derivatives, and the 3-deamino analog possess between 30% and 100% the potency of the native hormone[50]. S-Methylation, replacement of both Cys^3 and Cys^{14} by Ala[50,61], replacement of Trp^8 by Ala, and amidation of the COOH-terminal[62], all result in drastic loss of activity.

Clinical use of somatostatin may be expected in the treatment of juvenile-onset diabetes which is characterized by abnormally elevated secretions of glucagon and growth hormone and as a useful adjuvant to insulin in the treatment of diabetes mellitus[63]. Possible beneficial effects of somatostatin, in preventing the development of the classic microangiopathies (retinopathy) of diabetes and in treatment of acromegaly[64], remain to be established. Most importantly, long acting preparations of somatostatin will be needed for clinical therapeutic use, either as depot formulations or in form of synthetic analogs.

E. Thyrotropin Releasing Hormone (TRH) — The earliest known and one of the smallest of the hypothalamic hormones, TRH, <Glu-His-Pro-NH_2, continues to attract wide attention. Clinical use as a diagnostic, studies on CNS and behavioral effects in man, and conformational analysis have highlighted extensive medical, biological and chemical work[11,65-69].

TRH mediates release of TSH and PRL in animals and in man[7] and release of GH in acromegalic but not in normal humans[70]. It can be administered orally[71], an exceptional feature for a peptide hormone. The plasma half-life in rats is 2.2 min[72]; the hormone accumulates in kidney, liver, thyroid[73] and brain where highest concentrations occur in the hypothalamus[74]. Large amounts are excreted in the urine[75]. Deamidation and proline or prolinamide liberation result in <Glu-His-OH as major metabolite[76].

Several groups have reported on the efficacy of TRH in treatment of mental depression and schizophrenia[77]. Failure by other investigators to confirm these findings[78] has led to a controversy which may be difficult to resolve in view of great complexities in patient selection and evaluation of

effects. In mice and rats, TRH potentiates psychomotor stimulating actions of L-dopa and accelerates turnover rates of norepinephrine[79,80]. The tripeptide H-Pro-Leu-Gly-NH_2 (MIF) enhances dopamine synthesis in rat brain[81]. Much remains to be learned before the nature of the CNS effects of hypothalamic peptides[77] will be understood.

Synthetic work has somewhat abated due, perhaps, to the failure to find analogs[11,65] with inhibitory properties, with separated capacities for TSH or PRL release, with prolonged action, or with greatly enhanced potency (with the notable exception of [2-N^{τ}-methylhistidine]-TRH possessing 8-fold potency). An improved synthesis of TRH using carbodiimide along with N-hydroxy-5-norbornene-2,3-dicarboximide for condensation provided the first crystalline preparation of TRH as a tartrate[82]. Detailed conformational studies should eventually provide a clearer understanding of the molecular requirements for TRH activity. Recent ^1H NMR studies[83,84] showed TRH to be preferentially in extended conformation in polar solvents.

II. Pituitary Hormones

A. General — The spectacular recent increase in the use of synthetic ACTH-(1-24)-tetracosapeptide in human medicine certainly attests to the expertise and perseverance of the CIBA-GEIGY peptide group, but it also suggests great potential for clinical application of other pituitary hormones and analogs. Discoveries of highly active cores of human growth hormone and β-lipotropin may be advances in this direction. With increasing sensitivity of radioimmunoassays[2] multiple forms of almost every hormone have been found in circulation[85]. Comprehensive reviews and books have appeared[9,17,86-89].

B. Adrenocorticotropin (ACTH) and Melanotropin (MSH) — The amino acid sequences of human, bovine and ovine ACTH have been revised[90,91] and resemble each other even more closely; human ACTH has been synthesized according to the revised structure by conventional[92,93] and by solid phase techniques[94]. Reviews have appeared on the chemistry and biology[9,95-97] and on behavioral effects[98] of ACTH.

Results obtained by radioimmunoassay, bioassay and physicochemical methods indicated that the human pituitary does not normally produce α-MSH or β-MSH and it was suggested that these peptides may be artifacts formed by enzymatic degradation of β-lipotropin during extraction[99].

C. Growth Hormone (GH) and Prolactin (PRL) — Several reviews on the chemistry and biology of hGH and PRL[100-104] and somatomedin[100,103,105] have been published. Human PRL (lactogenic hormone) has only recently been identified as distinct from hGH[104,106].

Searches for an active core of hGH have been conducted for many years because (a) GH from domestic animals is inactive in man, (b) the supply of natural human hormone is obviously limited, and (c) commercial chemical syn-

thesis of the entire hormone is prohibitive due to its size of 191 amino acid residues. Recently, two active fragments have been isolated[107] after digestion with plasmin which were derived from sequence regions 1-134 (10-20% activity) and 141-191 (3-6% activity). Whether smaller fragments will exhibit similarly high activity remains to be established. A solid phase synthesis of N^{α}-acetyl-hGH-(95-136) possessing low activity in the rat tibia test has been reported[108]. — Circulating GH is heterogeneous in size and at least three components have been identified in man[70,104]. Whether big GH forms are biosynthetic precursors of the monomeric molecule remains undecided[109]. Growth promoting effects of bovine GH on mouse and rat tumors in vivo have been reported[110].

GH action on some tissues, e.g. skeletal tissue, is mediated by plasma factors, called somatomedins. Several distinct somatomedins seems to exist in man. They are insulin-like peptides of unknown structure possessing molecular weights of ca. 4-8000 daltons[100,103,105].

The clinical usefulness of hGH for hypopituitary children is well established[3]. In addition, beneficial effects were recently demonstrated in treatment of bleeding ulcers, muscular dystrophy, and osteoporosis[103].

D. Glycoprotein Hormones: Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Thyroid Stimulating Hormone (TSH) — Several comprehensive reviews have appeared on the chemistry, biology and immunology of FSH, LH and TSH[5a,30,111-114]. Primary structures of α and β subunits of both human LH and human TSH have been determined. Identity was established between their α subunits (lit. survey[115]). A simple improved procedure for separating LH subunits has been developed[115]. The disulfide bonds of ovine LHα have been assigned[116]. Sequences of the α and β subunits of human FSH have been determined[117]. The structures of the carbohydrate parts of FSH, LH and TSH are not yet known. Determination of those for α and β subunits of human chorionic gonadotropin[118] should direct efforts toward this end. — The nature and physiological actions of the pituitary gonadotropin hormones of non-mammalian vertebrates are still poorly understood. From both reptilian and amphibian sources two distinct gonadotropins each have been identified and isolated[119] which may correspond to mammalian FSH and LH.

E. Lipotropin — Lipotropin affects fat mobilization in intact adipose tissue as well as in isolated fat cells[120]. A solid phase synthesis of ovine β-lipotropin-(42-91)-pentakontapeptide led to the exciting discovery that this COOH-terminal part of the molecule exhibited 6-fold higher lipolytic activity in isolated fat cells than the native hormone on a weight basis[121]. The optimal active core giving rise to maximal biological activity remains to be determined.

III. Neurohypophyseal Hormones

A high state of expertise in synthesis of analogs of oxytocin [V] and the vasopressins [VIII, IX] and in assay of their biological activities has been developed since du Vigneaud's first oxytocin synthesis in 1953. Several reviews on the chemistry and biology of neurohypophyseal hormones have recently appeared[122-126].

H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2 (1 2 3 4 5 6 7 8 9)

[V] Oxytocin. [VI] 8 = Ile, Mesotocin.

[VII] 8 = Arg, Arginine-vasotocin (AVT).

H-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2 (1 2 3 4 5 6 7 8 9)

[VIII] Arginine-vasopressin (AVP).

[IX] 8 = Lys, Lysine-vasopressin (LVP).

Phylogenetic studies[127] revealed the presence of oxytocin [V] and arginine-vasopressin [VIII] in echidna (Australian spiney anteater, a monotreme and most primitive known protherian mammal); while mesotocin [VI] and vasotocin [VII] occur in nonmammalian tetrapods with great evolutionary stability. There appears to be a clear-cut division between mammals and the other tetrapods, pointing to a deep physiological meaning of the double change in neurohypophyseal hormones[127].

Correlations between solution conformations of oxytocin, vasopressins and analogs and their chemical and biological properties have been extensively investigated by ^1H and ^{13}C NMR, conformational energy calculations and model building (lit. survey[128]). In Me_2SO solution the 20-membered ring moieties of AVP [VIII], AVT [VII], LVP [IX], and oxytocin [V] all appear to possess a compact antiparallel β-pleated sheet conformation with a β-turn involving residues 2-5 (Fig. 1). The backbone NH of Asn^5 is intramolecularly hydrogen-bonded to the C=O of Tyr^2. The tail moiety (residues 7-9) in oxytocin forms a second β-turn stabilized by a hydrogen bond between the NH of Leu^8 and the side chain C=O of Asn^5. The charged tail portion of LVP seems to possess a slightly larger, those of AVP and AVT considerably larger, conformational freedom. The gross structure (Fig. 1) is presumed to be conserved in water although the hydrogen bonds may be loosened.

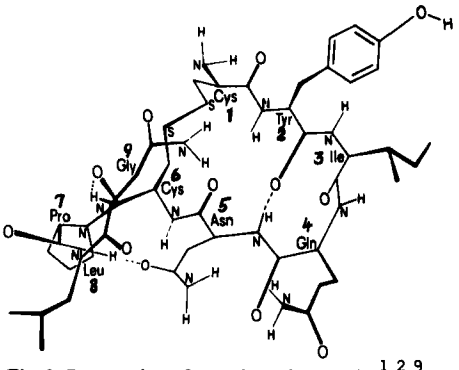


Fig. 1. Proposed conformation of oxytocin[129]

Novel rationales for analog design[130,131] have been based on the proposed oxytocin conformation (Fig. 1) as well as an interpretation[132] of the well known resistance or susceptibility of the hormone to degradation by certain proteolytic enzymes. Analogs with selectively modified activity profiles should result from amino acid substitutions in the corners of the β-turns (positions 3, 4, 7, 8 in Fig. 1) which are not primarily involved in the intramolecular stabilization of the peptide backbone and available

for intermolecular interactions. Indeed, [Gly^{7}]oxytocin and [Asu^{1,6},Gly^{7}]-oxytocin retained high oxytocic and milk-ejecting activities and lost almost completely the vasodepressor, antidiuretic and pressor activities[130]. Highly specific analogs, such as these, have long been a goal of synthetic efforts[122,125]. A remarkable recent example is [1-deamino,4-valine]-8-D-arginine-vasopressin (X)[133] which possesses an antidiuretic to pressor activity ratio of over 125,000[134], compared with a ratio of 1 for AVP. Favorable clinical results[135] with a less specific antidiuretic, [1-deamino]-8-D-arginine-vasopressin[136], indicate a high potential usefulness for X in the treatment of diabetes insipidus. Potent inhibitory analogs have recently been obtained by replacement of Cys^{1} with L-penicillamine and β-mercapto-β,β-dimethylpropionic acid[137-139] or by substitution in positions 9[140] or 2 of oxytocin, e.g. [2-o-iodotyrosine]oxytocin[141], (see also[122]).

The role of neurophysin[142] as a possible precursor or transport vehicle for neurohypophyseal hormones has not yet been clarified but impressive progress has been made[143] in sequence analysis of several neurophysins and studies on hormone binding properties.

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Section V - Topics in Biology

Editor: T. Y. Shen, Merck & Co., Rahway, New Jersey

Section Editorial

In selecting topics for the Biology Section, emphasis has been given to emerging new fields, particularly those with recent advances either in biological mechanism or biochemical characterizations. It is hoped that these reviews may stimulate the initiation of some exploratory synthetic studies to bridge the gap between basic sciences and biomedical applications.

Gamete biology, a potential approach to new fertility control agents, is gradually being unravelled in terms of biochemical processes and membrane receptors. The recognition of an immunological mechanism for periodontal disease, an inflammatory and degenerative disorder, suggests that this area may offer new opportunities to medicinal chemists already interested in the development of selective immunoregulants.

Several important aspects of immunology have been discussed in recent issues of the Annual Reports. This writer is particularly impressed by the frequent involvement of the function and transformation of cell membranes in the pathogenic processes of many immune and degenerative disorders. To provide an overview of the rapidly growing field of biomembrane studies, a broad survey of plasma membrane pathophysiology, complimentary to a chemical review of membrane function in Chapter 32, is presented here. More specific topics on biomembranes are planned for future issues. The pharmacological and antiparasitic properties of ionophore antibiotics illustrate some applications of membrane affectors. Undoubtedly other types of novel and selective membrane regulators may soon emerge as new classes of therapeutic agents.

Superoxide radicals and superoxide dismutase have received a great deal of attention in the past three years because of their conceptual as well as practical importance. An interesting review of the biological activities of organic silicon compounds was first considered last year. It reveals the biomedical potential of a large family of silicon derivatives hitherto overlooked by most medicinal chemists.

Chapter 22. Plasma Membrane Pathophysiology

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This article will document the involvement of the plasma membrane in the pathogenesis, pathophysiology and pharmacotherapy of some important disease processes. Because of the breadth and explosive growth of the field, its treatment here must be highly selective: Illustrations are deliberately drawn from very diverse disease processes and the emphasis is on topics where some specific biochemical information is available. More general reviews have been published[1,2] and a report on membrane biochemistry is presented by Baran in Chapter 32 of this Volume.

Cancer -(malignant neoplastic disease)- Cancer is characterized by a multitude of polymorphic defects, involving the plasma membrane and also intracellular membranes. This topic is under intense study and has been recently reviewed in detail.[3,4] I will here address only prominent plasma membrane alterations. It is important in this context to appreciate that the feature characteristic of malignant neoplasia is the propensity of the tumor cells to invade normal tissues and to spread (metastasize) to distant sites. Uncontrolled and rapid proliferation occurs also in benign neoplasms.

Composition - Virally-induced neoplastic fibroblasts generally exhibit a loss in high-molecular weight glycoprotein (> 200,000 D) present in (on) normal cells[3-12] probably a galactoprotein. Other data[13-16] indicate defective glycosylation of membrane proteins. On the other hand, wherever tested, tumor cells also exhibit new membrane proteins, which bear tumor specific transplantation antigens.[17,18] These proteins (M. Wt. 40,000-200,000) are the targets for cancer immunotherapy.

The plasma membranes of cancer cells generally also exhibit abnormal lipid composition. As far as phospholipids are concerned deviations in sphingomyelin content are common.[29,30] However, phospholipid alteration in intracellular membranes appears more common.[3,29] Hepatomas generally exhibit increased cholesterol in their plasma membranes[3,30] and whenever tested tumor cells exhibit abnormally high cholesterol biosynthesis and defective in vivo inhibition of sterol biosynthesis upon cholesterol feeding.[3,31,32] It now appears that certain oxidized cholesterol derivatives, e.g. 25-hydroxylcholesterol rather than cholesterol itself are the "feedback" regulators[33] and can regulate cholesterol biosynthesis even in neoplastic cells. As is true for phospholipids, the most profound cholesterol anomalies occur in intracellular membranes, e.g. mitochondria; endoplasmic reticulum.[3]

Very profound changes occur in the sphingo-glycolipid composition of tumor cells.[3,34,35] Both neutral glycolipids (cerebrosides) and acidic sialoglycolipids (gangliosides) are involved, although it is not clear that these lipids occur in the plasma membrane only. In neoplastic fibroblasts a common, but not consistent pattern is one of simplification, i.e.

depletion of glycolipids with complex headgroups and accumulation of glycolipids with shorter glycosyl residues, but this pattern is not seen in hepatomas. Whenever observed, the glycolipid anomalies can be related to deficiencies in glycosyltransferases involved in the "elongation" of the sugar chains. One of the most fascinating findings is the discovery of an apparently unique ceramide tetrasaccharide (lacto-N-neotetraosylceramide) in the membranes of polyoma-transformed hamster fibroblasts.[12] Another curious anomaly in neoplastic cells is the presence of substantial levels of polypeptide-glycosphingolipid complexes - the "eoproteolipids."[36]

It is difficult to evaluate the significance of reported glycolipid changes in tumor cells because glycolipids serve as both substrates and products. Accumulation of a given glycolipid thus depends not only upon the glycosyltransferases participating in its metabolism, but also upon the affinities of diverse membranes for this lipid. Also it is not known which enzymes in a glycosyltransferase are rate limiting and what factors regulate glycosyltransferase activity.

However, the altered glycolipid composition of tumor cells does account for some antigenic changes (non-tumor specific) observed since many of the glycolipids involved are antigenic (Forssman antigen; blood group antigens).

Plasma Membrane Enzymes - Tumor cells commonly show deviations in the activities of enzymes known or thought to be associated with the plasma membrane.[3] No consistent pattern has emerged except perhaps for enzymes and receptors involved in cyclic nucleotide metabolism. In general, this metabolism in neoplastic cells is less responsive to external stimuli - e.g. hormones, cell density. This unresponsiveness might be in part ascribed to a deficiency and/or altered distribution of specific receptors required for cyclase activation, but may also be associated with and derived in part from, modified adenylate cyclase activity and a shift in phosphodiesterase affinity for cyclic nucleotides. For the case of virally-induced tumors it has been proposed[37] that oncogenic viruses introduce different "transforming genes" which modify adenylate cyclase activity in various ways either directly or indirectly by changing a critical plasma membrane component (protein, lipid or carbohydrate), leading to a whole cascade of pleiomorphic anomalies.

Transport Anomalies - Fibroblasts neoplastically converted by tumorigenic viruses exhibit enhanced sugar and/or amino acid transport *in vitro*.[3] Anomalies in *sugar transport* have recently been reviewed in detail.[38] All available data point to enhanced uptake of sugars (glucose, galactose, 2-deoxyglucose) following neoplastic conversion. This phenomenon occurs at the transport level and is not an epiphenomenon of altered cellular metabolism. In the case of transformation with PAPOVA viruses, or chick embryo fibroblasts converted by Rous sarcoma virus, the enhanced uptake is due to either an increased number of turnover of carriers whereas with murine sarcoma viruses there may be a qualitative change in the transport system.[38] Experiments with viral mutants, temperature-sensitive for

tumorigenicity, show that enhanced transport occurs only upon neoplastic transformation not simply presence of the viral genome.[3,38] Moreover, the transport changes correlate strictly with morphologic alterations characteristic of neoplastic conversion. Certain tumor cells also exhibit enhanced amino acid uptake in vitro due to an increased number or turnover of transport carrier.[3,39,40] Convincing transport changes have not yet been discovered for other nutrients.

The significance of the transport enhancement observed in neoplastic cells remains to be established, but it is conceivable that cells unusually acquisitive for essential metabolites may "starve out" normal cells. However, considering the in vivo case, the normal concentration of glucose in the plasma ($\sim$ 5 mM) lies considerably above the K_m values observed in both normal and transformed cells and the alterations of sugar transport reported thus do not provide an obvious selective advantage for the tumor cells. Enhanced amino acid transport may have greater in vivo significance because the plasma concentrations of essential amino acids (0.02 to 0.2 mM) lie well below the K_m values for amino acid transport into cells.

An important aspect of membrane transport is its relevance to tumor chemotherapy, since some important anti-neoplastic drugs (nitrogen mustards, must be actively transported into cells to become biologically effective. Tumor variants that do not transport nitrogen mustard[41-44] or methotrexate[45-48] become drug resistant. Present data thus indicate that the membrane transport properties of tumor cells can be highly pertinent to the process of neoplastic transformation and tumor therapy.

Abnormal Enzyme Release - Tumor cells exhibit an unusual propensity to release diverse cytoplasmic enzymes into the extracellular space.[3] The mechanisms involved are not understood, but the phenomenon is not due to cell death or non-specific hyperpermeability. Indeed, tumor cells may leak large amounts of enzymes either in vivo or in vitro yet tightly regulate the transit of small molecules across the plasma membrane.

Of particular importance is the release of collagenase[49] and of plasminogen activator.[50-60] Collagenase is a lysosomal enzyme. Plasminogen activators may also be of lysosomal origin but it has also been suggested that the enzymes are latent in the plasma membrane. Plasminogen activators are serine-type proteases (M. Wt. $\sim$ 50,000-60,000) that activate plasma plasminogen into plasmin (fibrinolysin) by peptide cleavage. The enzymes are inhibited by diisopropyl phosphofluoridate. In vitro comparisons of normal with virally transformed neoplastic fibroblasts reveal an 8-30 fold increase in the release of plasminogen activator in the latter.[51] The tendency to release plasminogen activator correlates closely with tumorigenicity. The propensity of tumor cells to release proteases would appear to constitute a property that is extremely relevant to malignant behavior, in that it would lead to (a) polymorphic alteration of overall membrane behavior changing membrane cooperativity; (b) destruction of connective tissue allowing invasion by the tumor cells; (c) destruction of receptors for hormones and other regulators, releasing

the cells from physiological controls (d) destruction of tumor specific antigens creating a "bland" surface character, allowing the tumor cells to escape from immune surveillance and (e) interference with cell-mediated, or humorally mediated immune reactions. The development of agents that inhibit tumor proteases may therefore open a new dimension in tumor chemotherapy.

Hemolytic Diseases - Most hemolytic diseases involve a deficient stability of the erythrocyte membrane. This deficiency is often associated with transport abnormalities[61], but decreased *in vivo* survival of the erythrocytes probably derives primarily from their abnormal rheologic properties.[61] Three major categories of hemolytic disease exist, i.e. those due to abnormal hemoglobins, those due to an abnormal membrane lipid composition and those due to membrane protein deficiencies.

The impaired erythrocyte survival in sickle-cell disease, is due primarily to the precipitation of HbS at low pO_2 with resulting cell deformation. However, although sickle cells respond normally to agglutinating antisera at high pO_2, they do not do so at low pO_2, although antibody binding is equivalent. Also, homozygous sickle cells (SS) show an increase in K^+ and Na^+ permeability in N_2 and a net K^+ loss which can be reversed by "liganding" the hemoglobin with O_2 or CO.[62,63] A specific membrane effect appears involved since concentrated SS solutions have the same Na^+ and K^+ affinities in O_2 as in N_2. Apparently sickling accelerates the carrier-mediate transport of Na^+, K^+, at the same time opening diffusion paths for all of these cations.[62,63]

"Unstable" hemoglobins, e.g. Hb Köln, Hb Zürich, are also associated with decreased erythrocyte survival[61], but a different mechanism may be involved.[61,64,65] All of these hemoglobins have amino acid replacements in the β-chains and it is argued that these chains possess a hyper-reactive thiol (e.g. β93) that forms mixed -S-S- linkages with -SH groups on membrane proteins.[61, 64, 65] Indeed, an abnormal affinity for mixed -S-S- formation has been documented for the Hb Köln and Hb Zürich, as well as the synthetic hemoglobin, $\alpha_2^{heme}\beta_2^{o}$, and the latter protein forms -S-S- linkages with membrane proteins far more readily than normal hemoglobin.

Abnormally high membrane cholesterol produces acanthocytes ("spur" cells) that are rheologically fragile.[66] This may come about due to hereditary or secondary (hepatocellular disease) hypo- or α-betalipoproteinemia. It can also be induced by lithocholate which can be elevated in liver disease.[67]

A hereditary hemolytic anemia due to an increase (∿ 35%) of the amount of phosphatidylcholine per cell has been described.[68,69] This appears due to an abnormally low transfer of fatty acids from membrane phosphatidylcholine to phosphatidylethanolamine.[69]

Hereditary spherocytosis (HS) - This most common congenital hemolytic disease in caucasians is due to a membrane protein defect, which, by causing abnormal, rigid cell morphology and rheology, leads to accelerated

erythrocyte destruction *in vivo*. There is also an associated hyperpermeability to Na^+, with increased, glycolytically-supported Na^+-pumping, and a reversible depletion of cholesterol.[61] There is some dispute as to the protein defect. One proposal is that the disease is due to a genetic defect in microfilamentous (actomyosin-like) membrane-associated proteins.[61,70] Indeed, treatment of normal erythrocytes with agents (colchicine, strychnine, vinblastine), that precipitate microfilamentous proteins[71] reversibly induce the abnormal reigic morphology, increase Na^+-permeability, high glycolysis and impaired *in vivo* survival of HS cells.[72] However, it is possible that HS is more than one entity[73,74] and two reports describe a specific deletion of band IV b (defined by sodium dodecyl sulfate, polyacrylamide gel electrophoresis; SDS-PAGE) in HS[75,76] although this finding is not supported by another study using similar techniques.[77,78]

Another hypothesis is that spherocytosis derives from abnormally high *intracellular* Ca^{2+} [79] with secondary effects on microfilaments and a slight deficiency in Ca^{2+} ATPase (equated to the Ca^{2+} "pump") has been reported for HS cells.[80] Finally, a recent study[78] reveals that HS cells are deficient in the *cAMP-stimulated protein kinase* that phosphorylates membrane SDS-PAGE bands I, II (probably microfilaments) and band III.

Complement-Mediated Cytotoxicity - In higher animals a major defense against invasion by foreign agents and against authochthenous (self) parasitism, i.e. tumors, is provided by the *immune* system. Cells recognized as "non-self" are eliminated by destruction of their plasma membrane integrity. This can occur in processes mediated by circulating *antibody* and *complement*, or by lymphoid cells, apparently without participation of *free* antibody or complement.

The *complement reaction* involves the plasma membrane (a) as a reactive surface for the evolution of the complement cascade and (b) as the target of the final products of this cascade.[81] Complement lysis of an erythrocyte requires the attachment of only one molecule of immunoglobulin M (or two properly placed molecules of IgG) per cell. As a first step of complement activation antibody (A) becomes membrane (M) bound (AM), the C1 component of complement then attaches to AM, converting component C1s to an esterase C1. This complex may transfer to other membrane sites. C1 fosters membrane binding of components C4 as well as C2, and to C4a and C4b and cleavage of C4 to C4a and C4b, 5-10% of the latter remaining membrane associated. C2 is peptidolysed, and thereby activated. The larger fragment, C2a attaches to membrane-bound C4b, yielding C4b, 2a, which splits C3 into C3a and C3b, the latter binding firmly to the membrane, forming an AM C1, 4, 2, 3 complex, which regulates the subsequent steps of the cascade. C5 is cleaved into at least two components, C5a and C5b, of which 1% of C5b remains membrane bound and the rest is inactivated. Therefore C6 and C7 interact and attach to bound C5, followed by adsorption of C8. The cell membrane becomes fragile with or without component 9, which interacts with membrane bound C8. The final process preceeding cell lysis involves an increased permeability of the membrane to small molecules.

The terminal action of the complement system has been studied on model lipid membranes (liposomes) containing glycolipid antigens.[82-84] These membranes, just as biomembranes, become "leaky" to small molecules as a result of complement action, but no overall chemical changes of membrane lipid have been detected, suggesting that the end of the complement cascade involves formation of a membrane-active polypeptide (or polypeptide segment of a membrane bound complement component). This hypothesis is supported by recent studies on complement-mediated erythrocyte lysis, documenting that complement components 5, 6 and 9 form complexes that are in tight hydrophobic association with membrane proteins [85-86] and that the terminal action of complement involves a perturbation of the apolar membrane "core".[87]

Genetic Transport Defects - In man four hereditary, autosomal recessive conditions involving impaired amino acid transport have been characterized. Amino acid absorption in the intestine and the kidney are impaired concurrently. These are cystinuria[88], which can be a unique defect or accompany other abnormalities of amino acid transport[88], abnormal ab- and re-sorption of cystine, lysine, arginine and ornithine[88], Iminoprolinuria, affecting the transport of proline, hydroxyproline and glycine[89] and Hartnup's disease involving impaired transport of nearly all neutral amino acids.[90]

Renal glycosuria, an autosomal dominant with impaired glucose resorption in the proximal renal tubule, due to diminished T_{max} for glucose permeability of the luminal plasma membranes.[91] Renal aciduria is due to the inability of the tubular cells to maintain a steep pH gradient across their membranes because of abnormal permeability of the luminal membranes to H^+.[92] In hypophosphatemia the most important defect is an abnormally low T_{max} for phosphorous, leading to inadequate phosphate resorption from the glomerular filtrate and hence hypophosphatemia.[93]

Nephrogenic Diabetes Insipidus (NDI) - Due to an impaired response to antidiuretic hormone (ADH) which normally fosters water resorption is the distal nephron[94] ADH is known to act by binding to membrane receptors, leading to an activation of adenylate cyclase and an increase of cellular cAMP. In DI there is no change in the number of ADH receptors or their affinity for ADH, but ADH does not produce the normal increase of cAMP in collecting duct cells. DI can be induced by therapeutic agents. For example, Li^+ and dimethylchlortetracycline inhibit ADH induced cAMP production.[94,95]

Generalized Membrane Diseases - Cystic Fibrosis (CF) is a common hereditary condition that manifests itself in abnormal Na^+, Ca^{2+} and glycoprotein secretion by the epithelial cells of serous and mucous glands. Abnormal transport function has also been detected in non-secretory cells of patients and their parents[96-98], erythrocytes in particular.

It has been proposed that the disease derives from a generalized defect in Na^+ transport[99] but several authors argue that the primary defect lies in Ca^{2+}-pumping.[100-102] Decreased activities of Mg^{2+} activated membrane

ATPase has been reported for erythrocytes from patients [97,98] but other studies [103,104] demonstrate normal Mg^{2+} - and Ca^{2+}-activated ATPase and cAMP stimulated membrane protein kinase[105], but higher activity of the Ca^{2+} activated enzyme at low Ca^{2+} concentrations.[103] Importantly, the spent culture medium from CF fibroblasts, but not normal cells, depresses the Mg^{2+}-activated, Ca^{2+} activated and Mg^{2+}, Na^{+}, K^{+}-activated ATPase of normal erythrocyte membranes.[106]

Myotonic muscular dystrophy is an inherited (autosomal dominant) human disorder involving many tissues. The abnormality in muscle (repetitive depolarization of diseased muscle fibers, even after nerve or neuromuscular blockade) indicates a plasma membrane defect.[107,108]

Also, membranes isolated from dystrophic patients' erythrocytes lack the enhancement of protein kinase activity found in normal membranes after frozen storage.[109] Moreover, these membranes yielded unusual electron spin resonance spectra, when "doped" with nitroxide-labelled lipid analogues [110] leading to the proposal that myotonic muscular dystrophy may result from a generalized abnormality in plasma membrane structure.[110]

Intracellular Parasitism - Diseases due to parasites with an obligatory intracellular stage -- viruses, some mycobacteria, some protozoans -- constitute the major health problem on a world scale. In all of these conditions, the plasma membrane must be modified and broached to allow the entry and exist of the parasite. In viral and malarial infection, the membrane is also modified by insertion of new material. The complex and varied mechanisms by which viruses penetrate and modify host cell membranes are under intensive study and have just been reviewed.[111] I will restrict my comments to malaria, which despite its staggering incidence and impact (close to 1 billion cases and many millions of deaths per year) has received rather little attention.

During their intraerythrocytic replication, malarial parasites, Plasmodia, modify the host cell plasma membrane by (a) insertion of strain specific new antigen[112] (b) proteolytic degradation of the microfibrillar proteins of the host cells membrane[113] (c) increase of the phospholipid/protein ratio and the cholesterol/protein ratio of the membrane, decrease in membrane phosphatidylcholine and increase in phosphatidylethanolamine [113-116] and (d) alteration of membrane transport[117-119]. Particularly important is the fact that once erythrocytes are infected with strains of Plasmodium susceptible to the antimalarial drug chloroquine, they develop a $\sim$30-fold enhancement in their capacity to accumulate the drug, when this is presented at therapeutic (10^{-8}M) levels[120,121]. Since a similar, saturable high-affinity process follows protease-treatment of non-infected cells[111] and since malarial parasites release proteases[122] that attach the host-cell membrane[113] it has been inferred that the enhancement of chloroquine uptake represents activation of a "latent" membrane transport process.[121]

After intraerythrocytic maturation, parasites in the merozoite form are explosively released through the damaged cell membrane.[123] These collide randomly with uninfected cells and form a preinvasive contact by attachment to a specific surface receptor.[122,123] A localized, membrane perturbation may occur at the attachment site, and this may spread over much of the cell surface. Finally, the merozoite is interiorized by membrane invagination and the replicative cycle is reinitiated.

