Molecular Modification in Drug Design

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FOREWORD

ADVANCES IN CHEMISTRY SERIES was founded in 1949 by the American Chemical Society as an outlet for symposia and collections of data in special areas of topical interest that could not be accommodated in the Society's journals. It provides a medium for symposia that would otherwise be fragmented, their papers distributed among several journals or not published at all. Papers are reviewed critically according to ACS editorial standards and receive the careful attention and processing characteristic of ACS publications.

PREFACE

The specific aim of this symposium is a review of the salient features of molecular modification, which has become the hallmark of modern drug research. Molecular modification guided by deduction, induction, and serendipity via the prepared mind has produced in the last 25 years more potent and more useful drug agents in various areas of therapeutics than have been reported in all previous history.

But a brief cursory view of this two-day program reveals the unique, professionally synergistic character of modern drug research through presentations by some of the most outstanding medicinal chemists and clinicians that have contributed to the modern science of chemical therapeutics. Operating from the broad and deep bases of modern chemistry and pharmacology which are rooted in the enormous informational and technical resources of a score of other sciences, has evolved a new methodology, molecular modification, that is of the profoundest importance to all mankind.

As final attestors to the "magic ring" of informational feedback—that is, the spirit of molecular modification in the light of pharmacologic evaluation—are the clinicians who gather the ultimate evidence of its success. Thus, the present symposium may be viewed as a monument to the coming of age of a new science which has grown naturally—that is to say, organically—through integration and differentiation since its conception from those ancient parents—chemistry and medicine.

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Molecular Modification in Modern Drug Research

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Examples of molecular modification in nature include morphine, codeine, and thebaine among the opium alkaloids; atropine, scopolamine, and cocaine among the tropine alkaloids; and testosterone, progesterone, and estradiol among the sex hormones. Through structure modification the medicinal chemist has developed local anesthetics from cocaine, useful agents from atropine and scopolamine, MAP from progesterone, and Enovid from estradiol. Prontosil, the first sulfonamide of therapeutic significance, gave rise to a series of valuable sulfa drugs and drugs for tuberculosis, diabetes, gout, leprosy, hypertension, and cardiovascular disorders. Critics of the drug industry have found fault with the many congener drugs that offer choice to the clinician. The new drug regulations have created a new environment which will require medicinal chemists to choose compounds more selectively for development and studies in man.

A little over three years ago, at the 50th Anniversary Symposium of the Division of Medicinal Chemistry, I spoke of the sources of discoveries in chemistry which during the previous quarter century brought forth the greatest array of useful, chemical therapeutics ever known to the practice of medicine. The last two sentences of my talk (*6*) bespeak the boundless pride and peerless confidence that pervaded the halls of the symposium.

The accomplishments of the past gave us the strength and inspiration to meet the challenges of our time and the years ahead. Let us strengthen our dedication to the service of mankind so that those who inherit our responsibilities may derive even greater pride from their own achievements when they meet at the centennial anniversary 50 years from now.

Much has happened and crowded the past three years!

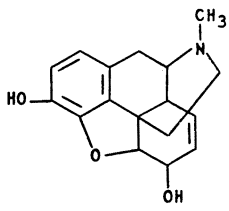
For those of us who have devoted our lives to medicinal chemistry, and particularly to the search for chemotherapeutic agents in the laboratories of the pharmaceutical industry, events of the past three years have brought bewilderment and chagrin. For the first time our objectives were questioned and our contributions were belittled. These criticisms of us as research workers in medicinal chemistry have left their mark on us—for how else can we explain a Symposium on the Influence of Molecular Modification on Drug Design?

If the soundness of molecular modification as a tool of research in medicinal chemistry has been questioned, let us remind ourselves and our critics that chemists have arrived at molecular modification by emulating nature itself. Molecular modification by nature has been going on since the beginning of life on this planet. In the relatively short time since chemistry has developed into a science, chemists had learned well from nature the significance of small changes in chemical structures of drugs to the biological activity in living organisms.

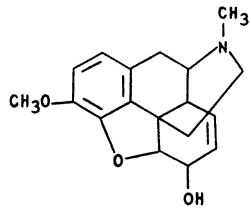
Let us recall a few examples of molecular modifications of drugs as they occur in the plant and animal kingdoms.

Opium Alkaloids

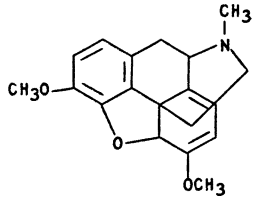
Opium, the sun-dried latex of the unripe fruit of *Papaver somniferum*, cultivated from early times for this drug, contains at least 23 alkaloids. Of the major alkaloids three—morphine, codeine, and thebaine—contain the morphinan ring system.



MORPHINE



CODEINE



THEBAINE

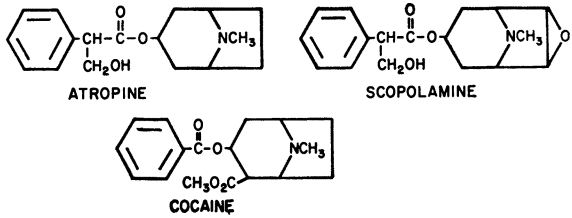
Morphine for over a century has been the most important agent for the relief of pain. Codeine, with its phenolic hydroxyl group protected by methyl, has about one tenth the analgesic activity of morphine, but as such has found its place in the relief of mild pain and as an antitussive agent.

Thebaine, which differs from codeine by the addition of methylene groups and the removal of two hydrogen atoms, is neither an analgesic nor an antitussive. Instead, it resembles strychnine and brucine in its spinal convulsant properties. It is not used in medicine; it has utility, however, in the synthesis of codeinone derivatives, some of which are useful as analgesics.

The chemist has unraveled the morphine-analgesic pattern and has gone far beyond nature in his quest for an analgesic with the power of morphine but without its liabilities.

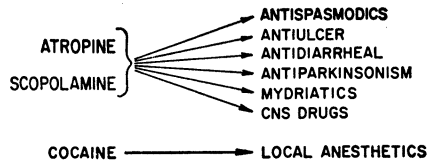
Tropine Alkaloids

The tropine alkaloids are another illustration. Atropine, scopolamine, and cocaine are structurally related, each having the tropine nucleus. While atropine and scopolamine overlap in pharmacodynamic activities, cocaine has uniqueness in this series, being a topical anesthetic and a potent addicting agent.



In this case, nature's molecular modification cannot be attributed to a special metabolism within a species. Cocaine and atropine are not related botanically. They originate from different plants.

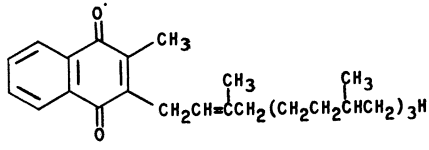
Extension of Tropine Alkaloids. Here again, medicinal chemists have gone beyond nature by molecular modification. While cocaine possesses topical anesthetic activity but no local infiltration value as an anesthetic, a number of very useful, local anesthetic agents such as procaine and lidocaine have been derived from our knowledge of the structure of cocaine. Chemists have broadened the scope of usefulness of the cocaine structure.



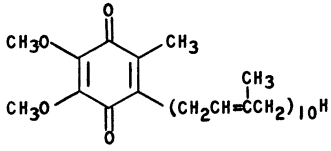
Atropine and its related oxide, scopolamine, have given rise to a number of important agents useful in the treatment of a number of disease conditions. The drugs applicable to each class of pharmacodynamic activity are all anticholinergics and block the parasympathetic nervous system with varying degrees of specificity. By molecular modification, it has been possible to produce a series of compounds having qualitative effects resembling cutting of the parasympathetic nerve to a particular organ.

Vitamin K and Coenzyme Q

Vitamins K_1 and K_2 exist in nature and are essential to the animal for their effect on the blood-clotting mechanism. While 2-methylnaphthoquinone itself and its related derivatives having isoprenoid side chains in position 3 also promote blood coagulation in varying degrees, coenzyme Q_{10} shows no activity in the blood-coagulating mechanism. Coenzyme Q_{10}, also called ubiquinone because of its ubiquitous occurrence in animal tissues, is important in its own right, since it plays an essential role in oxidative phosphorylation. As in the case of the vitamin K series, the size of the isoprenoid side chain does not appear to be critical with respect to the oxidative phosphorylation activity of the coenzyme Q series.



VITAMIN $K_{1(20)}$



COENZYME Q_{10}

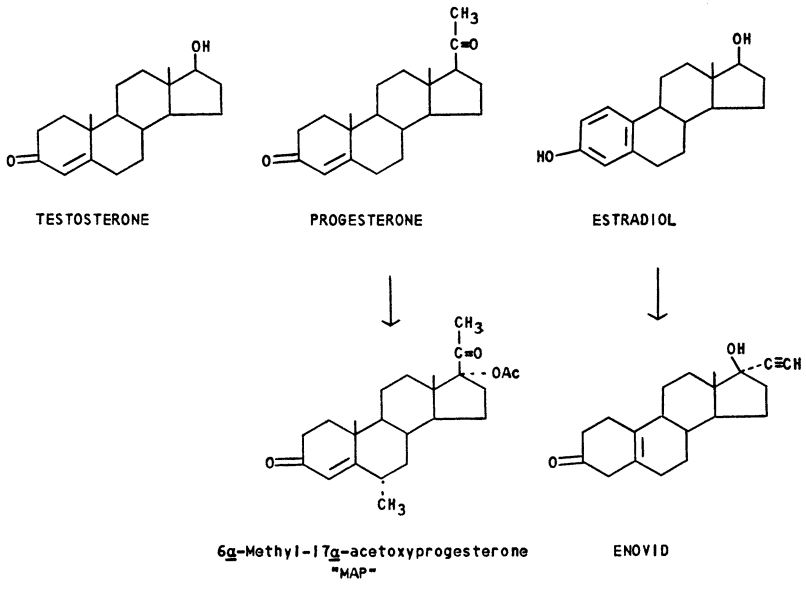
Sex Hormones

Nature is at its best in the sex hormone field. Here are three hormones containing steroid nuclei, each of which has a very specific pharmacological activity and a very specific role in sex physiology.

Testosterone, the testicular hormone, is identified as a male hormone. Estradiol (17β), which differs from testosterone only by loss of a methyl group and aromatization of ring A, is the ovarian hormone. Progesterone is the progestational hormone and is essential for fertilization and maintenance of pregnancy. Once again medicinal chemists, by further alteration of structures, have made more potent oral and more useful progestins.

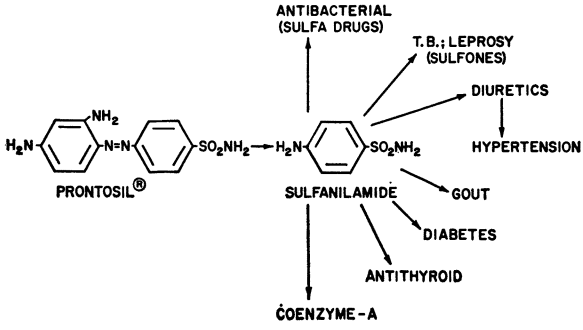
6α-Methyl-17α-acetoxyprogesterone is at least 100 times as potent as progesterone by the oral route. Norethynodrel (Enovid), also derived through molecular modification of the estradiol type structure, is the first significant orally active anovulatory agent and oral contraceptive.

Up to now, I have placed emphasis on molecular architecture in nature and how medicinal chemists elaborated on nature. Let us now turn to some structures discovered in chemical laboratories, structures conceived and synthesized by man.



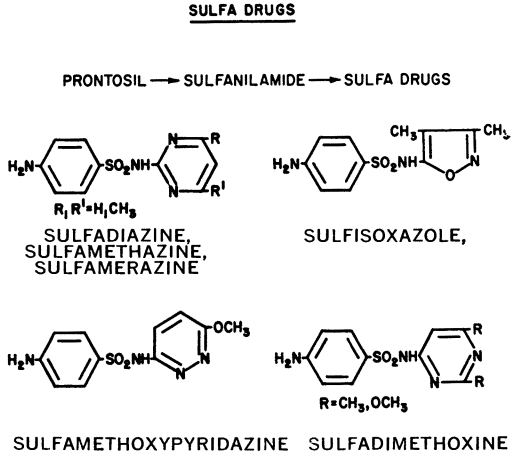
Sulfanilamide Developments

No review of medicinal chemistry and particularly of the utility of molecular modification in drug development is complete without at least a brief discussion of the sulfonamides. Domagk's discovery in 1934 of the therapeutic activity of the dye Prontosil rubrum, followed by the brilliant investigations of the French chemists Fourneau and the Tréfouëls, implicating the metabolite sulfanilamide as the active agent, gave new life to chemotherapy. Since then, medicinal chemists have synthesized literally thousands of compounds containing sulfonamide groupings in a search for better drugs and for a more complete understanding of molecular structure and biological activity. As this process went on, it became apparent to medicinal chemists and pharmacologists that the sulfonamide grouping had a rather exalted position, since new and unexpected biological effects were being uncovered, occasionally by accident. Let us briefly review where the initial observations of Domagk have taken us.



The foregoing diagram shows in a classical manner one of the greatest virtues of molecular modification. The medicinal chemists following Domagk's discovery have broadened the usefulness of the sulfanilamides and have created new drugs for conditions entirely unrelated to bacterial infections. Who in the early forties could have predicted that the sulfonamide grouping would have had so great an impact on the practice of medicine? Some of our best advances in the medical sciences are represented here.

Sulfa Drugs. The sulfa drugs are still important agents for the control of bacterial-infectious diseases.



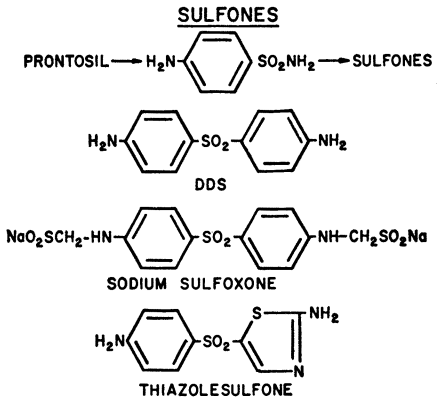
Over the years, however, the sulfa drugs used in medicine have not remained static, as one might have predicted in the early forties. Three of the most widely used sulfa drugs today have been developed rather recently: sulfisoxazole, sulfadimethoxine, and sulfamethoxypyridazine. Sulfanilamide, itself, sulfathiazole, and sulfapyridine have been displaced largely by these drugs in human therapy. This illustrates drug obsolescence.

Sulfones. In 1937, there were two distinct leads arising from the Prontosil discovery for agents active against bacterial infections, the sulfa drugs represented by sulfanilamide, and the sulfones, represented by 4,4'-diaminodiphenyl sulfone. Each had its own advantages and disadvantages. Medicinal chemists were divided into two groups and while the protagonists of the sulfa drug had written more exciting chapters, the sulfones did reach distinction.

4,4'-Diaminodiphenyl sulfone may be considered the first major advance in the chemotherapy of tuberculosis. However, it was overshadowed a few years later by streptomycin. DDS did find an important place in the treatment of leprosy following its first clinical use in 1943.

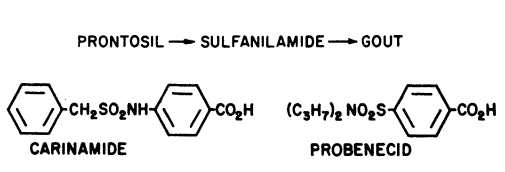
The initial clinical studies served as the first indication that Hansen's disease could be treated by means of a specific antibacterial agent. Up to then, leprosy had for centuries been regarded as incurable.

DDS is still used widely for leprosy, even though other related and possibly better tolerated compounds have been uncovered. Its low cost makes it ideal



for mass treatment in areas of the world where cost of medication is a serious problem.

Probenecid and Gout. The story of the discovery of probenecid and its usefulness in the treatment of gout is well known to medicinal chemists. Certain sulfanilamide compounds were observed to decrease the renal clearance of penicillin in a study aimed primarily at increasing the usefulness of penicillin during those days when this antibiotic was difficult and expensive to make.