Bacterial Toxins - Several bacterial toxins exert their action at the plasma membrane of susceptible cells.

Cholera is caused by choleragen, the choleratoxin secreted by Cholera vibrio. Choleragen is a protein with a molecular weight of 84,000, probably composed of six subunits linked by 5 to 6 intrachain disulphide bridges, and binding specifically to the monosialoganglioside GM_1[125] with subsequent complex formation with and activation of the plasma membrane adenylate cyclase.[126] In small intestinal cells the resulting cAMP level produces a massive secretion of isotonic fluid which is characteristic of the disease.[127-128] Certain strains of E. coli also produce enterotoxins with an action similar to choleragen[127-128] and prostaglandin E_1 causes an intestinal fluid secretion, similar to that produced by choleragen, by its stimulation of intestinal adenylate cyclase.[129]

Botulism, caused by the exotoxin of Cl. botulinum, is due to impaired transmission of neuro-impulses between the synaptic membranes of the entire nervous system. The active neurotoxin has a mol. wt. 150,000. It can be split into a 70,000 mol. wt. unit, and thereby activated.[130] The toxin is not an acetylcholine esterase inhibitor, but induces development of numerous ACh receptors on muscle membranes; these disappear after recovery.[131]

Tetanus is caused by the exotoxin of Cl. tetani, a protein with molecular weight of about 67,000 with a strong binding affinity for nervous system membrane trisialogangliosides.[125] It appears that tetanus toxin acts specifically on inhibitory synapses of the cerebrospinal axis, possibly blocking the release of glycine, the neurotransmitter in these synapses, from the presynaptic membrane.[132,133]

Metal Toxicity - Heavy metals present a very serious environmental hazard and exert many of their deleterious effects at the plasma membrane. This topic has been most extensively explored using erythrocytes.[134] Pb^{2+} and Hg^{2+} react very rapidly with sensitive membrane sites of erythrocytes before reaching into the cell interior. Cu^{2+} and Zn^{2+} permeate slowly and show no exceptional affinity for membrane components. Tl^{+} enters via the K^{+} carrier and uranyl ion complexes with membrane phosphate and carboxyl groups.

Inorganic and organic mercurials can profoundly impair active and passive membrane transport by reacting with membrane protein SH-groups. In erythrocytes mercurials cause both K^{+} efflux and Na^{+} influx across

the membrane. In other cells amino acid transport may also be affected. Because of the high affinity of inorganic Hg for -SH these groups are probably the metal's primary site of action. Inorganic Hg, as well as organic mercurials, inhibit both the active transport of Na^+ and K^+ and the Na^+, K^+-activated ATPase, but inorganic Hg produces a rapid permeability biphasic with regard to Hg concentration. It has been suggested[134] that this is due to the interaction of the bifunctional Hg^{2+} with closely-spaced -SH's. When $[SH] > [Hg^{2+}]$, the complex -S-Hg-Cl would predominate, giving way to -S-Hg-S- at intermediate Hg levels and returning to -S-Hg-Cl at maximum doses. The deleterious action of mercurials on cation transport has been recently implicated in the toxicity of these agents of aquatic animals.[135]

Lead severely impairs the functional and physical properties of plasma membranes[134] and erythrocyte membranes are particularly sensitive to the action of this metal. Low Pb^{2+} levels (10^{-7} moles Pb^{2+}/g cells) increases K^+ efflux by 10^3. This K^+ loss is very rapid at the start but then shifts to a slow rate. Isolated membranes required only 10^6 Pb^{2+} atoms/cell for a maximal response. Pb^{2+} increases only K^+ efflux, but not Na^+ influx, leading to cell shrinkage.[134] Moreover, increasing lead concentration causes an increasing proportion of the cells to shrink, the other cells retaining their original volume. The shrunken cells are osmotically less fragile and contain less K^+ than normal. Apparently, a given cell either leaks K^+ rapidly or stays intact. The former account for the high efflux rate, while the remaining cells, retaining normal K^+ concentration, retain the normal slow K^+ efflux, yielding the slow phase observed. At high Pb^{2+} levels, all cells in the population lose K^+ at the rapid rate and under this condition there is a massive loss of intracellular ATP. A 10^3 fold increment in K^+ or Rb^+ flux occurs without essential change in Na^+ flux. Also, immediately after Pb^{2+} addition $^{42}K^+$ enters the cells against its own concentration gradient. The ratio [$^{42}K^+$ inside/$^{42}K^+$ outside] increases rapidly toward 3.0 but then drops to 1.0. The energy for the transient $^{42}K^+$ accumulation derives from the simultaneous efflux of non-radioactive K^+ by counter transport. It has been proposed that the Pb^{2+} activates normally unused K^+ carriers.[134] Significant alterations in erythrocyte Na^+/K^+-stimulated ATPase have been found in individuals environmentally exposed to lead and this effect appears a more sensitive criterion of toxic lead exposure than serum levels.[136]

The toxicity of thallium derives from its competition for the physiological Na^+ energy. Moreover, Tl^+ uptake occurs in two phases, an initial, rapid one, which can be inhibited by cardiac glycosides (0.1 mM) or by addition of extracellular K^+, and a rather insensitive, slow phase.[137,138] The crystal radius of Tl^+ lies between those of K^+ and Rb^+ and, at low concentrations simulates K^+ in its action; at higher levels, Tl^+ becomes toxic. Tl^+ can replace K^+ in the Na^+, K^+ pump, but its affinity for the ATPase is about 10 times that of K^+ in both fragmented erythrocytes[139] and on brain preparations.[140]

Uranium toxicity is due primarily to UO_2^{2+}, which does not form at neutral pH, because of formation of complexes with OH .[141] For this reason, probably, the kidney is the most susceptible site in mammals. The normal acidification of urine in the renal tubules causes production of UO_2^{2+} which impairs glucose and amino acid resorption and eventually leads to destruction of renal tubules.

Lithium, widely used in salt substitutes and in the treatment of certain psychiatric disorders, can interfere with the activation of cAMP production by antidiuretic hormone at the plasma membrane of renal tubular cells, producing a reversible diabetes insipidus.[142]

Drug Interactions - The initial interaction of a pharmacologic agent with cells is at the plasma membrane. Some drugs permeate freely through the membrane and others are translocated into the cell by existing active transport processes (e.g. nitrogen mustards enter via the choline carrier system).

Certain drugs act specifically at the plasma membrane and modify its function. The cardiac glycosides are outstanding examples. These agents inhibit the Na^+, K^+ ATPase of susceptible cells.[143] Their receptors are accessible only from the external cell surface [143-145] but do not lie at the surface but within the membrane core. Other examples are inhibitors of membrane acetylcholine esterase.[146]

Pharmacologic agents can also act non-specifically with the plasma membrane; thus numerous lipophilic and amphiphilic drugs and anaesthetics, at low concentrations,protect erythrocytes against hypotonic lysis, but at high levels cause complete cell disruption.[147-150] In the case of anaesthetics, the bulk concentrations with induce osmotic stabilization correspond well to pharmacologic anaesthetic levels. The stabilization phase has been ascribed to a reversible "fluidization" of membrane lipids and there is evidence that such an effect can be induced in lipid model membranes by anaesthetics such as halothane.[151] The labilization phase of the stabilization-labilization sequence appears to be due to denaturation of membrane proteins.[152] However, recent data[153-155] indicate that osmotic stabilization at low concentrations may involve more than one mechanism. For example, several nitroxide-lipid analogues at concentrations which reduce osmotic fragility in hypotonic media, induce echinocytosis in iso-osmotic solutions[155]: i.e. these membrane-active agents perturb the erythrocyte membrane focally and, at non-lytic levels, do not cause uniform membrane expansion. Moreover, the nitroxide-lipid analogues, when present at concentrations causing osmotic stabilization, bind to membrane proteins. On the other hand, substances such as chloropromazine[153-154] do not produce echinocytosis, but induce a variety of morphologic alterations, including "cup-shapes."

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Chapter 23. Current Concepts in Periodontal Disease

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Introduction - Periodontal disease is one of the most prevalent diseases of man, affecting the vast majority of the adult population throughout the world.[1] It is a disease with serious consequences because it is the major cause of tooth loss in people over 35 years in age, usually involving most of the teeth in an individual's mouth. In reality, periodontal disease is not a single disorder but a series of conditions associated with the destruction of the tissues that anchor teeth in their bony sockets. The most prevalent form of periodontal disease is chronic periodontitis which is a slowly progressing inflammatory lesion. The purpose of this review is to highlight some of the mechanisms thought to be involved in the etiology and pathogenesis of chronic periodontitis.

The etiology of periodontal disease has been definitively linked to the deposition and accumulation of microbial colonies on the crowns of teeth in the region of the gingival sulcus, where the gingiva or gum meets the tooth (Fig. 1).[2,3,4] Bacterial aggregates adhere onto the tooth surface in a soft, sticky mass called "supragingival plaque" (Fig.1). As will be outlined below, plaque in some way triggers and sustains an inflammatory response in the underlying gingival tissues. During its earliest stages, periodontal disease is confined to the marginal gingiva (marginal gingivitis, Fig. 1). Diseased gingival tissues exhibit all the clinical and microscopic features characteristic of a chronic inflammatory process. In addition, an exudate, rich in plasma and tissue-derived proteins as well as white cells, drains from the affected gingiva into the crevice. This is the so-called crevicular or sulcular exudate, which can be detected throughout the course of periodontal disease.[5]

Gingivitis commonly begins during childhood or adolescence and can be readily cured by removing plaque deposits from the teeth. Furthermore, the reappearance of the condition can be prevented through judicious oral hygiene procedures which keep plaque off the teeth. Unfortunately, most patients are not aware of the condition in their mouths because gingivitis is usually associated with little or no subjective symptoms. If left untreated, gingivitis almost always progresses into periodontitis.[1] When this happens, the inflammatory process spreads into the deeper tissues (i.e., the periodontal membrane). As this occurs, one sees progressive osteoclastic resorption of the alveolar bone (Fig. 1). Thus, the tissues which actually hold the teeth in their sockets are gradually lost during the course of the disease. Coincident with these alterations, the gingival sulcus gets deeper and deeper resulting in the formation of a "periodontal pocket", which is teeming with bacteria (subgingival plaque) and inflammatory cells and fluids which have exuded from the diseased tissues (Fig. 1). With the progressive destruction of its supporting apparatus, the tooth eventually loosens and can no longer withstand masticatory

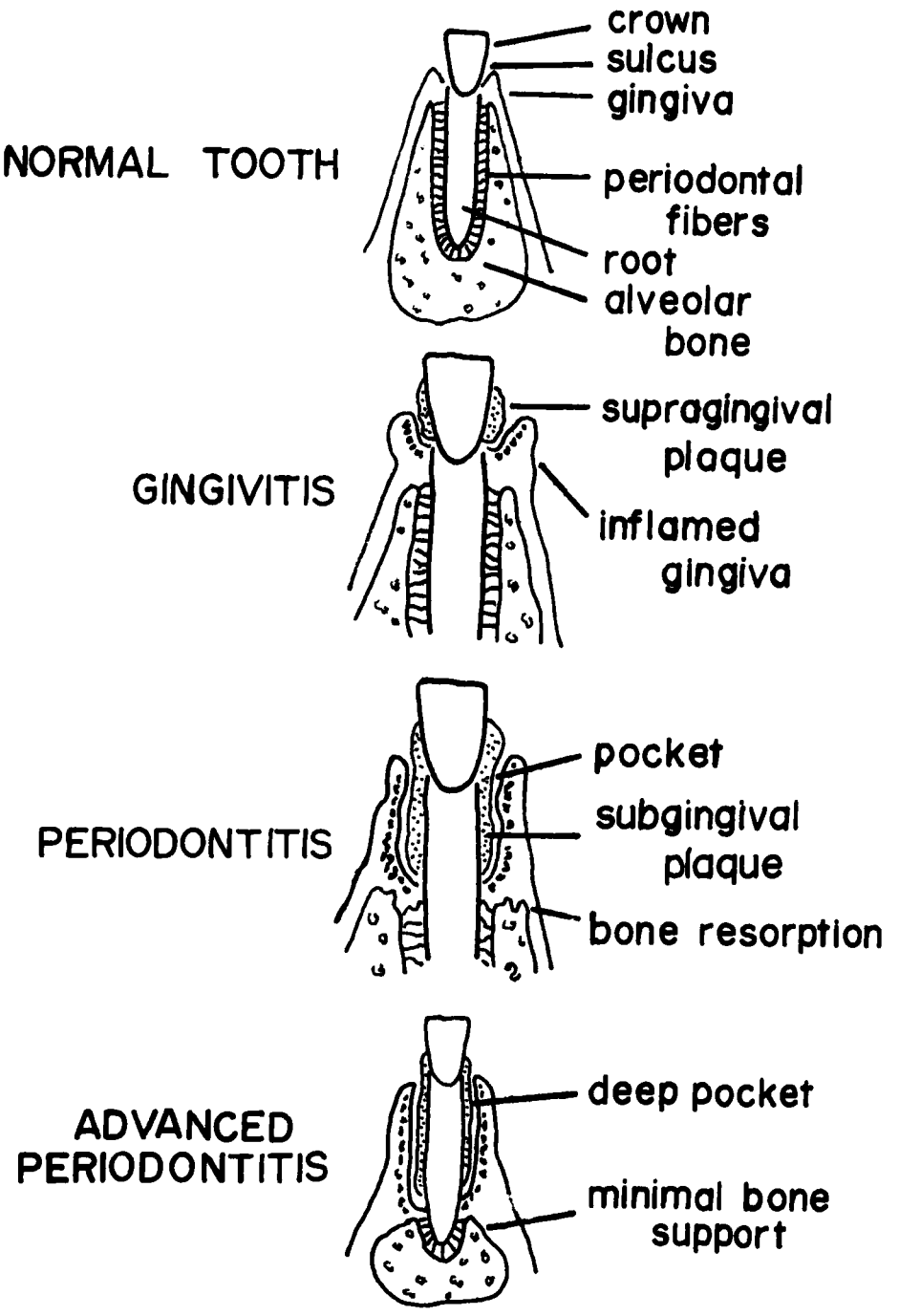
NORMAL TOOTH
crown
sulcus
gingiva
periodontal fibers
root
alveolar bone
GINGIVITIS
supragingival plaque
inflamed gingiva
PERIODONTITIS
pocket
subgingival plaque
bone resorption
ADVANCED PERIODONTITIS
deep pocket
minimal bone support

Figure 1.

stress. In other words, the normal bite or occlusal forces become pathologic or traumatic vectors, causing the teeth to migrate out of their normal position in the dental arch. Moreover, it has been suggested that such traumatic forces may hasten the destruction of the periodontal tissues by potentiating the injurious effects of the inflammatory process.[1] In the advanced stages of chronic periodontitis, the teeth can no longer be used effectively to chew food, and the patient may be restricted to a soft and often nutritionally-inadequate diet.

As with gingivitis, most patients with early or moderate periodontitis are ignorant of the disease in their mouths. Although they may be bothered by foul breath and some bleeding of the gums, these symptoms are often ignored. It is only in the relatively late stages of the condition that the patient usually experiences pain and inability to masticate properly. By that time, treatment is difficult and the prognosis is poor or hopeless. It should be mentioned that individuals with "infected gums" may be in a compromised position with respect to their general medical status. Patients with vascular abnormalities (e.g., rheumatic fever) may be in jeopardy due to systemic blood infections (bacteremia) emanating from organisms reaching the circulation from periodontal pockets.[6] It has also been said that chronic periodontal infection represents a "weak link" in the well-being and therapeutic control of diabetics.[1]

Bacterial Plaque as the Etiologic Agent in Periodontal Disease - As stated earlier, there is a direct correlation between the onset of gingivitis and the collection of microbial plaque on the cervical surfaces of teeth. This was convincingly demonstrated by Loe and his colleagues in a series of clinical studies involving groups of dental students.[7,8] These workers found that there was minimal evidence of gingival inflammation in students who kept their teeth free of plaque. But when students refrained from all oral hygiene procedures for periods ranging up to two weeks, plaque began to accumulate on their teeth and their gingiva became noticeably inflamed. With the reinstitution of home care and the removal of plaque deposits, the gingiva reverted to their normal, healthy clinical appearance. These clinical studies on the early development of gingivitis strongly implicate plaque in the initiation of gingivitis. There also appears to be a relationship between the presence of plaque and the later stages of periodontal disease (i.e., bone loss) because there is usually a close correlation between the extent of tissue damage and the amount of plaque that can be collected from the affected teeth.[9,10]

Additional evidence pertaining to the relationship of plaque to periodontal disease comes from the finding that antibiotics and other antimicrobial agents have been used with some success in reducing the amount of plaque and the degree of gingival inflammation in humans and experimental animals.[4,11] Further, germ-free animals do not generally exhibit any significant degree of alveolar bone loss.[3] But periodontal syndromes can be produced in hamsters and gnotobiotic rats by oral inoculation of certain bacteria isolated from human dental plaque.[12,13,14] It is not known, however, whether these same bacteria actually play a causal role in the development of human periodontal lesions. Finally,

periodontal disease can be transmitted by oral implantation of plaque organisms from diseased laboratory animals to healthy animals.[15,16,17] It should be emphasized, however, that the infectious process in humans is apparently noncontagious.

Having established that plaque seems to be necessary for the production of periodontal disease, much work is currently being done to better define the chemical and microbial constituents of plaque. Plaque is made up almost exclusively of an extremely heterogenous population of facultative and anaerobic gram-positive and gram-negative bacteria and their products. Both viable and dead organisms are embedded or held together by extracellular, sticky substances (see below). Gram-positive facultative cocci represent the dominant form (approximately 30%) of cultivatible organisms found in supragingival plaque deposits.[2,3,4] These bacteria colonize plaque during the initial hours following its formation on the tooth surface. The proportions of other types of organisms vary considerably. By 24 hours gram-positive rods make their appearance to be followed by gram-negative cocci and rods a few days later. Fusobacteria and filaments can be recognized by 5 to 7 days; vibrios and spirochetes come onto the scene at 7 to 14 days but form only a relatively small percentage of the total count. There are significant differences between bacterial populations in supra- and subgingival plaques; in particular, the latter contains a larger proportion of gram-negative anaerobic organisms than that seen in supragingival plaque.[18]

In spite of the valuable information obtained from these observations we have only scratched the surface as far as characterizing the total microbial population of plaque.[2,3,4] This is especially true in the case of subgingival deposits, which are probably more important than supragingival plaque in explaining the pathogenesis of long-standing disease. There are several reasons for this predicament; many organisms have defied all attempts at *in vitro* cultivation whilst others, particularly the anaerobes found in deep pockets, require sophisticated sampling and cultivation procedures. Moreover, a sample of plaque obtained at one moment of time during the course of a disease that develops over a period of years can only yield a limited amount of information. It is likely that the microbial composition and spatial arrangement of bacteria in plaque continually shifts as the disease progresses. Thus, organisms of etiological importance in the earliest stages may be of little significance during the later phases of the disease. It is extremely important to point out that the mere presence of an organism in plaque does not necessarily mean that it plays any role in the disease process. To complicate matters even further, there is every reason to suspect that plaque varies not only with its age and location but between subjects and even between different areas in the same subject.[4] To summarize thus far, periodontal disease represents a bacterial infection, but we have yet to identify the organisms in plaque directly responsible for the etiology and continued development of the disease.

In the formation of supragingival plaque, it is necessary for the organisms to adhere onto the tooth surface; otherwise, bacteria would pro-

bably be washed away by the flushing action of saliva.[19] There is evidence to indicate that the earliest microbial colonizers adhere onto the so-called "acquired enamel pellicle" which is derived from salivary glycoproteins absorbed onto the enamel.[20] The continued growth of the bacterial mass is then dependent upon the adhesion of microorganisms to one another. There are several ways in which this could occur. First, glycoproteins and other polymers originating from host secretions (saliva and crevicular fluid) may promote microbial aggregation.[21] Second, surface components of one bacterial species may enable them to attach to the surface of dissimilar organisms.[21]Third, the metabolic changes in plaque (e.g., lowered pH) also favor bacterial clumping.[22] Fourth, some organisms may synthesize extracellular polysaccharides, such as glucans (dextrans), which could stick bacteria to each other.[19] Currently, there is a great deal of interest in trying to better understand the mechanisms of interbacterial binding and adherance onto tooth surfaces because this may eventually result in new ways to control the build-up of plaques. Extracellular polysaccharide formation by S.mutans, for example, has been linked to the availability of sucrose in the diet.[19] It is conceivable that restrictions of various substrates in the diet may eventually prove to be therapeutically beneficial. Secretory (IgA) antibodies apparently inhibit bacterial colonization of certain surfaces[24]; thus manipulation of the immune response may eventually yield another means for controlling plaque formation. In the future chemotherapeutic agents capable of specifically preventing plaque accumulation may become available. Such agents could be incorporated into tooth pastes or mouth washes; this is an area that is under intensive investigation at the present time.[11]

The mechanisms of retention of subgingival plaque are not likely to be exactly the same as those described for supragingival plaque. For one thing, salivary components do not apparently enter periodontal pockets. But serum components derived from the crevicular fluid or exudate may promote bacterial clumping in such instances. Of course, the progressive deepening of the periodontal pocket would serve as a perfect environment for microorganisms, since they would be entirely protected against the flushing action of the saliva or mechanical removal by the action of the tongue or tooth brushing. Thus bacteria in pockets may not even have to stick as tenaciously to one another or to the root surface as that seen in supragingival deposits.

Bacterial Mechanisms of Tissue Destruction in Periodontal Disease - The precise manner in which the indigenous flora of plaque causes or promotes the breakdown of periodontal tissues is not known. Many theories are currently available. These can be divided into two major categories; bacterial-mediated and host-mediated tissue damage. The concept of bacterial-mediated damage is the traditional way of visualizing how plaque affects host structures. Under these circumstances, plaque would act as a continuous source of virulent pathogens which either invade or elaborate noxious products that diffuse into and destroy cells and tissues.[24] But at the present time it would seem that these exogenous mechanisms cannot fully account for the disease process. We can probably rule out overt microbial invasion of gingival tissues because electron microscopists

have repeatedly failed to identify bacteria in human and animal lesions.[25,26]

Nevertheless, it is highly likely that soluble bacterial products or constituents penetrate into the underlying tissues.[27,28] These would include an impressive array of enzymatic and non-enzymatic substances.[3] Such agents could, theoretically, adversely affect cells and tissue components, but no one has actually demonstrated that this occurs. We are not minimizing or discounting this possibility but merely pointing out that this has yet to be proven.

Host-Mediated Mechanisms of Tissue Destruction in Periodontal Disease - Within recent years there has been much interest in ascertaining whether the host's response contributes to the destruction of gingival and periodontal structures. In this context, plaque would not act as an overt irritant but would, instead, stimulate an inflammatory reaction which would serve as the principal effector of tissue damage. There are several immunologic and non-immunologic pathways whereby various cellular and humoral elements of inflammation could act in this deleterious manner. The discussion to follow will illustrate some of these potential pathways.

Immune Mechanisms of Injury - An increasing body of circumstantial evidence is becoming available to implicate immunopathologic reactions in the development of periodontal disease. Plaque probably acts as a reservoir of antigenic substances which continuously move into underlying host tissues.[27,28] The dense infiltrate of plasma cells, lymphocytes and macrophages in diseased gingiva is likely a manifestation of a local immune response to antigenic stimulation.[29] Indeed, one can demonstrate immunoglobulins (primarily IgG) in gingival plasma cells (B-cells) and in the crevicular fluids which exude from these tissues.[5,28-31] It seems reasonable to suspect that to some degree this reflects a regional synthesis and secretion of antibody in response to plaque constituents.[32] The adsorption of plaque antigens also induces an immune response in peripheral lymphoid organs as well. Thus, there is data to show that patients with gingivitis and periodontitis have elevated serum antibody titres to several plaque antigens.[33,34] Further, sensitized leukocytes which undergo blastogenic transformation upon exposure to insoluble and soluble extracts of plaque or isolated organisms can be identified in the circulation of patients with gingivitis and periodontitis but not generally in individuals with healthy tissues.[35-37]

Immune tissue damage in periodontal disease could be an expression of both delayed (cell-mediated) and immediate (antibody-mediated) hypersensitivity. In terms of implicating a delayed hypersensitivity-type vector one would visualize that inflamed gingiva would contain populations of lymphoid cells that were sensitized to several plaque antigens.[38] Upon contact with the appropriate antigen, sensitized cells would not only undergo blastogenesis and replication but would also secrete lymphokines into the surrounding environment. These soluble products of lymphoid activation have many profound biological properties. Of special interest are the findings of Horton and his colleagues.[39] They showed that plaque-

stimulated lymphocytes discharge "lymphotoxin" which can kill isolated fibroblasts and "osteoclast-activating factor" which induces osteoclastic resorption of bone cultures. If the release of such lymphokines occurs in vivo, it might provide a partial explanation for the mechanisms of collagen depletion and bone loss in the development of periodontal disease. The relative roles and interactions between lymphocytes (T-cells), plasma cells (B-cells) and macrophages in the production of the various lymphokines in response to plaque-stimulation has yet to be worked out completely. Recent findings suggest that plaque-stimulated B-cells may play a hitherto unanticipated function in the secretion of lymphokines.[40,41]

The presence of antibodies and complement components in inflamed gingiva and crevicular exudates point to the possibility that antibody-mediated injury may somehow be involved in the course of periodontal disease.[29,31] There are three fundamental forms of immediate hypersensitivity reactions: immune cytotoxicity, anaphylaxis and immune complex. On a theoretical basis all merit our attention, but none has actually been proven to be operative in periodontal disease. Immune cytotoxic reactions are triggered by the interaction of antibodies (IgG or IgM) with antigenic determinants situated on the cell surface. Such antigens may be an inherent component of the cell membrane or may be exogenous materials that have become bound to the membrane.[29] Once antibody combines with antigen in these instances, the complement system can be sequentially activated, leading to the formation of cytolytic factors capable of inflicting irreversible, lethal injury to the cell membrane. It is conceivable that complement activation in diseased gingiva may be a result of the interaction of antibodies with plaque antigens which have been adsorbed onto host cells. Dr. Theodor Dishon, a visiting scientist in our laboratories, has been able to induce immune cytolysis in vitro using nucleated and non-nucleated host cells coated with antigens derived from plaque bacteria (to be published). Immune cytotoxic phenomena in periodontal disease may be also set in motion by the formation of "auto-antibodies" that cross-react with bacterial antigens (which ellicited their formation) and with host constituents.

In his studies on inflamed gingiva, Nisengard has focused attention to the presence of relatively high numbers of mononuclear cells which demonstrate IgE staining by immunofluorescent techniques.[42] On the basis of this finding he has suggested that homocytotropic (IgE) antibodies may be locally available to sensitize host cells for anaphylactic-type reactions. Within this framework, bacterial-derived antigens would activate "target cells" that had previously been sensitized by the fixation of IgE antibodies to the cell membrane. As a consequence of such an event and depending upon the type of cell involved, various pharmacologically active materials could be liberated into the tissues and mediate, for example, increases in vascular permeability.

Immune complexes are involved in the pathogenesis of many inflammatory disorders. Injury in such diseases is often related to the activation of the complement system, resulting in the generation of inflammatory-promoting split products from individual complement components. These in-

clude chemotactic factors which attract neutrophils and macrophages to sites of antigen-antibody deposition. Polymorphonuclear neutrophils migrate towards and then interact with immune aggregates. Consequently, the cells release degradative lysosomal hydrolases, as well as other phlogistic agents which have the potential to inflict damage on surrounding structures.[43] Experimental forms of periodontal disease have been induced by the formation of immune complexes in gingiva.[44] It seems possible, therefore, that complement activation and neutrophil-macrophage infiltration in naturally occurring disease may be in part dependent upon the local accumulation of antigen-antibody complexes in the tissues or crevicular fluids.[45] We have recently found that certain gram-positive plaque bacteria can stimulate in vitro release of lysosomes from isolated rabbit polymorphonuclear leukocytes ("neutrophils"), whilst other organisms are not capable of inducing this reaction.[46] When these "inactive" bacteria were combined with specific antibody, the resulting immune aggregates became potent inducers of lysosome release. These observations illustrate how bacterial-antibody complexes might potentially act as pathogenetic stimuli in periodontal disease.

Non-Immune Mechanisms of Injury - Plaque bacteria or their products may directly activate inflammatory systems that could have detrimental effects on periodontal structures. There are numerous ways in which this could happen, but only a few will be outlined in this paper.

Bacterial constituents can trigger various humoral effector mechanisms of inflammation. For example, non-immunologic, sequential activation of the complement system can be achieved with bacterial endotoxins (see below), polysaccharides and enzymes.[47] In addition, our group has found that gram-positive plaque bacteria can activate the complement cascade.[48,49] Amongst the many functions of the complement system is the generation of factors that are chemotactic for white blood cells. The formation of such complement-derived substances in gingival tissues and exudates may help to account for the high numbers of neutrophils and macrophages that can be identified in these areas.[50]

Neutrophils and macrophages may contribute to the production of gingival injury by non-immunologic pathways. Neutrophils were first suggested as pathogenetic determinants on the basis of experiments in which viable and non-viable (heat-killed) dental plaques were injected into the skin of normal and leukopenic rabbits.[51,52] Local tissue injury was greater in normal than in leukopenic animals and was correlated with morphological and biochemical evidence for the release of neutrophil lysosomes in the lesions of normal rabbits. There is circumstantial data to suggest that lysosome release from neutrophils is occurring during the course of periodontal disease.[5,25,53,54] In this regard, we have noted that supra- and subgingival plaque as well as certain gram-positive plaque isolates can induce in vitro lysosome secretion from rabbit peritoneal polymorphonuclear leukocytes.[46,55,56] Related findings have been reported by Page and his colleagues who demonstrated that irradiated whole plaque induced in vitro synthesis and extracellular secretion of lysosomal hydrolases from mouse macrophages.[57] It seems conceivable, therefore, that lysosome

release from both neutrophils and macrophages may play some part in the destruction of host tissues in periodontal disease.

The destruction of alveolar supporting bone is perhaps the most crucial pathologic feature of periodontal disease. Obviously, we need to have a complete understanding of the mechanisms of bone depletion during the disease, but this has yet to be achieved. We do know that inflamed gingiva contain an unidentified factor or factors that promote bone loss in tissue culture systems.[58] These factors could possibly originate from plaque or be elaborated by cells of the host. Whole plaque extracts, endotoxins of gram-negative bacteria (see below) as well as lipoteichoic acid of gram positive bacteria appear to accelerate bone resorption *in vitro*.[59] The potential sources of endogenously-derived bone resorbing factors include agents released from inflammatory cells, such as lymphoid cells (see above). Moreover, the release of prostaglandins by inflammatory as well as resident tissue cells may represent another source of materials which are capable of modulating bone resorption.[58] This seems to be a distinct possibility in view of the finding that there is a substantial elevation in the level of prostaglandin in diseased human gingiva.[60]

A special word should be said about bacterial endotoxins because periodontal pockets contain a sizeable pool of gram-negative organisms.[18] Endotoxins derived from these bacteria are apparently able to diffuse into neighboring gingiva.[27] Once this occurs, it is conceivable that several, potentially harmful host effector systems could be set in motion.[61] For example, endotoxins could activate the clotting, kinin and complement systems, leading to profound changes in inflamed tissues.[62,63] Endotoxins also seem to act as mitogens stimulating lymphoid blastogenesis and lymphokine production.[64] Moreover, endotoxins are also stimulants for the release of lysosomal products from neutrophils and macrophages.[65] Lastly, bacterial endotoxins promote bone resorption in tissue culture.[66] From the foregoing it is easy to understand why there is so much interest in ascertaining whether endotoxins play an etiologic role in periodontal disease.