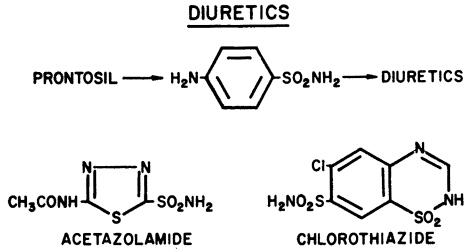


The first compound to arouse interest in the laboratory is the sulfamyl derivative of *p*-aminobenzoic acid. This was followed by carinamide, which was found to produce high plasma levels of penicillin for long periods of time even when relatively low doses of the antibiotic were administered orally. Probenecid, a structural modification of carinamide, was much more potent on a weight basis and on a dosage basis practical for oral administration. In fact, the combination of penicillin and probenecid was a practical oral formulation and was widely used for the treatment of infections until the costs and availability of penicillin were no longer factors and the better orally active penicillins became available.

Notwithstanding its obsolescence in the treatment of infectious diseases, probenecid found a very important place in the physician's armamentarium because of its uricosuric activity. Its interference with the reabsorption of uric acid through the renal tubular membrane was an unexpected observation. Probenecid is the first useful synthetic compound for the control of uric acid excretion and the age-old disease, gout.

Diuretics and Hypotensives. One of the side effects of sulfanilamide therapy recognized during the early development of the sulfa drugs as anti-infection agents was the loss of sodium in patients. This observation led to the discovery

that sulfanilamide inhibited the enzyme carbonic anhydrase in carrying out its function—namely, the catalysis of the exchange between carbon dioxide and bicarbonate. Medicinal chemists and pharmacologists quickly recognized the implication of these observations and directed efforts towards molecular modification of the sulfanilamide molecule in the hope of finding a superior and much needed diuretic.



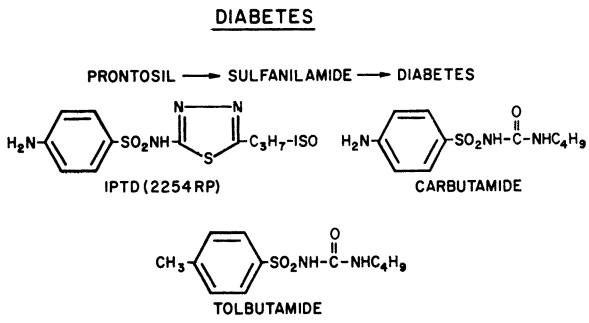
The search was rewarding and opened up new vistas. The first breakthrough in this field was acetazolamide, a potent inhibitor of carbonic anhydrase. This compound induces increased sodium ion excretion and diuresis. It found wide medicinal application in the treatment of cardiac edema, acting presumably by suppression of carbonic anhydrase activity in the renal tubules. Unfortunately, drug tolerance developed in patients, limiting its utility. This deficiency stimulated medicinal chemists to persist in the modification of the sulfanilamide molecule and led to the discovery of chlorothiazide and the thiazide family of drugs.

I need not dwell on the importance of the chlorothiazide discovery. The thiazides have not only proved to be useful diuretics but they have also found very wide application in the treatment of hypertension used either alone or in combination with other hypotensive drugs. As a class they probably represent the greatest chemotherapeutic advance in the cardiovascular diseases.

Contrary to the views expressed in many published reviews of this development, the hypotensive effect of chlorothiazide was predicted by those responsible for its discovery. On the basis of known effects of the "rice diet" in hypertensives, the chemists and pharmacologists almost from the beginning of their studies believed that an effective saluretic agent would control the disease more uniformly and effectively than diet.

Diabetes. It has been known since the early forties that some of the sulfa drugs produced hypoglycemia as a "side effect." One of the most potent hypoglycemic sulfa drugs, 2254RP, was described by Janbon in 1942.

Oddly enough, the isopropyl group in position 5 is essential for this effect, since the corresponding methyl or ethyl congeners are respectable sulfa drugs. 2254RP was abandoned and the significance of its hypoglycemic property lay dormant until Franke and Fuchs in 1955 reported on the oral hypoglycemic effects of carbutamide. Since the incidence of side effects, particularly blood dyscrasias, was considered excessive, it was never marketed in this country. The methyl isostere tolbutamide was found to be superior with respect to side effects and became the first orally effective agent for the control of diabetes in the "adult-onset" stable patient.



The long-sought "substitute for insulin" arose from the astute observation made in the clinic on a potential sulfa drug—a serious side effect turned into a valuable asset.

Accomplishments in Drug Research

The structures shown illustrate just a few of the important advances made through molecular modifications. Starting with Prontosil, we have come a long way. New chapters of greater dimensions have been added to our textbooks of medicinal chemistry. A red-orange dye, of little consequence to Bayer in 1934, has dramatically changed the treatment of important diseases and has saved an untold number of lives. The exciting drama of the intervening years flowed from the labor of countless medicinal chemists employing their most potent weapon of drug design.

Up to now, I have attempted to define molecular modification and its usefulness to the medicinal chemist with living examples. Although our knowledge of the relationship of molecular structure to biological activity has not reached a state where medicinal chemists can circumvent the arduous task of synthesizing large numbers of compounds, we are encouraged by the progress which has been made during the past two decades. We are beginning to sense a pattern which comes into play whenever an interesting observation is made in our screening programs. We have developed over the years a perspective of the field and a capacity to be more selective in our programs to modify structures. Medicinal chemistry stands in its development today where organic chemistry did 30 years ago.

Nevertheless, we have been proud as a group of the accomplishments of the past quarter century. Not only has our science advanced in its fundamentals, but it has had a tremendous impact on the practice of medicine. We were delighted with statements such as those which appeared in a recent report from the New York Academy of Medicine (5): "It is estimated that 90% of present prescriptions are for drugs that were unknown as therapeutic agents 15 years ago." Hence, it is readily understandable why we have become indignant in recent days with what some of us feel to be unfair and even irresponsible attacks on the fruits and methods of our research. It was all the more painful to us that among our critics are eminent scientists of varied medical disciplines, some of whom have shared with us the experiences in creating useful drugs.

Whether we like it or not, the criticisms are loud and clear. To a large measure they have had a traumatic impact on our lives as medicinal chemists and have precipitated a new era for the pharmaceutical industry and its research. An irreversible chasm now separates our past and future.

Let me quote a few of the statements about the effects of medicinal chemistry in the pharmaceutical industry on the practice of medicine.

From the report of the New York Academy of Medicine (5), an analysis of present trends in drug therapy, not meant to be an attack on research for new drugs:

> Now the stage was set and the performers were ready for growth, expansion, and extension. All this came with such a surge that new products appeared in what may fairly be called flood proportions. . . . It has been estimated that of the four hundred new products introduced by pharmaceutical companies each year, not more than forty are new entities. . . . The remainder are congeners—i.e., slight modifications of existing products. But it should be remembered that slight changes in a chemical compound alter its therapeutic properties, and most chemical compounds are capable of being modified in almost innumerable ways. Probably the actual new drugs that represent improvement in therapy are fewer than six a year.

From this same report (5) another quotation is pertinent.

> Formerly, the industry made what the physician prescribed; now the physician prescribes what the industry makes.

Wilkins (7) in his presidential address to the American Pediatric Society in May 1962:

> The structures of cortisone and testosterone have been altered with changes in side chains and double bonds, so that now we look for the new "steroid of the month" instead of the "book of the month."

He goes on to ask:

> How in the world can any physician be expected to keep up with the new pharmacy?
>
> How many of these new remedies are necessary or even bring improvements in treatment?

Finally, let me quote a few sentences from an editorial (2) in a recent issue of the *Journal of the American Medical Association*, entitled "Use and Misuse of Antibiotics." After stressing the dramatic benefits which followed the discovery of antibiotics, the editorial goes on to say:

> Unfortunately, however, the plethora of new drugs and combinations of drugs may be a mixed blessing to physicians who attempt to thread their way through a maze of conflicting claims concerning such complex matters as sensitivity spectra, blood levels, potency, and safety.
>
> Many of the new antimicrobials are clearly outstanding contributions to the art of chemotherapy and are, as such, real triumphs of pharmacological research. Others are molecular modifications of established compounds and are introduced with claims that some desired feature—such as greater potency, more rapid absorption, more sustained blood levels, reduced bacterial resistance, or fewer side effects—has been added to the more familiar properties. Since all such claims need to be viewed skeptically until they have been confirmed by impartial investigators and extended experience, compounds of this type often make only a numerical rather than a qualitative contribution to the list of available drugs, and thus add only confusion.

Our Reaction to Criticism

Although our normal reaction to this criticism is to shout that such statements indicate a lack of appreciation by our critics of what medicinal research is about, we cannot dismiss it so easily. When we point out that seemingly minute differences in structure can sometimes produce profound and beneficial physiologic differences, we are on firm ground. If we were not concerned with small differences in chemical structure, many of our essential drugs would have never been synthesized. It was Hench (2) who stressed this view when he pointed out at the Kefauver Committee Hearings in 1959 the very slight difference between 11-dehydrocorticosterone and cortisone. The latter compound, cortisone, "does all sorts of things in man," whereas 11-dehydrocorticosterone, having one less oxygen atom, "does absolutely nothing."

We are on less firm ground, however, when we as scientists insist that small differences in structure can sometimes bring about significant pharmacological differences in man which are:

Not always apparent in the same patient.
Often not definitive nor generally accepted.
Often extremely difficult to assess by the best-devised studies, but felt to be real.

In such complicated cases, it may not always be easy to maintain scientific objectivity—but we can accept no less than complete objectivity. Clinical studies must be designed and redesigned, extended and re-extended until the data are definitive and the evidence for claims is substantial enough to convince ourselves and those expert in the field. To bring out a new compound without such thorough-going studies, relying on the anticipated marketing response as the acid test for the usefulness of the drug, would be to abdicate our scientific and ethical responsibility.

The difficulty is—What can we accept as the acid test? Clinical experimentation is unlike laboratory experimentation in chemistry or even in biology. In the chemical laboratory variables can be controlled to a high degree. There is still a very large measure of control in the biological laboratory, particularly if statistical control procedures are used to balance the differences in biological variation. However, once out of the laboratory the problems of interpretation are much more difficult. Experiments take much longer, and results are much less certain. With the human patient as the subject of research, the situation is often frustrating to the laboratory scientist who is conditioned to relatively simple and controlled situations. Diagnoses, prognoses, and interpretations are complex and uncertain. Weeks and months can be consumed in obtaining answers to even simple questions, and years for complex ones. Take the field of antibiotics or of the cortical steroids, where clinical effects are dramatic—even today, after years of extensive use, there is no simple answer to such broad questions as which is the best and which is the safest.

Why have I chosen to raise these serious issues at a symposium devoted to scientific accomplishments? It is because I believe that all of us as medicinal chemists, individually and collectively, must do some earnest soul-searching in these times. We cannot ignore the criticisms that we have created an abundance of new drugs related structurally and therapeutically, which in the opinion of many serious-minded clinicians contribute little but confusion. It is difficult

to weigh on the scales of scientific objectivity the reasons for making available as drugs 13 phenothiazine tranquilizers and 11 thiazide diuretics in contrast to only two sulfonyl ureas for diabetes. As architects of new drugs, we can insist with justification that patient-to-patient variation in such physiological functions as absorption, metabolism, and excretion creates the need for a large number of congener drugs. We can even argue that the successful sale of so many congener drugs is a reflection of the physician's own research experiences on the grass-root level where critical and controlled studies cannot be made but where the only acid test of the drug is whether the patient gets well. If a patient feels better on a congener and not the parent steroid, is not this justification for making many congeners available, even though a sophisticated clinical study would rate them equal?

Notwithstanding these reasonable views, we must learn how to limit the number of congeners which are eventually offered to physicians as drugs. In my mind the only crucial question is whether the restraint should originate from commercial competitive considerations or should be exerted by us as architects of molecular modification. The answer is obvious. We are the ones with the power to create new congeners and play a decisive role in their development into new drugs. Our responsibility to society and to science demands that we act with complete objectivity and with the highest ethics. This view does not imply limitation of synthesis to accumulation of data on structure-biological activity relationship. As scientists we shall have no peace of mind until an interesting molecule is beaten into a pulp, because of our thirst to know and the urge to make the molecule do better. However, we must make a clear distinction—not every active compound should be considered a new drug. Let us not fail to exert objectivity and restraint in the area of this cutoff point. Let us be sure that before a decision is made to develop another congener into a new drug, we are convinced that a better drug is being created.

In viewing the congener-drug developments of recent years, I have often wondered why medicinal chemists in so many laboratories had chosen to direct their efforts into molecular modification of new drugs discovered by others. Although this massive surge has created new knowledge for the medicinal chemists at an almost explosive rate, its productiveness in the field of medicine can be questioned. In general, the discovery of a new and superior drug establishes a new order of feasibility for attacking a disease and frequently indicates the availability of superior animal methodology to guide the medicinal chemist. With such information, the medicinal chemist can choose to search for other structures completely different from the new drug. Admittedly it is difficult and daring to blaze a new trail when one has already been made and clearly marked. Since every useful drug has side effects or a limited spectrum of activity, this alternative has greater rewards when successful. Every drug, no matter how well it serves, can be displaced by a better one.

Future of Medicinal Chemistry Research

The events of the past year have given us much for reflection on the future of medicinal chemistry and drug research. While the print of the New Drug Regulations has scarcely dried, it is clear that our way of life will be different. While chemists will continue to sire new concepts and objectives

of synthesis at a rate even greater than in the past, progress in new drug development will be slower and more costly. The new degree of sophistication required for the pharmacological and clinical evaluation of safety and efficacy of potential drugs clearly demands serious readjustments on our part. It will mean that medicinal chemists will need to develop a greater degree of judgment, understanding, and patience and to be more selective in choosing fields of drug research. Unless very adequate scientific methods are available and are used for assessing potential drugs in animals and later in man for both efficacy and safety, drug research will be frustrating and unrewarding. Medicinal chemists would do well to select fields of research where definitive methodology exists and to stimulate more basic research in fields where methodology is inadequate.

The new environment also means that medicinal chemists will need to be more selective in choosing compounds for studies in man and for the protracted studies leading to a new drug application. The costs for such studies, in terms of manpower, facilities, and materials, are self-limiting. Undoubtedly, this factor will limit drug congener research, since it is becoming clear that the regulatory agency will frown on related compounds and their upgraded claims unless superiority can be clearly established. Within the practical level of dosage forms, the Food and Drug Administration and clinicians as a whole are unimpressed with claims for a congener of a greater potency on a milligram basis.

Whether we agree or not with the reason for the change, a new era is here. There is little we can do about it except to adjust ourselves to the change and hope that FDA will administer its regulations wisely and maturely. It is clear to me that research people in the pharmaceutical industry must meet these challenges determinedly, if the industry is to remain the important source of new drugs. May I quote Connor's statement (*1*) "Patent Protection and Research Incentive," presented before the Senate Subcommittee on Antitrust and Monopoly in December 1961:

> Of all the groups that support medical research, the pharmaceutical industry alone, in our society, has the special responsibility for converting knowledge into medicines. In carrying out this responsibility, it takes part in the search for basic understanding of life and life processes in health and disease. And it also seeks ways to apply this knowledge and the knowledge derived from all research everywhere, in the development and production of drugs and medicinals for the physician's use.
>
> Let's do everything possible to keep it this way.

On my part, I am convinced that the future of medicinal chemistry and drug research has never been brighter. We can now think less of quantity but more of quality with concomitant development of greater scientific acumen and judgment and with greater personal satisfaction from contributions which are real.

Modell (*4*) recently demonstrated concern lest a negative attitude toward drugs develop unless they are used more carefully. I should like to conclude my remarks by quoting a few sentences from this publication. Referring to this possible development of a negative attitude, he said:

There might have been some justification for such an attitude 50 years ago, but it would be an unforgivable disaster today, for never before has our ecologic balance been so dependent on drugs; never before in its history has medicine had so many useful, effective drugs on hand; and never before has there been such promise of even better ones to come.

In this we can agree with Modell (*4*) completely—we can also agree that in the search for better drugs, molecular modification will continue to serve the medicinal chemist handsomely.

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The Synthetic Penicillins

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Massachusetts Institute of Technology, Cambridge 39, Mass.

The semisynthetic penicillins offer an outstanding example of the role chemical modification can play in improving the medical properties of even a "wonder drug." Acylation of 6-aminopenicillanic acid, the "penicillin nucleus," has produced semisynthetic penicillins with demonstrated effectiveness against the troublesome penicillinase-producing clinically resistant staphylococcal infections. 6-Aminopenicillanic acid can be made by total synthesis or by biochemical techniques, and a chemical method has been devised for interchanging the side chain on the intact nucleus. The side chain of cephalosporin C, a closely related antibiotic, has been removed chemically and acylation of the resulting nucleus (7-aminosporanic acid) yields microbiologically active products.