Conclusions - We have certainly come a long way in our understanding of the nature of periodontal disease, but the story is far from complete. Many crucial issues remain unsolved. We still do not know the exact ways in which plaque initiates and sustains the development of the disease. And we still cannot precisely identify the operative mechanisms which mediate gingival and periodontal tissue injury. Although clinical and experimental observations have given us many important clues, most of this information can only be tenuously applied to the extremely complicated, chronic disease as it occurs in man. It seems reasonable to assume that periodontal disease is not due to a single etiologic vector originating in dental plaque. And it also seems that a single pathogenetic pathway is not going to completely explain the mechanism of tissue injury. Instead, the total story of periodontal disease will likely involve scores of interwoven, constantly changing interactions between the host and the microflora in dental plaque.[67] We have attempted to outline some

of these potential interactions but have had to limit the scope of our discussion. For example, we have given no consideration to the potential role of host resistance or defense in the etiology and pathogenesis of periodontal disease. Surely this must be taken into account in explaining the disease process.[68,69]

Periodontal disease fits into a broad family of poorly understood, crippling inflammatory diseases of man. More and more clinicians and scientists from various disciplines have become intrigued by this condition and many have instituted studies to better define the nature of the disease. There is every reason to believe that this multidisciplinary approach will yield important information on the pathogenesis, prevention and treatment of chronic periodontitis.

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Chapter 24. Recent Advances in Gamete Biology and Their Possible Applications to Fertility Control

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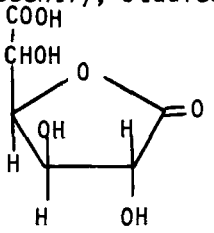
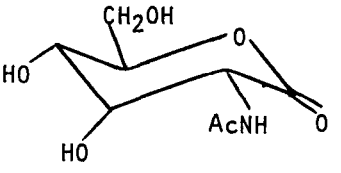
Contraception is now accomplished either by a physical barrier which prevents entry of spermatozoa into the uterus, by progestins which inhibit ovulation or by intrauterine devices which prevent implantation. All have limitations in efficacy or safety. To devise an improved contraceptive technology it will be necessary to discover other reproductive processes and ways of interrupting them in a controlled manner. Gamete biology is a promising area for exploration and in this chapter some recent advances will be reviewed. The research to be considered falls into five categories: (1) Sperm Capacitation; (2) Sperm Enzymes; (3) Sexing of Spermatozoa; (4) Surface Interactions between Gametes; (5) Immunization against Egg Components.

1) Sperm Capacitation - Capacitation, the process by which the inseminated spermatozoon acquires the ability to fertilize the ovum, has been proposed as an attractive target for fertility control ever since Bedford and Chang[1] showed that rabbit seminal plasma contains a non-dialyzable factor which interferes with this physiological process. Interest was increased by reports[2] that pronase released a 300-500 mol. wt. active component. However, interest has declined after studies by Davis[3] showed that this low molecular weight material is less active on a molar basis, by a factor of 10^{-5}, than the original 4 million mol.wt. material. Recently, studies with specific enzyme inhibitors (I & II)



I

Glucaro(1→4)lactone
(Specific Inhibitor of β-glucuronidase)

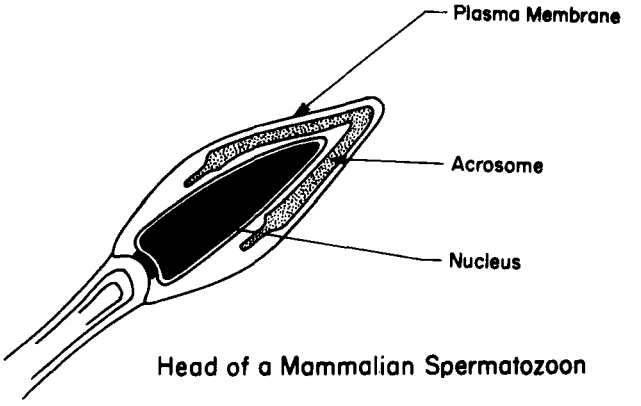


II

2-Acetamido-2-deoxy-D-gluconolactone
(Specific Inhibitor of β-N-Acetylglucosaminidase)

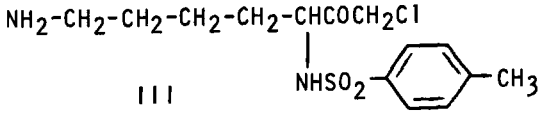
showed that the capacitation of hamster spermatozoa in vitro by the cumulus oophorus requires action by the glycosidases, β-glucuronidase and β-N-acetylglucosaminidase.[4,5,6] However, these enzymes are not unique to the cumulus cells, and so it seems doubtful that inhibitors will block capacitation in vivo without side effects. Furthermore, such an approach is unsatisfactory because the inhibitors would have to be administered in advance of coitus, and possibly continuously.

2) Sperm Enzymes - Acrosin, the acrosomal enzyme thought to be responsible for penetration of the zona pellucida, has recently been isolated from human spermatozoa[7,8] Since acrosin is trypsin-like in its substrate

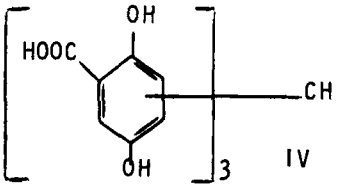


Head of a Mammalian Spermatozoon

specificity, attempts have been made to inhibit fertilization using the trypsin inhibitors, Nα-tosyl-L-lysyl chloromethane (TLCM, III) and soybean



III



IV

Tri-gentisic acid

trypsin inhibitor. At high concentrations these inhibitors prevented the fertilization of rabbit ova *in vitro*,[9,10] but with hamster ova only TLCM was effective.[11] The high concentrations which were used raise doubts about their specificity of action. Relatively large amounts of synthetic trypsin inhibitors were used to treat rabbit sperm prior to artificial insemination[12] or were placed in the rabbit vagina before natural mating.[13] Some reduction in fertilization was obtained, but this may have been due to effects on sperm viability.

High molecular weight protein inhibitors of acrosin, which also inhibit trypsin, have been isolated from boar[14] and human[15] seminal plasma. Fritz *et al.*[16] have suggested that the inhibition of acrosin by antibody or by an irreversible acrosin inhibitor, strongly adsorbed on the acrosomal membrane, could be a means of contraception. However, the difficulty of designing inhibitors with sufficient specificity and the need to administer them continuously, or at least in advance of coitus, present grave problems.

Hyaluronidase, an acrosomal enzyme which appears to be necessary for the sperm to traverse the cumulus oophorus, has been proposed as another target enzyme for contraception. Hyaluronidase inhibitors (e.g., trigentisic acid, IV), have blocked the fertilization of rabbit eggs in *vitro*, but were not effective *in vivo*.[17] Recent studies by Metz[18] indicate that it may be possible to immunize against sperm hyaluronidase, since he has shown that it is reproduction specific. Anti-rabbit sperm antisera inhibited rabbit sperm hyaluronidase but not the hyaluronidase from rabbit serum. Unfortunately, hyaluronidase also appears to be

species-specific: the antisera did not inhibit hyaluronidase from bull, mouse or guinea pig sperm. Thus, it would be necessary to prepare a vaccine from human sperm and this obviously would be impracticable.

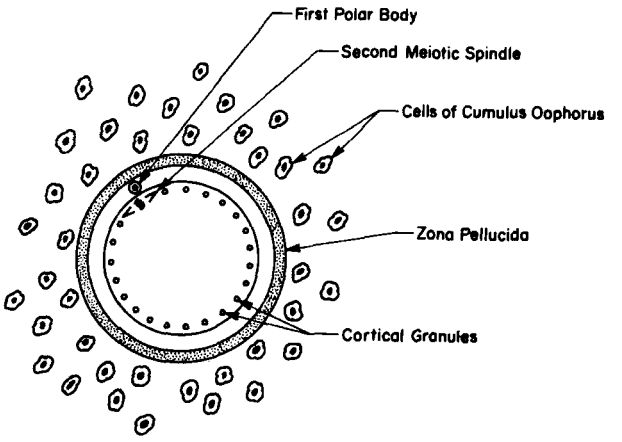
Although not directly involved in fertilization, the lactate dehydrogenase of spermatozoa, LDH-X, is distinct immunologically[19] and chemically[20] from either LDH-1 or LDH-5. Injection of highly purified mouse LDH-X into rabbits and mice reduced the number of embryos formed on subsequent mating by 58 percent[21] and 37 percent[22], respectively. Goldberg[19] has suggested that LDH-X may be of value as a contraceptive vaccine, but the fact that fertility is not completely suppressed is troublesome.

3) Sexing of Spermatozoa - Ability to control the sex of children would lower fertility rates by permitting parents to have the desired sex with the minimum number of pregnancies. Recently, advances have occurred in the sexing of spermatozoa by two distinctly different approaches. Bennett and Boyse[23] approached the problem by immunoselection. They prepared weakly cytotoxic anti-Y serum in inbred mice by grafting male skin onto females. Exposure of mouse epididymal sperm to this antiserum prior to insemination resulted in offspring with an 8 percent excess of females ($p < 0.003$). Since weak antibody may have left many Y sperm viable and non-specific antibody may have killed many X sperm, the authors feel that their approach could be improved. Even without such improvement, a small change in the sex of livestock may be economically important.

The second approach, which has so far been applied only to human spermatozoa, relies for assay on the fact that human Y sperm can be distinguished from X sperm by the presence of a bright fluorescent spot, the so-called F-body.[24] Washed ejaculated sperm were placed on columns containing varying concentrations of bovine serum albumin.[25] The front migrating into the albumin was found to contain up to 85 percent Y sperm, indicating that they are more motile than X spermatozoa. Considerable improvement in the technique will be required for practical application, since most of the sperm did not penetrate the albumin column and this fraction contained approximately the normal ratio of X and Y spermatozoa.

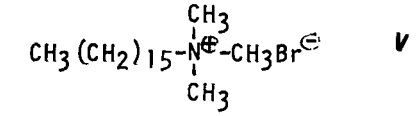
4) Surface Interactions between Gametes - The mechanism by which the capacitated spermatozoon forms a close association with the zona pellucida to initiate fertilization has been the subject of several recent investigations. Working with hamster gametes in vitro, Hartmann and Gwatkin[26] showed that the receptor on the zona pellucida, to which the capacitated sperm binds, is inactivated by crystalline trypsin. A protease from the cortical granules of the egg appears to be responsible for the zona reaction, a mechanism by which polyspermy is prevented.[27] The normal binding reaction leading to fertilization requires a factor emanating from the vitellus.[28] The chemical nature of this factor has yet to be determined, but if specific, it could be a target for contraception.

Studies by Schuel and his colleagues[29] have demonstrated the presence of sulfated acid mucopolysaccharides (SAMPS) in the cortical granules of sea urchin, amphibian, rat and human eggs. In sea urchin eggs



Ovulated Mammalian Egg
Surrounded by Cumulus Oophorus

SAMPS appear to play a role in preventing fertilization by more than one spermatozoon and quaternary ammonium salts, (e.g., CTMAB, V) which pre-



V

cipitate SAMPS, cause polyspermy. Oral administration of these compounds to female rats prior to mating reduced fertility and on this basis Schuel et al. have proposed that "quaternary ammonium drugs ought to be investigated as potential non-hormonal oral antifertility agents." Unfortunately such drugs would probably produce serious side effects, since they are known to inhibit synaptic transmission.

Plant agglutinins, which bind to specific saccharides,[30] were employed recently to explore for specific sugars on the surface of the zona pellucida.[31,32] Ricinus communis agglutinin (binds D-galactose), wheat germ agglutinin (binds N-acetyl-D-glucosamine) and Dolichos biflorus agglutinin (binds N-acetyl-α-D-galactosamine) attached to the zona surface of hamster eggs, where they scattered light under dark-field illumination. Such attachment was prevented in the presence of the specific binding sugar. Concanavalin A (binds α-D-mannose) did not induce light scattering indicating that it did not attach to the zona pellucida. Lectin attachment would be expected to cover up the receptor-for-sperm, thus preventing sperm binding and fertilization. This was the result obtained, but surprisingly Concanavalin A was also effective. A likely mechanism is that this lectin causes cortical granule discharge resulting in receptor removal. The authors state that agglutinins do not appear to cause release of cortical granules. However, a partial release, causing alteration of the zona pellucida,[33] would be difficult to detect. Experiments with agglutinins, unfortunately, do not yield information as to the nature of the receptor-for-sperm. They reveal only that sugars are present at the zona surface, where they could be a structural component or merely adsorbed glycoproteins. Nevertheless, the masking of receptors directly, or by

reaction with non-receptor sites on the zona surface, is a promising approach to fertility control.

5) Immunization against Egg Components - Shivers and his colleagues have prepared antisera in rabbits against saline extracts of homogenized hamster ovaries. After absorption with hamster intestine and lung, these antisera produced a single precipitin band against ovary extracts and formed a precipitate on the outer surface of the zona pellucida of hamster eggs.[34] Cross-reaction with the theca interna (inner wall of the ovarian follicle) indicated that the antiserum was not specific for the zona pellucida or was directed against ovarian material adsorbed to it. The reaction appeared to be species-specific, since it did not occur when the absorbed antiserum was applied to mouse or rat eggs.[35] Coating of the hamster zona with antiserum blocked the binding of sperm to it and prevented fertilization from occurring.[36] Evidence that the zona pellucida may be strongly antigenic is provided by the studies of Glass and Hanson,[37] who prepared antisera in rabbits against homogenates of mouse eggs in cumulus. These antisera reacted with the zonae pellucidae of the eggs and with their cumulus cells, but after absorption with mouse serum the zonae pellucidae alone reacted. Glass and Hanson suggest that immunization against the zona pellucida might be a good approach to contraception. However, to be practicable it would be necessary to obtain a non-species specific component.

As may be seen from this brief review, Gamete Biology is uncovering a series of novel reproductive processes of possible application to fertility control. Although most of these have serious drawbacks, it seems reasonable to expect that eventually some may be found which will lend themselves to contraceptive development.

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Chapter 25. The Polyether Antibiotics: Monocarboxylic acid ionophores

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Antibiotics that affect Membrane Function - Many antibiotics owe their biological activity to interference with the normal functioning of microbial membranes.[1] This disruption can take the form of a complete breakdown of membrane structure by detergent-like surfactant antibiotics, or more subtly by causing specific changes in membrane permeability towards specific ions. This latter mechanism is utilized by the ionophores. Ionophores are compounds which render cations lipid-soluble and so enable them to pass across membranes, either by the formation of channels or by acting as mobile carriers. Polyether antibiotics belong to the mobile carrier class of these cation conductors.

Examples of the surfactant type antibiotics are the tyrocidins (which include the cyclic peptide gramicidin S) and the polymixins, produced respectively by Bacillus brevis and Bacillus polymyxa. Although both classes are of the cyclic peptide type, tyrocidins are used topically for Gram-positive infections whereas the polymixins are used for the systemic treatment of infections caused by Gram-negative bacteria such as Pseudomonas.

The polyene macrolides form a third class of surfactant antibiotics. Typical examples are nystatin and amphotericin B which are effective fungicides against Candida albicans, Blastomyces dermatitidis, and other deep mycoses occurring in humans and animals. Polyene sensitive fungi, protozoa and higher algae all contain sterols in their membranes whereas bacteria and blue-green algae which are resistant to polyenes are free of sterols, suggesting that a polyene-sterol interaction is essential in the mechanism of action of this particular class of antibiotics.

In general, the result of treating sensitive organisms with a surfactant antibiotic is a non-specific release of the contents of the cell pool including metal cations, phosphate, amino acids and fatty acids. For instance, treatment of Pseudomonas aeruginosa with polymixin at concentrations as low as 25 micrograms per mg of dry cell weight causes loss of both small molecules and electron dense nucleic acids leading to the production of so-called "ghost" cells.

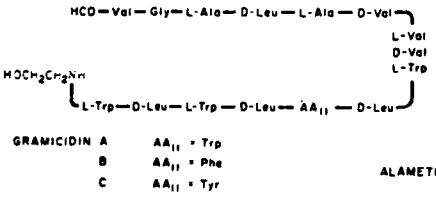
Ionophore Antibiotics - In contrast to the non-specific release of cell contents caused by the surfactant antibiotics, the ionophores cause specific changes in the

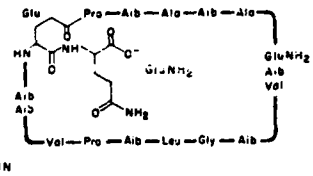
permeability of certain cations only. Ionophores were first described by Moore and Pressman[2] who demonstrated that certain peptide, depsipeptide and macrotetralide antibiotics (see Table 1) transport alkali metal cations across membranes. Gramicidins A, B, and C and alamethicin act by forming channels or pores through membranes whereas the other ionophores act by diffusing through membranes with the ion complexed in a central cavity of the ionophore molecule.

Pressman has shown[3] that coincident with the ionophore catalyzed uptake of cations by mitochondria, there is an ejection of protons and swelling of the mitochondrial particles due to uptake of water. The most effective ionophore in causing these changes is valinomycin (Table 1), a depsipeptide produced by Streptomyces fulvissimus. The linear gramicidins and nonactin, a macrotetralide antibiotic also cause mitochondrial swelling but are considerably less active in this respect than valinomycin.

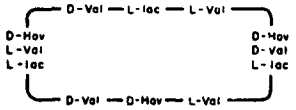
Table 1 - Ionophores other than the Polyether Antibiotics

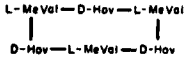
PEPTIDE





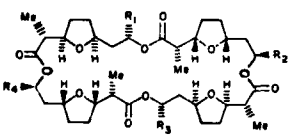
DEPSIPEPTIDE





MACROTETRALIDE

NONACTIN	$R_1 = R_2 = R_3 = R_4 = Me$
MONACTIN	$R_2 = Et, R_1 = R_3 = R_4 = Me$
DINACTIN	$R_2 = R_4 = Et, R_1 = R_3 = Me$
TRINACTIN	$R_2 = R_3 = R_4 = Et, R_1 = Me$
TETRANACTIN	$R_1 = R_2 = R_3 = R_4 = Et$



The structures of the linear pentadecapeptide gramicidins[4] (see Table 1) have recently been confirmed by synthesis[5] using the solid-phase Merrifield technique. Synthetic gramicidins B and C caused mitochondrial swelling[6] to an identical extent to that observed for the natural products. D.W. Urry[7] has demonstrated the helical conformation which these peptides are capable of forming and that a head-to-head dimer of two gramicidin molecules spans lipid bilayers and acts as a transmembrane channel affecting ion transport. Harold and Baarda[8] have shown that inhibition of Streptococcus faecalis by gramicidins or valinomycin was reversed by the addition of excess K+ ions.

Alamethicin (Table 1) is toxic to Gram-positive[9] bacteria and this is also true of the macrotetralide nonactins which have been demonstrated to uncouple oxidative phosphorylation, induce uptake of K+ and simultaneously eject protons from mitochondria.[10] Although the term "macrotetralide" has been used to include polyether antibiotics[1], in this review it is restricted solely to the "actins" illustrated in Table 1.

The molecular basis of action of these cation conductors resides in their ability to form coordination complexes with cations by multiple hydrogen bonds, or dipole interactions with carbonyl oxygen atoms projected towards the center of the complex. Such a complex has a hydrophobic exterior capable of solution in the hydrocarbon side-chains of the membrane lipids. The existence of such complexes has been demonstrated for the nonactin[11], valinomycin[12] and another depsipeptide, enniatin[13].

Polyether Antibiotics - Under normal conditions, the inner membrane of the mitochondrial particle is quite impermeable to cations, but the ionophores just discussed (depsipeptides, macrotetralides and peptides) induce active transport of cations into the mitochondria at the expense of energy generated by electron transport. A fourth class of ionophore, the polyether antibiotics also render mitochondrial membranes permeable to cations but by a different mechanism, passive diffusion down the concentration gradient.[14,15] For instance, mitochondria loaded with potassium ions from previous treatment with valinomycin immediately contract on exposure to a polyether antibiotic (e.g. nigericin) due to the loss of K+ and the accompanying release of water molecules involved in the hydration of the cations within the mitochondrial matrix.

The polyether antibiotics are produced by various streptomycetes. Although compounds of this type were first isolated[16-18] twenty years ago, it was not until 1967 that the first structure, that of monensin, [19] was solved. Later reports[20-22] stating

that monensin and three other polyether antibiotics (X-206,[16] nigericin,[17,18] and dianemycin[23]) were effective per os in poultry coccidiosis, created considerable interest in this class of antibiotic. By early 1975, twenty distinct compounds of the polyether type had been reported.

Table 2 - Structures of Polyether Antibiotics

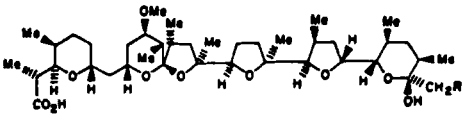
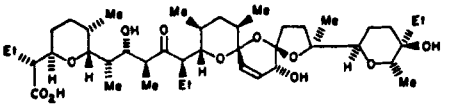
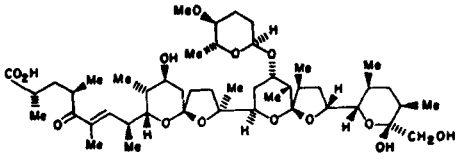
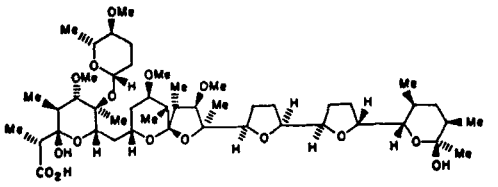
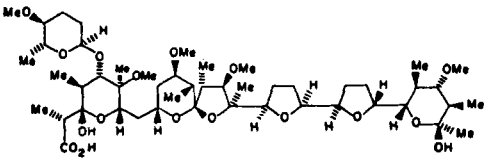
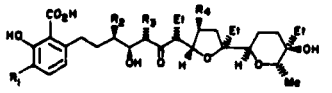
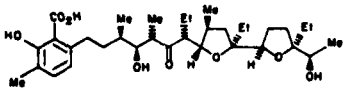
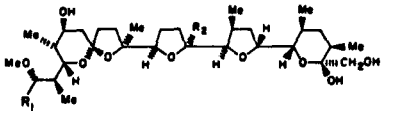
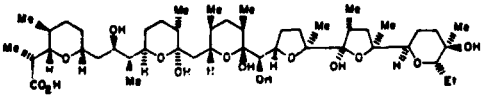
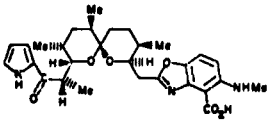
BACKBONE LENGTH			
C_{30}	Nigericin	R=OH	
	Grisorixin	R=H	
	Salinomycin		
	Dianemycin		
	Septamycin		
	Antibiotic A204A		

Table 2 (continued)

C_{24}	Lasalocid A	R_1=R_2=R_3=R_4=Me	
	Lasalocid B	R_1=Et, R_2=R_3=R_4=Me	
	C	R_2=Et, R_1=R_3=R_4=Me	
	D	R_3=Et, R_1=R_2=R_4=Me	
	E	R_4=Et, R_1=R_2=R_3=Me	
	iso-Lasalocid A		
C_{26}	Monensin	R_1=CH(Me)CO_2H, R_2=Et	
	Factor B	R_1=CH(Me)CO_2H, R_2=Me	
	Factor C	R_1=$(CH_2)_3CO_2$H, R_2=Me	
C_{36}	Antibiotic X-206		
—	Antibiotic A23187		

The antibiotics are characterized by good in vitro activity against many Gram-positive bacteria and mycobacteria, but are in general inactive against Gram-negative bacteria. They may also have certain specific antifungal or insecticidal activity, but parenteral toxicity has prevented their use in humans.

The structures of these compounds (Table 2) have been elucidated almost entirely by X-ray analysis of their heavy atom salts. Microanalysis reveals a high oxygen content, but the compounds contain few hydroxyl groups. The oxygen atoms are accounted for by a number of cyclic ether functions. In addition, the compounds are all monocarboxylic acids ranging in molecular weight from 500 to 1000. They are

characterized by a large number of C-alkyl groups.

In the crystalline state, all polyether antibiotics analyzed so far exist in a cyclic conformation with a hydrogen bond connecting the carboxyl at one end of the molecule to a hydroxyl at the opposite end. The oxygen functions are concentrated in the center of the molecule, and the hydrophobic alkyl groups are all on the surface of the complex. This accounts for the unusual solubility properties of the antibiotic salts. They are virtually insoluble in water, but soluble in solvents such as benzene, ether, and chloroform.

About one year after the structure of monensin was revealed, simultaneous papers on the X-ray analysis of nigericin[24] and polyetherin A[25] appeared. Soon afterwards nigericin, polyetherin A, and X-464, were conclusively shown[26] to be identical. Three other compounds (helixin C,[27] K-178,[28] and azalomycin M[29]) are not separable from nigericin by paper chromatography. Nigericin and antibiotic X-464,[16] now shown to be identical, were amongst the original compounds of this class isolated in 1950.

The structure of lasalocid A (formerly known as antibiotic X-537A) which was also isolated at that time, was solved[30,31] in 1970 and was the first antibiotic in the polyether class shown to contain an aromatic chromophore. The other unique structural feature of this antibiotic was the presence of three C-ethyl groups, which prompted an investigation of its biosynthesis. This in turn produced[32,33] the first illustration of the incorporation of a complete butyric acid unit to form a C-ethyl group.

The next structure to appear in the literature was grisorixin,[34] which was found to differ by only a single oxygen atom from nigericin.

The structure of antibiotic X-206[16] was solved[35] by X-ray analyses of the silver salt and the hydrated free acid form. The X-206 molecule is distinguished from the other polyether antibiotics by its three lactol rings. Dianemycin was shown[36] to contain an α-β-unsaturated carbonyl group and a 1,2-glycol.

Recent antibiotics in this class to be reported[37-39] were A-204A and A-204B, which were isolated as the complex A-204. They behaved identically to X-206[35] on TLC, but NMR revealed that A-204A has four or five methoxyls and A-204B has three, whereas X-206 contains no methoxyls. Although extremely toxic, A-204 was reported to be effective in the treatment of coccidial infections in poultry, as shown earlier[20] for monensin, nigericin[22] (polyetherin A[40]), X-206 and dianemycin[41] and more recently for lasalocid[42] and salinomycin[43].

The structure of dianemycin was solved by X-ray analyses of the potassium, sodium and thallium complexes of the antibiotic,[41] which revealed two sets of spiroketal linked rings and for the first time, a tetrahydropyranyl ring linked through an acetal-ether bond to the rest of the molecule. A second example of this glycoside-like moiety in a polyether antibiotic was demonstrated on X-ray analysis of the silver salt[44] of antibiotic A-204A. The structure of the A-204A salt was found to be very similar to grisorixin and in fact, all four polyether antibiotics, A-204A, nigericin, grisorixin, and dianemycin have in common a 30-carbon backbone. A fifth antibiotic with a 30 carbon backbone, salinomycin, was recently reported from Japan[43]. The structure of this antibiotic was solved by X-ray analysis of the p-iodophenacyl ester, which revealed a unique tricyclic spiroketal ring system containing an unsaturated cyclic ether. X-ray analysis of a p-bromophenacyl ester, derived from the novel polyether antibiotic septamycin[45] revealed a structure very similar to A-204A, making septamycin the sixth polyether antibiotic shown to have a 30-carbon backbone. The structural differences between septamycin[45] and the earlier reported[44] A-204A are the loss of one of the five methoxyls present in A-204A and a change in the configuration and point of attachment of the glycoside-like branched tetrahydropyranyl ring (Table 2).

During last year (1974), further studies on the biosynthesis of lasalocid A[46] and the structure of five natural analogs of the antibiotic[47,48] have been reported. Iso-lasalocid A was isolated from S. lasaliensis fermentations at a level of only one part in five thousand relative to lasalocid A, and was found to differ from the major lasalocid component in both the size and configuration of the terminal cyclic ether. These differences in structure led to speculation on the nature of the cyclization systems involved in the biosynthesis of lasalocid A and other polyether antibiotics. Four isomeric homologs of lasalocid A have also been isolated from cultures of S. lasaliensis[48]. The homologs each arise by replacement of one of the four propionate derived methyls in lasalocid A by an ethyl group, which results in each homolog molecule containing four C-ethyls.

A study on the biosynthesis of monensin[49] by S. cinnamonensis revealed the second example of incorporation of a butyrate unit to form a C-ethyl group.

Laidlomycin was the first polyether antibiotic reported to exhibit activity against Mycoplasmas, in particular Acholeplasma laidlawii.[50] In addition to the anti-mycoplasma activity, laidlomycin was stated to be a coccidiostat in spite of being quite

toxic [mouse LD_{50}(i.p.) 5 mg/kg].

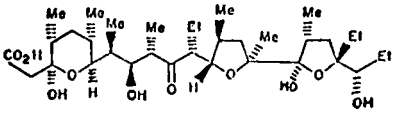
The nineteen polyether antibiotics discussed up to this point all contain only three elements: carbon, hydrogen and oxygen in the approximate range represented by the empirical formula $(C_{3-4}H_{5-7}O)_n$. In 1974, however, a new divalent-cation ionophore A-23187 containing two cyclic ethers in a spiro ring system and a carboxylic acid function was reported[51] to have a molecular formula $C_{29}H_{37}N_3O_6$. The three nitrogens in the molecule were shown by X-ray analysis to be parts of a substituted benzoxazole and an α-keto-pyrrole (see Table 2).

Coccidiostat activity - Effective levels in feed vs. Eimeria tenella, and LD_{50}(p.o.) in mice have been published for several polyether antibiotics. Values vary from 0.0017% for A-204[44,55] (LD_{50} 8 mg/kg) through 0.004% for dianemycin[36], 0.0075% for lasalocid[42,53] (LD_{50} 146 mg/kg), 0.008% for X-206[53] (LD_{50} 17 mg/kg) and 0.011% for monensin[20,54] (LD_{50} 44 mg/kg) up to 0.02% for nigericin[22,53] (LD_{50} 190 mg/kg). Coccidiostat activity is also claimed for laidlomycin[50] and lysocellin[64,65] but no data have been published. The success of these compounds as coccidiostats depends on their selective toxicity against the parasitic protozoa in the intestinal tract of the chicken coupled with relatively poor absorption of drug by the host.

Although interest in the polyether antibiotics was initiated by the discovery of the coccidiostat activity of monensin[20], considerable attention at present is directed towards the possible application of these ionophores as cardivascular agents. In this area of pharmacological research, the two antibiotics under most thorough study are lasalocid and A-23187[52].

In addition to the twenty polyether antibiotics discussed so far, more have appeared in the patent literature. Antibacterial activity is claimed for A-218[56], and K-5610[58] (lysocellin), but duamycin[59] also exhibits antifungal activity and A-130A[60] has anti-protozoal activity. Antibiotics A-28695A and B[61]; BL-580α and β[62]; salinomycin[63] and lysocellin[64,65] (see structure below), are all claimed as coccidiostats and in the case of BL-580, antimalarial activity is also noted.

Lysocellin



A possible clue to the mechanism involved in the cardiac sympathetic effects of the polyethers was the close correlation reported recently[66] between the cardiac effects[67,68] and the ability of a number of lasalocid derivatives[69], to facilitate permeation of norepinephrine across artificial membranes.

The versatility of the polyether antibiotics goes beyond their cardiotonic and anti-coccidial activity. For instance during the last year, reports have been published of the influence of these compounds on the extrusion of granules from peritoneal mast cells[70], release of acetylcholine from nerve terminals[71], ion movement across leukocyte plasma membranes[72], calcium release from neurohypophysis[73], platelet secretion of ATP[74], and effects on skeletal muscle of the frog[75].

Clearly the investigation of these interesting compounds is at a very early stage, but the results already noted indicate possible utility in a number of different areas of medicinal chemistry.

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Chapter 26. A Free Radical Pathology: Superoxide Radical and Superoxide Dismutases

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Introduction - Recent work has shown that the univalent reduction of molecular oxygen to the superoxide radical (O_2^-) is a commonplace event in both biological and non-biological oxidations. Since free radicals are very reactive, the generation of O_2^- in living systems must be viewed as a threat to their integrity. Cells which respire and which can thus produce O_2^-, must have evolved suitable defenses against this radical. The keystone of such defenses is provided by enzymes which scavenge O_2^-, by catalyzing the reaction: I. $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$. These enzymes, which have been named superoxide dismutases, are essential for the survival of respiring organisms. They have been extensively studied during the past six years and have been the subject of several reviews[1-6]. It would not now be worthwhile to write yet another review on superoxide dismutases.

There is, however, an aspect of superoxide biology which has very recently received a great deal of attention, which has obvious clinical significance and which has not been summarized. I refer to the superoxide pathologies. It is perhaps not surprising that a finely tuned balance between reactions which generate O_2^- and the enzymes which scavenge this radical could, on occasion, be upset, with pathological consequences. We will begin with the briefest possible introduction to superoxide and superoxide dismutases and then will examine a few free radical pathologies.

The Dangers of Superoxide - O_2^- can act both as an oxidant and a reductant. Thus it oxidizes thiols [7], polyphenols[8-10] and dehydrogenase-bound NADH[11] and it reduces cytochrome c[8], tetranitromethane[12] and nitroblue tetrazolium[13]. Perhaps the cytotoxicity of O_2^- depends upon such direct oxidations or reductions, but there is another and a more interesting possibility. Thus, O_2^- might give rise to the awesomely reactive hydroxyl radical (OH·) by reacting with H_2O_2, as follows: II. $O_2^- + H_2O_2 \rightarrow OH^- + OH\cdot + O_2$. This has been called the Haber-Weiss reaction. There have been several indications of the reality of this reaction. Thus the conversion of methional to ethylene could be caused by both O_2^- and H_2O_2, acting together, but not by O_2^- or H_2O_2 acting separately. Furthermore, compounds which scavenge OH·, but not O_2^- or H_2O_2, prevented this ethylene production[14]. Such results which are most easily explained in terms of the Haber-Weiss reactions, have been obtained with several different reaction systems[15-17].

All reactions which generate O_2^- will, by virtue of the dismutation reaction (I), also produce H_2O_2. The continuing flux of O_2^- could then react with the accumulating H_2O_2 to generate OH· (II). In this way it

is possible, during normal oxidative metabolism, to generate the hydroxyl radical. Superoxide dismutases would serve to prevent reaction II by keeping the steady-state concentration of O_2^- vanishingly low. Catalases and peroxidases would prevent reaction II by minimizing the steady-state concentration of H_2O_2. The concerted action of superoxide dismutases, catalases and peroxidases thus prevents the Haber-Weiss reaction and protects against this aspect of oxygen toxicity. Assuming that O_2^- is a dangerous species and that superoxide dismutases have evolved to eliminate these radicals, we must next inquire after the actual sources of O_2^-.

The Sources of O_2^- - The aerobic actions of a number of enzymes produce O_2^-. Among these are the xanthine oxidase from bovine milk, the aldehyde oxidase of rabbit liver, the dihydroorotic dehydrogenase from Zymobacterium orotlcum and several flavin dehydrogenases[4]. The electron transport systems of several organelles leak electrons to oxygen and thus produce O_2^-. This has been demonstrated with mitochondria[18,19], microsomes[20,21] and chloroplasts[22-25]. Indeed whole cells of the leukocyte series have been shown to liberate O_2^- into the suspending medium when they have been activated to engulf bacteria and other paticles[26-29]. In addition to these obviously biological sources of O_2^-, there is a host of autoxidations which produce O_2^-. Among these are the autoxidations of hemoglobins[30,31], reduced ferredoxins[32], cytochrome P_{450} [33], catecholamines[9,16], pyrogallol[10], hydroquinones[34] and leuko dyes [13,35,36]. Given these numerous and diverse sources of O_2^-, we may confidently anticipate some production of O_2^- in all respiring cells. The ubiquity of superoxide dismutases in such cells makes it difficult to estimate the rate of production of O_2^- in cells or in crude extracts of cells. We therefore remain ignorant of the quantitative aspects of the generation of O_2^- by aerobic cells.

The Distribution of Superoxide Dismutases - All respiring organisms which have been examined have been found to contain superoxide dismutases. This statement applies to bacteria, algae, protozoa, fungi, plants, insects, birds and mammals. Even moderately sensitive anaerobes contain these enzymes. Sensitive anaerobes, which really cannot tolerate exposure to oxygen, do not contain readily measurable levels of superoxide dismutase.

There are two classes of superoxide dismutases. One of these has a subunit weight of 20,000 and contains Fe(III) or Mn(III). The manganisuperoxide dismutase has been found in bacteria and in the matrix of mitochondria. The ferri-enzyme has been found in the periplasmic space of Escherichia coli and in blue-green algae. The other class of superoxide dismutases contain both Cu(II) and Zn(II), have a subunit weight of 16,000 and are found in the cytosols of eukaryotic cells. The evolutionary implications of the sequence homologies among the various superoxide dismutases have been discussed[38].

For the purposes of this discussion, the salient fact about the distribution of superoxide dismutase is that it is abundant within the

cells of the diverse tissues of mammals[38-41]; but is virtually absent from extracellular fluids[42]. This has the consequence that O_2^- generated inside cells is not likely to cause acute pathology; whereas O_2^- generated in the extracellular space is likely to damage cell membranes and connective tissue, because of the lack of superoxide dismutase in this compartment[42].

Examples of Free Radical Pathologies

A. Oxygen Toxicity - Exposure of rats to 1 atm. O_2 causes progressive lung damage which culminates in death. If the rats are first exposed to 0.85 atm. O_2, for a few days, they become adapted to oxygen, such that they can then tolerate 1.0 atm. O_2. This adaptation towards oxygen has been found to correlate with an increase in the level of superoxide dismutase in lung[43]. Bacteria[44,45] and yeast[46] have similarly been found to respond to oxygen by increasing their content of superoxide dismutase and these inductions have been shown to impart enhanced resistance towards the lethality of hyperbaric oxygen. There is a strain of the green alga chlorella (HPO) which is notably resistant towards oxygen and which has been found to contain an unusually high level of superoxide dismutase[47]. Conversely, mutants of E. coli B, which had a temperature-sensitive defect in superoxide dismutase, had a parallel defect in tolerance for oxygen[2].

It follows that the acute oxygen toxicity seen upon exposure to elevated concentrations of oxygen must be, at least partially, due to the actions of O_2^-. Otherwise we could not account for the protective action of superoxide dismutase. Oxygen toxicity is therefore an acute free radical pathology. It is tempting to speculate that we suffer a chronic free radical pathology as an inescapable condition of our aerobic lives and that this is one of the causes of senescence.

B. Streptonigrin and Paraquat Toxicity - The normal flux of O_2^- in respiring cells must represent a small fraction of the total oxygen consumption. Thus most of the respiration in aerobic cells is due to the action of cytochrome oxidase, which is known to reduce oxygen to water without releasing intermediates such as O_2^- or H_2O_2[48]. We can imagine compounds which would increase the net flux of O_2^- by shunting electrons from the normal electron transport pathways to oxygen in univalent steps. Streptonigrin appears to be such a compound and paraquat (methyl viologen) is another.

Streptonigrin is more toxic to E. coli in the presence of oxygen and it does increase the rate of respiration and change the pathway of electron flow to oxygen[49]. A source of electrons, such as glucose, is essential for full expression of the aerobic toxicity of streptonigrin and inhibition of cytochrome oxidase, with cyanide, enhances this toxicity. In addition shunting electrons to oxygen, with phenazinium methyl sulfate, protected against the toxicity of streptonigrin[50]. The reduction of streptonigrin,

which is a paraquinone in its oxidized form, to the semiquinone radical, by E. coli, was demonstrated by epr spectrometry[51]. In vitro, streptonigrin caused single strand breaks in DNA, when incubated with a reductant plus oxygen[52] and superoxide dismutase protected the DNA against this attack[53]. It is clear that streptonigrin readily cycles between the quinone and semiquinone forms, in the presence of a reductant and of oxygen and that, in so doing, it produces O_2^-. Is this the basis of its oxygen-dependent toxicity in vivo? An affirmative answer is provided by the reports that increased intracellular levels of superoxide dismutase protect E. coli B[44] and the ORS strain of chlorella[47] against streptonigrin.

Paraquat (1,1'dimethyl-4,4'bipyridylium chloride) has been used as a herbicide. It is toxic and this toxicity is enhanced by oxygen[54]. Compounds closely related to paraquat have been shown to generate O_2^- when treated with a reductant plus oxygen[55]. Anaerobic incubation of paraquat, with NADPH plus mouse lung microsomes, led to its reduction, while aerobic incubation led to a peroxidation of the microsomal lipids, which could be prevented by superoxide dismutase[56]. The notion that paraquat acts as an effective source of O_2^- fits nicely with the fact that paraquat toxicity is most evident in the lung, no matter what the route of administration. It has been reported that repeated intravenous doses of superoxide dismutase prolonged the survival of rats given a lethal dose of paraquat[57].

C. 6-Hydroxydopamine, Dialuric Acid, etc. - When given to rats, 6-hydroxydopamine accumulates in catecholamine-containing nerve terminals and destroys them[58]. Related polyhydroxy compounds have neurotoxic effects which correlate with ease of autoxidation[59]. 6,7-Dihydroxy tryptamine is also readily oxidizable and it exerts selective toxicity on serotonin nerve terminals, whereas dialuric acid affects the β cells of the pancreas. The cytotoxicity of these compounds seems to relate to their ability to cause the production of O_2^- in the target cells. The autoxidations of 6-hydroxydopamine, 6-aminodopamine, 6,7-dihydroxy tryptamine and dialuric acid do all produce O_2^- [16]. In addition, these autoxidations generate an oxidant capable of producing ethylene from methional. This oxidant was produced only when both O_2^- and H_2O_2 were present in the autoxidizing reaction mixtures and it is presumed to be OH·, generated by the Haber-Weiss reaction[60]. Ethanol or benzoate, which scavenge OH·, prevented this ethylene production. Indeed ethanol given at a level of 4 g/kg protected mice against the toxicity of alloxan[16].

D. Radiation - The passage of ionizing radiation into oxygenated aqueous media generates several radicals including O_2^-. When formate is present O_2^- is the major product and this fact has been extensively exploited in the use of pulse radiolysis to study the mechanism of superoxide dismutase. It is possible that some portion of the damage sustained by cells exposed to such radiation, under aerobic conditions, may be mediated by O_2^-. One test of this proposal employed a comparison of the radiation sensitivities of E. coli B, containing different levels of superoxide dismutase[61]. No significant differences were observed and O_2^-

was thus excluded as an agent of radiation lethality, inside of E. coli. Given the advantage of hindsight, this experiment was doomed from the outset. All of these E. coli, even the ones with the relatively low level of enzyme, contained high concentrations of superoxide dismutase. Hence damage by internally-generated O_2^- could not have been significant in any case. In contrast, the suspending medium was devoid of superoxide dismutase and most of the O_2^- must have been produced in the medium, whose volume exceeded the cell volume by a factor of 10,000. It follows that superoxide dismutase would be most likely to protect against radiation lethality when added to the suspending medium.

In fact, foetal calf myoblasts have been reported to be protected against ionizing radiation by superoxide dismutase placed in the medium. Controls showed that the enzyme had no effect in the absnece of a radiation challenge[62]. Acholeplasma laidlawii were similarly protected. Furthermore, it was shown that this protection occurred only under aerobic conditions[63]. The application of photochemical or enzymatic sources of O_2^- gave results similar to those obtained with ionizing radiation. Thus the periplasmic superoxide dismutase of E. coli B protected these cells against exogenous sources of O_2^- [45]. Photobacterium leiognathi and Bacteriophage R-17 were protected against a photochemical source of O_2^- by superoxide dismutase[64].

It appears that O_2^-, whether generated inside the cell by enzymatic reactions or the application of hyperbaric oxygen, streptonigrin, paraquat, 6-hydroxydopamine, etc., or generated outside the cell by ionizing radiation or by photochemical or enzymatic processes, is a deleterious agent. It seems likely that at least part of its cytotoxicity is actually due to OH· generated by interaction of O_2^- with H_2O_2. In all of these free radical pathologies, superoxide dismutase provided protection.

Superoxide Dismutase Pharamcology - Since exogenous O_2^- can damage enzymes, viruses, mycoplasmas, bacteria and mammalian cells, we must inquire whether there are extracellular sources of O_2^- in mammals. Of course, ionizing radiation is such a source but exposure to it is infrequent. Similarly, exposure to intense light in the presence of metabolic or dietary photosensitizers is an infrequent source. These are special cases and will not be further considered. In contrast, the production of substantial amounts of O_2^- by activated phagocytes[26-29] is an everyday source.

Consider the following chain of events. Local sepsis or trauma results in the release of substances which attract phagocytes[65]. These leukocytes migrate to the site, become activated and so proceed to secrete O_2^- and H_2O_2. It has been shown that O_2^- and H_2O_2, generated enzymatically, depolymerizes the hyaluronic acid, which is responsible for the viscosity of synovial fluid[42]. Superoxide dismutase or catalase or hydroxy radical scavengers, such as mannitol, prevented this depolymerization. The localized production of O_2^- and H_2O_2 thus damages adjacent cell membranes

and connective tissue. This damage leads to a further influx of phagocytes which in turn become activated and release more O_2^- and H_2O_2. This self-exacerbating process results in inflammation. In such a case we might expect that superoxide dismutase, injected into the affected area, would minimize the damage caused by the O_2^-, secreted by the phagocytes. It might thus exert an anti-inflammatory activity.

It is extraordinary, but true, that prolonged attempts to isolate an anti-inflammatory protein from bovine liver finally resulted in the isolation of superoxide dismutase, without knowledge of its enzymatic activity[66]. This protein was named orgotein and Palosein and it has been used in the treatment of a variety of inflammatory processes[67-69]. Orgotein or Palosein (really superoxide dismutase) is a poor antigen and appears to be safe for repeated parenteral application[70]. It is being tested in humans and has been reported to relieve osteoarthritis[71]. Clinical results such as these support the notion that O_2^- is involved in the inflammatory process. Superoxide dismutase appears to constitute a new way of interceding and of decreasing the severity of this process.

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Chapter 27. Silicon in Biology and Medicine

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In the world we live in, the basis of inanimate nature is silicon. Inorganic silicon compounds are involved in silicate building materials (cement, brick), glass and ceramics. Organosilicon compounds, silicones, are used in many branches of human practice. We are in constant contact with silicon compounds in drinking water and in inspired air (as silicon-containing dust). In addition, man ingests daily about 0.5 g of silicon from foods[1,2], especially those of vegetable origin. Despite this, silicon does not seem to accumulate in the organism (its content in the human body does not exceed several grams) and seems to produce no effect upon it. All these facts led scientists to believe silicon to be inert and useless[1,3].

This prevailing conviction was shaken by a discovery made in 1963 in the laboratory of the present author. We showed that within a new and at that time unstudied class of organosilicon compounds with general formula $RSi(OCH_2CH_2)_3N$, called by us "silatranes", there were substances with very high and specific biological activity[1]. These substances were 1-arylsilatranes (R = aromatic substituent) which exhibited a strong analeptic effect and were highly toxic for warm-blooded animals (LD_{50} = 0.1 - 10 mg/kg). The high toxicity of 1-arylsilatranes[1,4,5] facilitated the marketing of a new rodenticide, 1-(p-chlorophenyl)silatrane[5]. This compound is rapidly and completely inactivated in poisoned rodents, so their corpses are not harmful to other animals.

The striking biological action of 1-arylsilatranes was pointed out by us several times[1,6-9]. Not only 1-aryl- but also some 1-heteryl- and 1-aralkylsilatranes as well as certain carbofunctional derivatives of 1-alkyl-silatranes are highly toxic. 1-aryl-silatranes induce a high activation of the background electrical activity of the cortex and some subcortex structures of the brain. The toxicity of silatranes ranges greatly depending on the nature of R substituent (0.1-5,000 mg/kg). We have prepared silatranes of low toxicity posessing a wide spectrum of physiological effects (for example, narcotic or depressant). This wide range of biological activity of silatranes, mainly due to the nature of the R substituent at the silicon atom, as well as their high permeability through the cell membranes, resulting possibly from a high dipole moment and spherical shape of the silatrane system, make silatranes a rather promising object of pharmacological and biochemical studies.

These findings convinced us that a number of derivatives of this element could play a significant role in vital processes. Our preliminary results were reported in the monograph "Silicon and Life" in 1971[1]. The book deals with a thorough analysis of highly scattered data in the world literature concerning the silicon content of microorganisms, plants and

animals, the possible role of this element in their life activity, and the biological action of silicon compounds.

Science-fiction writers have suggested that somewhere in the universe there exists a silicon-based life instead of a carbon-based life. Recent scientific data, however, do not support such a concept. We may say with confidence that silicon-based life is impossible on planets with environments similar to those on the Earth. But perhaps somewhere in the farthest corners of the Cosmos--for example, on compartively cold planets with ammonia atmosphere or on planets burned by their sun to temperatures ruinous for all life-forms known. The possibility of the existence of living material based on certain silicon polymer compounds cannot be excluded. Indeed on earth natural silicon compounds played a significant role in the origin of life. First, due to their adsorption and catalytic properties, these compounds served as a matrix on which the simplest organic molecules taken from the world ocean evolved to complex protein bodies. The sun and cosmic rays or even impact waves[10] might have played an important role in this process. There is a hypothesis that the phosphorus comprising the living material originated from the penetrating radiation affecting the silica via the following nuclear reaction that occurred at the prebiological stage of our planet's existence[11]. The

$$^{30}Si \xrightarrow{\gamma n,} {}^{31}Si \xrightarrow{\beta^-} {}^{31}P,$$

optically active quartz forms might have given rise to the present asymmetry of natural organic compounds. Finally, highly porous silica and silicates enabled microorganisms inhabiting their pores to survive environmental effects. In the course of biogenesis on the surface of silica and silicates, the silicon could not but intrude into the protein structure under formation and thus comprise one of the principal elements of living nature and its protoorganisms. This is confirmed by the fact that even nowadays silicon is of great importance for many organisms at a low stage of evolutionary development[1,2].

Many representatives of the simplest life forms use silicon from the environment, for example, to form shell, test and skeleton. The silicon content of existing so called "prehistoric" plants (horse-tails, bryophytes, ferns) is very high. There even exist "silicate bacteria" which inhabit soil and destroy rocks. In the processes of life activity these bacteria release potash, phosphorus, silicon and some trace elements absorbed by plants; this action explains their application in agriculture as fine fertilizers[12]. Of special interest are bacteria which synthesize organosilicon derivatives, including those containing a Si-C bond from mineral compounds of silicon. The silicate bacteria are possibly the first living beings that appeared on land. It was they who, having destroyed the silicate cover of the earth, prepared the ground for life of higher organisms[7]. Some bacteria are able to substitute their phosphorus for silicon. Some plants also exhibit similar ability. This enabled us to

advance the hypothesis of "non-phosphorus life" where the hereditary information is recorded and realized via the silicon analogues of DNA and RNA. It is most likely that silicic acid is able to partially replace phosphoric acid from nucleic acids, which play the main role in both the protein biosynthesis and hereditary character transmission[6,7]. The possible existence and role in vital processes of such partially "silicified" nucleic acids, are being investigated. We have already shown that the NDA and RNA preparations contain appreciable amounts of silicon.

Silicon compounds also play an important role in the organisms at higher stage of evolutionary development. These include, for example, higher cultural plants, herbs and trees. The silicon contents of these species amount to 15-20% of the dry plant weight[2]. The plant species which contain relatively high proportions of silicon and effeciently assimilate it from soil were even called "siliceous" species. Silicon not only imparts mechanical strength (for instance, to cereal stalks) but also promotes the important protective functions of plants. Silicon compounds added to culture solutions increase the growth of plants, their viability, crop capacity and stability to lodging, drought, frost, radiation and fungal infections, improve assimilation of phosphorus and other nutritious elements from soil. At the same time, a silicon content of soil below normal make the plants sensitive to fungal and bacterial infections and decreases cropping power.

The role of silicon in vital activity of plants has been little studied. It is known, however, that this element is present in plant tissues partly as organosilicon compounds (silicic esters of carbohydrates and proteins),[1,2,13] and partly as solid mineral compounds - silica, silicic acids and silicates including insoluble particles, "phytoliths"[2,14,15]. There were also found in plants specific enzymes--"silicases"--which promote the conversion of mineral silicon to organosilicon compounds.

In higher animals and man, silicon is present only in slight amounts (ca. 10^{-3}% of body weight), although it is contained in almost all tissues and organs. Among other bio-elements found in mammals, silicon holds a modest fifteenth place, giving way to magnesium, fluorine, iron and zink. The highest silicon content is found in connective tissues, skin (especially in epidermis), lungs, glands (adrenal, thyroid and pancreas, lymph nodes), bone, dental enamel and hair.

In mammals silicon compounds are present in three principal forms:

1. Water-soluble inorganic compounds capable of passing through cell walls which can be readily eliminated from the organism (orthosilicic acid and ortho- and oligo-silicate ions).
2. Organosilicon compounds and complexes soluble in organic solvents and containing Si-O-C groups (ortho- and oligo-silicic esters of carbohydrates, proteins, steroids, choline, lipids, and phospholipids).
3. Insoluble silicon polymers (polysilicic acids, silica, silicates) whose surface is always covered with a chemisorbed layer of

organic substances.

Silicon is essential for normal functioning of epithelial and connective tissues, to which it imparts strength, elasticity and permeability by serving as an agent which holds the keratin and collagen macromolecules together. In blood-vessels silicon prevents the deposition of lipids, normalizes the permeability of walls and increases their elasticity. Silicon compounds play an important role in many physiological, immunological, pathological and gerontological processes. For example, silicon induces the biosynthesis of collagen and the formation of bony tissues[19]. Silicon compounds are also of great importance for the growth of hair and nails in man, feathers in birds, and hair, horns and hoofs in animals. Silicon compounds increase the stability of cell membranes, affect lysosomes[24,25] and accumulate in mitochondrions.

Most of the silicon passing in the alimentary tract is eliminated in the feces. However, a portion of this silicon is absorbed in the duodenum and then enters the blood. This absorption is controlled by the steroid and thyroid hormones. The concentration of silicon in the blood is maintained at a constant level by the kidneys. Very low amounts of silicon are excreted in the bile[16]. The synthesis of vitally important organosilicon derivatives occurs in the liver under the conditions of exogenous feeding[15]. The metabolism of silicon in the organism is regulated by the endocrine[26] and nervous[16] systems. The hormonal regulation of silicon metabolism is determined by sex and age; the reduction of hormonal activity with age seems to be associated with a general decrease in the silicon content of the organism. When the nervous system is excited the silicon content of the blood flowing from the brain increases, and when it is inhibited the silicon concentration in the blood drops. It seems likely then that silicon participates in the transmission and enhancement of nervous fiber excitation[16]. The silicon accumulation in the optic nerve tissue with age seems to be caused by an adaptation mechanism which counterbalances the alteration of the eye medium with age.

Silicon takes an active part in the metabolism of calcium and some other elements (P,Cl,F,Na,S,Al,Mo,Co) and lipids as well. The metabolism of silicon compounds releases silicic acid which links physiologically important cations (Mg,Cu,Fe etc.) by forming insoluble silicates. This process may be related to the effect of silicon on a number of enzymes systems in plants and animals.

The disruption of silicon metabolism is associated with some diseases such as atherosclerosis, cancer,[20] leprosy, tuberculosis, diabetes, hepatitis, encephalitis,[16] goitre, certain types of dermatites, skin erysipelas[16], and with some processes of senescence[1,21]. For example, in the case of malignant neoplasm, the silicon content of the tumor is much higher than that of unaffected tissues, the silicon being transported to the tumor from other organs which become depleting in this element[1,16,20-22]. The liver proteins of patients suffering from cancer display an increased ability to link silicon[23]. In this connection, the proposed application

of silicon compounds for chemotherapy of wounds[1,9,31-33] seems rather promising. In the case of atherosclerosis the silicon content in the connective tissue decreases sharply. This results in a drop in elasticity of arterial walls due to the disappearance of elastin responsible for their resilience and in the increase of their permeability to lipids. The silicon content of skin and arterial vessels as well as bony tissues and the ability of the organism to assimilate silicon decrease with age and are closely connected with the processes of senescence. At the same time, the extracellular and plasma silicon content of the organism increases with age[26]. The silicon concentration in the blood of pregnant women, nursing mothers and new-born children is extremely high.

A lack of silicon intake (man daily assimilates 20-30 mg SiO_2) causes "silicotic anaemia". This may be associated with a high calcium content (the antagonist of silicon) in drinking water or a lack of biologically utilizable silicon in food, as in refined foods. Chickens fed on silicon-free diet for two weeks display poor development of feather and skeleton and very thin leg bones. 0.003% of silicon added to the diet increases the growth of chicks by as much as 35% and normalizes the development of feather and skeleton[27,28]. The addition of "soluble silica" to drinking water at a concentration of 800 mg of SiO_2 per liter increases body weight gains of male lambs as well as feeding effectivity[29]. Analogous experiments show increased body weight gains of weanling male rats by about 6%. These all suggest that readily assimilated silicon preparations added to food and drinking water might be very useful not only to young stock, pregnant women, nursing mothers and rachitic children, but also for healthy people, especially if they are old. There are reasons to suggest that in the near future compounds containing silicon (mainly organosilicon compounds) will become effective medicinal preparations. In France, for instance, certain organosilicon medicinal preparation, DNR and RDN, proposed by Prof. N. Duffaut have begun to be used. They are likely to be rather effective in the treatment of cardiovascular diseases, cancer and virus infections.

An intensive search for biologically active organosilicon compounds carried out under the author's guidance has led to the discovery of silicon derivatives which exhibit antiblastic, antisclerotic, anticoagulative, analeptic, narcotic, psychotropic, antipyretic, ganglion-blocking, bactericidal, fungicidal, zoocidal, insecticidal, chemosterilizing and insect repelling effects[1,6-9]. Some substances which greatly intensify the growth of connective tissue and hair have also been found[58,59]. Of special interest are absolutely non-toxic organosilicon compounds ("mival" "migugen") having specific and effective antitumor effects on animals[31]. The mechanism of the antiblastic activity is quite different from that of all previously known anticancer drugs. The new silicon compounds stimulate the protective reactions of the organism and cause a strictly local formation of collagen, thus intensifying the development of connective stroma which inhibit the development of tumorous parenchyma. It is interesting that the effectiveness of antitumorigenic preparations is considerably raised when used together with migugen and its analogs. We have also

found organosilicon compounds effectively accelerating the genesis of other connective tissue, especially in pathogenic processes.

We are searching for organosilicon compounds having a favorable effect on the metabolism of calcium and lipids which will be used in prophylaxis and therapy of atherosclerosis. Studies in this field are also being carried out by other authors[39].

A new class of antimicrobial substances of the $XYZSiCH_2CH_2NRR'$ type distinguished for their wide range of activity and effective suppression of pathogenic microorganisms were also found[54]. Some of these compounds were rather more active than widely used antibiotics such as streptomycin and nystatin and affect the strains of pathogenic microorganisms resistant to well-known antibiotics. On the basis of compounds of this type containing three or two readily hydrolyzing substituents on the silicon atom, we[8] and later American scientists[35] obtained new preparations able to form stable antimicrobic coatings on the surface of materials.

Among compounds of the $RR'NCH_2(OR'')_3$ type and their derivatives we found substances repelling bloodsuckers. 1,3,5,7-Tetrasylaadamantane and its derivatives also possess an insect-repellent effect (on mosquitoes)[40].

Triorganylsilanols and triorganylisocyanatosilanes[57] as well as some silatrane derivatives were found to display an inhibiting (narcotic) effect. Organosilicon compounds affecting coagulative and anticoagulative blood systems were discovered. They are believed to affect the surface of biological membranes. An anticoagulative effect of inorganic compounds of silicon has been reported[41,42]. Several reviews in this field were published in 1971,[4,36] and hundreds of papers have appeared after the publication of our book[1]. Among the latest studies on this problem, a discovery made in the USA is worth special notice; it deals with the depression of male reproductive function of mammals under the effect of phenylmethyl substituted silanes and siloxanes (in particular, heptamethylphenyl- and hexamethyl-2,6-cis-diphenylcyclotetrasiloxane)[37,38]. These results are consistent with the data which show that sodium silicate administered per os to rats reduces the birth rate by 70-80%[30].

Much attention has been given recently to the synthesis of steroid organosilicon derivatives[43,44]. New organosilicon derivatives of cholesterol have been synthesized[45]. Much work is being done in search for organosilicon antiradiation agents,[46] coronary vasodilators of the $R_3Si(CH_2)_nONO_2$ type,[47] antiinflammation drugs and analgesics (trialkylsilylmethyl isothiuronium and guanidinium salts),[48] insecticides (substituted trisilylamines of the $(R_3Si)_nN(SiR_2CH_2X)_{3-n}$ type),[49] preparations against stomach ulcer,[59] and insect-repellents[60].

It has been recently shown that silico-tungstic acid salts inhibit oncogenous viruses[50]. However, despite the available data on the antitumor effect of a number of silicon compounds it was firmly established

that liquid polyorganylsiloxanes had a carcinogenic effect[51,52]. This discovery has dampered the use of injections of liquid silicones to increase female breast size.

The studies of the metabolism of silicon compounds carried out recently in the USA[53-56] indicate that compounds of this type undergo in the organism a biological oxidation and oxidizing dealkylation[53]. On systemic administration of various organosilicon compounds almost all the dose is eliminated in the first 48 hours,[56] suggesting that accumulation of this element in the organism is unlikely.

Organosilicon medicinal preparations of the future will include substances isostructural to known biologically active organic compounds. The corresponding organosilicon compounds are often easier to synthesize and cheaper than their organic analogs. Some organosilicon O- and N-derivatives of well-known drugs are beginning to come into practice. Preparation of this type[61] may differ from their predecessors in their prolonged action, absence of bitter taste, better permeability through cell membranes, etc.

We are witnesses to the birth of a new branch of silicon chemistry - the chemistry, biochemistry and pharmacology of biologically active organosilicon compounds, i.e., bio-organosilicon chemistry. Its aims should include a detailed study of the role of silicon in living organisms and in the physiological processes and pathological and geronthological changes occurring in them, the search for new compounds of this element with high and specific biological activity, and the study of the possible practical application of such compounds in medicine, veterinary practice, plant growing and other branches of human activity.

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Section VI - Topics in Chemistry

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Chapter 28. Reactions of Interest in Medicinal Chemistry

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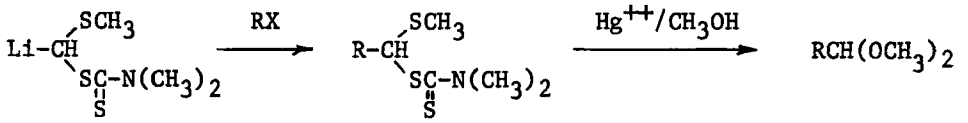
Publication of the following useful reference books of general interest in 1974 includes: Volume 4 of Reagents for Organic Synthesis by Mary Fieser and Louis F. Fieser, Organic Syntheses, Collective Volume 5, edited by Henry E. Baumgarten, Heterocycles in Organic Synthesis by A. J. Meyer, Volume 2 of Compendium of Organic Synthetic Methods by Ian T. Harrison and Shuyen Harrison, Organometallics in Organic Synthesis by J. M. Swan and D. St. C. Black, Volume 21 of Organic Reactions edited by W. G. Dauben, and Volume 28 of Synthetic Methods edited by W. Theilheimer S. and A. G. Karger.

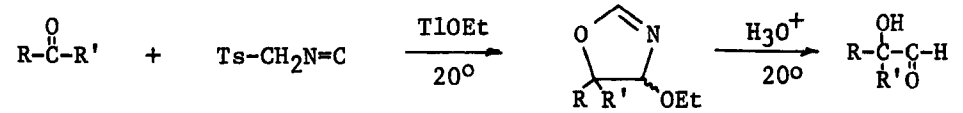
Reviews - New alkylation methods,[1] the use of oxyphosphoranes,[2] chromium(II) salts,[3] sodamide-containing 'complex bases',[4] carbon suboxide,[5] and thexylborane[6] in organic synthesis have been reviewed. Of particular interest is a monthly abstract entitled, Current Highlights in Heterocyclic Chemistry, which was initiated in 1974. The abstract is published under the auspices of the International Society of Heterocyclic Chemistry, W. W. Paudler and F. D. Popp, editors.