Treatment of a penicillin sulfoxide with acetic anhydride affords the cephalosporin C ring system, although with different substituents. Medically useful drugs with a modified penicillin ring system will probably be found.

The semisynthetic penicillins provide one of the best modern examples of how the medical properties of a natural product can be improved by chemical modification of the molecule. Although the discovery of penicillin ushered in a new area of chemotherapy, it has been noted for some years that fermentation-produced penicillins are less than ideal in several major as well as minor respects. Five desirable properties which one might hope to incorporate into a chemically altered penicillin are:

1. Acid stability
2. Broadened microbiological spectrum
3. Activity against resistant organisms
4. Less allergenicity
5. Greater metabolic efficiency (better oral absorption, slower excretion)

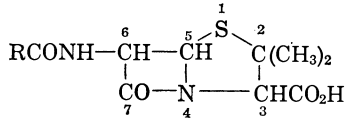
This list was not devised especially for this occasion; it was prepared by this author in 1953.

These improved properties have all been observed in the semisynthetic penicillins to a greater or lesser degree, with the possible exception of the lowered allergenicity. In at least one category—activity against penicillin-resistant organisms (notably penicillinase-producing staphylococci)—the results have been dramatic. According to published clinical reports infections have been successfully treated with at least two of the new penicillins in cases which medical experience and controls indicated would not have yielded to the penicillins produced directly by fermentation. This change from failure to success is an outstanding example of the medical advantages that chemical modification can achieve even in the case of a "wonder drug."

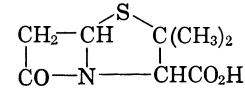
In general, three methods can be employed to produce structural variations in the penicillin molecule: addition of precursors to the fermentation media, chemical modification of the fermentation-produced antibiotic or intermediate, and total synthesis.

The addition of precursors during the fermentation has thus far been very limited in scope, although the acid-stable penicillin V (phenoxymethylpenicillin) is produced by this method. This review deals principally with chemical alteration of the fermentation-produced antibiotic or intermediate.

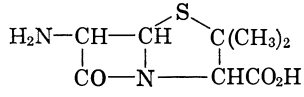
In Figure 1 penicillin nomenclature is presented (*5, 19*). A total synthesis of penicillin V (1957) and a total general synthesis of penicillins (1959) have been reported by Sheehan and Henery-Logan (*16, 17, 18*). Although total synthesis is the most elegant from an organic chemical point of view, also in principle the most flexible, the numerous steps involved (albeit each proceeds in good yield) render total synthesis noncompetitive industrially with partially synthetic techniques at the present time.



R-Penicillinic acid (*5, 19*)
R-Penicillin (salt)



Penicillanic acid (*16, 17, 18*)



6-Aminopenicillanic acid

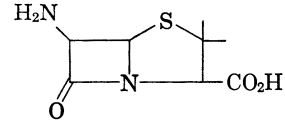


Figure 1. Penicillin nomenclature

One should note particularly the structure of 6-aminopenicillanic acid (6APA), which has been termed the "penicillin nucleus." Our announcement (*13, 14*) in March 1958 that ". . . we have prepared this compound [6-aminopenicillanic acid (6APA)] via a totally synthetic route . . . We have shown that one can acylate with various acid chlorides and obtain the corresponding penicillins," together with the subsequent development of commercially attrac-

tive biochemical routes to 6APA has been followed by the remarkable story of the "semisynthetic" penicillins. In addition to our original totally synthetic route and a chemical partial synthesis from penicillin G (Figure 2), 6APA has been obtained subsequently by direct fermentation and by enzymatic methods from the "natural" penicillins V and G in four independent laboratories (*2*, *3*, *7*, *9*). The semisynthetic penicillins now available commercially are manufactured by one of these biochemical preparations of 6APA followed by the chemical acylation of this key intermediate as first reported by our laboratory. Two independent Japanese laboratories (*8*, *12*) had previously obtained evidence for a penicillin nucleus, but their experiments were not followed up.

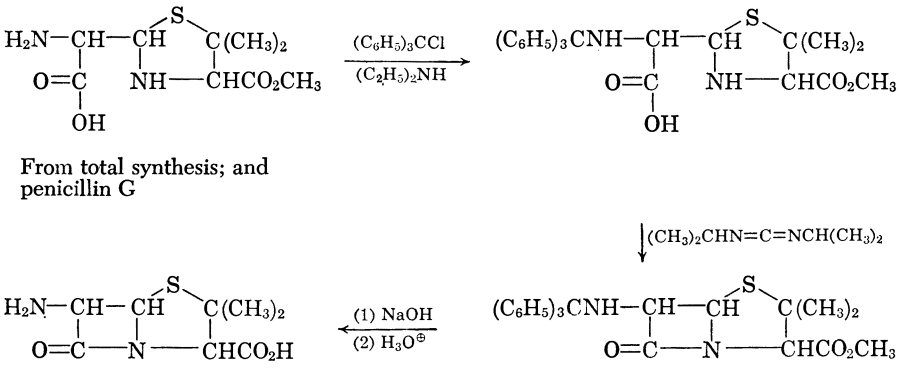
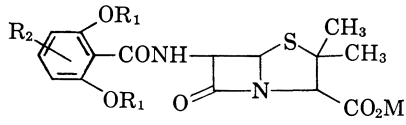


Figure 2. Total and partial synthesis of 6-aminopenicillanic acid

Perhaps the most striking and medically useful change in pharmacological properties of the semisynthetic penicillins as compared with penicillin G is in the 2,6-dialkoxyphenylpenicillin area (*6*) (Table I). For example, 2,6-dimethoxy-

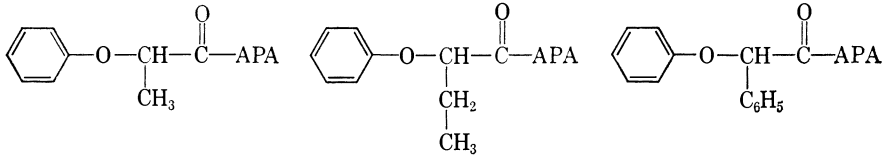
Table I. 2,6-Dialkoxyphenylpenicillins



			MIC, μg./Ml. vs. S. aureus			
			Sensitive		*Resistant*	
No.	R_1	R_2	*−Serum*	*+Serum*	*−Serum*	*+Serum*
Methicillin	CH_3-	H	1.6	1.6	3.2	3.2
1	C_2H_5-	H	6.2	6.2	12.5	12.5
2	C_6H_5-	H	1.6	>12.5	3.1	100
3	$C_6H_5CH_2$-	H	1.2	>12.5	3.1	100
4	$C_6H_5CH_2CH_2$-	H	3.1	>12.5	6.2	400
5	CH_3-	3-Cl-	0.8	6.2	3.1	6.2
6	CH_3-	3-CH_3O-	3.1	6.2	3.1	6.2
7	CH_3-	4-CH_3O-	3.1	>12.5	12.5	50
	Benzylpenicillin		0.05	0.1	50	100

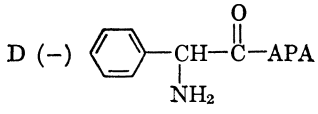
phenylpenicillin (methicillin) has an MIC, micrograms per milliter *vs.* resistant *S. aureus*, in the clinically useful range of 3.2 (even in the presence of serum), while penicillin G has risen to the medically impractical level of 50 to 100. Another striking change in microbiological properties can be observed in the case of D(−)-α-aminobenzylpenicillin (ampicillin) (Table II). The D-epimer is approximately an order of magnitude more effective against certain Gram-negative organisms than is benzylpenicillin, and the low MIC values indicate that ampicillin should be clinically effective against infections due to these strains. In Figure 3 are listed by name and formula the semisynthetic penicillins available commercially at the present time.

Penicillin G spectrum



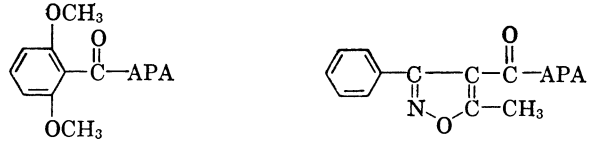
Phenethicillin Propicillin Phenbenicillin

Increased Gram-negative activity



Ampicillin

Penicillinase resistant



Methicillin Oxacillin

Figure 3. Semisynthetic penicillins commercially available

It is gratifying to review these past achievements, but it is also of interest to note what future developments may be expected in the penicillin modification field. Although the enzymatic splitting of a natural penicillin (G or V) to give 6APA is reported to be an efficient process, our laboratory undertook an investigation of purely chemical means for the conversion of penicillin G to 6APA derivatives carrying other side chains. The β-lactam function of penicillin G is much more susceptible to solvolysis than the phenylacetamido side chain; therefore the chemical replacement of the side chain without disruption of the β-lactam ring appears to be an unusually difficult problem. However, the side chain is a mono-substituted amide, whereas the β-lactam is di-substituted. Ad-

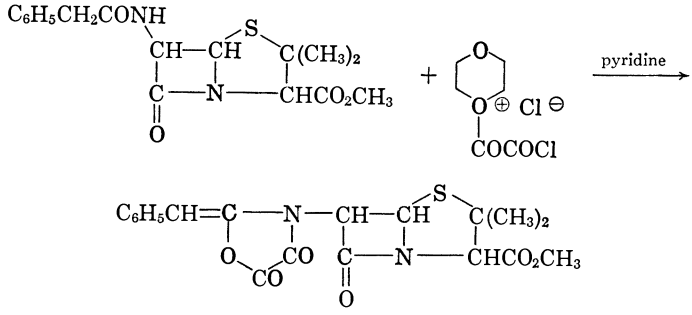
Table II. Activity of Ampicillin,[a] Its Epimer, and Benzylpenicillin against Gram-Negative Organisms

		MIC, μg./Ml.		
	Organism	Ampicillin (D−)	Epimer (L+)	Benzyl-penicillin
1.	*Aerobacter aerogenes*	100	100	100
2.	*Alkaligenes faecalis*	0.4	3.1	6.2
3.	*Escherichia coli*	6.2	25	100
4.	*Klebsiella pneumonia*	1.6	12.5	12.5
5.	*Salmonella enteriditis*	0.4	0.8	1.6
6.	*Salmonella typhosa*	1.6	12.5	12.5
7.	*Shigella dysenteria*	0.4	3.1	12.5
8.	*Shigella sonnei*	1.6	3.1	12.5

[a] D (−)-α-aminobenzylpenicillin.

vantage can be taken of this difference in the two functions by interaction with oxalyl chloride. This reaction had been tried previously as a part of the structural studies on penicillin carried out by the World War II Cooperative Project (*4*). The reaction as reported produced a crystalline compound of unknown structure, but all evidence indicated that the β-lactam function had been destroyed.

This reaction was reinvestigated in our laboratory by W. von Phillipsborn (*15*). In the presence of pyridine and using the dioxane complex of oxalyl chloride, the desired oxazolidine-4,5-dione was produced (Figure 4). This oxazolidine was reduced with zinc in buffered acetic acid to a dihydro derivative, which was cleaved under very mild conditions to phenylacetaldehyde and the benzyl ester of 6-oxamidopenicillanic acid. Hydrogenolysis yielded 6-oxamidopenicillanic acid (Figure 5), thus completing the first chemical interchange of side chains on the intact penicillin nucleus. Our laboratory previously reported the conversion of penicillin G into 6APA by a partially synthetic scheme which involved the opening and reclosing of the β-lactam ring.



M.p. 182–83°; 50–60%. Analysis corresponds to $C_{19}H_{18}O_6N_2S$.
UV (ether) 2.42 mμ (log ε = 4.22); 336 mμ (log ε = 4.04).
IR (chloroform) strong 1827 cm.$^{-1}$ (lactone); 1795 cm.$^{-1}$ (β-lactam); 1755-1740 cm.$^{-1}$ (ester and γ-lactone) and 1682 cm.$^{-1}$ (C_6H_5CH=C—O).

Figure 4. Conversion of methyl benzylpenicillinate to an oxazolidine-4,5-dione

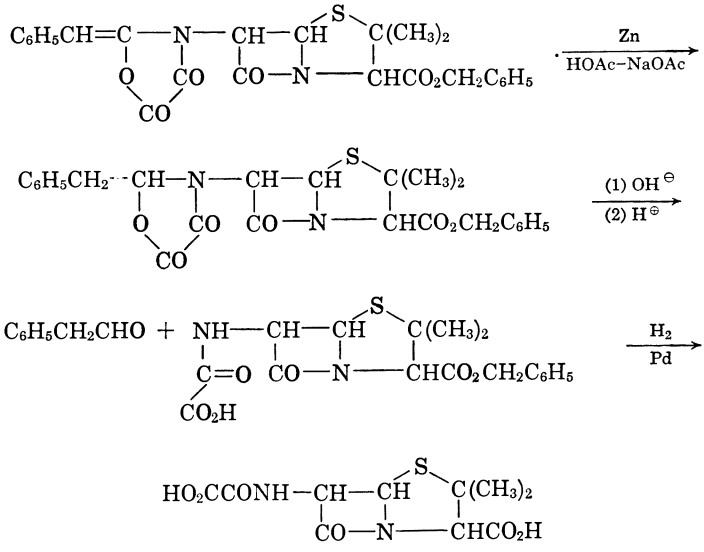
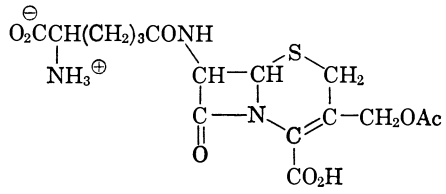


Figure 5. Chemical interchange of penicillin G side chain

One of the surprising features to this reviewer has been the useful alteration of pharmacological properties observed by variation only in the side chain. However, with the preparation of over 1000 side-chain variants in the 6APA series it is possible that the law of diminishing returns is beginning to apply. More deep-seated chemical modification—for example, of the ring system—offers a promising field for future investigation.

The structure (*1*) of cephalosporin C



demonstrates that this antibiotic can be regarded as a close relative of the penicillins in which the five-membered thiazolidine ring has been expanded to a six-membered ring. Recently (*10*) the side chain of cephalosporin C has been removed by an ingenious chemical method to produce 7-aminocephalosporanic acid, the cephalosporin C nucleus. Discussion of acylated derivatives of 7-aminocephalosporanic acid is beyond the scope of this review, but the high antibiotic activity reported in this series makes it clear that the thiazolidine ring of the penicillins is not necessary to the antibiotic action and suggests that other

modifications of the penicillin nucleus will be fruitful. In Figure 6 is outlined a novel rearrangement (*20*) of penicillin G acid chloride in which the normally reactive β-lactam ring is unaltered. The product, termed "anhydropenicillin G,"

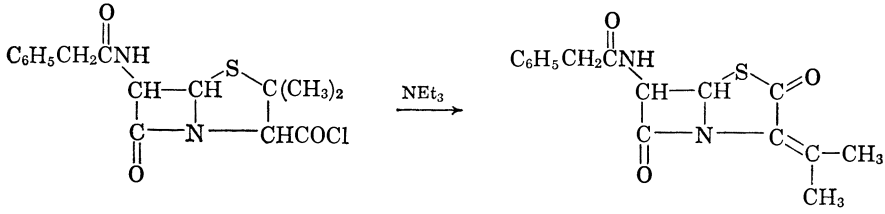


Figure 6. Anhydropenicillin G

offers further possibilities for chemical modification, including the possibility of rearrangement to the cephalosporin C ring system after allylic attack on the methyl groups.