Aldehydes and Ketones - Et_3SiH effectively reduces N-alkylnitrilium ions, prepared *in situ* from nitriles and $Et_3O^+BF_4^-$, to N-alkylaldimines (RCH=NEt) which upon mild acid hydrolysis affords aldehydes in good yields.[7] Aldehydes are also obtained from alkyl halides *via* alkylation of the lithium salt of 1-methylthiomethyl N,N-dimethyldithiocarbamates and subsequent hydrolysis of the alkylated dithiocarbamates in the presence of Hg^{++}.[8]

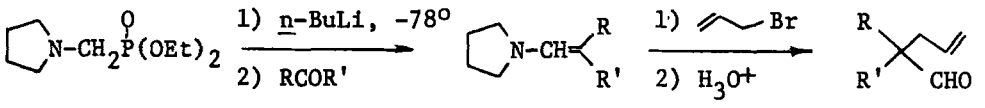


Both 1° and 2° alkyl bromides containing other sensitive functionality such as unsaturation are oxidized in excellent yields to aldehydes or ketones by $AgBF_4$ in DMSO.[9] A very simple, efficient and selective method for the transformation of acyl chlorides into aldehydes involving triazolium salts has been described.[10]

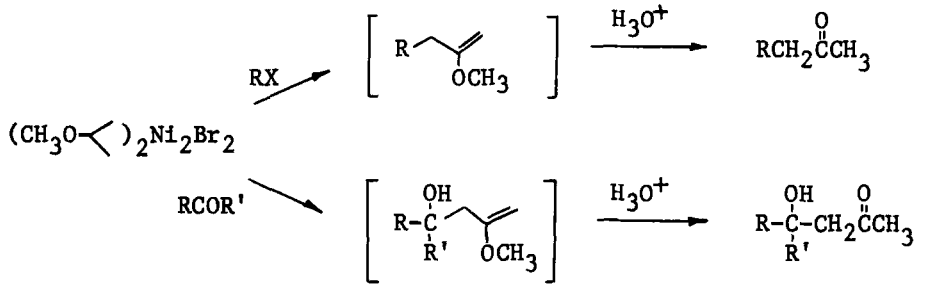
Treatment of ketones with tosylmethylisocyanide in the presence of thallium ethoxide followed by acid hydrolysis of oxazoline intermediates provides a nice entry for the formation of α-hydroxy aldehydes.[11]



A facile method for the one-carbon homologation of ketones to α-alkyl aldehydes which may be extended to the synthesis of other α-substituted aldehydes has been reported.[12]



π-(2-Methoxyallyl)nickel bromide, prepared from 2-methoxyallyl bromide and nickel carbonyl, is an excellent reagent for introducing the acetonyl moiety into reactive organic substrates under mild neutral conditions.[13]



Several new or improved methods for the preparation of α,β-unsaturated aldehydes[14-16] and ketones[17] have appeared as well as methods for the synthesis of 1,4-diketones,[18] 4-ketoaldehydes[19] and γ-ketoesters.[20]

Reductions - Aldehydes and ketones are reduced by alkylsilanes to alcohols in aqueous acid media; in carboxylic acid as solvent, carboxylate esters and symmetrical ethers are formed.[21]

2-Aminoalcohols can be easily prepared from cyanohydrins,[22] or α-amino acids[23] by diborane reduction without loss of the halogen substituents or hydroxyl groups.

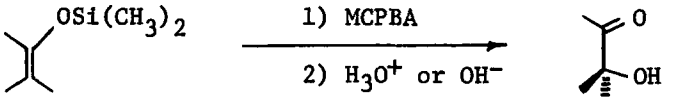
Selective reduction of the benzene ring in quinolines and isoquinolines can be achieved with PtO_2 in a strongly acidic medium (such as conc-HCl, H_2SO_4 or CF_3COOH) in yields ranging from 53 to 98%.[24]

2-Phenoxy-1,3,2-benzodioxaphosphole represents a new reducing agent that readily converts sulfoxides to sulfides under mild conditions and high yields (87-95%).[25]

Oxidations - With steroidal alcohols, specific oxidation of 2° hydroxyl groups in the presence of 1° hydroxyl groups with chlorine and pyridine has been reported.[26] A convenient one-step synthesis of epoxides and other cyclic ethers involves treatment of diol with diaryldialkoxysulfurane $Ph_2S(OR_F)_2$, where $R_F = C_6H_5C(CF_3)_2$ at room temperature for 10-20 min.[27] Alternatively, epoxides can be prepared in excellent yield via epoxidation of olefins with peroxycarbonic acids[28] or substituted peroxycarbamic acids.[29]

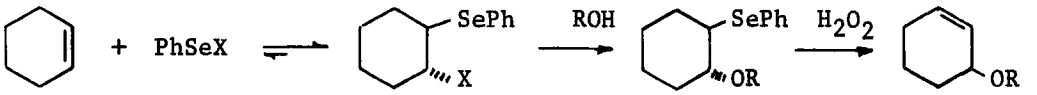
These agents have the advantage over that of peroxycarboxylic acid in that the reaction medium remains essentially neutral during the oxidation reaction. Two new routes to arene oxides of carcinogenic aromatic hydrocarbons in the previously inaccessible "K-region" have been reported.[30,31]

The oxidation of trimethylsilyl enol ethers, in hexane with m-chloroperbenzoic acid, followed by treatment of the crude reaction mixture with acid or base, provides a facile method for the specific α-hydroxylation of ketones.[32] Similarly, esters, lactones, and ketones having an enolizable



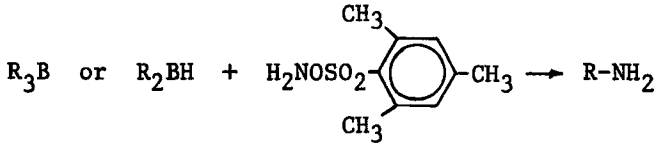
methine or methylene group are α-hydroxylated in good yield (80-85%) and without contamination by oxidative cleavage by-products with molybdenum peroxide $MoO_5 \cdot Py \cdot HMPA$ complex.[33]

Organometallic Reagents - Phenylselenyl bromide and acetate undergo electrophilic trans-1,2 addition to olefins.[34] The adducts solvolyze readily in acetic acid or alcohols and oxidation of the resulting products affords allylic acetates and ethers in high overall yield.



The addition of lithium and Grignard reagents to isocyanides not containing α-hydrogens proceeds by an α-addition to produce metallo-aldimines.[35,36] The lithium aldimines are versatile reagents which can be used as precursors for the preparation of aldehydes, ketones, α-diketones, α-hydroxyketones, α-keto acids, α- and β-hydroxy acids, and nitriles.

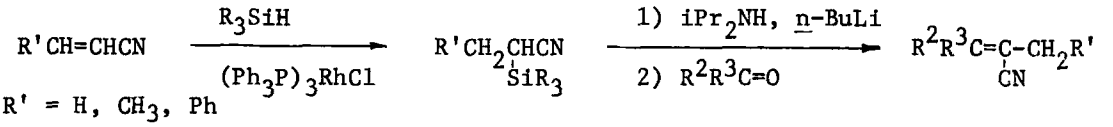
Amines and Nitriles - Amines can be prepared by the reaction of O-mesitylenesulfonylhydroxamine and a variety of organoboranes under very mild conditions.[37]



Several new reagents for demethylation or dealkylation of 3° amines or 4° ammonium compounds have appeared in the literature.[38-41] Relatively milder conditions and better yields are reported.

Aldehydes when treated with NH_2OSO_3H at room temperature, give high yields of the corresponding oximino-O-sulfonic acid ($RCH{=}NOSO_3H$) from which sulfuric acid is eliminated to furnish nitriles.[42] Similarly, acid-catalyzed

reaction of aldoximes and ortho esters affords the nitriles usually in high yield.[43] Addition of α-silylpropionitriles, easily prepared by a rhodium complex-catalyzed hydrosilylation of acrylonitrile and its derivatives, to aldehydes or ketones provides an excellent route for the synthesis of α,β-unsaturated nitriles (92-98% yields).[44]



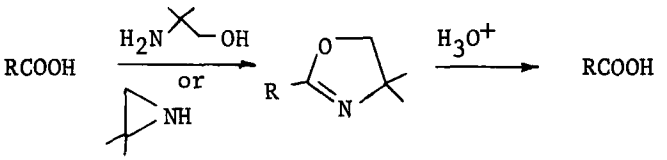
Crown Ethers and Their Synthetic Applications - Crown ethers, a class of macrocyclic polyethers which have a remarkable ability to solubilize alkali metals in non-polar aprotic solvents have found new and useful synthetic applications.[45-48] For instance, poor nucleophiles such as fluorides, solubilized as the potassium salt in CH_3CN or benzene containing 1,4,7,10,13,16-hexaoxacyclooctadecane (18-crown-6) becomes sufficiently nucleophilic to react smoothly and quantitatively, at room temperature, with a variety of organic substrates.[45] This 'naked' fluoride reagent, thus, provides a facile and efficient means of obtaining organic fluorine compounds in high yield. In a similar fashion, the 'naked' acetate[46] and cyanide[47] are excellent reagents for preparing organic acetates and nitriles, respectively.

Reduction of alkoxysulfonium salts formed by alkylation of sulfoxides with Magic Methyl® (methyl fluorosulfonate) proceeds readily with $NaBH_3CN$ in the presence of crown ethers to give the sulfides in excellent yield (85-91%).[49]

Crown ethers also find application in many two-phase reactions of carbanions and halocarbenes.[50-53] In these reactions crown ether is used as phase transfer catalyst.

Protective Groups and Their Removal - t-Butyldimethylsilyl and tri-isopropylsilyl protecting groups for the protection of 2'- and 2',5'-positions in ribonucleosides are readily prepared, are nicely crystalline, and overcome most of the problems associated with currently used protecting groups.[54]

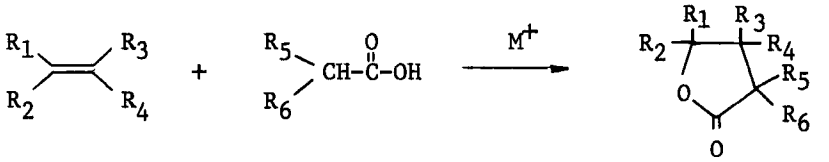
Carboxyl groups masked as an oxazoline are inert to Grignard reagents and lithium aluminum hydride. The carboxyl function may be masked starting from either the acid or ester by treatment with 1,1-dimethylaziridine and subsequent rearrangement or by direct formation with 2-amino-2-methyl-1-propanol. The carboxyl group is regenerated by acid hydrolysis.[55]



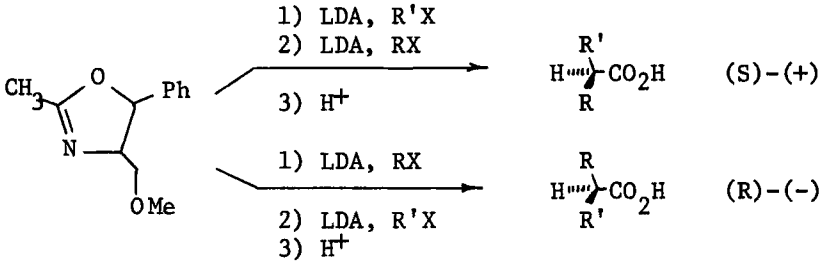
α,α-Dicyanoethyl acetate, readily accessible from the reaction of acetic anhydride with alkali cyanide, effects smooth N-acetylation and is capable of discriminative action between hydroxyl and amino functions.[56]

Several useful methods for removal of protecting groups have been reported in 1974. Basic nitrogens protected by benzyl groups allows deblocking with lithium n-propyl mercaptide in HMPA with few side reactions.[57] The reaction of anhydrous ferric chloride in acetic anhydride converts a variety of ethers to the corresponding acetates, affording a mild versatile reagent for the cleavage of ethers.[58] Tosylhydrazones should be more useful in the characterization and purification of carbonyl substances with the finding that they are readily removed in one step by treatment with commercial bleach solution,[59] or with N-bromosuccinimide in methanol.[60] Cleavage of the benzyloxycarbonylamine protecting groups from S-benzylcysteine-containing peptides can be attained by palladium-catalyzed hydrogenation when liquid ammonia is used as solvent.[61,62]

Carboxylic Acids and Derivatives - A general synthesis of γ-butyrolactones from readily available olefins and carboxylic acids in a simple one-step process has been reported. The general reaction consists of the addition of a carboxylic acid having an α-hydrogen atom across the double bond of an olefin in the presence of stoichiometric amounts of metal oxidants.[63]



Oxazolines may be used to homologate acetic acids[64] and to prepare either R or S dialkylacetic acids from a readily available oxazoline by merely reversing the order of alkyl introduction.[65]



The use of t-butyl-α-lithio esters shows promise for the general synthesis of α-mono- and dialkylated ketones that are difficult to prepare by other means.[66] A facile synthesis of acid-esters via monodemethylation of a dimethyl ester by 1,1-dimethylhydrazine and subsequent conversion of the resulting trimethylhydrazonium ester has been reported.[67] The use of α-carbanions of carboxylic acids and esters has been extended to the synthesis of malonates, phosphonoacetates, α-selenyl and sulfinyl esters;[68] α,β-unsaturated carboxylic esters;[69] and monoesters of malonic acids.[70]

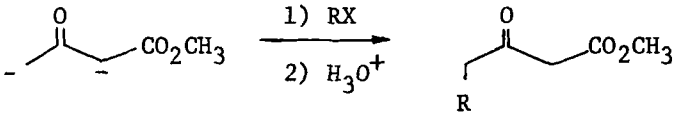
Boron trihalides catalyze the conversion of esters to amides in high yield,[71] while the use of boron trifluoride etherate-alcohol system for esterification has been reviewed.[72] Oxidative decarboxylation of primary acids with lead tetraacetate in the presence of cupric

acetate affords a new route to α-methylene lactones.[73]

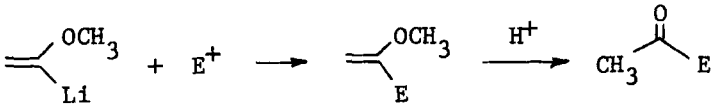
A mild and selective means for effecting the hydrolysis of nitriles to amides in near neutral media at room temperature using chloropentaaminerutthenium(III) chloride $[(NH_3)_5RuCl]Cl_2$ in which the resulting amides require no further purification has been reported.[74] Isomerization of aldoximes to amides is effected by heating the former in the presence of silica-gel and appears to give better yields and cleaner products than the nickel acetate procedure.[75] Polystyrene resins bearing mixed carbonic carboxylic anhydride functions have been synthesized and utilized to prepare amides and anhydrides from amines and carboxylic acids, respectively, in high yields under mild conditions.[76]

C-C and C=C Bond Formation - A general process has been developed for the ortho alkylation of aromatic amines. Starting with anilines or N-alkylated anilines bearing ortho, meta, or para substituents (electron-donating or electron-withdrawing), ortho-alkylated anilines are prepared via the intermediacy of an o-alkyl-α-thioalkoxy substituent.[77] A new procedure for direct introduction of alkyl and alkenyl substituents into heterocyclic nuclei has been reported. A suitable leaving group on the heterocycle is displaced by an alkylidinephosphorane and the resulting heterocyclic ylide converted in situ either by hydrolysis into an alkyl derivative of the heterocycle or by reaction with a carbonyl compound into an alkenyl derivative of the heterocycle.[78]

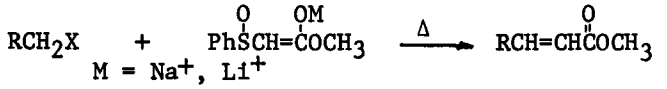
Under the appropriate conditions of time and temperature, dianions from a variety of β-keto esters generated using 1 equiv of sodium hydride and 1 equiv of n-butyllithium or methyllithium or 2 equiv of lithium diisopropylamide react with a range of alkylating agents to produce γ-alkylated products exclusively in good yield.[79]



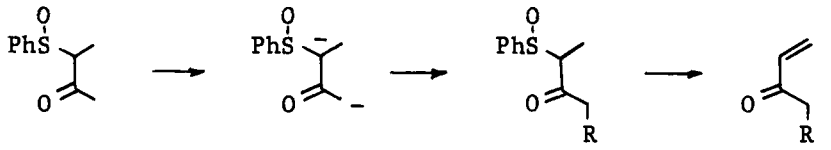
α-Phenylthio ketones and aldehydes may be alkylated at carbon bearing sulfur and subsequently the sulfide function of the alkylated phenylthio ketones may be regiospecifically replaced by an alkyl substituent through reductive alkylation as a means of effecting geminal dialkylation at a methylene adjacent to a carbonyl function.[80] α-Methoxyvinyllithium and related species can be used to prepare acyl anion equivalents of useful synthetic value. The reaction products of these species with electrophiles (E^+) contain vinyl ethers which may be further elaborated or, more usually, converted by mild acid treatment to their corresponding carbonyl compounds in high yield.[81]



Elimination of α-sulfinyl carbonyl compounds to their α,β-unsaturated derivatives forms the basis for the synthesis of α,β-unsaturated esters utilizing the anion of methyl 2-phenylsulfinylacetate and the corresponding halides. Use of π-allylpalladium complexes, directly available from the corresponding olefin, offers a novel substitution at the allylic position of an olefin.[82]

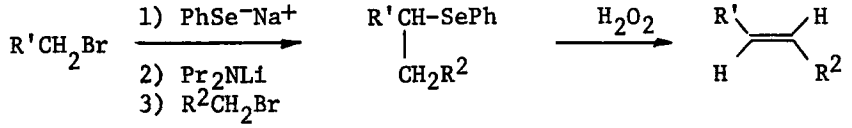


The dianion of the β-keto sulfoxide can be alkylated exclusively on the γ-carbon atom with a variety of alkyl halides; the resultant α-phenylsulfinyl ketones undergo ready elimination of benzenesulfenic acid providing a new general route to alkyl vinyl ketones.[83]



A new method for the preparation of diallylic sulfones from allyl alcohols allows extension of the Ramberg-Backlund olefin synthesis to synthesis of the corresponding trienes.[84] Coupling of allylic sulfones with allylic halides followed by reductive cleavage of allylated sulfones offers a new method for the preparation of geometrically pure 1,5-dienes.[85] Alkylation of ketone and carboxylate enolates with benzyl sulfide, followed by sulfoxide elimination, gives α-methylene ketones and carboxylic acids.[86]

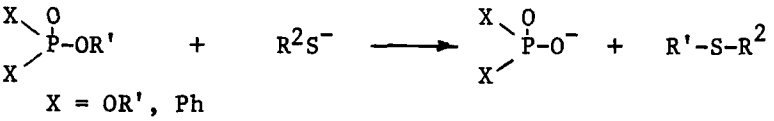
Treatment of epoxides with Ph_2PLi-THF followed by H_2O_2-AcOH gives, by a single inversion, β-hydroxydiphenylphosphine oxide. The latter can be fragmented stereospecifically to olefins upon treatment with an appropriate base.[87] A second method involves thermal decomposition of sulfoximines prepared from the appropriate epoxides.[88] Alkylation of benzyl phenyl selenides (prepared from benzyl halides) with alkyl or arylalkyl halides followed by oxidation to the selenoxide and elimination results in high yields of trans-alkenes.[89]



1-Chloro-4-chloro(bromo)methoxybutane and 1,4-bis[chloro(bromo)methoxy]-butane are effective chloro(bromo)-methylating agents of wide utility circumventing the need to use the carcinogenic chloromethyl ethers.[90]

Miscellaneous - A new synthetic procedure for the direct preparation of thiol esters from carboxylic acids and thiols using diethyl phosphorocyanidate [$NCPO(OEt)_2$] or diphenyl phosphorazidate [$N_3PO(OPh)_2$] as a condensing agent has been described.[91] Unsymmetrical sulfides are easily prepared in one step

under mild conditions from the reaction of trialkyl phosphates or phosphinate and alkyl mercaptide.[92]



X = OR', Ph

Conversion of primary alcohols to alkyl chlorides[93] and fluorides[94] in the presence of phase-transfer catalysts has been reported.

A novel and covenient conversion of 17-ketosteroids into their 18-nor derivatives via their oximes in three steps has been described.[95] Improved methods for the side-chain degradation of lanosterol were reported which preserve the acid-sensitive Δ^8-olefin.[96,97]

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Chapter 29. Radioimmunoassays

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The technique of radioimmunoassay (RIA) introduced in 1960 by Yalow and Berson[1] for the measurement of plasma insulin has now been expanded to include the detection of minute amounts of a wide variety of substances of biological interest, including steroid hormones and drugs that are not antigenic per se. It has become the method of choice for the measurement of most protein and polypeptide hormones. Thus, a number of complete commercial kits and antisera are now available for clinical use. Several comprehensive books and reviews of this technique have been written[2-10]. This review will focus on the methods and applications of RIA.

Fundamental considerations - RIA is a saturation assay technique, using the antibody (Ab) as a specific binding agent. In this method the antigen (Ag) to be measured competes with a labeled derivative (Ag^*) for the binding sites of the antibody. After equilibration, the antigen bound to antibody is physically separated from the free antigen, and the label on the antigen either in the bound (B) or free state (F) is measured and compared with the corresponding value for standard solutions of the antigen. The competing reactions can be summarized as shown below:

$$\underset{(F)}{Ag^*} + Ab \rightleftharpoons \underset{(B)}{Ag^*Ab}$$
$$Ag$$
$$\downarrow\uparrow$$
$$AgAb$$

RIA procedures depend on the following prerequisites: (i) production of antisera with a high affinity and specificity for the antigen; (ii) preparation of labeled antigens; (iii) standardization procedures; (iv) separation techniques; (v) assay validation and expression of the results.

(i) Production of antisera - Antibody production depends, among other factors, on the nature of the immunogen and the animal species used for immunization.

The immunogen - Proteins of molecular weight in excess of 5,000 are immunogenic without modification. The smaller polypeptides (mol. wt. 1,000-5,000, e.g. oxytocin, gastrin, vasopressin) have low immunogenicity and may be conjugated to carrier molecules such as synthetic polypeptides (polylysine),[11] or polymers (polyvinyl pyrrolidone)[12] or natural protein molecules[13] (albumins, thyroglobulins). Smaller molecules (steroids,[14] drugs,[15] hypothalamic releasing hormones[16]) with a molecular weight of $< 1,000$ are not antigenic but can function as haptens, i.e. they may be rendered antigenic by covalent attachment to a macromolecule. Most conjugation methods involve the formation of a peptide bond between the hapten and

the carrier molecule. If the hapten already contains a free amino group (e.g. amphetamines[17]) or a free carboxyl group (barbiturates[18], prostaglandins[19]), it can be linked unchanged to the protein. Alternatively, a carboxyl group can be introduced easily into the molecule by using a symmetrical or ambidentate reagent (succinic anhydride, carboxymethyl oxime, thioacids), one end of which attacks a reactive position on the hapten (e.g. OH, C=O, halogen, double bond conjugated with a keto group, phenolic ring) while the other serves to fashion an amide bond with the carrier[14]. However, convenient general methods for preparing amino derivatives of haptens have not been elaborated. The mixed anhydride[20], the Shotten-Baumann[21] or the carbodiimide[22] reactions have been used for the condensation of carboxyl derivatives with carrier. The glutaraldehyde condensation reaction[23] has been described for amino derivatives. Other condensation reactions that have been used include the Mannich reaction[24]. For drugs that are totally unreactive in their native forms (e.g. diphenylhydantoin) their reactive congeners (in this case p-hydroxyphenytoin) have been employed as antigen[25].

Species immunized - Guinea pigs or rabbits have been commonly used. Other species such as rats, chickens, sheep, ewes, goats have also been immunized, and the bigger species are usually used for the production of second antibodies for assays of the double antibody type which require relatively large volumes of serum[26]. The general method of inducing antibody formation is to inject by various routes (intraperitoneally, subcutaneously, intravenously or directly into the lymph nodes) the pure antigen mixed with Freund's adjuvant into a number of animals[27]. Several immunization schedules have been described[28]. The antisera that are thus generated are tested for their titer, affinity and specificity[28].

(ii) Preparation of labeled antigen - A prerequisite for a sensitive RIA is the availability of a purified antigen which has been labeled with a radioactive nuclide, though many alternative types of label are now being used for immunoassay (enzymes[29], spin-label[30], phage[31], etc). The isotopes of iodine, particularly ^{125}I and ^{131}I, being γ-emitters, have been used for iodinating the tyrosyl or histidyl residues of highly purified and preferably homologous peptide hormones. The iodination procedure involves release of nascent iodine from $Na^{125}I$ or $Na^{131}I$ which is brought about by the use of oxidizing agents such as chloramine T[32], iodine monochloride[33], chlorine or sodium hypochlorite[34], by an electrolytic process[35] or by enzymic iodination using lactoperoxidase[36].

Recently the use of a solid phase iodination technique using lactoperoxidase coupled to polyacrylamide has been reported[37]. Alternatively, the iodination can be carried out on an active acylated agent like 3-(p-hydroxyphenyl)propionic acid N-hydroxysuccinimide ester which is then conjugated to the protein[38]. The labeled antigen can be purified by electrophoresis, gel-filtration, or ion-exchange chromatography[39]. Haptens, such as steroids, prostaglandins and various drugs are available commercially labeled with β emitters (^{3}H or ^{14}C). The low specific activity of ^{14}C-labelled compounds severely limits their usefulness. There has

also been an interest in the provision of γ-emitters for some of these assays, since sensitivity can be improved by the use of a labeled antigen with high specific activity. The requirement is to introduce a γ-ray emitting radionuclide into the hapten. Advantage has been taken of the availability of the carboxyl derivatives of haptens which were linked to the carrier to form the immunogen. These carboxyl derivatives were first conjugated to tyrosine methyl ester, tyramine or histamine and subsequently iodinated and purified. These iodinated tracers were then used as labels in RIA provided that they showed an affinity to the antibody close to that of unaltered hapten[40]. For phenolic steroids, histamine was first iodinated and then conjugated to estradiol-6-(O)-carboxymethyloxime[40]. A new development in this area is the use of ^{75}Se labeled steroids[41] as an alternative to ^{125}I-labeled steroids.

(iii) Standardization procedures - Quantitation of the results of a given RIA is based upon comparison of responses given by test materials with those given by a standard examined in the same assay. Validity of such assays require that the standards show immunochemical identity with the substances assayed. With standards that can be obtained in a pure form (drugs, steroids) results obtained with any pure preparation as standard are comparable. However, with those substances not completely characterized (peptide hormones), disparate assay results can be obtained because of dissimilarity between the substance in the test sample and the standard. Non-identity can occur either because of natural variations of the substance[42] or because of artifacts produced from in-vitro handling of the standard[42]. Thus, for assays applied routinely in a clinical laboratory it is important to evaluate what molecular forms of a hormone an assay is intended to measure, to develop assays with defined specificity and to relate the estimates to a particular reference material. A service of standards and reference material is provided by the World Health Organization (WHO), by the National Pituitary Agency (Bethesda, Maryland) and by the National Institute for Biological Standards and Control (Hampstead, London).

(iv) Separation techniques - The end point of saturation analysis involves the determination of the relative proportion of labeled antigen that is free and antigen that is bound to the antibody. Advantage has been taken of the physiochemical or immunological differences between free and bound moieties. Separation of the two moieties can be accomplished in the following ways:[43]

(a) Fractional precipitation of the bound fraction using organic solvents (ethanol, dioxane or polyethylene glycol) or salts (ammonium sulphate);

(b) separation on the basis of molecular size (gel filtration) or electrophoretic mobility (electrophoresis on starch gel, cellulose acetate, etc);

(c) adsorption of free fraction (dextran-coated charcoal, talc, silicates);

(d) immunological precipitation of the bound fraction using an antiserum directed against γ-globulin (double antibody techniques).

An alternative to these methods is the use of solid-phase RIA wherein the antibody is introduced either in the form of a coating on discs or tubes or linked to a finely divided solid phase or as a copolymer[43].

(v) Assay validation and expression of results - A reliable antiserum is the ultimate requirement for any RIA. Five main criteria have been used to assess the reliability of RIA: precision, sensitivity, specificity, accuracy and reproducibility. These aspects of RIA have been described in detail by Ekins[44] and Midgley[45]. Several co-ordinate systems for dose response curves in RIA are in common use. A number of calculator and computer programs[46,47] have been written for the statistical analysis of assay data and for the optimal assay design. Of these programs, the Rodbard[47] program is widely used, and is applicable to any saturation analysis assay. The results are usually expressed in terms of mass, molarity or of a particular reference preparation.

Applications

RIA has been applied as an analytical tool in various fields such as endocrinology, clinical pharmacology, haematology, virology and oncology. Since it is not possible in the space available to cover all the applications of this method, emphasis will be given only to those assays that proved of clinical value.

Endocrinology - The substances that have been assayed in the whole endocrine system can be categorized in the following way: (i) peptide and protein hormones; (ii) non-peptide hormones; (iii) non-hormonal substances.

(i) Peptide and Protein Hormones

Several review articles on RIA methods of hypothalamic peptides[48,49], human pituitary hormones[50,51], placental hormones[50], thyroid[52] and parathyroid hormones[53], gastrointestinal hormones[54], and vasoactive peptide hormones[55,56] have been published. Each subject will be treated very briefly and only those methods that have a direct impact on human medicine will be given.

Hypothalamic Peptides - The physiological aspects of the hypothalamic peptides have been discussed elsewhere in this volume (Chapter 21). So far, specific RIA methods have been described only for two hypothalamic releasing hormones (luteinizing hormone-releasing hormone, LH-RH[16,48] and thyrotropin releasing hormone, TRH[49]). Basal values for serum LH-RH have been reported[57]. However, attempts to measure TRH in human plasma have so far proved unsuccessful[49].

Pituitary Hormones

The chemistry and physiology of these hormones are covered in another survey in this volume (Chapter 21). The heterogeneity of the protein hormones[58], the poor immunogenicity of the peptide hormones[59], and the difficulty in preparing

pure labeled antigens[39] have been some of the major problems in the development of specific RIA methods for these hormones.

Glycoprotein Hormones: Thyroid Stimulating Hormone, TSH, Luteinizing Hormone, LH, Follicle Stimulating Hormone, FSH - Antisera raised against the intact hormones were non-specific due to the similarity of the α subunits of these hormones[50]. Recently specific RIA methods using the β subunit as an immunogen have been described for LH[60], TSH[50] and FSH[61]. The RIA of LH and FSH has been applied in studies in reproductive physiology[62].

Adrenocorticotropin Hormone, ACTH - The problems involved in the development of a RIA method for ACTH has been reviewed[63]. The RIA of ACTH in plasma has applications in the diagnosis of Cushing's syndrome and in cases of primary and secondary adrenal deficiency[63].

Prolactin - The RIA of human prolactin has been reviewed[64]. The immunoassay of prolactin has been used in the investigation of patients with pituitary tumors and with inappropriate lactation[64].

Human Growth Hormone, HGH - The RIA of HGH provided the first quantitative estimates of basal HGH concentrations in plasma[65]. The measurement of HGH by RIA has been used in the diagnosis of acromegaly[65] and in screening patients with suspected HGH deficiency[65].

Posterior Pituitary Peptides: Vasopressin, Oxytocin, Neurophysin - The RIA of vasopressin provided basal values of this hormone both in plasma[66] and in urine[67]. The RIA of oxytocin has been reviewed[68]. The RIA of vasopressin has potential applications in the diagnosis of diabetes insipidus[69] and that of oxytocin in studies on animal[69] and human parturition[70]. The RIA of neurophysin[69] is relatively recent and has been confided to studies in which there was a release of oxytocin and/or vasopressin.

Placental Hormones: Human Chorionic Gonadotropin, HCG, Human Placental Lactogen, HPL

The structure, function and relationship of HCG to the glycoprotein hormones (LH, TSH, FSH) have been reviewed [71]. Thus, antisera raised against intact HCG[50] reacted with all the glycoprotein hormones whereas those raised against HCG β subunit[72] were specific, and were used for the measurement of HCG by RIA in the presence of physiological amounts of LH. The estimation of HCG by RIA has been applied in the early detection of pregnancy[50] and in the diagnosis of choriocarcinoma[50]. The estimation of HPL by RIA has been used in monitoring placental function and fetal well-being[50].