An extremely interesting and potentially useful rearrangement of penicillin sulfoxides has recently been reported (*11*) (Figure 7). Penicillin V sulfoxide

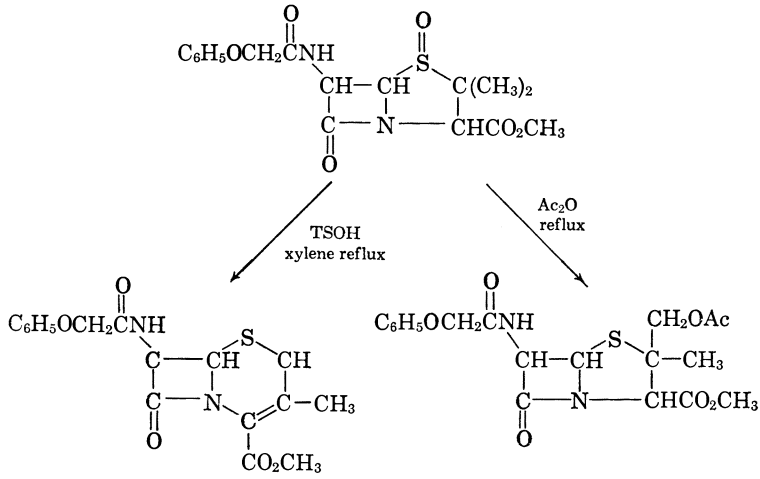
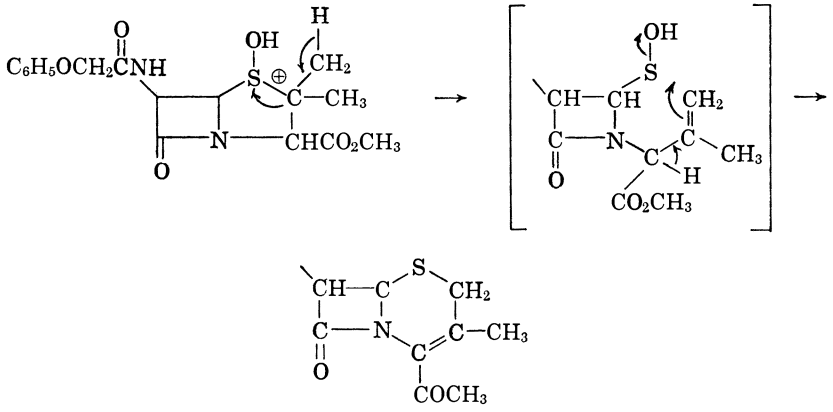
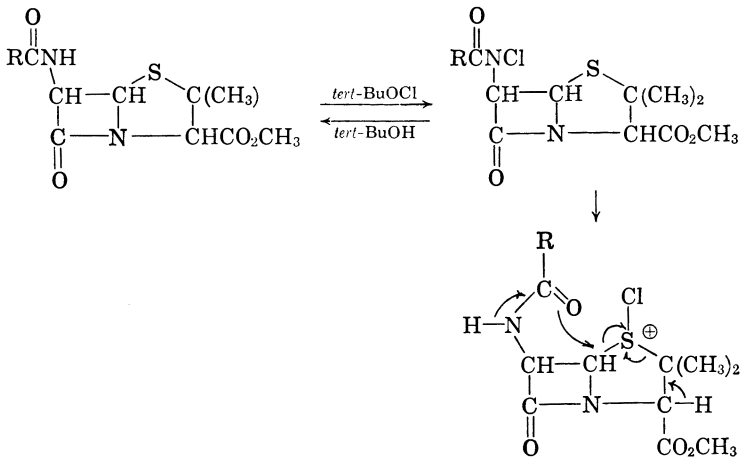


Figure 7. Rearrangement of penicillin sulfoxides

methyl ester can be caused to rearrange into cephalosporin C ring system (although with different substituents) by heating in the presence of acid. Alternatively, reflux with acetic anhydride leads to acetoxylation of one of the geminal methyl groups. The mechanism proposed by the authors is:



An interesting reaction which likewise causes disruption of the thiazolidine ring without cleavage of the β-lactam has been studied in our laboratory by C. Abshire and M. H. Forbes. As outlined in Figure 8, treatment of penicillin G methyl ester with *tert*-butyl hypochlorite, followed by triethylamine, results in elimination of the sulfur atom and formation of an oxazoline. The structural assignment is based on analytical data and in particular on infrared and NMR spectral studies. Surprisingly, hydrogen over palladium causes hydrogenolysis rather than saturation of the fully substituted carbon-carbon double bond. A possible mechanism for this unusual reaction is:



These few examples illustrate that properly designed reactions can be carried out on the penicillin nucleus without destruction of the sensitive β-lactam, and the development of medically useful compounds by the chemical alteration of the penicillin ring system can be anticipated. The chemical conversion of the 6APA structure into the 7-cephalosporanic acid system by chemical or biochemical means presents an intriguing scientific challenge.

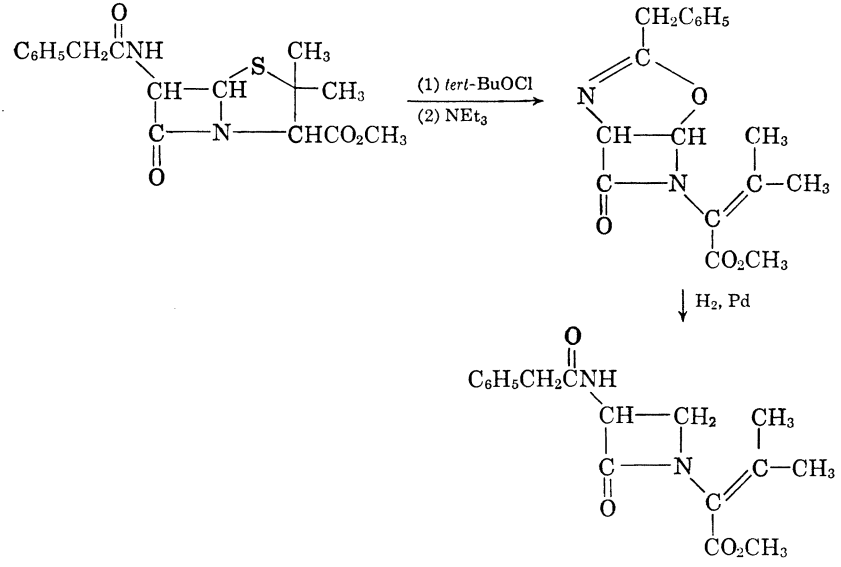


Figure 8. Opening the thiazolidine ring

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RECEIVED December 9, 1963.

Discussion

L. C. CHENEY, presiding

Manfred E. Wolff (University of California): I would like to ask Dr. Sheehan concerning the allergenicity of these synthetic penicillins. There was a theory that the allergenicity of penicillin itself is due to the carry-over of the fermentation, some minute amounts of fermentation by-products. Does the allergenicity of the synthetic penicillin have some bearing on this?

Dr. Sheehan: Totally synthetic samples of Penicillin G and V from our laboratory have been tested for allergenicity, and they definitely do react in a sensitive patient fully as much as does the material from fermentation.

However, what I had in mind was that some of the chemically altered penicillins might be either better tolerated by patients already sensitive, or show less tendency to induce the allergic response in patients who are not already sensitive to antibiotics. It is probably too early to say whether this is going to be true. Certain of them do show lowered properties, but I do not think anyone is willing to say that they have been abolished.

Dr. Fellano (Ferris State College, Michigan): I notice that in most of your experimental work you worked with an aryl side chain. What is the effectiveness of groups of the alkyl type in the sixth position? Has any work been done in that area?

Dr. Sheehan: Yes, both semisynthetic preparations have been prepared. As a matter of fact, the earliest penicillins, originally observed by Fleming, were the aliphatic side chain type, so called Penicillin F and Penicillin K. Penicillin F had an unsaturated side chain but straight chain, and K was fully saturated with eight carbons.

Dr. Garrod: Dr. Sheehan, to go back to the subject of allergenicity, may I ask you this: It has been suggested by workers in this country that the haptene, which combines with protein to form the sensitizing compound, is not penicillin itself, but penicillinic acid. If that is true, it should be possible to make a nonallergenic penicillin which does not degrade in that direction.

What are your views on the possibility of doing that?

Dr. Sheehan: Some of our early totally synthetic penicillins, which were produced as early as 1958, had a side chain which could not produce a penicillinic acid. That has an unsaturated azlactone structure with a free thiol group. As a matter of fact, the benzylsulfonyl side chain could not produce a benzyl penicillinic acid.

I am not sure which of these types have been investigated thoroughly enough to make any comments about the effect. Most of the testing has actually been done on sensitive patients, with patch tests and so forth.

But the haptene production would be in the induction of the sensitivity in de novo patients, some one who had not been exposed to penicillin, and at any rate did not have the sensitivity. That is where you would expect it to be effective.

I do not know how far these experiments have progressed, although the concept that you did outline does make a great deal of chemical sense.

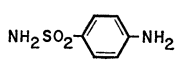
Molecular Modification in the Development of Newer Anti-infective Agents

The Sulfa Drugs

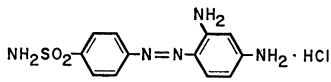
GERHARD ZBINDEN
Research Division, Hoffmann-La Roche Inc., Nutley, N. J.

The $-SO_2N<$ group has interesting functions in several areas of medicinal chemistry. Its most important application lies in the field of antibacterials, where derivatives of sulfanilamide maintain an important position. Thousands of congeners of the sulfonamide molecule have been prepared. The most fruitful changes were achieved by substitution on the N^1 atom, which yielded compounds of higher chemotherapeutic activity. Although the antibacterial spectrum was not significantly broadened, many chemical properties were changed, which resulted in differences in pharmacological behavior—e.g., solubility of the sulfonamides and their metabolites, protein binding, speed and pathway of metabolism, tissue distribution, and mechanism of elimination. These and other factors determine toxicity, half life, and effectiveness of a sulfonamide and greatly influence its clinical usefulness.

Domagk's (7) discovery that Prontosil protected mice against lethal infections with streptococci and the findings of Fourneau and his group (8) that sulfanilamide was the chemotherapeutically active moiety of Prontosil initiated a



SULFANILAMIDE



PRONTOSIL

most important breakthrough in modern drug research. No other drug, with the possible exception of barbituric acid, has been as extensively modified as sul-

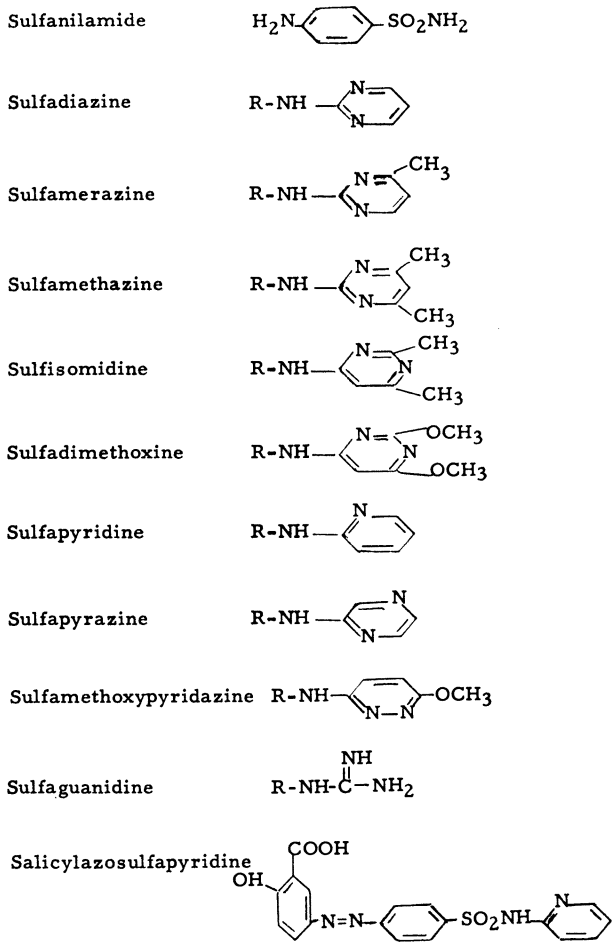
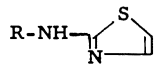
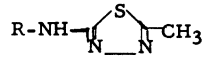


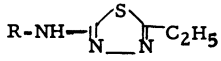
Figure 1. Sulfonamides commercially available

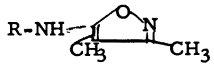
fanilamide. In 1948, Northey (*17*) listed over 5000 derivatives, and several thousands more were synthesized and studied in the past 15 years.

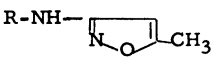
The chemical aspects of this impressive synthetic program are perhaps best illustrated by the few compounds which have been introduced in human therapy. Figure 1 contains the formulas of sulfonamides which became available on the American market. In Figure 2 some newer drugs marketed abroad or still under clinical investigation are listed. It is evident that the chemotherapeutically useful agents are all derivatives of sulfanilamide, with the NH_2 group in the para position occasionally replaced by a radical which can be readily converted into a free amino group in the body. The main differences center around the substitution of a hydrogen at the N^1 atom, where aromatic heterocycles have yielded the most useful agents. Among these, additional changes of the biological properties were achieved by substitution on the heterocycles with methyl, ethyl, methoxy, or phenyl groups.

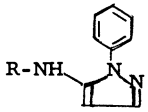
Sulfathiazole 

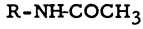
Sulfamethizole 

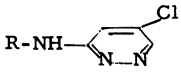
Sulfaethidole 

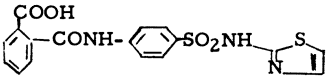
Sulfisoxazole 

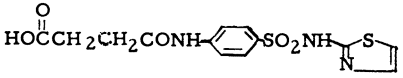
Sulfamethoxazole 

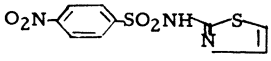
Sulfaphenazole 

Sulfacetamide 

Sulfachloropyridazine 

Phthalylsulfathiazole 

Succinylsulfathiazole 

p-Nitrosulfathiazole 

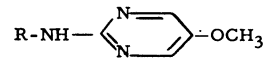
for human therapy in the United States

From the chemical point of view, this small crop of closely related compounds derived from over 10,000 new agents does not look too exciting. The question then arises whether conservative variations of the sulfanilamide substituents have provoked significant changes of the biological properties, which in their turn proved to be responsible for an improved performance of the therapeutic agents in man. The person most qualified to answer this question is the physician, who constantly observes and compares the effects of drugs in his patients. His reaction to the sulfonamides which became available over the past 26 years is therefore analyzed.

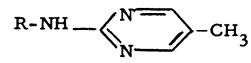
After sulfanilamide and Prontosil, sulfapyridine became the drug of choice in 1938. It was soon replaced by sulfathiazole, which had to yield its favored position to sulfadiazine and its methylated derivatives, sulfamerazine and sulfamethazine, which were predominantly used for an extended period of time (*10*). Further changes, however, occurred in recent years. These are sum-

Figure 2. Newer sulfonamides marketed in Europe or under clinical investigation

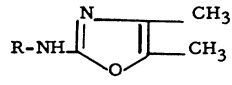
Sulfamethoxydiazine



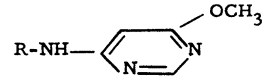
Sulfamethyldiazine



Sulfadimethyloxazole



Sulfamonomethoxine



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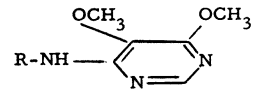

marized in Figure 3, which demonstrates the estimated number of new prescriptions for various sulfonamides per year in the United States. Sulfisoxazole, introduced in 1948, soon became and still is the leading sulfonamide. In 1957, the first long-acting agent, sulfamethoxypyridazine, appeared, followed by the also long-acting sulfadimethoxine, a compound now widely used. The recently introduced intermediately acting sulfamethoxazole has also been well accepted. Although still available under various trade-marks, the older sulfonamides are

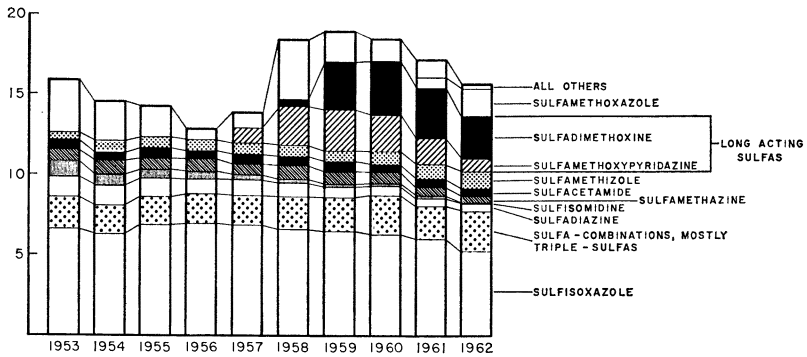

Figure 3. Approximate millions of new prescriptions for sulfonamides in U. S.

According to independent surveys

only rarely used. Experience with salicylates, phenothiazines, barbiturates, and many other agents has shown that small but significant differences between drugs of similar structure and activity may be recognized by the practicing physicians who are constantly exploring and comparing the clinical usefulness of older and newer drugs. It is therefore probable that this definite trend away from the older agents indicates that the newer sulfonamides offer some real advantages in clinical practice.

Effect of Molecular Modification on Biological Properties

Chemotherapeutic Activity. It is characteristic of the sulfonamides that even minor chemical variations may lead to remarkable changes of their biological properties. To the chemist this becomes first evident when he submits his compounds to the chemotherapeutic screening laboratory. To illustrate this point, the in vivo screening results of the 60 sulfonamides most recently synthesized in our laboratories are summarized in Figure 4. It shows that significant activity is present with 21 compounds, but very often it is restricted to one or a few infections, sometimes limited to Gram-positives or Gram-negatives, sometimes scattered throughout the spectrum. Figure 4 therefore demonstrates very marked qualitative and quantitative differences in chemotherapeutic activity among this randomly selected sample of sulfonamides. But even if closely related compounds with high chemotherapeutic activity are compared, substantial differences in antibacterial potency are observed, as reported for the recently most extensively studied sulfapyrimidines and related compounds (*1*, *12*).

Figure 5 demonstrates the chemotherapeutic screening results obtained with three representatives of this class which are all highly active but still exhibit remarkable quantitative differences in most of the experimental infections. It is evident from this example that the monomethoxy compound has the highest activity in most of the infections. However, clinical testing indicated that this agent was considerably less active against common sulfonamide-sensitive infections than other modern sulfas, including the two compounds listed in Figure 5 (*18*). This would indicate that factors other than the chemotherapeutic activity are also important for the clinical efficacy and usefulness of a new sulfonamide. It is in this area of additional biochemical and pharmacological properties where most of the modern research efforts have been invested. However, for the evaluation and the ultimate clinical usefulness of a sulfonamide all antibacterial, pharmacological, and biochemical properties have to be taken into consideration together. The best sulfonamide is not the one that excels in one particular area but the one that combines the maximum of all positive assets with a relatively insignificant incidence of unfavorable factors (*23*).

Absorption, Metabolism, Distribution, and Excretion. In the following paragraphs, the many biochemical and pharmacological processes which determine the fate of sulfonamides in the body are explored, and to this end various sulfonamide derivatives are followed on their journey through the human organism. The first stop is in the stomach and small intestine, where the compounds are dissolved and prepared for absorption. Here we already distinguish a special class of agents rendered insoluble by chemical modification. Since these agents are not absorbed readily, they continue their journey down the intestinal tract. This property makes them useful as intestinal disinfectants and for

Number of compounds	IN VIVO ACTIVITY AGAINST								
	Strept. hemol.	Dipl. pneum.	Microc. pyog.	S. typhosa	S. schott.	E. coli	K. pneum.	Ps. aerug.	P. vulgar.
39	0	0	0	0	0	0	0	0	0
4	0	0	X	0	0	0	0	0	0
1	0	0	X X	0	0	0	0	0	0
2	0	0	0	X	0	0	0	0	0
1	0	0	X	0	0	0	0	0	X
1	X	0	X X	0	0	0	0	0	0
1	X	0	XXX	0	0	0	0	0	0
1	X	0	X	0	0	0	0	0	X
1	0	0	X X	X	X	0	0	0	0
1	0	0	X X	X X	0	0	0	0	X X
1	X	0	0	X	0	X X	X X	0	0
1	0	0	0	X	X	X X	0	X	X X
1	X	0	XXX	XXX	0	0	0	XXX	XXX
1	X	0	0	X	0	X	0	X	X
1	X	XXX	XXX	0	X	0	0	X X	X X
1	X	0	X X	X	X	X	X	X	X
1	X	0	X X	X X	X	X X	X	X	X
1	X	X X	XXX	X X	X	XXX	X X	X X	X X

0 – NO CHEMOTHERAPEUTIC ACTIVITY
X – LESS ACTIVE THAN SULFADIAZINE (CD_{50} more than twice the CD_{50} of sulfadiazine)
XX – ACTIVITY IN THE RANGE OF SULFADIAZINE
XXX – MORE ACTIVE THAN SULFADIAZINE (CD_{50} less than half of CD_{50} of sulfadiazine)

Figure 4. In vivo screening results obtained with 60 randomly selected sulfonamides in mice
Experiments of E. Grunberg

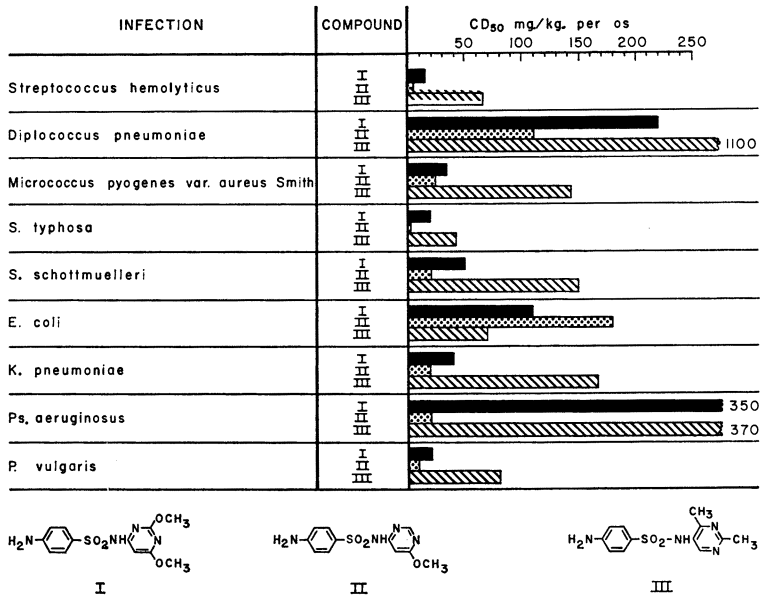

Figure 5. In vivo activity of three newer sulfapyrimidines against nine infections in mice
Experiments of E. Grunberg

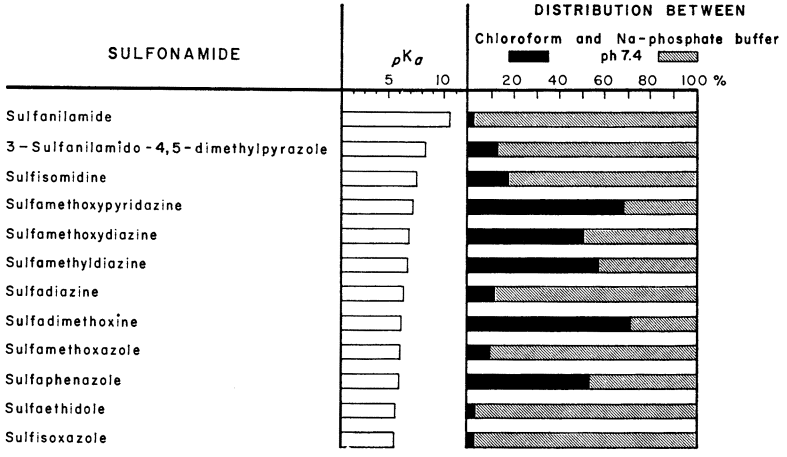

Figure 6. pK_a values and distribution between chloroform and water of a selected number of sulfonamides
Based on results reported by Rieder (20)

the therapy of various forms of enterocolitis. A larger group of sulfonamides, however, is used for the treatment of systemic infections and therefore has to be absorbed. Absorption of most drugs is a physicochemical process which is markedly influenced by the chemical structure. The most important factors are lipide solubility and the pK_a, which determines how much of a drug is present in the better absorbable nonionized form at a given pH (*4, 21*).

Lipide solubility and pK_a values of various sulfonamides differ over a considerable range (*4, 20*), as is demonstrated for selected compounds in Figure 6. These differences unquestionably influence the kinetics of drug absorption, which in humans is often judged according to the time interval between oral administration and the occurrence of maximum sulfonamide levels in the plasma. With sulfadiazine, for example, maximum plasma levels are reached after 6 to 8 hours, whereas with sulfamoxole peak levels appear 2 hours after ingestion (*23*). These determinations, however, do not represent the speed of absorption only, since the plasma levels also depend on inactivation and disappearance of the drug into the tissues. The speed of absorption can be measured more precisely in animals by determination of the sulfonamide content in the small intestine after intraduodenal administration. With this technique, considerable differences between various derivatives were found (*13*). Complete absorption is a prerequisite of a good systemic sulfonamide. Fast absorption is considered an added advantage.

The moment a sulfonamide reaches the blood stream, many biological and biochemical processes take place simultaneously, as indicated in simplified form in Figure 7.

As soon as the sulfonamide is in circulation, it is exposed to the metabolic attacks of the liver. One of the principal routes of biotransformation in man is N^4-acetylation. However, there are other pathways, and it is noteworthy that chemical variation of the substituents markedly influences the metabolic fate of

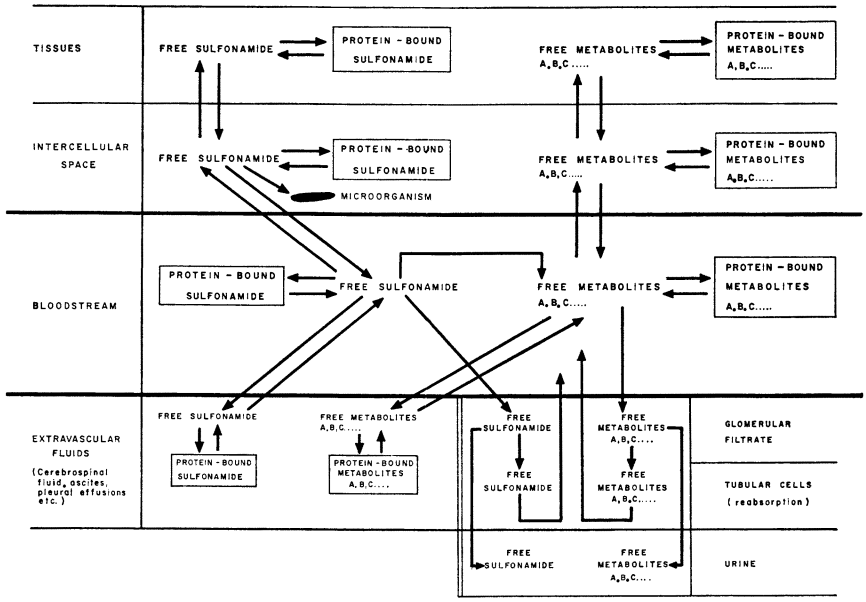

Figure 7. Distribution of sulfonamides in the body

sulfonamide derivatives (Figure 8). Intact drug and metabolites are removed from the blood stream by the kidneys at different rates. This, together with the rate of metabolism in the liver, accounts for a certain accumulation of metabolites in the blood which is characteristic for each sulfonamide. For example, sulfapyridine and sulfathiazole are distinguished by a high degree of acetylation

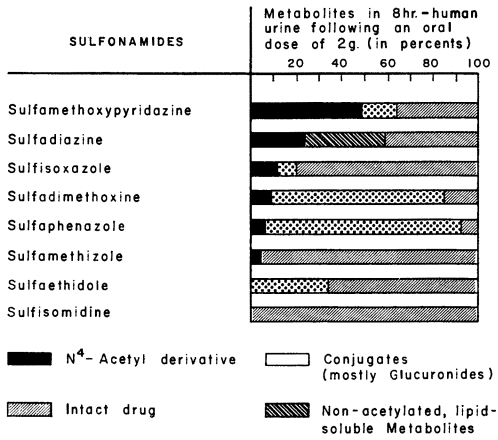

Figure 8. Metabolites of eight sulfonamides in human urine
Analytical results of B. Koechlin

in the blood (28 to 30%), whereas most of the modern sulfonamides have a low acetylation rate (5 to 15%) (*16*, *23*). Since the N^4-acetyl derivatives are inactive chemotherapeutically and more toxic, a lower accumulation of metabolites in the blood is certainly preferable (*23*).

An important property of sulfonamides is their ability to penetrate into the tissues and extravascular fluids where they can exert their bacteriostatic function. This can best be demonstrated in animal experiments. If the sulfonamide is injected intravenously into an animal whose excretory functions have been markedly reduced by extirpation of the kidneys and ligation of the bile duct, one finds much lower blood levels than are calculated from the total blood volume and the total amount of drug administered. This proves that a substantial part of the drug has disappeared into the tissues (*13*, *21*).

As an example of how the chemical modification can influence the affinity of sulfonamides to certain tissues, the results of another model experiment are briefly reviewed. Groups of four rabbits received a single oral dose of 500 mg. per kg. of five different sulfonamides and were sacrificed 3 hours later. Free and total sulfonamide were determined in the cortex and medulla of the kidneys as well as in the blood plasma. A summary of the results limited to the free sulfonamide is presented in Figure 9. The most pronounced differences are seen between sulfisoxazole and sulfamethoxazole, two agents which are chemically similar. With sulfisoxazole, the kidney levels are much higher than the plasma levels and the sulfonamide content in the medulla of the kidney markedly surpasses the one in the cortex (index: medulla/cortex = 1.6). With sulfamethoxazole, almost identical sulfonamide levels are found in kidney cortex, medulla, and blood plasma.

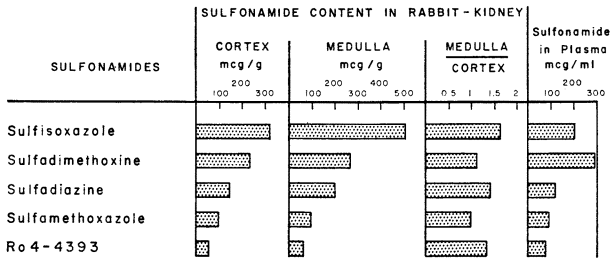

Figure 9. Distribution of free sulfonamide in kidney cortex, kidney medulla, and blood plasma of rabbits 3 hours after oral administration of 500 mg. per kg. of five sulfonamides
Experiments by J. Fellig and W. Marusich

Although the determination of the tissue levels gives important information about distribution of the sulfonamides in various organs (*15*), it furnishes no indication as to the local active concentration of the antibacterial. According to modern views, sulfonamides are bound reversibly to blood proteins, mostly the albumins. The degree of protein binding varies considerably among various

derivatives and, in most cases, also depends on the sulfonamide concentration (Figure 10) (*9, 12, 20, 21*). The N^4-acetyl compounds have a higher degree of protein binding than the corresponding free sulfonamides, which may lead to some competition for binding sites (*6, 20*). The free sulfonamide dialyzes through the capillary wall into the intercellular space and on into the cell water until a dynamic equilibrium is established (*6*). In the intercellular space, the protein content is generally low, with the exception of inflamed areas. Therefore, there are usually only small amounts of protein-bound sulfonamides there. In the cells, however, a relatively large amount of structural protein is available to which sulfonamides may be bound (*5, 13*). But here, as well as in the blood, the protein binding is reversible and as soon as the free sulfonamide levels in the blood are lowered, the free sulfonamide in the tissues shifts back to the blood and the sulfonamides are released from their protein-binding sites (*13*).

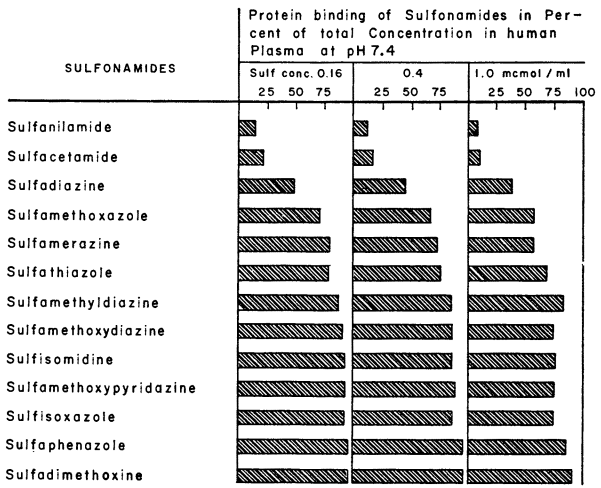

Figure 10. Protein binding of various sulfonamides in human plasma
Based mostly on results reported by Rieder (20)

There has been considerable controversy recently as to the significance of protein binding for chemotherapeutic activity (*2, 3, 14, 21*). Since protein binding inactivates sulfonamides in vitro, it was claimed that highly protein-bound compounds would often not reach effective bacteriostatic levels in the intercellular spaces, despite considerable plasma levels. However, this notion has not been supported by clinical evidence and it is possible that the in vivo chemotherapeutic effect is also accompanied by a certain binding of the drugs to the microorganisms (*21*), which can be demonstrated experimentally (*24*). Sulfonamides penetrate not only into the intercellular space but into extravascular fluids such as cerebrospinal fluids, ascites, pleural effusions, etc. (*22, 23*), and since in meningitis, pleuritis, and peritonitis the microorganisms are present in these fluids, a high concentration of the antibacterials is desirable.