Thyroid and Parathyroid Hormones: Calcitonin, CT, Parathyroid Hormone, PTH - The estimation of CT in plasma by RIA has been applied in the diagnosis of hypercalcemia[73]. High levels of CT have been reported in patients with medullary carcinoma of the thyroid gland[73]. The development of a RIA for human PTH has been rather difficult due to the heterogeneity of PTH[53] and to the lack of a human reference preparation. The RIA of the plasma concentration of human PTH has many applications in the differential diagnosis of hypercalciuria and the management of renal failure by dialysis[53].

Hormones of the Gastrointestinal Tract

RIA methods have been published for some of the peptide hormones of gastrointestinal tract whose amino acid sequence has been elucidated (secretin, gastrin, cholecystekinin, glucagon and C-peptide)[54] and for the protein hormones[74] (insulin, and pro-insulin) that are secreted by the pancreas. Of these hormones, the estimation of human insulin[74] is widely used for the routine investigation and diagnosis of hypoglycaemia. The RIA of serum gastrin is of diagnostic value in patients with suspected Zollinger-Ellison syndrome[75].

Vasoactive Peptide Hormones: Bradykinin, Angiotensin I and Angiotensin II - RIA methods have been reported for the vasoactive peptide hormones bradykinin[55], angiotensin I[56] and angiotensin II[56]. Of these, the RIA of angiotensin I is used clinically for estimation of plasma renin activity[56,76]. However, the lack of an internationally accepted standard for renin makes it difficult to compare results obtained in various laboratories[56].

(ii) Non-Peptide Hormones:

This group consists of the steroids, prostaglandins and hormones of the thyroid gland.

Steroids - Several RIA methods[77,78] have been published for the determination of natural steroids secreted by the adrenal (mineralcorticosteroids[79] and glucocorticosteroids)[80] and by the gonads[81,82] (estrogens, androgens, progestagens). Recently RIA methods have been reported for synthetic steroids which are used as drugs (contraceptive[83] and anti-inflammatory[84] steroids). Earlier antisera were generated by using as immunogen steroids coupled to the carrier via their existing functional groups, and antisera thus produced were non-specific[20,21]. However, more specific antisera were obtained when the antigen used for immunization was coupled to the carrier molecule through reactive groups introduced into the steroid skeleton at positions remote from the existing functional groups[14,81,82]. In spite of good specificity, conventional methods of purification, including solvent partition and chromatography have to be carried out for the measurement of some of these steroids from biological fluids. Recently RIA methods without extraction and chromatography have been reported for estriol[85] and estradiol[86].

Prostaglandins - RIA methods have been published for prostaglandins such as E, A and F[87] and for some of the metabolites (15-keto, 13,14-dihydro-PGE_2[88], and 15-keto, 13,14-dihydro-$PGF_{2\alpha}$[89]). An inclusion of a chromatographic step proved to be necessary for the measurement of these prostaglandins from biological fluids[87].

Hormones of the Thyroid Gland - RIA methods have been developed recently for the two thyroid hormones, thyroxine (T_4)[90] and triidothyronine (T_3)[90,91]. The estimation of both of these hormones by RIA is useful in evaluating thyroid function.

(iii) Non-Hormonal Substances

RIA techniques for cyclic nucleotides have been reported by Steiner et al.[92]. Low concentrations of these nucleotides in body fluids and tissues have been measured by RIA methods[92].

Clinical Pharmacology - The application of RIA to pharmacology has been reviewed[15,93]. RIA methods have been published for the cardiac glycosides[94], for morphine and opiate alkaloids[95], for lysergic acid derivatives[96], for tetrahydrocannabinol[15], for anti-convulsants (barbiturates[18] and phenytoin[25]), for amphetamines[17] and for phenothiazines[15]. It has, thus, been possible to measure by RIA low concentrations of these drugs in blood, and in some cases to monitor treatment.

Haematology - RIA methods have been applied to the measurement of some of the lytic factors[97] (fibrinogen, fibrinogen degradation products, fibrinopeptides, plasminogen, plasmin and prothrombin), of vitamin B_{12},[98] of ferritin[99] and erythropoietin[100]. The determination of serum ferritin concentrations by RIA[99] promises the most convenient, direct assessment of body iron stores.

Virology - RIA techniques have been applied for the detection and meaurement of viral antigens[101] (hepatitis B antigen) and antiviral antibodies[101]. These methods have been in the order of 10^2 - 10^3 times more sensitive than other laboratory tests commonly used for diagnostic purposes (e.g. complement fixation).

Oncology - RIA techniques have been applied for the detection of tumor secreting antigens[102]. Of these, the RIA of fetal antigens [α-fetoprotein[102] and carcinoembryonic antigen (CEA)][103] is quite promising. There are indications that estimation of plasma CEA in patients with gastrointestinal carcinoma can provide a guide for monitoring treatment[103].

Conclusion

The number of applications of RIA is growing at a fast rate. Extensions and modifications of RIA include the immunoradiometric and "sandwich" or "2-site" assays which utilize labeled antibodies[104]. Future changes in RIA will be, whenever possible, to substitute specific biological receptors for the antibody in the assay.

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Chapter 30. Vitamin D and its Metabolites

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Introduction - In the last decade there has been intense interest in the study of vitamin D's metabolism and the function of its metabolites in the maintenance of calcium homeostasis. Research has focused on elucidating the role of the various D metabolites in mediating specific aspects of calcium uptake and mobilization; and on investigating the biosynthetic control of these metabolites by calcium, phosphate and parathyroid hormone. Interest in vitamin D's metabolism is rooted in Carlsson's[1] observation of a time lag between dosage with vitamin D and onset of physiological response. However, early investigations failed to uncover any active metabolites of vitamin D,[2-4] which implied that the vitamin worked directly to elicit physiological responses. This conclusion was proven erroneous upon the advent and use of powerful new tools which permitted the investigation of vitamin D at physiological doses. These were: the synthesis of vitamin D_3 of high specific activity;[5,6] the development of new chromatographic methods which were capable of separating vitamin D and its metabolites;[7-9] and the coming of age of nmr and mass spectrometry. Several excellent reviews cover these aspects.[10-18]

Metabolites - There are two natural sources of vitamin D_3-diet and endogenous synthesis. In mammals, intestinal absorption of cholecalciferol (D_3) and 7-dehydrocholesterol (7-DHC) is a facile process, whereas ergocalciferol (D_2) is only poorly absorbed.[11,19] 7-DHC is also an intermediate in the biosynthesis of cholesterol and thus occurs in all tissues which synthesize cholesterol. Mammalian tissue can not dehydrogenate cholesterol to 7-DHC.[20] In the epidermis, 7-DHC is irradiated to D_3. Epidermis synthesizes 7-DHC, but the predominant source of this sterol is most likely liver and gastrointestinal tract - tissues that are the major sites of cholesterogenesis.[21]

Work by DeLuca's group in the 1960's demonstrated the existence of biologically active metabolites of D_3[22] and established that D_3 is metabolized to 25-hydroxy-D_3 (25-OH-D_3).[23,24] Presently, 25-OH-D_3 is considered the major circulating form of D_3.[10-18]

The 25-hydroxylation of D_3 occurs in hepatic microsomes[25,26] and is catalyzed by an enzyme which requires molecular oxygen and NADPH,[25] but inhibitor studies failed to demonstrate that this mixed-function oxidase is cytochrome P-450 dependent.[27] The D_3-25-hydroxylase is stimulated by a specific cytoplasmic protein, which probably protects D_3 from oxidative degradation by non-specific peroxidases. This supposition is supported by the observation that the antioxidant diphenyl-p-phenylenediamine promotes 25-hydroxylation in the absence of cytoplasmic protein.[28]

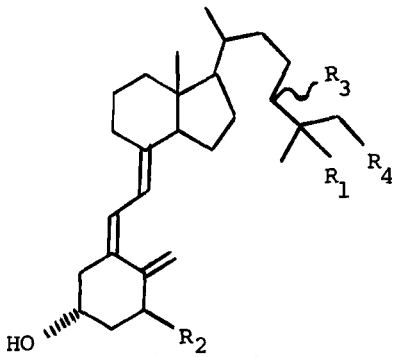
Physiological amounts of D_3 dramatically decrease the activity of rat 25-hydroxylase within three hours after dosage.[25-29] The rate of

onset, magnitude, and duration of this effect is dose dependent; however, very large doses of D_3 can overcome this effect.[25] In contrast to the rat, Haussler and co-workers[30] reported that chick 25-hydroxylase activity was not diminished by dosage with 10 IU of D_3 (10 IU=0.65 nmol). They also reported that chick kidney and intestine metabolized D_3 to a compound which co-migrated with 25-OH-D_3, and that these hydroxylations were unregulated. DeLuca's group[14] confirmed the appearance of the kidney and intestinal moiety which co-migrated with 25-OH-D_3, but questioned the identity of the compound. These further experiments demonstrated that rat kidney and intestine did not metabolize D_3 since the plasma of hepatectomized rats remained free of [^{3}H]25-OH-D_3 for as long as 12 hours after dosage with [^{3}H]D_3. Bhattacharyya and DeLuca[29] confirmed that 10 IU of D_3 did not inhibit the chick 25-hydroxylase, but went on to establish that larger doses (20 and 50 IU) did result in diminution (63 and 83%, respectively) of 25-hydroxylase activity. Thus, presently, the mammalian 25-hydroxylation of D_3 must be considered a regulated process which occurs only in liver. The mechanism and physiological significance of the regulation are unknown.

Both D_3 and 25-OH-D_3 are bound to specific carrier proteins in chick, rat and human plasma.[31] In the rat, one protein, distinct from albumin, binds both D_3 and 25-OH-D_3. This protein demonstrates an overwhelming preference for 25-OH-D_3, *in vitro*. The binding half-time of 25-OH-D_3 is 9 minutes, whereas that of D_3 is about 2.5 days. Human[31,32] and chick[31,33] plasma contain two different carrier proteins. One, which is distinct from albumin, binds both D_3 and 25-OH-D_3. A second, which co-migrates with albumin on acrylamide gel electrophoresis, binds only D itself. *In vitro*, 25-OH-D_3 is preferentially bound by the 25-OH-D/D protein, whereas the D protein binds the parent vitamin and the metabolite equivalently. In the chick, D_2 and 25-OH-D_2 are bound poorly to the 25-OH-D/D protein relative to 25-OH-D_3 and D_3. Rat and human 25-OH-D/D carrier proteins are about equally receptive to D_3 and D_2 and their 25-hydroxylated metabolites. According to DeLuca,[31] the lower affinity of the chick carrier protein to D_2 and 25-OH-D_2 may explain, in part, the increased metabolic degradation and the consequent lower efficacy of these compounds with respect to the D_3 compounds in curing chick rickets.

There is no indication that physiological concentrations of 25-OH-D_3 act directly to mediate calcium or phosphate homeostasis. The metabolite is, however, 2-5 times more active than D_3 in curing rickets and acts faster than D_3.[34]

In the kidney cortex mitochondria, 25-OH-D_3 is hydroxylated to either 1α,25-dihydroxy-D_3 (1,25-diOH-D_3)[35-38] or 24,25-dihydroxy-D_3 (24,25-diOH-D_3)[39-41] depending on physiological circumstances. Both 1,25-diOH-D_3[42] and 24,25-diOH-D_3[43,44] undergo further hydroxylation on the 24 and 1α positions, respectively, to give 1α,24,25-trihydroxy-D_3 (1,24,25-triOH-D_3). The 1α-hydroxylase is a cytochrome P-450 dependent mixed-function oxidase,[39,45,46] which is phylogenetically widely distributed. Studies have demonstrated its presence in various species of Amphibia, Aves, Mammalia, Osteichthyes, and Reptilia.[18] The 24-hydroxylase remains to be studied.



D_3: $R_1=R_2=R_3=R_4=H$

25-OH-D_3: $R_1=OH$, $R_2=R_3=R_4=H$

1,25-diOH-D_3: $R_1=R_2=OH$, $R_3=R_4=H$

24,25-diOH-D_3: $R_1=R_3=OH$, $R_2=R_4=H$

25,26-diOH-D_3: $R_1=R_4=OH$, $R_2=R_3=H$

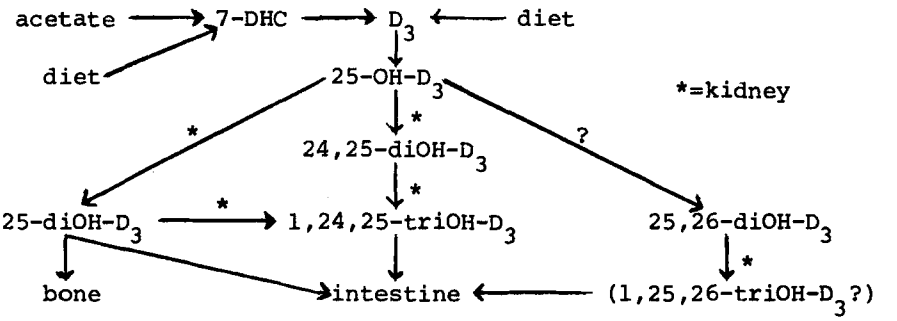
1,24,25-triOH-D_3: $R_1=R_2=R_3=OH$, $R_4=H$

1,25-DiOH-D_3 is the most physiologically significant form of D_3.[10-18] This metabolite acts directly on all known vitamin D mediated calcium homeostatic control points. It promotes intestinal calcium absorption, increases serum phosphate concentrations,[47] and mobilizes calcium from bone. With respect to the latter, it is literally infinitely more effective than D_3 itself in isolated bone cultures. It acts more rapidly than D_3 and when parenterally administered is 10-15 times more potent than D_3.[16] The biological activity of 1,25-diOH-D_3 has been demonstrated in chick, rat, dog, and man.[18]

The dihydroxylated metabolite found in the highest concentration in the blood of rat[48] and man[24] is 24,25-diOH-D_3. The biological activity of this metabolite is manifested subsequent to its hydroxylation to 1,24,25-triOH-D_3.[44] The latter metabolite is about 60% as effective as D_3 in reversing the course of rickets. It acts by preferentially stimulating intestinal calcium absorption; and has little activity in promoting bone calcium mobilization. It is about 67% as effective as 1,25-diOH-D_3 in its intestinal activity.[43] The significance of both 24,25-diOH-D_3 and 1,24,25-triOH-D_3 is unknown.

Other metabolites of D_3 exist. At least seven metabolites more polar than 25-OH-D_3 can be detected in the plasma of pigs fed large dietary amounts of D_3 (2.5 mg/day). Only one other than 24,25-diOH-D_3 has been isolated and identified as 25,26-dihydroxy-D_3 (25,26-diOH-D_3).[49] This metabolite has no rachitic healing activity[50] but is about 12.5% as active as D_3 in promoting intestinal calcium absorption.[50,51] It is ineffective in promoting bone calcium mobilization.[49] Significantly, 25,26-diOH-D_3 is inactive in bilaterally nephrectomized rats, indicating that this metabolite, like 24,25-diOH-D_3, must undergo 1α-hydroxylation before it can exert physiological action.[51] No direct attempt to prove that this hydroxylation occurs has been reported. Nor is the significance of this metabolite known.

1,25-DiOH-D_3 and Calcium Homeostasis - Both ionic and hormonal control of the 1α-hydroxylase have been demonstrated. The renal tubule production of 1,25-diOH-D_3 is closely regulated by calcium,[52-56] phosphate,[47] parathyroid hormone (PTH)[57,58] calcitonin,[59] and 1,25-diOH-D_3 itself.[60-62]



Hypocalcemia stimulates the 1α-hydroxylase to produce 1,25-diOH-D_3,[52-54] whereas normal and hypercalcemia decrease the activity of the hydroxylase so that 24,25-diOH-D_3 becomes the major metabolite of 25-OH-D_3.[60] Parathyroidectomized animals lose the ability to respond to hypocalcemia.[57] This can be corrected by administration of PTH. The link between PTH and 1,25-diOH-D_3 was strengthened by the demonstration that PTH or cyclic AMP stimulate the synthesis of 1,25-diOH-D_3 in isolated renal tubule cells.[17]

1,25-DiOH-D_3 may inhibit its own synthesis by more than one mechanism. Vitamin D deficient animals do not respond to hypercalcemia by deactivating the 1α-hydroxylase.[60] But a time lag of 150 minutes between administration of 1,25-diOH-D_3 and decreased 1α-hydroxylase activity argues against a direct effect of the metabolite on the hydroxylase.[61] In addition, actinomycin D prevents the inhibition of the hydroxylase by 1,25-diOH-D_3. Thus Larkins et al.,[61] postulate that 1,25-diOH-D_3 stimulates the production of the calcium binding protein (CaBP) known to be present in kidney.[63] Since the 1α-hydroxylase is known to be sensitive to calcium,[55-57] enhanced calcium entry into renal cells mediated by newly synthesized CaBP may explain the inhibitory effect of 1,25-diOH-D_3 on its own synthesis.

The demonstration of selective accumulation of 1,25-diOH-D_3 in the parathyroid glands of D deficient chicks was considered a preliminary indication of negative feedback control of this metabolite on PTH secretion.[64] The crucial test of whether 1,25-diOH-D_3 actually decreases PTH secretion has not yet been reported.

DeLuca and Tanaka[47] have developed evidence which suggests, at least in the rat, that 1,25-diOH-D_3 is also involved in phosphate transport acting independently of its involvement in calcium transport. Vitamin D deficient, hypophosphatemic rats with normal calcium levels responded to dosage with either 25-OH-D_3 or 1,25-diOH-D_3 by increasing serum phosphate concentrations. With a single dose there was no concommitant calcium mobilization but daily dosage stimulated this effect. The intestinal calcium transport system was also stimulated, but continued to function several days after serum phosphate levels dropped. Thyroparathyroidectomized rats fed phosphate-deficient diets also demonstrated an increase in serum phosphate. In contrast, the sensitivity of nephrectomized rats to the phosphate concentrating ability of 1,25-diOH-D_3 was severely decreased.

It was concluded that 1,25-diOH-D_3 acts in the absence of PTH to increase serum phosphate by stimulating the renal tubular reabsorption of phosphate. This is in contrast to the conjunctive function of 1,25-diOH-D_3 and PTH which, while promoting serum calcium increases, stimulate the renal excretion of phosphate.[16,65]

PTH and 1,25-diOH-D_3 act in conjunction to promote bone calcium mobilization.[10,11,14-16] Little is known about the molecular mechanisms of this process; but since several hours elapse between exposure of bone to PTH and 1,25-diOH-D_3 and observable physiological effects, bone calcium mobilization is thought to involve a complex biochemical action - perhaps calcium transport protein induction. This speculation is supported by the observed prevention of bone calcium mobilization in rats by actinomycin D.[66] Facilitated transport is also indicated by an increase in cyclic AMP levels in bone cells upon dosage with PTH and 1,25-diOH-D_3.[67]

1,25-DiOH-D_3 acts without PTH to stimulate calcium uptake from intestine to blood.[10-18] Upon kidney excretion, this metabolite associates with a carrier protein[31] and translocates to the intestine where it appears to concentrate in the order: duodenum>ileum>jejunum.[68] In the intestinal cell, 1,25-diOH-D_3 binds with a cytoplasmic receptor protein[69-71] which transports it to the nucleus.[72] Some evidence is available which suggests that in the nucleus the metabolite is associated with the chromatin.[12,69,72,73] This complex may be in equilibrium with a nuclear receptor protein different from the cytoplasmic receptor.[74] Further, evidence has been produced which suggests that the primary action of 1,25-diOH-D_3 in the intestine is to stimulate mRNA and protein synthesis.[75-77] Specifically, 1,25-diOH-D_3 has been linked with the appearance of an intestinal CaBP.[76-78]

Several of the criteria which define hormones are satisfied by 1,25-diOH-D_3. It is biosynthesized in minute amounts in one organ in response to specific physiological stimuli. It then travels to specific target organs where it elicits specific responses. Consequently 1,25-diOH-D_3 is presently considered a kidney hormone.[10-18]

Analogs - The elucidation of the metabolic fate of D_3 prior to the appearance of its physiological effects has had tremendous practical applications. Vitamin D deficiency diseases resulting from liver malfunction or the induction of the hepatic microsomal xenobiotic metabolizing system by drugs can now be successfully treated with 25-OH-D_3.[15] Likewise, D deficiency syndromes resulting from chronic renal failure can be successfully treated with 1,25-diOH-D_3.[15] However, the expense and limited availability of 1,25-diOH-D_3 has made substitutes for this hormone extremely desirable. Several analogs are already in use. The synthetic approaches to[13,79,80] and methods of testing[81,82] of these analogs have been thoroughly reviewed.

Perhaps the most important analog of the hormone is 1α-hydroxy-D_3 (1-OH-D_3). This analog was first synthesized and evaluated by DeLuca's group.[83] Since then several other reports have been published.[84-88] In the chick, 1-OH-D_3 is reportedly as effective as the hormone in all phases

of calcium and phosphate homeostasis.[89] In contrast, in the rat 1-OH-D_3 is about 0.5 times as effective as 1,25-diOH-D_3 and 2.5 times more effective than D_3.[90] Therefore, species differences, compound purity, and test conditions are under further evaluation.[90] It is not known if 1-OH-D_3 undergoes 25-hydroxylation prior to acting. 1-OH-D_3 is active in anephric rats.[83] In comparison to the hormone, 1-OH-D_3 is less prone to produce hypercalcemia, more active orally and, relatively easy to synthesize.[90] Currently 1-OH-D_3 is successful in the treatment of human osteomalacia stemming from a variety of causes.[91-93]

Lam, Schnoes and DeLuca[94] have also synthesized and tested 1α-hydroxy-D_2 (1-OH-D_2). This agent is similar in activity to 1-OH-D_3 in rat. Orally, it is equipotent with D_2. Intraperitoneally, it is 3 times more effective than D_2 in both bone and intestine.

A third potent analog of 1,25-diOH-D_3 is 3-deoxy-1α-hydroxy-D_3 (3-deoxy-1-OH-D_3). In rats, this agent is as effective as 1-OH-D_3 in promoting intestinal calcium absorption, but only about 86% as effective with respect to bone calcium mobilization.[95] In chicks, it was 1.5 times more effective than 1,25-diOH-D_3 in both bone and intestinal responses.[96] It is not known if this compound undergoes 25-hydroxylation prior to manifesting biological activity. It almost certainly does not undergo 3-hydroxylation.[96]

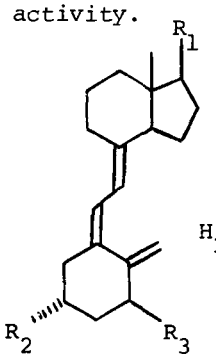
The isomeric 24-hydroxy-D_3's (24-OH-D_3) were synthesized and resolved, but no structural assignments were made.[97] Both possess activity nearly equal to that of 25-OH-D_3 in rats. Since nephrectomized rats do not respond to these agents, one concludes that they must undergo 1α-hydroxylation prior to producing physiological responses.

Two other less potent analogs have been in use for some time. The dihydrotachysterols (DHT_2 and DHT_3) are considered analogs of 1,25-diOH-D_3 because they have a hydroxyl group in a locus similar to the 1α-position of the hormone.[13] In the case of DHT_3 the 25-hydroxylated metabolite, 25-OH-DHT_3 has been shown to be the active form.[98] 25-OH-DHT_3 is 100 times less effective than 1,25-diOH-D_3. Nevertheless, DHT_3 is useful in raising blood calcium levels since its metabolism to 25-OH-DHT_3 is not feedback regulated.[98] Bone calcium mobilization responds linearly to increasing doses of 25-OH-DHT_3, whereas 20 IU of this agent is optimal in promoting intestinal calcium absorption in the rat.

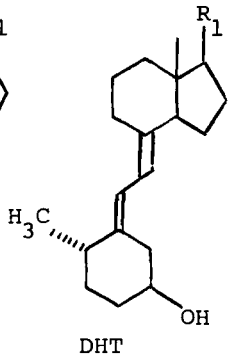
Finally, 5,6-trans-D_3 is effective in increasing blood calcium levels in humans with chronic renal failure but is about 100 times less potent than 1,25-diOH-D_3 and slightly less potent than DHT_3.[99,100] It too is an analog of 1,25-diOH-D_3 since it has a hydroxy group in the approximate locus of the 1α-hydroxy group of 1,25-diOH-D_3.

A series of analogs showed that biological activity is sensitive to changes in the side-chain of 25-OH-D_3.[101,102] Both 26-nor-25-OH-D_3 and 26,27-bisnor-25-OH-D_3 were 10 times less effective than 25-OH-D_3 in stimulating intestinal transport and bone calcium mobilization. They were

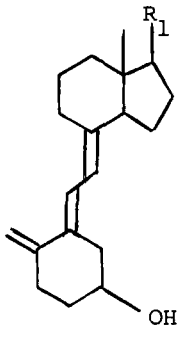
virtually inactive in anephric rats. In contrast, the 26-nor and 26,27-bis-nor analogs of 5,6-trans-D_3 were active but were 100 times less potent than 25-OH-D_3. An analog of 25-OH-D_3 with the side-chain shortened by one carbon, 24-nor-25-OH-D_3, was poor in stimulating intestinal processes, but was active in bone. Compounds with the side-chain reduced to that of a 20-hydroxylated-pregnane (pregcalciferol) were almost devoid of activity.



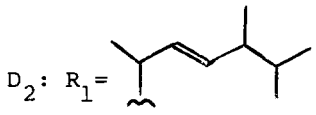
1-OH-D: R_2=R_3=OH
3-deoxy-1-OH-D: R_2=H, R_3=OH



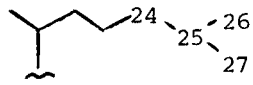
DHT



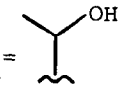
5,6-trans-D



D_2: R_1=



D_3: R_1=



pregcalciferol: R_1=

It is tempting to draw conclusions about the structural requirements of D3 metabolites and analogs necessary to elicit biological responses at tissue receptor sites from the above information. However, the situation is much too complex. Belsey et al.,[31] have shown that various D_3 metabolites and analogs have widely differing affinities for the rat plasma 25-OH-D/D carrier protein in vitro. The order of affinity is: 25-OH-D_3(1) > 25-OH-D_2 > 24-nor-25-OH-D_3 > 26,27-bis-nor-25-OH-D_3 > 5,6-trans-27-nor-25-OH-D_3 > 25-OH-DHT_3(6) > 1,25-diOH-D_3(35) ≃ 5,6-trans-25-OH-D_3 > D_3(100) > D_2 > DHT_3(1000) >> 5,6-trans-D_3. The numbers following several of the entries represent the amount needed relative to 25-OH-D_3 to displace [^{3}H]-25-OH-D_3 completely from the carrier protein. Even at concentrations 1000 times greater than 25-OH-D_3 5,6-trans-D_3 cannot fully displace the metabolite.

Despite the complexities, some correlations can be drawn. The activity of 3-deoxy-1-OH-D_3 suggests that the 3-hydroxyl group is not important to the ultimate expression of biological activity, but perhaps serves only as a recognition factor in intermediary metabolism.[95] The nature of the activity of the pseudo-1-hydroxy compounds (5,6-trans-D_3 and 25-OH-DHT_3) suggests the importance of a particular attitude in space for the 1α-hydroxyl group. That is, although liberties can be taken with the configuration in the A ring, the most potent compounds presently known are those with the A ring of D. D_2 and D_3 side-chains appear to be functionally equivalent in man. A hydroxyl at the 24-position can be substituted for a hydroxyl at the 25-position. But decreases in the length of the side-chain alter the magnitude and the spectrum of activity. Partial removal of the side-chain results in an inactive agent. The apparent importance of the 25- and 1α-hydroxyl groups needs further clarification.

Future research in the area of the action of vitamin D's metabolites and analogs at the molecular level will undoubtedly address itself to the many questions raised in the last decade.

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Chapter 31. Prodrug Approach in Drug Design

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General - Three approaches traditionally have been utilized in the search for new drugs[1] and all embrace the concept of the lead compound. The first is the general screen approach in which substances from a wide variety of sources are tested for general pharmacological effect. Any promising leads are further tested for specific bioactivity. The second involves mimicking nature by designing compounds that exert an effect similar to a naturally occurring substance. The synthetic steroids, prostaglandins and antimetabolites evolved from this approach. The third approach is chemical in nature and involves modifying a known drug to improve one property or minimize an undesirable one. The medicinal chemist has two options in this regard: (1) In conjunction with the biologist, he can prepare analogs of a lead compound in an attempt to increase potency, broaden or narrow the spectrum of therapeutic activity, decrease or eliminate toxicity and side effects and/or enhance other desirable properties absent in the lead compound. Preparation of such analogs can be considered chemically and biologically irreversible since the lead compound is not regenerated in vivo or (2) Preparation of prodrug derivatives of a parent drug molecule can be undertaken to modify some undesirable property of that molecule. In this instance, however, the parent molecule is regenerated in vivo. Properties that can be modified using the prodrug approach include (a) alteration of the time course of the drug in vivo such that transport, tissue distribution, excretion and metabolism are modified, (b) enhancement of bioavailability by increasing aqueous solubility, (c) increasing patient acceptance by eliminating pain on injection, bitterness, tartness, odor and gastrointestinal irritation caused by drugs (d) enhancement of stability of the final drug product and (e) improvement of drug formulation.

This review will focus on use of the prodrug concept in the design of drugs with improved pharmaceutical, physicochemical and biological properties. The emphasis of this area of medicinal chemistry has primarily been chemical in nature but with rapid advances in the areas of quantitated SAR's[2,3] and the biological and clinical-pharmacokinetic disciplines, the thrust in present and future efforts in this field logically will shift to an interdisciplinary approach. A prodrug has been defined by Albert[4] as "a substance which is converted after administration to the actual substance which combines with receptors". This definition includes metabolites of administered drugs that are the true active drug species as well as latentiated drugs. In this context then, prior chemical modification is not required and in all probability the metabolite could be administered directly. This is indeed the case with oxyphenbutazone which is a bioactive metabolite of phenylbutazone and responsible for its uricosuric effect[5].

Harper further elaborated on the prodrug concept by defining the term drug latentiation as "the chemical modification of a biologically active compound to form a new compound, which upon in vivo enzymatic attack will liberate the parent compound"[6]. He also stressed the necessity of a

knowledge of biology in the rational design of such compounds. Prodrugs have also been called reversible derivatives[7-12] and bioreversible derivatives[13] but for purposes of simplicity the term prodrug will be used throughout the discussion. Several reviews have been written dealing, in part, with prodrugs[14-16]. Recent reviews concerning rational prodrug design have stressed an interdisciplinary approach involving Hansch correlations, chemistry, biology and clinical pharmacokinetics[12,17].