Excretion of a sulfonamide occurs chiefly through the kidneys. The free, non-protein-bound drugs and their metabolic products are ultrafiltered in the glomeruli and then partly reabsorbed. The renal clearance rate of the metabolites is generally higher than that of the intact drug (*12, 16*). Studies in animals and human subjects furthermore indicate that the N^4-acetylated metabolites are often also eliminated through tubular excretion (*19*). There are very marked differences among chemically similar sulfonamides with regard to the degree of reabsorption, a fact which is of great practical importance, since it determines the clinical dosage schedule and duration of action for each compound.

Figure 11 shows the half lives of various sulfonamides in human blood. They range from a few hours for compounds which have to be given at high doses every 4 to 6 hours, to the intermediately acting agents which are administered twice a day, to the long-acting "one-a-day" sulfas, and finally to the ultralong-acting drugs which require only one dose of 1 to 1.5 grams a week. Protein binding has little effect on the duration of action of the sulfonamides (*22*), with the exception of those rare compounds which are chiefly eliminated by ultrafiltration without appreciable tubular reabsorption (*19*). However, it has been noted by Rieder (*21*) that long-acting compounds with a high tubular reabsorption rate are generally distinguished by a high degree of lipide solubility, which facilitates penetration through the cell membranes of the tubular epithelia.

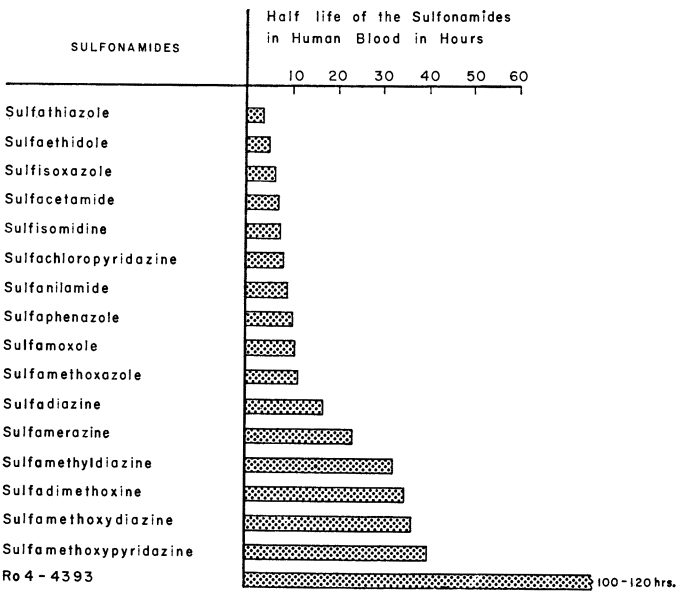

Figure 11. Half lives of sulfonamides in human blood
Based on results reported by Rieder (20) and Walter (23)

An important function of the kidneys is reabsorption of water from the ultrafiltrate. As the urine descends through the renal tubules, it is concentrated considerably and the pH falls. The possibility therefore exists that the sulfonamides and their metabolites may precipitate in the tubules and cause dangerous renal blockade. Determination of the solubility of any new sulfonamide and its principal metabolites is therefore of vital importance. Various sulfonamides differ considerably in their solubility characteristics at the physiological pH of the urine. A few examples are demonstrated in Figure 12, showing highly soluble derivatives (compounds 1 and 2), substances with low solubility at acid pH and high solubility at pH 6.5 and higher (compounds 3, 4, and 5), and analogs with low solubility even at pH 7 (compound 6). Another precautionary measure consists in the administration of relatively large doses of the new drug to animals and careful examination of fresh kidney slices under polarized light for the presence of crystals. Of the five substances listed in Figure 12, only No. 6 caused precipitate formation in rat kidney.

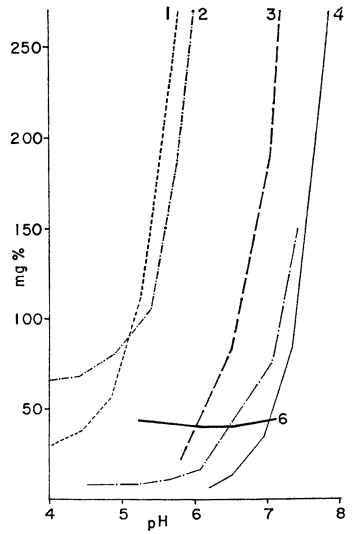

Figure 12. Solubility of sulfonamides at various pH's

1. *Sulfisoxazole*
2. *Sulfamethoxazole*
3. *Sulfaphenazole*
4. *Sulfadimethoxine*
5. *Sulfamonomethoxine Figure 5 Compound II*
6. *Ro 1-8228 = N^1-(4,5-dimethyl-3-pyrazolyl) sulfanilamide*

Based on results by B. Schmidli and A. Motchane

From the clinical point of view, kidney blockade during sulfonamide therapy is much less a problem now than it was years ago. This is mostly due to the discovery of agents which are highly soluble at the pH of the urine (pH 4.8 to 7.4) and the development of long-acting sulfas which can be given

at such a low dose that crystals rarely precipitate, despite the rather low solubility, and finally the discovery of compounds which are chiefly excreted in the form of highly soluble glucuronides. Cyanosis was another toxic effect very frequently seen in patients treated with sulfanilamide and its first derivatives. This symptom was due mostly to methemoglobin formation. It is very rarely, if ever, observed with the newer drugs. Thus, molecular modification has all but eliminated two major toxic effects of the original sulfonamides.

Conclusions

During the past 25 years a generation of chemists and biologists has intensively built upon Domagk's original discovery. One big hope to find analogs with an even broader chemotherapeutic spectrum has not been fulfilled. Painstaking research, however, has led to refined experimental techniques which permit a detailed and meaningful evaluation of new sulfonamide derivatives in the laboratory. With the help of these methods, new compounds have been screened out which, in many respects, surpass previously developed analogs. However, intensive clinical research has shown that there are still many properties contributing to the efficacy and safety of the sulfonamides which cannot yet be evaluated in animals.

Clinical effectiveness does not always parallel efficacy in the animal screening experiment. Differences in the metabolic fate, protein binding, and tissue distribution between various animal species and man may, at least partly, be responsible for such discrepancies. Furthermore, general tolerance and toxicity of a new agent can hardly be predicted from experimental results. This does not apply to the possibility of crystalluria, which can be reasonably well predicted from physicochemical and biological experiments, but other typical side effects of the sulfonamides, such as nausea, vomiting, leukopenia, and particularly the sensitivity reactions, can be discovered only in humans. Sometimes a low degree of efficacy and high incidence of side effects becomes evident in the first hundred patients treated with a new agent, and it is our experience that in such cases the frequency of toxic symptoms and the clinical and bacteriological response rates are repeated in every new trial with amazing constancy. Among the best compounds, however, these differences are not so obvious and it may take years of clinical experience to pinpoint advantages, special properties, and weak points of these analogs (*11*).

For those scientists who have been working in the not too spectacular field of sulfonamides, it is a great satisfaction that the newer compounds retain their firm place in the chemotherapy of bacterial infections, despite the considerable number of new antibiotics which have given the physician new and powerful tools for the treatment of the same diseases for which sulfonamides are used. In recent years and months, an increased interest in sulfonamides is evident all over the world. Many new compounds have been developed and tried in the laboratory and the clinic, and it is expected that new knowledge about metabolism and tissue distribution of sulfonamides will contribute to the discovery of more selectively acting agents of low toxicity.

Acknowledgment

This paper is based on many contributions by members of the Research Divisions of F. Hoffmann-La Roche & Co., Ltd., Basle, Switzerland, and Hoffmann-La Roche Inc., Nutley, N. J. Their help and guidance are gratefully acknowledged.

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Impact of the Synthetic Anti-infectives on the Therapy of Bacterial Infection

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The chemotherapy of bacterial infections dates from the introduction of the sulfonamides in 1935. Examination of mortality statistics in England and Wales from that date onwards shows that these drugs had reduced the mortality from hemolytic streptococcal infections (typified by puerperal fever), pneumonia (except in the highest age groups), and cerebrospinal fever almost to the present low levels before antibiotics were introduced. The steep fall in mortality from tuberculosis began later, and was initially due to streptomycin, but subsequently also to synthetic compounds. Synthetic derivatives of penicillin possess three new properties, of which resistance to penicillinase is the most valuable. Although effective treatment is now possible for most specific infections, there has been an increase in nonspecific infections, due largely to bacteria of the coliform group, which are difficult to treat. Some types of chronic infection also present difficulty.

Apart from the action of arsenobenzyl compounds in anthrax and that of certain drugs in the urinary tract, the chemotherapy of bacterial infections dates from 1935. I was present at the meeting in London in March of that year at which Hörlein of Elberfeld announced the discovery of Prontosil. I am sure that no one there can have had any conception of the developments to which this would lead. Moreover, no one at the present day without a long memory can have much idea of what the practice of medicine and surgery was like before this discovery. Nowadays the vast majority of deaths are due to degenerative or malignant disease. Thirty years ago cancer was certainly one of the three major causes of death, but the others were pneumonia and tuberculosis, and it will be profitable to examine the effect of later developments on mortality from these two diseases.

The sole original claim made for Prontosil was that it would control infection by the hemolytic streptococcus. Although numerically less important, deaths from streptococcal septicemia—a condition which most present-day doctors have never seen—were peculiarly tragic because of their suddenness, and often the youth and the previous good health of the victim. Many were women after childbirth, and fatal puerperal fever is perhaps the most poignant tragedy in the whole of medicine. Some were members of the medical or nursing professions. In April 1913, a brilliant young surgeon at my hospital, who had rowed for the university when he was at Cambridge, and was still, at the age of 35, of fine physique and in perfect health, was accidentally infected from a septic case. Only two days later he was dead. Two more surgeons at the hospital fell victims to such infection during may own time there, the site of entry in one being a needle prick, and in the other a small accidental burn with a cautery. The explanation of these highly virulent infections in hospital personnel is apt to be forgotten. The hemolytic streptococcus, more than any other organism, gains virulence by "passage": Its capacity to attack the fresh host is multiplied at least 10-fold. Hence the paradox of rapidly fatal infection in a previously healthy person, acquired from a patient who may himself recover.

To fulfill what I conceive to be my task here I must examine the trends in mortality from these and other infections from 1935 until the present day. Here it is difficult to keep within the strict limits of the subject, because what has happened is due not only to sulfonamides and other synthetic drugs, but to antibiotics, most of which are natural products and thus outside our scope. From about 1945, when penicillin came into general use in most countries, the share of the sulfonamides in what has been achieved can no longer be assessed. Thereafter the choice of effective drugs, and particularly antibiotics, became so wide that their individual effects, or indeed those of antibiotics and synthetics as groups, are impossible to distinguish. The result can only be credited to chemotherapeutics as a whole, scores of which are now available, any one of which would have been a godsend in earlier days.

The following data are all derived from the Reports of the Registrar-General in London. It is likely that experience in Great Britain would be closely paralleled in any similar country.

Hemolytic Streptococcus Infections

Puerperal Fever. Puerperal fever may be taken as the leading example of these infections. Almost all fatal cases were streptococcal, and since the disease is notifiable, accurate data are readily available. It was regularly causing nearly 1000 deaths annually in England and Wales until 1935 despite intensive study, streneous efforts at prevention by hygienic measures, and attempts at immunotherapy.

The effect of Prontosil and shortly after that of sulfanilamide, first thoroughly studied at Queen Charlotte's Hospital in London by Colebrook, is evident from Figure 1. Mortality, until then nearly constant, fell steeply from the crucial year 1935, and continued to diminish more slowly thereafter. The contribution of penicillin to this fall was probably small: On the other hand, the influence of penicillin may perhaps be seen in the reduced incidence after 1945, because its widespread use creates an environment in which hemolytic strep-

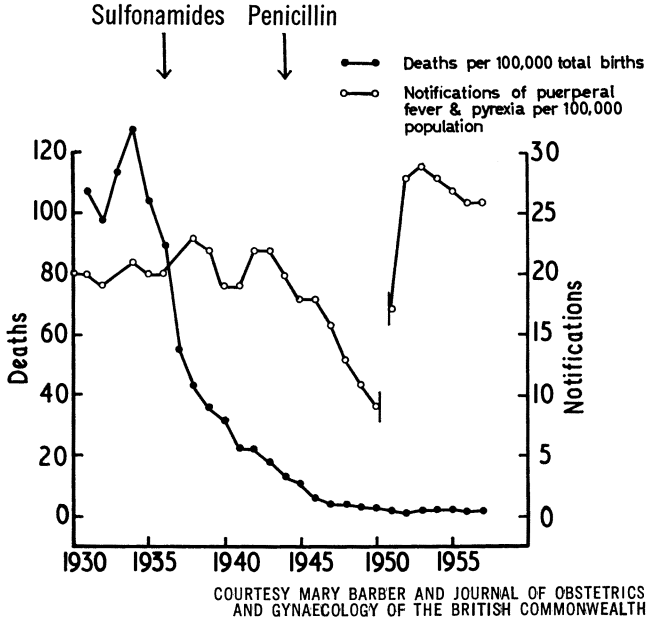

Figure 1. Infection during childbirth and puerperium

tococcus cannot exist, and even potential sources of the infection are eliminated. The apparent rise in incidence after 1950 is an artefact, due to a change in the official definition of puerperal pyrexia.

What is true of puerperal fever applies to acute streptococcal sepsis of other kinds. This most dangerous infection of all kinds of wound, from those sustained in battle to the trivial pricks and scratches which could once lead to septicemia, has lost its terrors.

Pneumonia. Standardized death rates per million from pneumonia in England and Wales in the first 60 years of this century and at different ages are shown in Figures 2, 3, and 4. For the first half of this period they are 5-year averages, and thereafter annual. The fluctuations in this later period are attributable to influenza epidemics.

There was no specific treatment for pneumonia in the early part of this period. The steady fall in mortality seen in Figure 2 from about 1901 to 1931 must be attributed to improved nutrition and general health and to better nursing and supportive treatment. Antipneumococcal serum became available about 1931, but its use was limited. Chemotherapy became possible in 1938 with the introduction of sulfapyridine, shortly to be succeeded by other more effective and less toxic sulfonamides, and the steep fall in mortality which followed, obviously breaking the previous trend, is wholly attributable to these drugs until the mid forties. The continuing steep fall thereafter is doubtless attributable in part to penicillin. In the past few years there has been little change, and the possible effects of more recently introduced antibiotics are not discernible. The present mortality may represent an irreducible minimum, most of the deaths being due to associated disease rather than the pneumonic process itself.

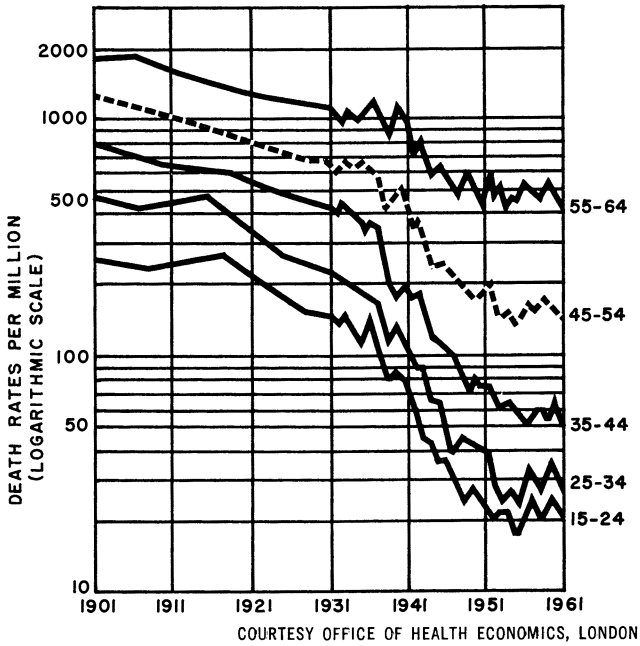

Figure 2. Standardized death rates from pneumonia in England and Wales

Ages 15 to 64, 1901 to 1960

The pneumonia in the age periods shown in Figure 2 is mainly lobar, and it is interesting that mortality increases progressively with age, and that the fall in mortality is least in the highest of these age groups. Corresponding data for the two extremes of life, when bronchopneumonia is commoner, are presented in Figures 3 and 4. In children there has been a reduction in mortality of about 90%, although that in the 0 to 4 group still leaves room for improvement. In the 65 to 74 group there has been little reduction, and at the age of 75 and over mortality has actually increased.