Prior to embarking on a synthetic prodrug program, one should consider the following factors: (1) What functional groups on the parent drug molecule are amenable to chemical modification? Molecules containing alcoholic hydroxyl, thiol, and amine groups, carboxylic acids and ketones are customarily utilized for this purpose. (2) Are synthetic methods available for selectively modifying a particular drug molecule? Many drugs contain a variety of functional groups having similar chemical reactivities toward the modifying intermediate. Advantage may have to be taken of differences in stereochemistry or chemical reactivity of the functional groups involved. (3) Are chemical intermediates available at reasonable costs? Drugs are frequently very expensive to produce and further chemical modifications can become economically prohibitive if starting materials are expensive. Fortunately, most commonly used intermediates are available at reasonable costs. (4) Synthesis and purification of the prodrug should ideally be simple. Syntheses involving many steps ultimately decrease yields of prodrug and increase yields of undesirable side products. Unequivocal one or two step syntheses permit optimal yields that involve a minimum of purification. (5) The prodrug should be chemically stable in bulk form and compatible with ingredients in the dosage formulation. Problems that may occur include drug polymorph changes in suspension formulations, solution degradation phenomena in liquid formulations, melt back and caking in sterile products and interactions in solid dosage formulations (eutectic mixtures, chemical interactions of prodrug with excipients, lubricants, etc.). (6) Toxicity of the derivative portion of the prodrug must be considered due to the advent of increased emphasis by the various federal regulatory agencies of multispecies toxicological testing of all new drug entities. A number of relatively nontoxic derivatives include a variety of aliphatic and aromatic alcohols, amines and carboxylic acids as well as inorganic and organic acid and base salt combinations. Amino acid derivatives have also been commonly used for this purpose. (7) The parent drug molecule must be regenerated from the prodrug *in vivo*. The exclusive purpose of the derivative moiety is to modify some unwanted physicochemical or pharmacologic property of the parent molecule. *In vivo* lability can be enhanced by judicious choice of derivative. In the case of esters or amides, chemically activated derivatives can be made containing electron withdrawing substituents to promote ease of hydrolysis. The propensity of the prodrug toward *in vivo* enzymatic hydrolysis is critical since most prodrugs per se are devoid of bioactivity. Further, in certain situations (antibiotic chemotherapy), regeneration to the parent bioactive species is important for early onset of action. In cases where the prodrug is used to mask bitter taste or odor, improve absorption, eliminate pain on injection, decrease or eliminate gastric distress, etc., rapid regeneration to the parent drug is essential since the pharmacokinetic behavior of the parent molecule

should be maintained as closely as possible. The only difference between the prodrug and parent drug should be the elimination of the undesirable property in question. Thus, situations of this type necessitate a short half-life for the prodrug. The alteration of the pharmacokinetics of the parent drug via the prodrug becomes necessary when the transport, distribution, site or tissue localization, metabolism or excretion of the parent molecule necessitate modification. This occurs when one wants increased depot bioavailability (long acting antibiotics, neuroleptics, steroids) or localized drug activity (cancer chemotherapy). In these cases, an extended half-life for the prodrug is desirable. For prodrugs intended to be hydrolyzed in the gastric contents, acetals, ketals and ethers can be utilized that hydrolyze in acidic media. Prodrugs intended to exhibit slow hydrolysis (depot activity) include long chain aliphatic esters and derivatives sterically hindered at or near the site of hydrolysis (vitamin A α,α-dimethylpalmitate, steroid esters). Water soluble prodrugs can be synthesized to enhance bioavailability of the parent drug. Molecules containing carboxyl or amine moieties are easily converted to water soluble salts or complexes. Alcoholic hydroxyl functions can be esterified with derivatives containing amine or carboxyl functions that can be solubilized via a salt. This technique allows the chemist to adjust the hydrophilic/lipophilic balance of the parent molecule to arrive at the proper solubility both in water and tissue membrane. Phosphate or sulfate esters can be made that ensure adequate aqueous solubility (steroids, clindamycin). A wide variety and combination of derivatives are available to the medicinal chemist for modifying the physicochemical characteristics of the parent drug molecule.

Physicochemical - The chemical modification of a parent drug molecule to form its prodrug involves altering certain physicochemical properties of that molecule. Alteration of aqueous solubility of the parent molecule via the prodrug approach is the primary method whereby bioavailability (absorption) is enhanced. The addition of lipophilic (hydrophobic) groups to a molecule frequently improves drug passive absorption through epithelial tissue and is due to the increase in the biological membrane-water partition coefficient. The studies of Hansch and coworkers correlating *in vitro* partitioning behavior (octanol-water) with the biological response have been extensively reviewed[18,19]. Using the octanol-water model system, these correlations between the logarithms of the biological response (BR) and lipid-water partition coefficients (PC) can be either linear or parabolic in nature. The simplest form of this relationship is

$$\log (BR) = a + b \log (PC) \qquad \text{Eq. 1}$$

and has been proposed by several workers[20-22]. Equation 1 can be rearranged to a form of equation 2

$$(BR) = \frac{1}{a + b/PC} \qquad \text{Eq. 2}$$

and provides a better statistical fit for much of Hansch's data. It can be applied to linear as well as nonlinear data[23]. To realistically evaluate the role of lipophilicity on drug absorption and transport, a wide range of partition coefficients should be considered. In a study of the structure-

activity relationships of a series of triazenoimidazoles and nitrosoureas, Hansch et al[24] found a parabolic relationship between activity (1/C) and lipophilic character of the prodrug derivatives. For the nitrosoureas, it was found that when log P (P = octanol-water partition coefficient) was plotted vs. log 1/C (C = molar concentration of drug producing a 75% increase of lifespan of mice inoculated with L1210 leukemia cells), nitrosourea prodrugs having values of log P near zero or negative were the most bioactive. An extension of this study by Montgomery et al[25], using fourteen nitrosoureas and Lewis lung carcinoma (in mice), found the highest activity when log P was between -0.20 and +1.34. This study did not consider electronic and steric effects and their role in antitumor activity. A similar parabolic relationship was found for the triazenoimidazoles. Khan and Ross[26] demonstrated a linear relationship between log PC (ether-water) and carcinostatic activity of a series of monosubstituted 5-aziridino-2,4-dinitrobenzamides. The highest activity was found with the more hydrophilic and easily hydrolyzed derivatives. One further study illustrating the utility of the Hansch approach in prodrug design was carried out with a homologous series of nandrolone aliphatic esters[27,28]. A binomial relationship was found between log anabolic activity (weight increase in levator ani) and log partition coefficient (ethyl oleate-H_2O). The relationship was parabolic and bioactivity was related to distribution (hydrophilic-lipophilic balance) of the esters. These esters are known to hydrolyze (via esterase) prior to exhibiting anabolic bioactivity[29]. A study of testosterone esters revealed similar correlations[30]. It can thus be seen that modification of a parent molecule to alter its hydrophilic/lipophilic balance plays an important role in the ultimate bioactivity of the parent molecule. This bioactivity is a reflection of the absorption and transport (with subsequent bioconversion to the active parent molecule) of the prodrug molecule to the site of action. A more detailed discussion of the influence of prodrug lipophilicity on absorption has been written[12].

Solubility - Since most drug molecules are either weak acids or bases, dissociation and solubility of the drug in biological fluids and absorption are all interrelated phenomena. Usually the unionized form of a drug is absorbed more efficiently than its ionic species[31,32] and is due to the fact that the increase in the partition coefficient of the free acid or base vs. the prodrug derivative is much greater than the corresponding decrease in aqueous solubility. In a homologous series, aqueous solubility of the prodrug (e.g. a series of prodrug esters) would decrease by a factor of 4.0 for each additional methylene unit. Branched chain derivatives decrease solubility less than do straight chain derivatives[33].

The choice of modifying group and type of chemical linkage becomes important if one desires to rationally design a prodrug for a specific indication. Much valuable *in vitro* information can be obtained by the determination of solubilities and partition coefficients in a homologous series of prodrug derivatives.

Biological - While little is known concerning the variety and complexity of enzymes and enzyme systems in the human organism and their effect on drug hydrolysis and metabolism, there remains a body of literature available that

allows for qualitative predictions concerning drug substrate-enzyme interactions. Certain enzymes are ubiquitous in their distribution (e.g. esterases) while others are localized (amidases). The liver, the most important organ involved in drug metabolism, contains a variety of esterases, phosphatases, reductases, amidases, etc. that constitute the organisms most important line of defense (metabolism) to administered foreign substances (in this case drugs). Other organs and tissue contain certain specific enzyme systems indigenous to that tissue. Several enzymes responsible for the hydrolysis of a variety of prodrug linkages have been reviewed[12]. Perhaps the earliest indication of enzymatic activity toward a series of prodrug substrates can be obtained using in vitro enzyme preparations. Von Daehne et al[34] utilized the serum enzymes from a variety of animal species to determine the comparative half-life of hydrolysis of the prodrug pivampicillin HCl. Also studied were the gastric and intestinal mucosal homogenates from the dog and the human. A representative series of acyloxymethyl esters of ampicillin was studied using 10% human serum[35]as the enzyme source. Similar studies have been performed by Dittert et al[36] with a series of carbonate esters of salicylic acid.

Prodrugs and Protective Groups - The medicinal chemist is frequently confronted with the synthesis of an interesting drug molecule that involves several steps. In many cases it becomes necessary to enlist the aid of a protective group. The judicious use of protective groups frequently determines the difference between success or failure for a given synthesis. In the same way the medicinal chemist utilizes his knowledge of chemical reactivity differences within functional groups in a molecule to achieve synthetic success, so also can he utilize his knowledge of chemistry and biology in the rational approach to the design of prodrugs. Elements of this approach include chemical synthesis, in vitro studies (chemical and biological lability of the prodrug), physicochemical considerations (solubility, partitioning studies), choice of appropriate animal models for in vivo evaluation of pertinent prodrug biopharmaceutic and pharmacokinetic parameters and ultimately clinical pharmacokinetic considerations. Prodrugs can thus be considered as drugs containing specialized nontoxic protective groups utilized in a transient manner to alter certain properties not desired in the parent molecule. Several similarities and dissimilarities exist between the rationale for the use of prodrugs and protective groups. The chemistry of protective groups has been extensively reviewed[37-39] and many of these same groups have been utilized in the chemical design of prodrugs.

Types of Prodrugs - Aliphatic-aromatic esters - Esters can be formed in which the parent molecule constitutes the acyl portion or the alcoholic portion. Many classes of drugs administered via several routes of administration for a multitude of reasons have been modified using the ester prodrug approach. Table I illustrates some examples of this type of prodrug.

Table I

Prodrug Esters Used to Improve Various Properties of Parent Drug

Parent Drug	Ester Type	Route Administered	Property Modified	Ref.
1. Acetylsalicylic acid	Acetamidophenyl,	Oral	Absorption	40
	Carbonate anhydride	Oral	Sustained release	41
2. Prostaglandin	Glyceride	Oral	Increased half-life	42
	Alkyl		Duration of activity	43-47
	Anhydride		Duration of activity	48
3. Gitoxin	Pentaacetate	Oral	GI irritation	49
4. Nandrolone	Phenylpropionate, decanoate	IM	Duration of activity	29
5. Clindamycin	Palmitate	Oral	Taste	10
6. Hydrocortisone	Acetate	Topical	Absorption, duration of activity	50
7. Fluphenazine	Alkyl	IM	Duration of activity	51
8. Clofibrate	Ethyl ester	Oral	Absorption	52
9. Erythromycin	Thiolester	Oral	Taste, absorption	11

Carbonate ester prodrugs have been used for a variety of reasons with a number of drugs. Alkylcarbonate esters of salicylic acid[53] exhibited a greatly reduced incidence of gastric irritation compared to aspirin. The sedative trichloroethanol[54] was modified by forming its carbonate derivative which resulted in an odorless, tasteless, non-irritating solid that could conveniently be administered orally. Other carbonate esters include those of lincomycin[7-9] and clindamycin[10] and were prepared as tasteless forms of these extremely bitter antibiotics.

Hemiesters - Frequently the need exists for a water soluble form of a drug to enhance bioavailability or minimize pain on injection. One of the primary means of increasing aqueous solubility is through formation of hemiester derivatives. Thus, enhanced blood levels of hydrocortisone and prednisolone are observed after intramuscular and subcutaneous administration of their 21-hemisuccinate derivatives[55]. The esters are rapidly absorbed from the injection site and peak levels are seen at 60 minutes. Similarly, the hemisuccinate ester of 3-(o-tolyloxy)-1,2-propanediol has been utilized as a muscle relaxant and possesses greater water solubility, longer duration of activity and lower toxicity than the parent compound[56]. The 3-hemisuccinate ester of chloramphenicol has been advantageously used when bacteriostatic blood levels of antibiotic are needed shortly after administration. The ester afforded peak levels intramuscularly greater than twice those seen with chloramphenicol itself and occurred in three hours vs. six hours[57,58].

Phosphate esters - The abundance of phosphatases (orthoester phosphohydrolases) in the human organism[12] make phosphate ester prodrugs the logical choice for a variety of uses in drug therapy. This type of ester is usually highly water soluble, making it a suitable candidate for solubilizing lipophilic drugs via the covalent phosphate ester bond. These prodrug esters have been used for several indications including enhanced bioavailability and rapid onset of activity with steroids[55], localization of activity in cancer chemotherapy with diethylstilbestrol[14], decrease of bitterness of antibiotics[59], elimination of pain on injection[60-62], and decrease of GI side effects[63,64].

Nitrate and sulfate esters have found limited use in prodrug chemotherapy but examples exist where their use seems warranted. Pentaerythritol tetranitrate, a coronary vasodilator, is metabolized in man to pentaerythritol trinitrate and possesses a greater vasodilator activity than the parent molecule[65]. The transformation from the tetra to the triester occurs primarily in the liver and is catalyzed by the enzyme glutathione-organic nitrate reductase[66]. Various aspects of nitrate ester metabolism have been reviewed[67]. Sulfate ester prodrugs are rare but have been used on occasion with questionable results. Thus, sulfate esters of steroids[68] and the narcotic antagonist naloxone[69] have been synthesized but exhibited no advantages over the parent molecules. Corticosteroid sulfate esters[64,70] have been used for parenteral administration of poorly soluble steroids and when used orally, exhibited a significantly diminished incidence of gastric hemorrhaging over the parent drug.

Special esters of antibiotics have been prepared to take advantage of the excellent GI absorption exhibited by such prodrugs. Thus, acyloxymethyl esters of a wide variety of penicillins and cephalosporins have been prepared. The chemistry and biology of these unique prodrugs has been reviewed[17].

Amide - Amide prodrugs have been used only to a limited extent due to their relative stability in vivo. The distribution of amidases is localized in certain GI microorganisms[71], the liver[72] and certain types of neoplastic tissue[73,74]. Advantage can be taken of this enzyme localization in the design of prodrugs used in site-specific chemotherapy. This approach was employed by Tsou et al[72] in the synthesis of a series of nitrogen mustard amide (carbamate) prodrugs. One derivative, 1,1'-(2,2-dimethylpropylene) bisaziridinyl formate, was found to possess good antitumor activity in the Dunning leukemia system. Several mixed amide-ester prodrugs of erythromycin have been prepared in an effort to increase the bioavailability as well as eliminate the bitter taste properties of this useful antibiotic[11]. Several of the derivatives exhibited improved bioavailability and taste characteristics but at least one derivative exhibited an unusual drug-induced enteropathy syndrome (accumulation of lipid in macrophages)[75]. Other studies utilizing the amide prodrug approach have been undertaken[76,77].

Peptide (amino acid) - This type of prodrug linkage is bioreversible due to the presence in vivo of a class of hydrolases known as proteolytic enzymes or peptidases. These enzymes are present in duodenal and intestinal epi-

thelium and catalyze the hydrolysis of ester and amide as well as peptide bonds[78,79]. Certain of these enzymes are known to form enzyme-substrate complexes with specific amino acids. Thus, trypsin acts exclusively on peptide, amide, and ester bonds formed with arginine and lysine. The carboxypeptidases are known to hydrolyze both ester and amide linkages. Peptidases are formed in neoplastic tissue and glycineamide nitrogen mustard prodrugs have been made to exploit this fact[80,81]. A tryptophan nitrogen mustard derivative has also been studied[82] as a means of localizing the mustard "warhead" in neoplastic tissue. Glycine and alanine esters of chlorphenesin were made to provide water soluble derivatives suitable for injection[83].

Carbamate prodrugs have been used primarily as transport groups for drugs exhibiting restricted distribution in the body. Although carbamate hydrolyzing enzymes per se probably do not occur in vivo[84], non-specific amidases and esterases are probably responsible for hydrolysis. In an attempt to penetrate the blood-brain barrier, Verbiscar and Abood[84] prepared a series of carbamate esters of phenethylamine, amphetamine, ephedrine and hydroxyamphetamine. The o-carbomethoxyphenyl and o-nitrophenyl carbamate esters of amphetamine were shown to rapidly enter mouse brain and readily hydrolyze. A similar rationale has been used with diethyl stilbestrol[85] and alkylating agents[86].

Azo - Prodrugs containing the azo linkage have been used to some extent in drug therapy. The most famous example is the conversion of the prodrug Prontosil(p-[(2,4-diaminophenyl)azo]benzenesulfonamide) to sulfanilamide[87]. Conversion of prodrug to bioactive species occurs mainly through the intervention of gut microflora, the azo linkage being reduced by azoreductase enzymes. This same phenomenon is also known to occur with salicylazosulfapyridine, an agent used in the chronic treatment of ulcerative colitis. The azo linkage is split in the GI tract releasing sulfapyridine and 5-aminosalicylic acid[88,89]. The active agent appears to be sulfapyridine. Azoreductase enzymes are also present in the liver and several nitrogen mustard azobenzene derivatives were prepared as selective cytotoxic agents for use in liver cancer[90]. It was thought that the active alkylating agent would be enzymatically released in this specific tissue. This is an important consideration in certain types of neoplastic disease in which the relatively toxic alkylating agent must be localized to prevent systemic toxicity to the organism. Azo reductase enzymes are found as well in neoplastic tissue such as sarcoma 180[91], adenocarcinoma 755[92] and lymphoid leukemia L1210.

Phosphamide - Such derivatives have been utilized virtually exclusively in cancer chemotherapy in a number of variations (cyclic phosphoramide, phosphorodiamidic acid). Notable among the prodrugs in this area are cyclophosphamide [2-(bis(2-chloroethyl)amino)tetrahydro-2H-1,3,2-oxazaphosphorine-2-oxide] and isophosphamide[3-(2-chloroethyl)2-(2-chloroethyl)amino tetrahydro-1,3,2-oxazaphosphorine-2-oxide]. The metabolism of these compounds is complex and several metabolites have been suggested as responsible for cytotoxic activity including 4-hydroxy-and 4-hydroperoxycyclophosphamide[93,94], 4-ketocyclophosphamide[95], N,N-bis(2-chloroethyl)phosphorodiamidic acid[96], chloroacetaldehyde[97] and acrolein[98-100]. These drugs appear to have selective activity on a variety of neoplastic tissue and their pharmacokinetics

have been studied[101-103]. Enzymes involved in metabolism are found in liver microsomes and probably consist of a variety of amidases, oxidoreductases (aldehyde oxidase)[94], NADPH, N and O dealkylases[104], and phosphoroamidase[105]. Cyclophosphamide metabolism has recently been reviewed [106].

Glycoside - Prodrugs containing this linkage are found in nature as well as designed synthetically. This type of prodrug is employed for a number of uses including enhancement of resorption[107,108] and absorption[109], increased aqueous solubility[110], facilitate transport[111,112], and as protective groups[107]. Naturally occurring glycosides such as amygdalin and cycasin are hydrolyzed by gut microorganisms to mandelonitrile and methylazoxymethanol respectively[71]. The cardiac glycoside lanatoside C is hydrolyzed after oral administration to bioactive digoxin and involves both acidic and bacterial hydrolysis[113]. An excellent review of semisynthetic cardiac glycosides (including prodrugs) has recently appeared[114].

Ether - Ethers have not been used extensively in prodrug design because of their high resistance to enzymatic hydrolysis and metabolism. Several classes of drugs however have been modified using an ether linkage. Trimethylsilyl ethers have been made of chloramphenicol[115], prostaglandins[116] and norepinephrine[117]. A series of prostaglandin alkoxytetrahydropyranyl ethers have been prepared for controlled release of parent prostaglandin to avoid gastric side effects[118]. Several 1,3,5(10)-estratrien-17β-yl enol ethers exhibited oral activity equal to or greater than estradiol and displayed prolonged parenteral uterotropic activity[119]. Ether hydrolysis is acid catalyzed (gastric pH).

Acetal and ketal - These prodrug derivatives have found some utility for oral administration since they revert to the parent compound under acidic conditions. Convallotoxin acetals and ketals were prepared to enhance oral resorption of this drug[108]. Ketals of digitoxigenone and digoxigenone and acetals of digitoxigenin and digoxigenin were synthesized in an attempt to reduce toxicity of the parent glycosides[120]. Other acetals have been employed as protective groups in prodrug synthesis[121]. Normally, acetal, ketal and ether derivatives find limited utility in prodrug design.

Conclusion - This review demonstrates that it is frequently possible to design reversible drug derivatives (prodrugs) on a rational basis. These derivatives can be designed so that they are free of some undesirable property of the parent molecule and yet retain its desired biological activity. Two major factors are important in the design of such prodrugs (1) determination of what physicochemical properties are required in order to eliminate the undesired effect in the parent molecule; and (2) selection of a chemical linkage that alters this effect. Selection of a potential prodrug linkage also requires a knowledge of those enzymes or enzyme systems that catalyze their hydrolysis. Orally administered prodrugs rely on enzymes present in the gut, blood and liver to regenerate the parent drug whereas parenterally administered prodrugs encounter enzymes found in the circulation and liver. Enzymes indigenous to specific tissue provide the rationale for the design of site activated prodrugs.

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Chapter 32 The Molecular Aspects of Membrane Function

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The primary purpose of this report is to expose the medicinal chemist to the recent evidence for the molecular nature of cell membranes and their components. Since molecules which are associated with or are a part of cell membranes can be receptors for regulatory agents, important regulator-receptor interactions which were recently studied will be identified. Finally the most recent highlights advancing the understanding of the biosynthesis and chemistry of selected membrane components will be discussed. Evidence for membrane-associated inherited or acquired diseases is reported by Wallach in chapter 22 and elsewhere.[1-3]

In relating molecular aspects of cellular membranes to cellular processes, one must realize that a medicinal chemist is ultimately concerned with preparing substances that will change the rate of a given enzyme reaction usually found within a cell. If the enzymatic reaction which is ultimately to be controlled is represented simply by equation (1),

$$\underline{A} \xrightarrow[\text{enzyme}]{\text{cofactors}} \underline{B} \qquad (1)$$

its rate can be changed for a given amount of substrate A by

(a) altering the absolute quantity of the enzyme or by
(b) regulating the energy required (i.e. entropy or enthalpy) to place that system in the activated state.[4-7]

It has been established that process (a) can be effected either by substrates and end products associated with that enzyme reaction or by inducer hormones such as the steroid hormones and thyroxine. These substances enter the cell and in combination with receptors eventually act on the nuclear material to induce protein synthesis via increased synthesis of m-RNA and r-RNA. Process (b), which provides the necessary signal for proper energy requirements to activate a particular intracellular enzyme system, is, in addition to cofactors, controlled by

1. ligands such as ions, neurotransmitters such as catecholamines, acetycholine, tripeptides, macromolecular peptide hormones and antigens,
2. simple nutrients such as sugars, amino acids, fatty acids,
3. supramolecular complexes such as viruses, bacteria, fungi, other cells,
4. and other molecules such as toxins, lectins, and nucleic acids.

Each of these ligands, 1 through 4, express their action by first binding to a complementary molecule called a receptor, found on a cellular or intracellular membrane. The initial interaction (ligand & receptor), alone or in combination with another molecule, initiates a complex programmed response exemplified by the activation of the adenyl or guanyl cyclase system, which generates the energy requirements for the particular enzymatic reaction represented by equation (1). This report will be re-

stricted to the novel aspects of the structure, biosynthesis and chemistry of molecules associated with or contained in membranes and primarily with those that bind the regulatory ligands identified in process (b).

Membrane Structure and Components - The presently accepted working model for the cytoplasmic cell membrane of an eukaryotic cell is based primarily on studies of the red blood cell (RBC) membrane.[1,2,8,9] The membrane composition of leukocytes, cells of the liver, pancreas and other tissues have also been studied. The model depicted in Figure 1,

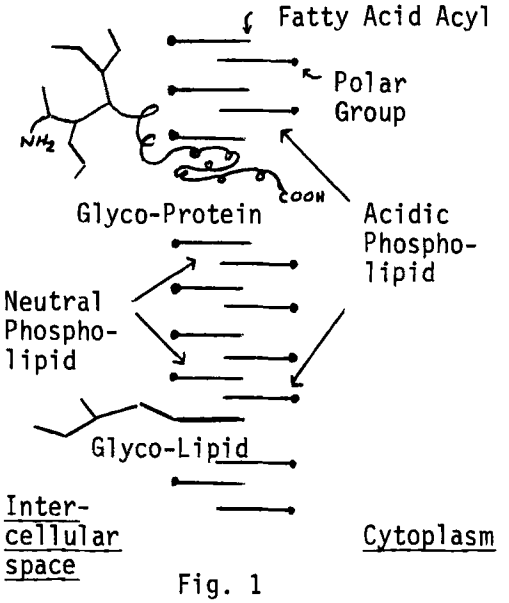
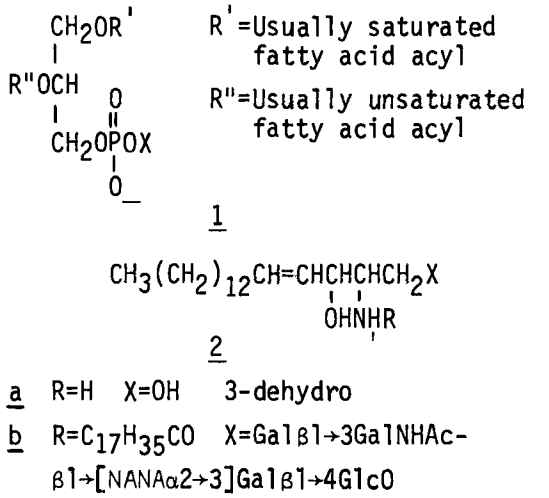
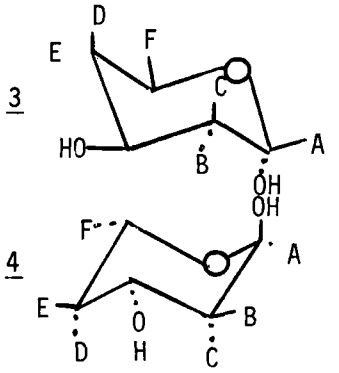


Fig. 1



which fits most of the experimental evidence, shows a cell membrane as an organized boundary. The external monolayer of a lipid bilayer is composed of glycerophospholipids of the choline type [1, $X=CH_2CH_2N^+(CH_3)_3$]and glycolipids like 2b which are derived from sphingosine. The inner, in this case cytoplasmic, monolayer of the membrane lipid bilayer is made mostly of acidic glycerophospholipids exemplified by 1, where $x=CH_2CH_2N^+H_3$, $CH_2CH_2N^+(CH_3)_3$, $CH_2CH(NH_3)^+(COOH)$, $CH_2CHOHCH_2OH$, and inositol. Imbedded and partially imbedded proteins, which have often been designated as integral and peripheral proteins respectively, are composed of amino acids and carbohydrates. The relatively less polar amino acids are mostly incorporated into the integral proteins. The more polar amino acids, like serine, threonine, hydroxylysine, hydroxyproline, cysteine and asparaginine, which form glycosidic linkages with carbohydrates, are found more abundantly in the protein portion which is exposed on the outer layer of the membrane. When glycolipids and glycoproteins form a part of the membrane protein, the oligosaccharide portion is found on the external

surface of the membrane. The carbohydrates which are incorporated into the glycolipids and glycoproteins are D-glucose (3a), N-acetyl-D-glucosamine (3b), D-xylose (3c), D-galactose (3d), N-acetyl-D-galactoseamine (3e), L-arabinose (3f), D-mannose (3g), L-fucose (4a), and N-acetylneuraminic acid (4b). Cholesterol, other minor lipids and water are also contained in cell membranes. Recent reports on their structure or function are available.[1,10]



3		A	B	C	D	E	F
a	α-D-Glc	H	OH	H	H	OH	CH_2OH
b	α-D-GlcNHAc	H	NHAc	H	H	OH	CH_2OH
c	α-D-Xyl	H	OH	H	H	OH	H
d	α-D-Gal	H	OH	H	OH	H	CH_2OH
e	α-D-GalNHAc	H	NHAc	H	OH	H	CH_2OH
f	α-L-Ara	H	OH	H	OH	H	H
g	α-D-Man	H	H	OH	H	OH	CH_2OH
4							
(a)	L-Fuc	H	OH	H	OH	H	CH_3
(b)	NANA	CO O H	H	H	H	NH Ac	CHOH CHOH CH_2OH

Ligand-Membrane Receptor Interaction - The report will continue by concerning itself particularly with structural aspects of the oligosaccharide portions of glycoproteins and glycolipids associated with membranes.

The glycoproteins and glycolipids associated with membranes are known to be involved with ligand-receptor actions and in many cases the carbohydrate structure of the macromolecule is at least partially known (Table 1).

TABLE 1

GLYCOLIPID OR GLYCOPROTEIN	LIGAND	FUNCTION
Glycolipid (RBC)	Cholera Toxin	Macromolecular recognition
Glycophorin-glycoprotein of erythrocyte	Flu virus, Lectin	Recognition, infectivity, cell division, contains MN and ABCH blood group antigen
Component a (or Band III) glycoprotein of erythrocyte	Ion	Transport
Glycoprotein	Insulin	Hormone Receptor[11]
Glycoproteins of nerve cells	Choline, 5-Hydroxy-tryptamine	Neurotransmitter receptors[12,13]

Ligand - Membrane Receptor Structure - Structure elucidation of animal cell glycoproteins indicates that certain predicitions can be made for the position of a specific carbohydrate in a given oligosaccharide chain. Fuc and NANA, which are linked to other hexoses at the 1α and 2α anomeric hydroxyl position respectively, have only been found at the terminal position in the oligosaccharide portion of the glycoprotein or glycolipid of

animal cells. The other carbohydrates have been found at the terminal or internal position of oligosaccharides and linked α or β at the anomeric hydroxyl group, depending on the carbohydrate to which it is attached. GlcNHAc, Gal, GalNHAc, and Man, when linked internally, can participate in branching at more than one position other than the anomeric hydroxyl. These hydroxylic positions have been found to be 4 for Glc, 3, 4 or 6 for GlcNHAc, 2, 3, 4 or 6 for Gal, 3 and 6 for GalNHAc, and 2, 3 and 6 for Man. Xyl and Ara have reported to be found rarely in animal glycoproteins, abundantly in the plant kingdom. Carbohydrates are linked to proteins or lipids at the anomeric C-atom and the O-atom of the hydroxyaminoacid or lipid. When Asn or Cys participate in the linkage to the carbohydrate, the attachment is at the N-atom of the amide or S-atom, respectively. The following glycoaminoacid linkages have been demonstrated: Glc to ceramide or S-cysteine; GlcNHAc to Asn; Xyl to Ser; Gal to HydroxyLys, Ser, Threo or Cys; GalNAc to Ser or Threo; Ara to HydroxyPro; and Man to Ser or Threo. Recent excellent reviews, which describe the detailed molecular aspects of the carbohydrate and peptide portion of glycoproteins and receptor structure and interactions, are available.[14-18,78]

Advances have recently been made in the elucidation of the molecular aspects of membrane-associated glycolipids and glycoproteins. The ganglioside 2b and related gangliosides may normally have important roles in the regulation of cell growth and c-AMP mediated responses.[19] It was confirmed that the ganglioside 2b specifically interacts with choleraexoenterotoxin by precipitate formation and toxicity.[20] The isolated carbohydrate moiety of the ganglioside, i.e., 2b minus ceramide, neither precipitated choleragen nor interfered with the reaction of the toxin and its antibody. However, polyacrylamide gel electrophoresis revealed a specific interaction between the sialooligosaccharide and the choleragen. Identical results were found with sialo sugar derivative prepared by reductive amination of 2b, followed by N-acetylation of the glucose unit at the reducing end.

A novel, physiochemically homogeneous lipoglycoprotein with MW 256,000 was isolated from human RBC membranes.[21] It is rich in NANA, Gal and hexoseamines. The intact substance prevented attachment to erythrocytes of unheated and heated, smooth and rough lipopolysaccharide and protein lipopolysaccharide of all gram-negative bacteria tested. It did not react with other bacterial antigens and therefore is referred to as "lipopolysaccharide receptor." Strong evidence has accumulated that the lipid A part of the lipopolysaccharide is responsible for attachment to tissue components.

Glycophorin, the major sialoglycoprotein of the human RBC, has yielded four unique glycopeptides after tryptic digestion which were characterized with respect to its amino acid and carbohydrate composition and receptor activities for phytohemeagglutinins and influenza virus.[22] The physiochemical properties and molecular weight of the monomeric unit of the major glycoprotein were described.[23] Isolation and partial characterization of the lipophilic fragment of human RBC membrane and the

monomeric unit of the major glycoprotein were reported.