Whatever the explanation of this last finding, it means that modern treatment for pneumonia is saving useful and active lives and not those of aged dependents. It has in fact reduced premature mortality by four fifths, and it can also be calculated that had the death rates of the early thirties continued, 320,000 people in England and Wales now alive would have died from pneumonia. This is a massive achievement, in terms of lives saved much the greatest in the whole field here under review.

Tuberculosis. In the terms referred to, this disease ranks next to pneumonia. Figure 5 compares the mortality rates from tuberculosis in the United States and in England and Wales and shows that they correspond closely. Both World Wars caused an increase in mortality in England, whereas the first had only a slight effect in America and the second none. Our figures show an average annual reduction of 3% in mortality up to 1948, since when it has been 15%. The change at that time is obvious in the graph. This is a disease in

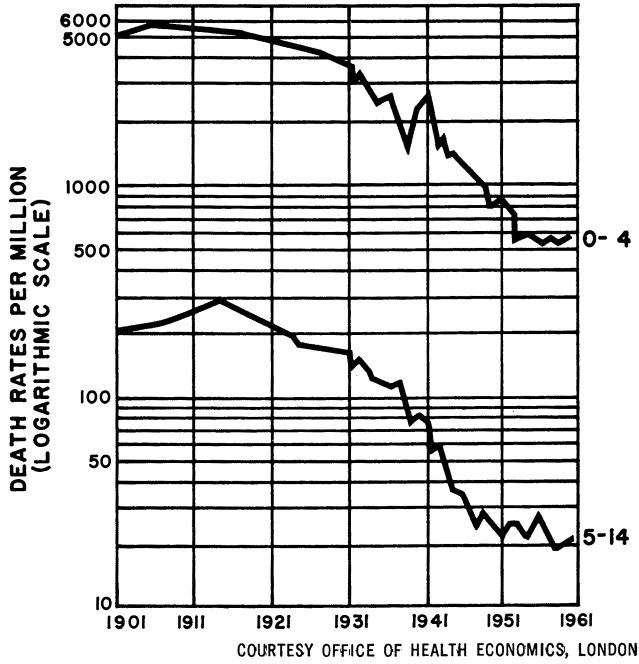

Figure 3. Standardized death rates from pneumonia in England and Wales

Ages 0 to 14, 1901 to 1960

which chemotherapy first became possible not with a synthetic drug but with an antibiotic, streptomycin, but PAS and isoniazid were soon brought in to reinforce its action. Among many drugs of both classes now employed, the synthetic take at least an equal if not a larger share in what is being achieved.

As in pneumonia, the benefits of chemotherapy are greatest in younger people. Figure 6 shows that mortality from tuberculosis in the elderly has been least affected. In contrast to this, deaths in the age group 15 to 29, which were 14,010 in 1930, fell to only 75 in 1960, a reduction of 99.5%. This astonishing figure is of course not wholly attributable to successful treatment of patients in this age group, but also results from the progressive elimination of sources of infection. Notifications of new cases fell by 60% between 1950 and 1960, and to this extent reduced mortality is attributable to lower morbidity. It is safe to predict that in ten years tuberculosis will have become a curiosity.

Cerebrospinal Fever. Meningitis is much the commonest serious manifestation of meningococcal infection, although it can also take the form of a hyperacute or frankly chronic septicemia. Meningococcal infections as a whole are listed in the Registrar-General's Reports for England and Wales, and Figure 7 shows the annual notifications and percentage mortalities from 1931 to 1961. Total mortality increased sharply in 1940, when notifications in fact rose to 12,771 from only 1500 in the previous year. This was a repetition of our experience in the winters of 1914–18, when overcrowding, particularly in mili-

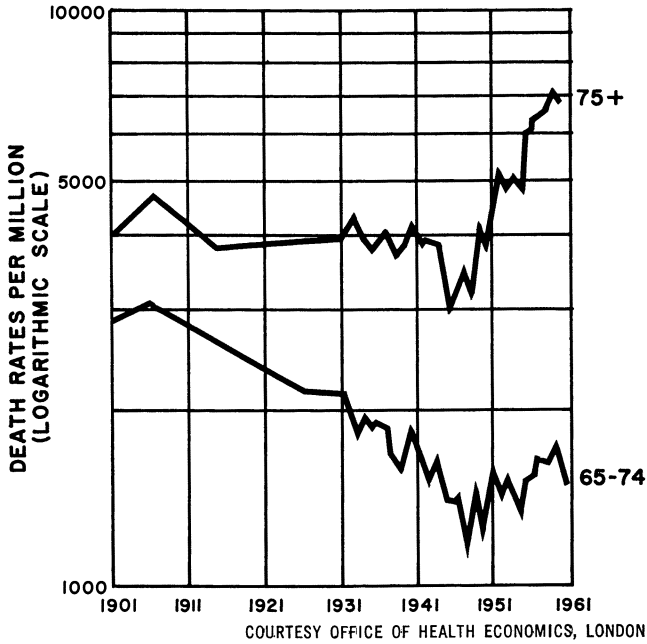

Figure 4. Standardized death rates from pneumonia in England and Wales

Age 65 or more, 1901 to 1960

tary barracks, favored the spread of the infection. Incidence did not fall to the prewar level until 1948, but has since diminished further.

The meningococcus was first shown to be sensitive to sulfanilamide by English workers in 1936, and reports of successful treatment with it of cerebrospinal fever appeared in 1937. Mortality, which averaged 63% in the years 1931–36, fell more gradually than might have been expected: The figures for the next five years are 61, 51, 34, 22, and 19% (Figure 7). It thus took about four years for the profession to learn to use sulfonamides for this disease. There have been fluctuations since, with a maximum of 30% in 1949. The figures for the late forties give no indication that the use of penicillin produced any improvement; whether other antibiotics contributed to lower figures in the fifties it seems impossible to say. Chemotherapy alone may not reduce the mortality further: Another vital factor is sufficiently early diagnosis to ensure success.

Whatever antibiotics may have achieved, it is a tenable view that sulfonamides given alone afford perfectly adequate treatment for this disease. They have the advantage over most antibiotics of attaining high concentrations in the cerebrospinal fluid: Moreover, they are then uniformly distributed, whereas an antibiotic injected intrathecally may fail to reach parts of the cerebral meninges covered with thick exudate. If this view is correct, this is the one acute bacterial infection for which sulfonamides are still indicated in preference to any of the otherwise all-conquering antibiotics.

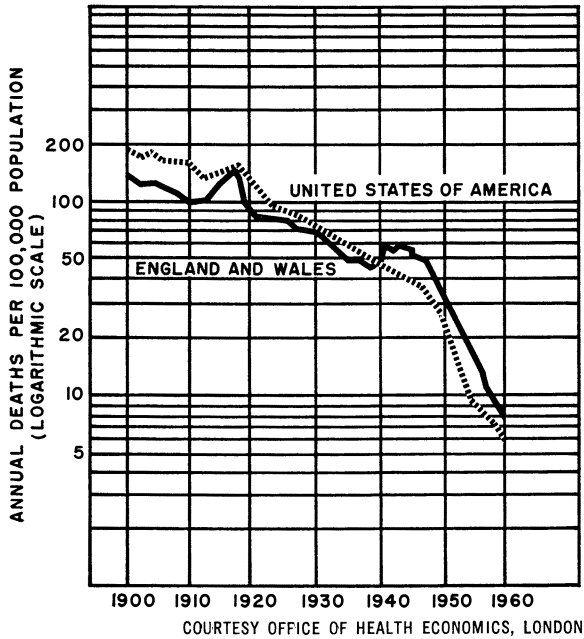

Figure 5. Mortality from tuberculosis in England, Wales, and the United States

1900 to 1960

Achievements of Structurally Changed Antibiotics

The foregoing are the main achievements of chemotherapy in bacterial infections in the past 28 years. I conceive it to be part of my task also to examine briefly the contribution to improved therapy which has resulted from modifying the molecules of antibiotics.

The possible effects of such modifications are of several kinds, some not directly concerned with therapeutic efficacy. In its new form the antibiotic may be more stable, more soluble, more palatable (chloramphenicol palmitate), better absorbed (erythromycin estolate), or less irritant to the tissues (polymyxin methane sulfonate). Some of the most far-reaching effects produced by a minor structural change are seen in demethylchlortetracycline, which, as compared with tetracycline, is more stable, antibacterially more active, and much more slowly excreted.

To find more profound changes than these we must look first to the semisynthetic penicillins (below). The three main defects of benzylpenicillin are

	Acid Resistance	*Penicillinase Resistance*	*"Broad Spectrum"*
Penicillin V	+		
Phenethicillin	+		
Propicillin	+		
Phenbenicillin	+		
Methicillin		+	
(Cl)oxacillin	+	+	
Ampicillin	+		+

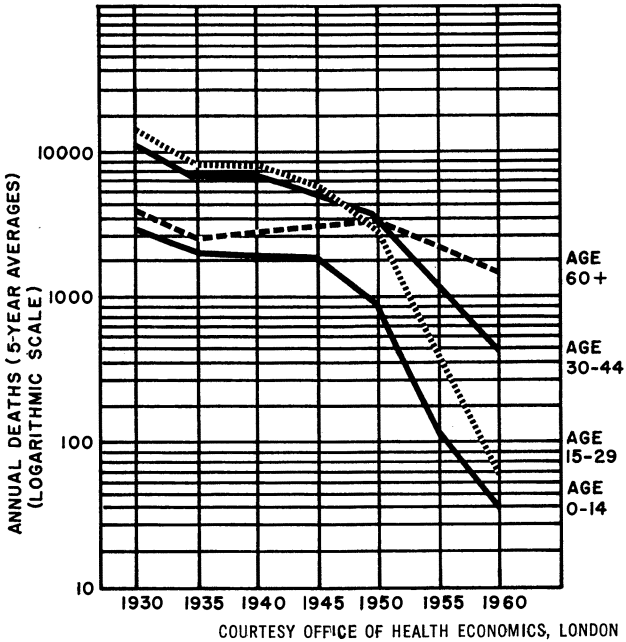

Figure 6. Deaths from tuberculosis in England and Wales by age groups

1930 to 1960

its acid instability, precluding effective oral medication, its susceptibility to penicillinase, depriving it of adequate activity in resistant staphylococcal infections, and its lack of activity against many Gram-negative species. Each of these defects has been to some extent overcome, not always without loss in other directions, but no new penicillin overcomes all of them. Acid stability was achieved years before these developments in phenoxymethylpenicillin. We now have several others with this property—e.g., phenethicillin, propicillin, phenbenicillin—but all this group are rather less active than benzylpenicillin against streptococci and considerably less so against the more sensitive Gram-negative species. Stability to penicillinase has been achieved in methicillin and the isoxazolylpenicillins, and activity against a wider range of Gram-negative species in ampicillin. Two of these products overcome not merely one defect but two. The isoxazolyl penicillins and ampicillin not only have the properties here stated but are also acid-stable.

A personal view is that the most important of these new properties is resistance to penicillinase. Staphylococcal infections are now the most serious complications of surgery, and moreover staphylococci have an unequaled facility for developing resistance to every antibiotic in turn which is brought up to attack them. Not long ago there was an early prospect of seeing the surgeon having to stand with folded hands before the bed of a patient whose life was endangered by one of these infections, because there was nothing that he could do. That prospect has been removed, at least for the present, by methicillin

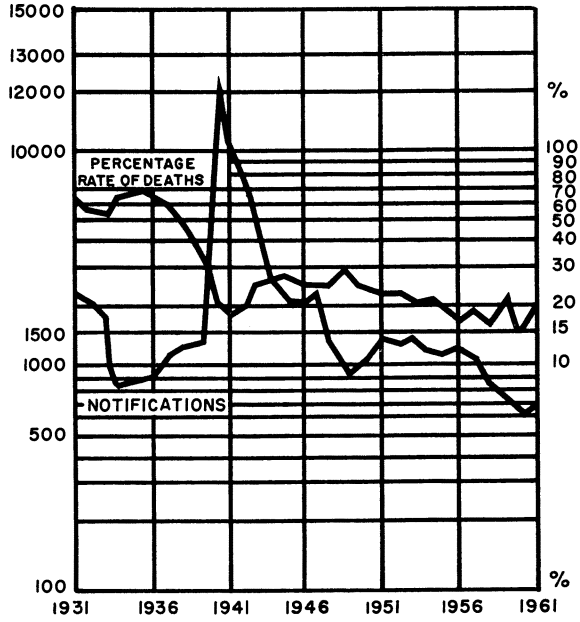

Figure 7. Meningococcal infections
Notifications and percentage rate of deaths, 1931 to 1961

and the isoxazolylpenicillins, and the relief afforded by this resource is immense. Nothing so far apparently achieved by ampicillin can rank with it. If ampicillin had the effect which we seemed entitled to hope for in typhoid fever, the foregoing statement would be untrue, but its effect in this disease is disappointing.

Similar treatment of the nucleus of cephalosporin C (7-aminocephalosporanic acid) has greatly increased the antibacterial activity of this antibiotic, while retaining its high resistance to staphylococcal penicillinase. Thus phenylacetylcephalosporin, as prepared in England, and the related product known as cephalothin in the United States, are further alternatives for the treatment of resistant staphylococcal infections. Their utility for this and other purposes—including some Gram-negative infections—is now being studied.

Future Prospects

Although much has been achieved, we have little right to be complacent. What has been achieved is a fairly firm control over most specific infections communicable from man to man, of which the more important have been examined here, and over some derived from animal sources, such as plague, typhus, and anthrax. In connection with this second group it is a reassuring thought that acquired microbic resistance, resulting from previous exposure to the drug, is unlikely to become a problem.

But while specific infections have been brought under control, nonspecific,

those caused by what have been called "opportunist" bacteria, have correspondingly increased. The findings of Finland and his colleagues on the frequency and bacterial causation of septicemia, meningitis, and empyema at the Boston City Hospital from 1935 until the present day are the best illustration of this. While deaths from hemolytic streptococcal and pneumococcal infections have been much reduced, staphylococci and various coliform bacilli have been increasingly prominent, particularly as causes of septicemia, and the mortality from this cause is actually greater now than in 1935.

Few of these infections are primary. They have always been liable to occur in patients predisposed to them by serious disease of other kinds, such as cancer, leukemia, uncontrolled diabetes, and alcohol and drug addition. To these predisposing causes have been added in recent years more adventurous surgery, more massive radiotherapy, antimitotic drugs, and corticosteroids. In patients whose normal defenses are impaired or shattered in these various ways, such infections are not surprising, nor is it surprising that their response to treatment is poor.

There is another class of bacterial infections in which the response to chemotherapy has been disappointing. These are chronic infections of wounds and sinuses, catarrhs of mucous surfaces, notably nasal and bronchial, and some infections of the skin and urinary tract. Chemotherapy is in fact better at saving lives from acute infections than at relieving ill health due to chronic ones. Some of these conditions have underlying causes with which antimicrobic treatment alone cannot deal. Others, so far as is known, have not. Most of these drugs act best, and some only, on multiplying bacteria, and in these chronic processes a proportion of the bacteria may be dormant, organisms which Bigger called "persisters." We evidently need some other way of dealing with these, and now that this limitation of chemotherapy has been defined, the time has perhaps come for a fresh study of the pathology and immunology of intractable chronic infections.

RECEIVED December 9, 1963.

Discussion

L. C. CHENEY, presiding

Floyd E. Anderson (Northeastern University): I would like to direct the question to both Dr. Zbinden and Professor Garrod, because they have clinical knowledge. Do these longer acting drugs, which are either not excreted or metabolized, constitute a greater hazard to the patient who develops a sensitivity to the hemotherapeutic agent?