The principal high affinity receptor for Concanavalin A is the minor glycoprotein of human RBC.[24] Freeze fracture and freeze-etch and molecular labelling studies elucidated the nature of interaction of the minor glycoprotein and the protein spectrin.[25]

A hypothetical model for the carbohydrate chain of H-2 glycoproteins was presented on the basis of sequence analysis of carbohydrates in H-2b and H-2d glycopeptides.[26] In this case, evidence is presented which indicates H-2 glycoproteins do not need carbohydrate moieties to retain antigenic activity. Heterogeneity of the HL-A antigen may be due to variable sialic acid content.[27] It was found that an IgE myeloma protein contains 4 oligosaccharide units per heavy chain, one of which contained only mannose and N-acetylglucoseamine, and the other three B-glycopeptides contained neuraminic acid, galactose, fucose, mannose and N-acetylglucoseamine. The complete sequencing of these oligosaccharides was reported.[28] The oligosaccharide units of the B-glycopeptides appear to be closely related to the neuraminic acid-containing glycopeptides of IgG, IgM and IgA.

Structural studies on an acetylcholine receptor from Torpedo californica show that it contains 5-6% carbohydrate and has two binding sites.[12] Dopamine-β-hydroxylase, which is partly membrane bound, was found to be a tetrameric glycoprotein.[29,30] Studies of its binding to Con A indicate that it has a highly branched polysaccharide portion which contains mannose and glucose as terminal residues.

Lectin-Receptor Binding and Structure - The ability of lectins, which are cell-agglutinating proteins occurring primarily in plants, to participate in selective binding to carbohydrate units of receptors on cell membranes has been well established.[31] Recent studies center around elucidation of the nature of binding to lectins and structures which serve as receptors for them.

Receptor glycopeptides for pea hemagglutinin of Group B human RBC and for Con A from calf thymocytes were isolated and characterized with respect to their carbohydrate content.[32,33] In the former case, the receptor, which has a molecular weight of about 4000, shows a 32 times higher receptor site binding activity than does the monomer glucose. The glycopeptide receptor for pea hemagglutinin from rabbit RBC was isolated and a partial structure was assigned to it. About 90% of the receptor-site activity for the pea hemagglutinin is due to the presence of two galactose units on non-reducing terminals of the saccharide chain, in spite of the fact that the pea agglutinin is not inhibited by this sugar. In addition, the nature of Con A binding to receptors has been based on studies of nmr spectra with N-trifluoracetyl α and β-glucoseamine,[34] and specificity of binding to particular hydroxyl groups on the carbohydrate moiety.[35,36] The mechanism of action of the toxic lectins abrin and ricin have been elucidated.[37] The toxins appear to have one binding site for

galactose-containing carbohydrates, whereas nontoxic agglutinins present in the same seeds have two binding sites. It appears that these lectins have a haptomer moiety responsible for binding and an effector moiety responsible for specific toxication. Bandeiraea simplicifolia lectin has been the first α-D-galactopyranosyl binding lectin (anti-B lectin) to be purified and characterized.[38,39] The nontoxic agglutinins present in the seeds of Abrus precatorius and Ricinus communis were separated from ricin and abrin and the nature of their carbohydrate binding was studied. Excellent immunochemical studies on the specificity of soybean agglutinin with selected mono- , di- , and oligosaccharides were reported.[40]

Glycerophospholipids and Lipid-Protein Interactions - An abundance of research has been reported in recent years on the function of phospholipids. References to earlier reviews are available.[41-43] Phospholipids have been directly implicated in supporting the activity of enzymes (phospholipid dependence) and restraining their maximum activities (phospholipid constraint).[44] It is still unclear how phospholipids support enzyme activities at the molecular level. In this regard, D-β-hydroxybutyrate dehydrogenase, which has been purified to homogeneity, was shown for the first time to specifically require phosphatidyl choline [1,X=$CH_2CH_2N^+(CH_3)_3$] for enzyme activity.[45] The apoenzyme which is devoid of lipid is incapable of binding DPNH, its coenzyme. It was also reported that the nature of interactions between lipid and protein in plasma lipoproteins is such that phospholipids are an absolute requirement for combination with apolipoprotein in order to carry fat in serum lipoproteins.[46] Phospholipid binding to human apo-LDL was also studied.[47,48] Sodium ion transport was demonstrated with Na^+-K^+ ATPase in phospholipid vesicles.[49]

Biosynthesis of Glycoproteins - After the primary assembly of a peptide chain which is under RNA control, the attachment of the first sugar via transferases occurs while the peptide is attached to the polyribosome or shortly thereafter. This is an outcome of the kinetics and high specificity of the transferase for an acceptor molecule.[16-18,50] It is not known if the carbohydrate transferases responsible for the peripheral sequences are exclusively Golgi enzymes. Since the Golgi membrane may fuse with the plasma membrane, it is not unlikely that glycosyl transferases exist at the surface of the cell. From an overall point of view, oligosaccharides of glycoproteins can be synthesized in three known ways:[1,50-53]

1) Carbohydrates are transferred stepwise from their nucleotide derivatives to growing carbohydrate chains of proteins.
2) Carbohydrates are transferred to a growing oligomeric carbohydrate which is linked to a polyprenol (see Table 2) intermediate until a final transfer to the protein moiety.
3) Carbohydrate nucleotides as well as polyprenol linked carbohydrates serve as immediate precursors for a stepwise formation of the glycoprotein.

Evidence for the existence of the third possibility was provided for mannose in yeast cells.[53] The enzymatic synthesis of $(Man)_5$-$(GlcNAc)_2$-PP-Dolichol and (GlcNAc)-PP-Dolichol by mammalian, microsomal systems and evidence for the transfer of the oligosaccharide portion of the glycolipid to protein was reported.[54-56] A system in human lymphocytes that utilizes dolichol phosphate as a carrier of mannosyl residues for the synthesis of

a glycoprotein or complex polysaccharides was studied.[57] GDP-mannose is the mannosyldonor, but other sugars can also be transferred to lipid intermediates.

In regard to the synthesis of glycolipids of the sphingolipid type found as receptors in cell membranes, results show that gangliosides are not synthesized at the surface membrane. As with membrane glycoproteins, these glycolipids appear to be glycosylated in the endoplasmic reticulum and Golgi apparatus during transport to the surface membrane.[58,59]

Progress in Chemistry - It is beyond the scope of this chapter to review advances in the synthesis of all membrane components such as proteins, oligosaccharides, glycopeptides and lipids. However, references to important review articles and selected highlights in the chemistry of membrane components important to the medicinal chemist are given.

A review on the synthesis of phospholipids is available.[60] Within the last year a brief, versatile synthesis of 3-dehydrosphinganine[61] and the synthesis of Tay-Sachs globioside,[62] which can represent a model for glycolipid synthesis, were reported. Structural studies using 220 Hz PMR proved useful in analysis of glycolipids.[63] Combined mass spectrometry and vapor phase chromatography of methylated ganglioside derivatives afforded structural information on the entire molecule.[64] The preparation and properties of phosphatidal ethanolamine (1,R'=CH=CHR, R''=fatty acid acyl, X=$CH_2CH_2N^+H_3$), an important intermediate in plasmalogen synthesis, was described.[65] The novel displacement of cyclic sulfite and phosphate esters of 2-O-benzylglycerol provided a facile route to the silver salts of dl-1-acyl-2-O-benzyl-glycerol-3-phosphates, which are important intermediates in phospholipid synthesis.[66] The synthesis of 1-stearoyl-2-oleyl-phosphatidyl inositol was achieved.[67]

Oligosaccharides and glycopeptides, which form a segment of natural glycoproteins and whose synthesis were reported during the last year, are 2-acetamido-2-deoxy-[3-O-α-L-fucopyranosyl]-D-glucose, 6-O-α-L-fucopyranosyl-D-galactose,[68] 6-O-β-D-galactofuranosyl-D-galactopyranose,[69] 4-O-β-D-mannosyl-L-rhamnopyranose,[70] 3-and 4-O-β-D-galactopyranosyl-L-rhamnose,[71] 3-glycosyloxyprolines,[72] and selected β-D-glucopyranosyl dipeptides utilizing serine and threonine.[73] Important advances in carbohydrate chemistry which should be included are reports on homogenous Koenigs-Knorr reaction with cyclic polyethers,[74] stereoselectivity of the reaction alkyl-β-galactopyranoside and β-D-xylopyranoside peracetates promoted by mercuric bromide and oxide,[75] and complete ^{13}C and H nmr spectroscopy of permethylated α and β-D-galactopyranose and disaccharides.[76] Additional reviews in carbohydrate chemistry are available.[77,78,82]

The isolation and structural characterization of the polyprenols, (also called antigen carrier lipids), which play an important role in oligosaccharide and glycoprotein biosynthesis, were reported by several research groups.[79-80] The polyprenols fall into several groups, depending on their source and on the number and stereochemistry of isoprene units in

the oligomeric chain (see Table 2). Controlled prenol homologation which now allows the sequential synthesis of E or Z prenol units was reported.[81]

TABLE 2

POLYPRENOL	STRUCTURE	ISOPRENE UNIT
Betulaprenol	ω-T-T-C-$(C)_{1-4}$-C-OH	ω=Terminal
Ficaprenol	ω-T-T-T-$(C)_{5-7}$-C-OH	T=trans(E)
Dolichol	ω-T-T-C-$(C)_{12-16}$-S-OH	C=cis (Z)
		S=Saturated

In summary then, the increased knowledge of the molecular aspects of membrane components should allow the medicinal chemist to utilize models in the design of new substances. These would control ligand-receptor binding, and thus important biological reactions relating to hormonal control, cell-cell and cell-macromolecule interactions. Instead of modifying the ligand, as has been successfully done in the past, the chemist can concern himself with receptor modification. The immunologist has been successful in this sense by providing us active and passive immunity. On the other hand, the chemist can now design substances which could control glycoprotein or glycolipid synthesis and interactions by

1) attempting to control specific glycosyltransferases or glycosidases with synthetic acceptors for which they may be highly specific,
2) attempting to control ligand-receptor interactions by providing false exogenous receptors which would compete with the intact membrane receptor for different ligands, and
3) controlling glycoprotein synthesis with specific carbohydrate donor nucleotides or polyprenols.

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Chapter 33. Organocopper Reagents

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Several very comprehensive reviews on various aspects of organocopper reagents have appeared in the period between 1972-1973. Normant[1] has covered both organocopper(I) compounds and organocuprates in synthesis, while G. H. Posner has reviewed the conjugate additions of organocuprates in 1972.[2] In 1973, A. E. Jukes[3] reviewed the Organic Chemistry of Copper, and H. O. House surveyed a number of important reaction types of organocopper reagents in his Robert Welch Lecture.[4] The intent of this chapter is to minimize duplication of material covered in the above reviews and to concentrate on the chemistry of organocopper reagents which has emerged since 1973. The presentation of this material will be organized on the basis of structural-types of copper reagents.

General Comments - The versatile and sometimes uniquely selective behavior of organocopper reagents renders them comparable in synthetic utility to Grignard and organolithium reagents. It is already recognized that the problems of thermal instability,[3] oxygen sensitivity and structural complexity associated with the use of organocopper reagents require the utmost of care in experimental handling. Despite these complexities, organocopper reagents have emerged as a unique and indispensable class of synthetic reagents.

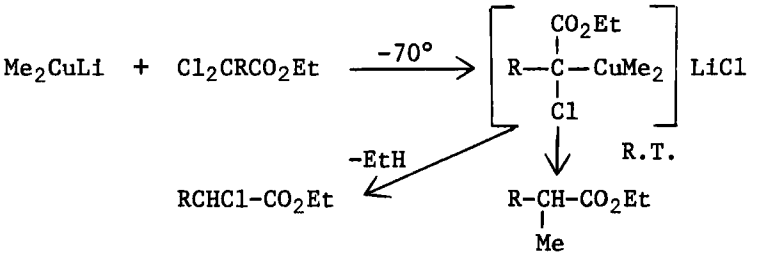
Alkylcopper Reagents - The most significant and recent contribution to the understanding of conjugate additions of dialkyl cuprates to enones comes from House's correlation of enone-reduction potentials with 1,4-additions.[4] As a qualitative rule, unsaturated esters and ketones which have reduction potentials lying in the range of minus 1.64 up to 2.3 volts react rapidly with lithium dimethylcuprate to form conjugate addition products. It has also been pointed out by House that with less reactive enones, raising the reaction temperatures increases the competitive deprotonation to form the corresponding enolates.

From a synthetic standpoint, reports of dialkylcuprate reactions have emphasized a 1:1 stoichiometry of copper reagent to substrate. A general ketone synthesis has appeared which involves the reaction of dialkylcuprates with S-alkyl and S'-arylthioesters.[5] The yields of ketones are quite good (60-90%), and complete utilization of both alkyl groups of the organocuprate is accomplished.

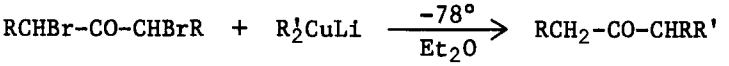
$$R_2CuLi + 2R'COSR'' \xrightarrow[-10^{\circ}]{Et_2O} 2R'-CO-R$$

Two groups have reported on the reactions of α-halocarbonyl compounds with dialkylcuprates in 1973. Normant has studied the reaction of lithium dimethylcuprate with α-dichloroesters[6] as well as a comparison of

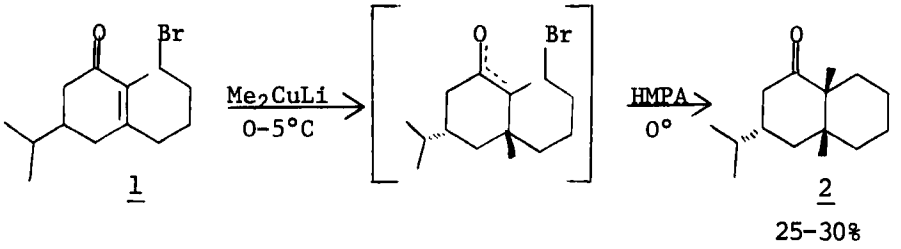
copper enolates of α-chloroesters with their magnesium and lithium counterparts.[7] The action of lithium dimethylcuprate on α,α-dichloroesters at room temperature leads to the formation of α-chloro-α-methylesters together with equal amounts of nonchlorinated α-methylesters. The same reaction performed at -70° produces α-chloro ester enolates which can be quenched to the α-chloroester, or upon heating to room temperature can be transformed into α-methyl ester enolates.[6]



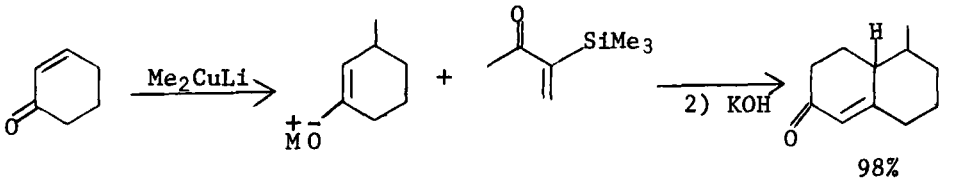
The reaction of dialkylcuprates with α,α'-dibromoketones at -78° produces high yields of α-monoalkylketones, presumably via cyclopropanone intermediates. This procedure is mild but unfortunately is limited to symmetrical dibromoketones.[8]



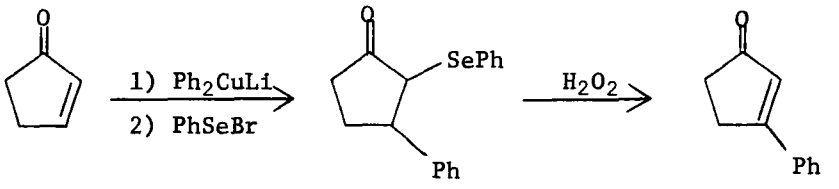
Considerable interest has been generated in achieving the alkylation of enolate ions generated regiospecifically via organocopper conjugate addition reactions. Posner[9] has used the intramolecular alkylation of the enolate generated from the conjugate addition to enone 1 in the construction of the valeranone skeleton 2. This two-step sequence is highly stereoselective but the overall yield for this process is low.



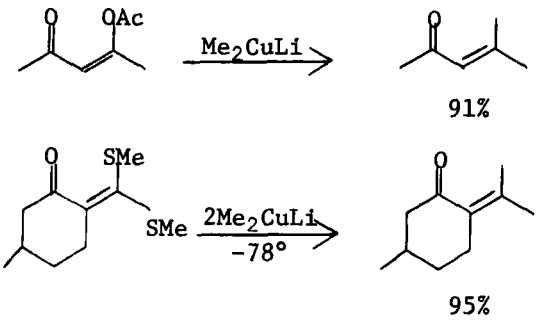
As part of an application to syntheses of decalins, copper enolates have also been trapped by α-silylvinyl ketones in a Michael type addition.[10,11]



A facile synthesis of β-substituted enones from enones utilizes the selenoxide elimination method after trapping a copper enolate with phenylselenenyl bromide.[12]



Lithium dialkylcuprates react well with enol acetates of β-diketones[13] and α-dithiomethylene ketones[14] to yield the corresponding β-alkyl-α,β-unsaturated ketone.



Methylcopper(I) derivatives which contain an α-substitutuent such as cyano[15] or carbethoxy[16] react selectively with allyl halides to give, respectively, γ,δ-unsaturated nitriles and γ,δ-unsaturated esters in good yields.

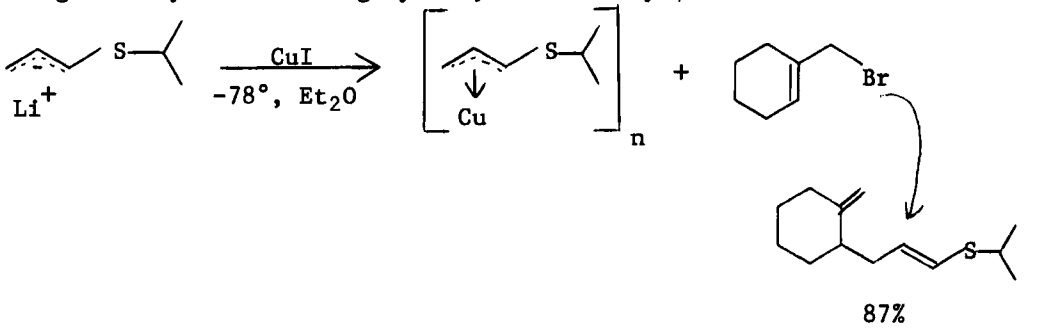
$$CuCH_2E + R_2C{=}CHCH_2Br \longrightarrow R_2C{=}CHCH_2CH_2E$$

E=CN or CO_2Et

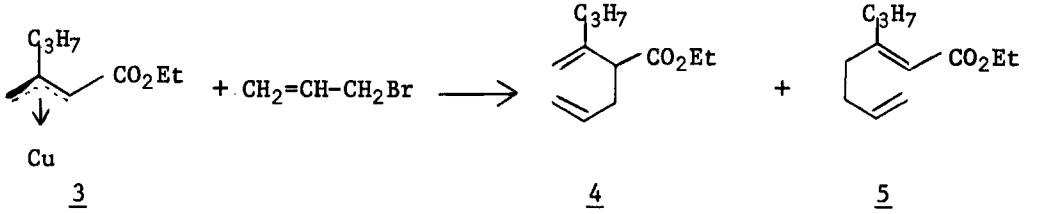
Full details have been reported on the mechanism and synthetic aspects of reactions of dialkylcuprates with tosylates.[17] Lithium dimethylcuprate adds to both primary and secondary tosylates in high yield while more substituted alkylcuprates give high yields only with primary tosylates (70-100%).[17] The opening of simple epoxides with organocuprates

usually gives the alcohol derived from attack at the least substituted carbon.[18] Such epoxide openings are usually sluggish and go best at 25° in ether as the solvent. Epoxides of cycloalkenes yield predominately the trans-alkylcycloalkanols in good to excellent yields.

Allylcopper Reagents - The role of sulfur-stabilized allyl anions in synthesis has gained considerable momentum in recent years. While the allyllithium reagents have received the most attention, much less chemistry has been reported on the corresponding allylcopper species. Yamamoto has reported the preparation of a polymeric isopropylthioallylcopper reagent which was generated from the corresponding lithium species.[19] This allylcopper reagent reacts with allyl halides via a S_N2' type mechanism and undergoes alkylation in high yield, exclusively γ to sulfur.

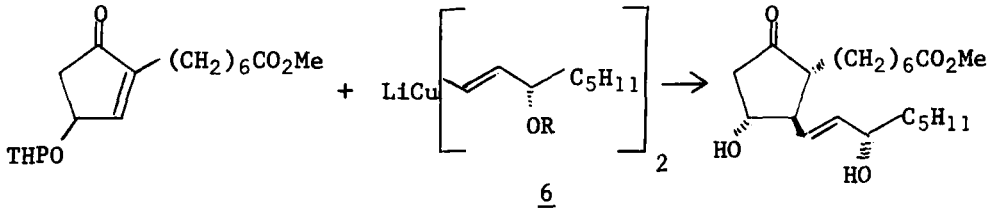


A partial solution to the age-old problem of preferential α-alkylation of anions derived from unsaturated esters was reported recently. Copper dienolate 3, formed from the corresponding lithio species and cuprous iodide, gave substantially greater amounts of γ-alkylation (4:5, 44:56) with simple allyl bromides.[20]



3 4 5

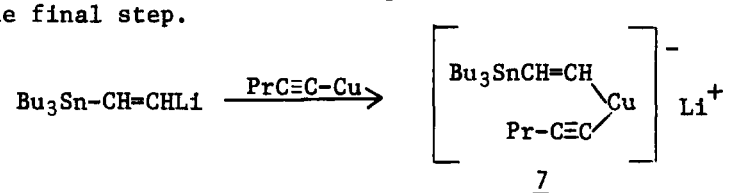
Vinylcopper Reagents - During the past few years, the intense interest in terminal-vinylcopper reagents revolved around synthetic approaches to prostaglandins. Sih has reported full details on the reactions of 2-alkylcyclopentenones with vinylcuprates such as 6.[21] The vinylcuprate is prepared from the corresponding lithium species which in turn are obtained by metallation of the trans-vinyl iodide. The conjugate addition is highly stereospecific and yields diastereomeric trans-isomers.



6

A very efficient conjugate addition of the analogous cis-divinylcuprate to prostanoid precursors was utilized in another total synthesis of PGE_1. In this case the resulting 13-cis-prostaglandins were stereospecifically transformed into the natural 13-trans-15α-PGE's.[22]

Several new vinylcopper synthons were described in 1974. Corey reported a mixed vinylcuprate 7 which accomplishes the overall synthetic feat of conjugate addition of acetylene to an enone.[23] Reagent 7 is prepared from the corresponding vinyllithium species and a copper acetylide. Oxidative elimination of a tri-n-butyl tin moiety releases the acetylene in the final step.



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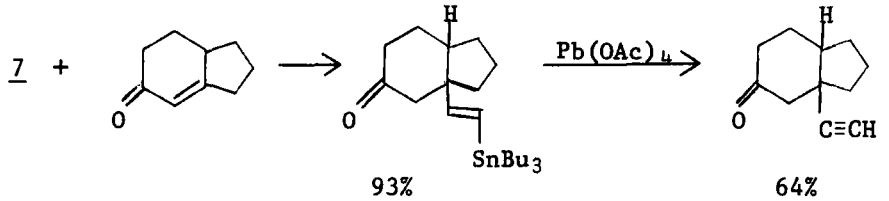
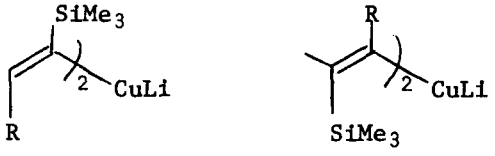


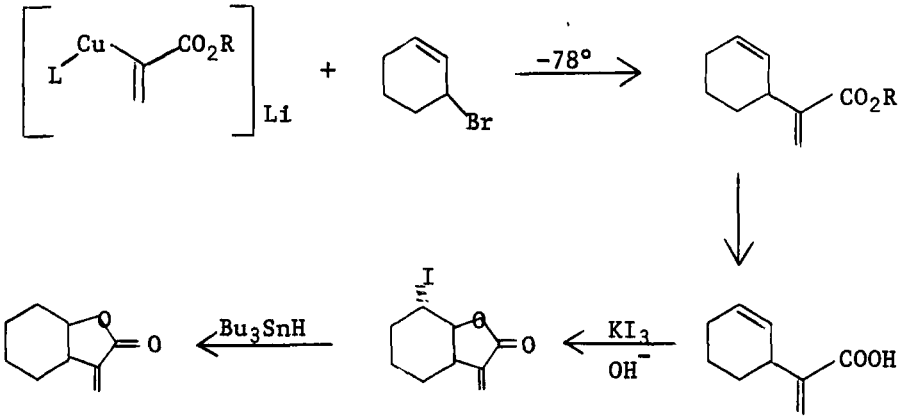
93% 64%

Vinylcuprates which contain an α-trimethylsilyl moiety have been reported and used as latent acyl anions and enolate carbanion equivalents. Thus, cuprates of general structures 8 and 9 have been prepared from their lithio precursors and added in high yields to enones.[24] The subsequent conversion of the resulting vinyl silanes to carbonyl functions is, however, still a difficult process to effect in high yield.

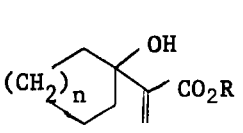


8 9

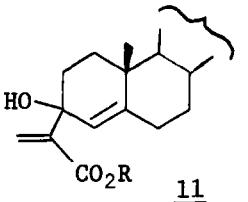
A new mixed cuprate containing an acrylate ligand has been shown to specifically alkylate allyl halides to yield nonconjugated dienic esters.[25] The adduct from cyclohexenyl bromide serves as a precursor to cis-α-methylenebutyrolactones.



This same acrylate synthon can be added in high yields to the carbonyl group of most enones and simple ketones to give adducts such as 10 and 11.[26]

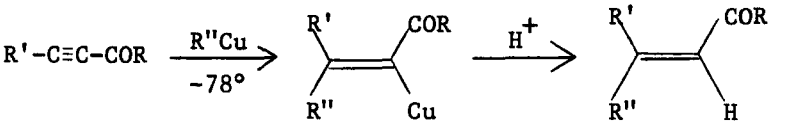


10

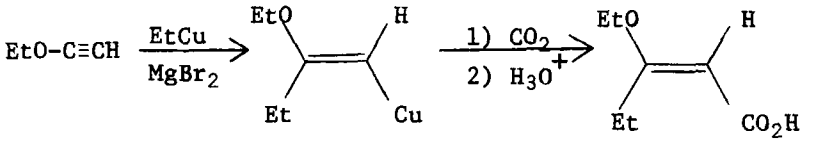


11

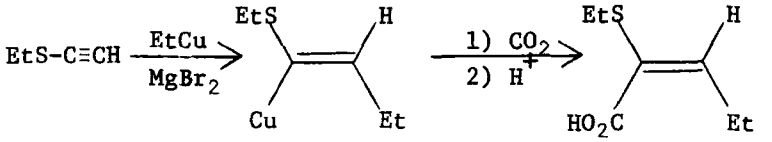
The generation of functionalized vinylcopper reagents by the addition of alkyl cuprates to acetylenes has received considerable attention in recent years. The conjugate addition of polymeric organocopper reagents as well as organocuprates to acetylenic esters, ketones, amides and acids has been reported to proceed via cis-addition. The resulting vinylcopper species can be quenched at low temperatures to yield trisubstituted alkenes stereospecifically.[2,27]



The stereoselective syn-addition of alkylcopper reagents to various propargylic alcohols, ethers, bromides and acetates has been reported.[28] The regiospecific and stereospecific addition of alkylcopper reagents to alkoxy and thioalkoxyacetylenes has led to the formation of vinylcopper reagents. Carbonation of these vinylcopper intermediates leads to high yields of unsaturated carboxylic acids.[29]

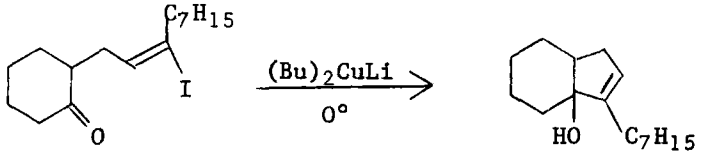


86%



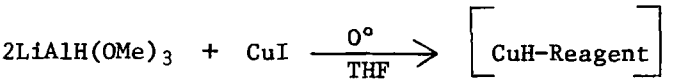
82%

The intramolecular addition of an organometallic reagent to a carbonyl group is a formidable synthetic operation because of generation of the metal-carbon bond in the presence of a ketone. When di-n-butylcopper lithium is treated with keto-vinyl iodides such as 12, a vinylcopper reagent is generated which is capable of intramolecular cyclization to form an allylic alcohol.[30]



12 90%

Copper Hydrides - The use of copper hydrides as reducing agents has received some attention in recent years. Initially, it was shown that solubilized $Cu(I)H \cdot PR_3$ was capable of functioning as a reducing agent.[31] Masamune has demonstrated that a mixed copper hydride reagent, 13, prepared from lithium trimethoxyaluminum hydride and cuprous iodide cleanly affected the reductive removal of halide and tosylate groups.[32] This reagent was shown to displace bromide with retention and mesylate groups with inversion of configuration.



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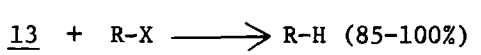


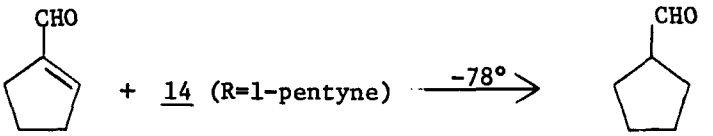
X=Cl, Br, OMs

Selective reductions of α,β-unsaturated carbonyl compounds have been accomplished with several classes of mixed copper hydride "ate" complexes of general structure 14.[33,34]

$$RLI + CuH \xrightarrow{Et_2O} LiCuHR$$

14

When R=n-Bu, t-Bu, i-Pr, an excess of the reagent reductively removes allylic tosylates in high yield and reduces cyclohexanone to cyclohexanol quantitatively.[33] The conjugate reduction of enones proceeds in high yield at -40°C and does not reduce the ketone or ester groups under such conditions. An analogous set of hetero-substituted and acetylenic mixed cuprates has also been used for selective enone reductions (14, R = O-t-Bu, -S-Ph, 1-pentyne).[34]



86%

Mixed Cuprate (I) Reagents - Some of the practical limitations of various organocuprate reagents includes the use of thermally unstable sec- and tert-alkyl cuprates and the need for a large excess of copper reagent to obtain high conversions. The advent of using inert ligands as part of the mixed cuprates enhances the stability of the cuprates in some cases and maximizes the efficiency of transferring desired organic ligands. The preparation of various hetero-substituted mixed alkyl cuprates can easily be accomplished by the following scheme.[35]

$$Het\text{-}H + BuLi \xrightarrow{THF} Het\text{-}Li \xrightarrow{CuI} Het\text{-}Cu$$

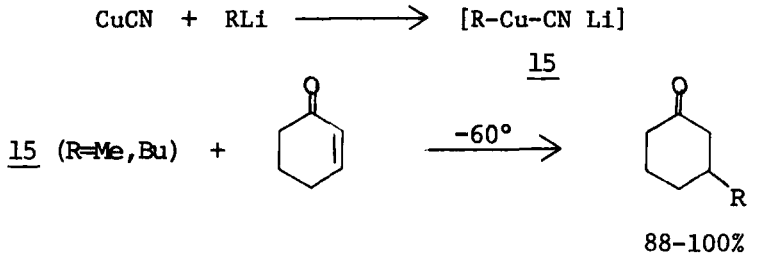
$$Het\text{-}Cu + RLi \xrightarrow[-78°]{THF} [Het\text{-}CuR]^- Li^+$$

Het≡ t-BuO; PhO; t-BuS; PhS; Et_2N-

The stability of these mixed cuprates follows an order of PhS>PhO>t-BuO>t-BuS~Et_2N. The (PhSCuR)Li reagent is the most effective for selective conjugate addition and substitution reactions using secondary and tertiary alkyl R-groups.

Mixed cuprates in which R and R' groups are differently hybridized carbon ligands also show selective transfer of one ligand.[36,37] Mixed

cyanoalkyl cuprates[15] have been prepared from cuprous cyanide and alkyllithium reagents.[38]



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