Dr. Zbinden: Everybody would agree that once a sensitivity reaction occurs we should try to get rid of the compound left in the body as soon as possible. If you give a drug which is excreted in 40 hours and an acute skin sensitivity reaction occurs, this sensitivity reaction does not disappear faster than if you have a skin reaction to a shorter-acting drug. On the other hand, the depot of course is dangerous, particularly if you give injections which form a depot. There, always new material can be released and prolonged sensitivity reactions can occur.

5

Hypertension, an Important Disease of Regulation

IRVINE H. PAGE

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Next to coronary artery disease, hypertension is numerically the most important cardiovascular disease afflicting civilized man. Several of the barostats controlling arterial blood pressure are known; important ones are in the carotid sinus and in the renal artery. These can be reset at higher levels in hypertension. Many substances control cardiac output, peripheral resistance, and hence arterial pressure levels. Among these are serotonin, norepinephrine, and acetylcholine. Currently under most active investigation is angiotensin, which acts to regulate aldosterone secretion besides being associated with marked and sustained hypertension. Since there are many mechanisms controlling blood pressure, there are many ways to control it by drugs. Prolonged drug therapy results in some patients in reversal of the disease and probable reset of the barostats to normal blood pressure levels. Thus, some patients with hypertension are reversible.

Among the diseases of the heart and circulation, only arteriosclerotic disease of the blood vessels exceeds hypertension as a cause of death. This dubious distinction of hypertension is too seldom recognized by the research public. Years ago we all thought a small amount of high blood pressure was a good thing because it acted as a mild warning to "slow down." But as Figure 1 shows, it takes only a small rise in pressure to influence mortality statistics. One way these small rises in blood pressure help to do you in is by increasing the rate at which arteriosclerosis forms. Make no mistake about it, hypertension is one of the nation's most important diseases and it has, until very recently, been largely ignored. Although it kills more than cancer, there is not a single "institute" in this country devoted exclusively to the study of hypertension. Actually, modern knowledge of the subject began only about 35 years ago.

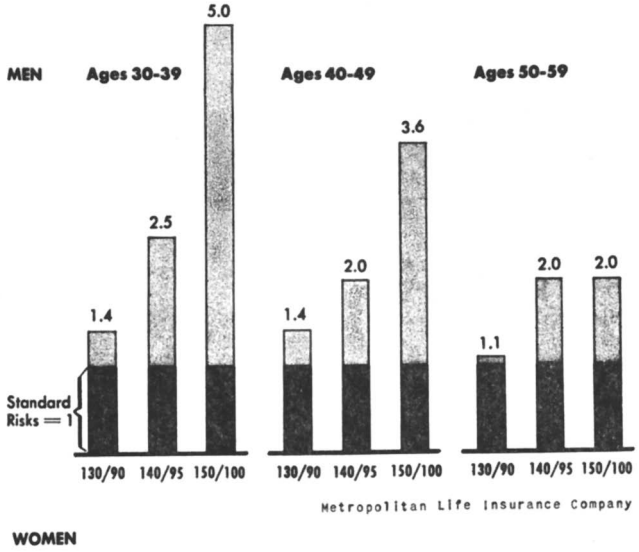

Figure 1. Effect of moderate elevation of arterial pressure on relative mortality over 20 years

Many gifted investigators have built the body of knowledge which I will present. Since science is made of individuals, their names should be given. But on this occasion it is clearly not possible.

Chemists have had an important stake in the field chiefly on two fronts: the determination of structure and synthesis of the several humoral agents concerned in the regulation of blood pressure and the synthesis of large numbers of antihypertensive chemicals now widely used by patients.

Background of Mechanisms Controlling Blood Pressure Levels

Blood pressure is only a part of the mechanism that controls the perfusion of blood through the various tissues, and elevated blood pressure itself does not cause death; rather it is the deleterious effects it has on the smooth muscle of the body, whether the muscle is in the heart or the blood vessels. It is for the latter reason that stroke, representing damage to cerebral blood vessels, heart failure, the inability of the heart muscle to cope with the demand for blood, and kidney failure (uremia), the inability of the kidneys to remove waste products effectively, are the three common, immediate causes of death in the hypertensive population.

There is another broad problem of the greatest importance. When I was an interne in 1926, it was the accepted teaching that elevation of blood pressure was an attempt on the part of the body to force blood through thickened inelastic arteries and arterioles. In other words, thickening of the blood vessels with reduced lumens preceded the rise in blood pressure. To lower blood pressure was to court disaster, because then the tissues would not receive enough blood. For this reason the first research I ever did was to study this problem by means of Van Slyke's clearance method elaborated about 1928.

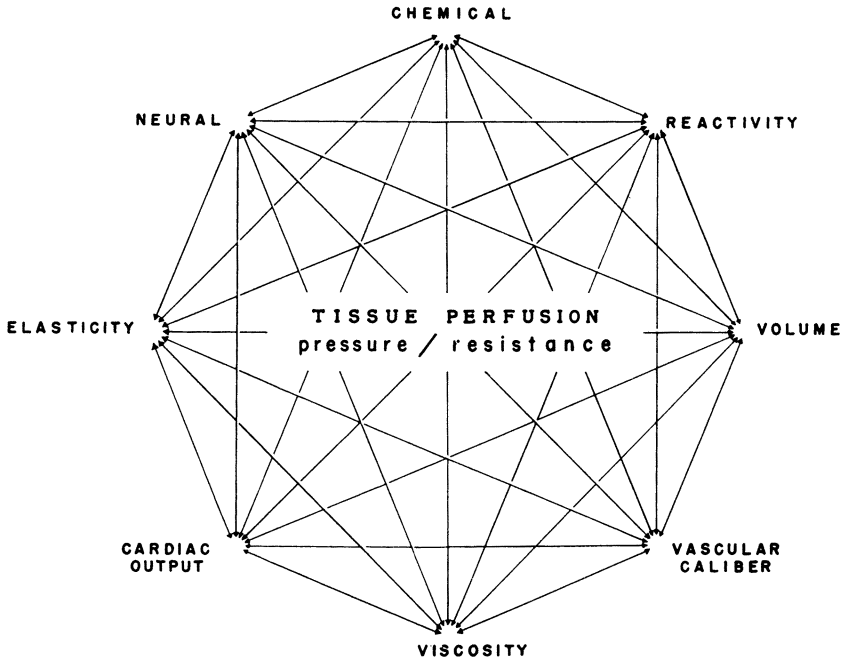

Figure 2. Mosaic theory of hypertension, an equilibrated system for tissue perfusion

This ingenious method gave a rough index of blood flow through the kidneys. It was subsequently greatly developed by my old friend and former associate, Homer Smith. Measurements of discrete function of glomerular filtration rate and renal blood flow using mannitol, creatinine, and *p*-aminohippurate were developed later.

If the theory was correct, lowering the blood pressure of hypertensive patients should reduce renal blood flow. I found this was not true. Lowering of blood pressure to normal levels resulted in no change. Clearly, it was safe to lower the patient's blood pressure in the hope that it might stop the damaging effects of hypertension. Now let us look at several of the mechanisms that control blood pressure levels.

A Disease of Regulation

The body faces an extraordinarily difficult problem, in that a limited amount of blood must be gotten to a great variety of areas, all with different demands. Blood must be withdrawn from one area to supply another and it must be the right area, lest bloodlessness with resultant loss of function result. It should not be surprising that a highly complex regulatory system has been elaborated. Sensing devices widespread throughout the body warn of change. They may be baroreceptors that notify the brain of change in pressure or wave form or chemoreceptors that notify it of change in environmental chemical

composition. Indeed, there are so many facets to the problem that a good many years ago I proposed that the components be looked at as a mosaic (Figure 2) in which all of the varied parts of the regulatory mechanism are in equilibrium with one another.

I want to stress the equilibria in this system. If the body is to maintain any sort of stable blood pressure with the incessant and varying demands of tissue perfusion, a steady state can be had only by closely maintained equilibria. This was all just an idea until recently, when Olmsted (*13*) in our laboratory developed an electromagnetic sensing device for measuring blood flow at the base of the aorta. Concurrently, arterial pressure was measured from a catheter in the aortic arch. Such a system could all be implanted so dogs could be studied in an essentially free-living state. Cardiac output, stroke volume, heart rate, arterial mean pressure, and total peripheral resistance could all be registered on a recorder. Rushmer has used the same general approach but with different instrumentation. In my opinion, these are great advances in the study of the circulation, because normal unanesthetized animals can be used, and a far cry from the highly artificial preparations so dear to the hearts of many pharmacologists.

The important thing to point out is that blood pressure can remain at quite constant levels, but that the mechanisms which maintain it constant change spontaneously. At one time peripheral resistance may be more important, or cardiac output, or stroke volume. The upshot of it is that if you do not happen to be recording all these hemodynamic variables, you might well suppose that a normal blood pressure is a constant always maintained by the same mechanisms.

The next general problem is: What is normal blood pressure? It makes little sense to say that hypertension is present when arterial pressure is 141/91 and is normal at 140/90 mm. of mercury. The set of the blood pressure levels depends on a great variety of things.

Normal blood pressure has a wide range of variability. But when the diastolic pressure is fairly regularly elevated above a statistical norm, it is reasonable to assume and has been proved in insurance experience that damage is being done. Elevation of blood pressure above the normal is "hypertension," but the disease "hypertension" implies more than just elevation of blood pressure. Damage to the heart and blood vessels is the other important facet that must be included in consideration of the elevated blood pressure.

In most patients with hypertension, the disease, cardiac output is about normal while resistance to the flow of blood in the tissues is raised. Blood viscosity and blood volume are also normal. The question is: Where is the resistance increased which results in elevated blood pressure? Most of the older evidence pointed to the arterioles or small arteries, and this is still believed. But it should be extended farther down the line to the precapillary sphincter area, a region that has much to do with the control of the exit of fluid from the blood.

Chemical and Neural Regulation of Tissue Perfusion

For purposes of exposition it seems useful to think of the regulators of tissue perfusion as being in two separate categories, which, of course,

in reality they are not. The chemical controls seem to be the more primitive ones, since phylogenetically they appeared before the organized and integrated nervous system. I can only touch on these briefly, and naturally they will be the ones on which our own group has worked.

Serotonin

In a way, the most intriguing is serotonin—5-hydroxytryptamine—because it illustrates the great economy with which the body seems to use the same substance, or its derivatives, for a great variety of purposes. Our interest began when I found that a vasoconstrictor substance present in serum was forever getting in the way of the search for pressor substances from the kidneys or for those in the blood of hypertensives (*14, 15*). M. M. Rapport and A. A. Green played key roles in isolating and determining the structure of this material. Once it was in our hands and synthesized by K. Hamlin, the study of this material by others became phenomenal. I can only give you an inkling of its possible role in the circulation, which has been so importantly contributed to by my close associate, James W. McCubbin. A very gifted Italian biochemist, V. Erspamer, recognized long before the isolation the existence of what subsequently turned out to be serotonin in extracts of several marine invertebrates. Until the structure was elucidated, we had no notion that his extracts contained serotonin, nor did he.

Serotonin seems to live at least two different lives, one in the brain, and the other in the rest of the body. The story of brain serotonin is fascinating but much too complicated for quick description. That contained in the body may well have a regulatory function on the smooth muscle of the circulation. In clams it seems to cause muscle relaxation and there is evidence that it may act as a neurotransmitter.

The problem of the regulation of the caliber of blood vessels in mammals is a most complex one. Most of us were brought up on very simple ideas of vasoconstrictor and vasodilator substances which had an effect, at least when given as a "slug" by a pharmacologist.

Serotonin is usually a pressor substance, but there are times when it is a pure depressor agent. To get around this difficulty, McCubbin and I felt ourselves forced into creating the word "amphibaric" to describe this situation. Then we found that whether it raises, or lowers, blood pressure depends on the neurogenic tone at the time. If it is high, as after stimulation of the sympathetic chain or induction of neurogenic hypertension, serotonin is a depressor. In short, there was clear sympathetic inhibition.

On the contrary, when blood pressure is low, along with low neurogenic tone, as after destruction of the spinal cord, it has a pure pressor action. The response, then, to a large degree reflects neurogenic tone.

We are faced with one of those interesting situations where it is hard to believe that such an ingenious arrangement for vascular control would not be used by the body. Does serotonin, then, have something to do with blood pressure regulation? This is just one facet of the problem and it is difficult enough. I think it does, but I have no proof for it, only some provocative observations. I am confident that some younger investigator will be "provoked" and will provide the answers.

My guess is that serotonin will be found to be part of the equilibrated system of chemical controls of smooth muscle and that the gross effects most of us study with present crude methods bear little relationship to the physiological mechanisms of the circulation. The story may well have a parallel with norepinephrine. Until von Euler got the idea that norepinephrine was a transmitter, and then distinguished between exogenous and endogenous stores, the understanding of this catecholamine was pretty muddy as well.

Norepinephrine and Histamine

The story of norepinephrine, which is so inextricably associated with the names of my friends, Ulf v. Euler (*2*) and Peter Holtz (*6*), needs no telling again by me. It has grown in our lifetime from very uncertain observations to a great body of fact. Preventing its transmitter function at the myoneural junction in patients has proved one of the key mechanisms by which elevated blood pressure can be lowered. Clearly, the catecholamines occupy a critical position in the chemical regulation of tissue perfusion.

In the past few years, the chemistry and pharmacology of the catecholamines and their metabolic inhibitors have been the subject of intensive investigation in many parts of the world but especially by Sjoerdsma and Axelrod at the National Heart Institute. Monoamine oxidase inhibitors have been tried in the treatment of hypertension with both uncertain results and uncertain rationale. Recently, the decarboxylase inhibitor, methyldopa, has been widely recommended in the treatment of hypertension, though, again, the evidence gives no clear idea as to how it works.

As knowledge of this field expands, it is becoming clear that the relatively simple explanations of the metabolism of the catecholamines of only a year or so ago do not hold. For example, most would agree that the main pathway is tyrosine→dopa→dopamine→norepinephrine→epinephrine. Axelrod, Armstrong, Udenfriend, Sjoerdsma, and others have pointed out that there are many alternative pathways for their formation. Much remains to be done to understand the metabolic transformations in normal animals, quite aside from conditions in which regulation is disturbed. This is an area of knowledge in which very competent investigators are involved and hasty conclusions had best be avoided.

Histamine is still another vasoactive, regulatory substance with a long, and highly respectable, history, so respectable indeed that most investigators lost interest until its revival by Kahlson and Schayer (*8*, *22*). The same is true of brodykinin and kallidin. There is just too much to say to include it in one lecture. Fortunately the subject has been recently reviewed.

Angiotensin

With the great acceleration in the rate of scientific research, investigators have little time for such things as the history of the discovery of angiotensin (*16*). Most of the literature references in current papers cover a maximum of five years. Much of the early work must seem simple and pedestrian to the younger generation.

Angiotensin was discovered in 1939, by two different groups and by

1956 the amino acid sequences of both the deca- and octapeptide were known. Angiotensin was synthesized concurrently by our group with Bumpus and Schwarz playing the leading role and by Schwyzer's group at Ciba in Basel. Now that angiotensin is readily available in ampoules, a blizzard of papers has appeared and is still appearing, covering great sweeps of scientific interest.

A Singular Hormonal Mechanism. I suppose one can debate whether or not angiotensin should be called a hormone. It is an internal secretion from the kidneys and functions, at least in part, as a regulator not only of smooth muscle but of aldosterone secretion. If the catecholamines are considered hormones, why not angiotensin?

What is odd about the renal pressor system is that it originates with the synthesis of an enzyme called renin, presumably in the granules of the juxtaglomerular cells (*5, 27*). Change in intraluminal pressure seems to be a sufficient cause for release of renin into the blood stream, which then acts on renin substrate, synthesized by the liver, to produce first a decapeptide which seems to have little activity. But if histidylleucine is split off it by the "converting enzyme" or what Helmer and I formerly called "angiotonin activator," it becomes highly active. The converting enzyme is highly chloride ion–dependent (*23*). There also is uncertain evidence of some substance contained in plasma which is necessary for the action of angiotensin on smooth muscle (*17*). This can be shown by perfusing a rabbit ear vessel with Ringer's solution. After a time the vasoconstrictor response diminishes and finally disappears. If now a small amount of plasma is added, the vasoconstrictor response returns.

Chemistry of Angiotensin. Most of the work on the isolation, identification, and synthesis of angiotensin was done before the current methods for such purposes were available. With large teams of peptide chemists, synthesizing peptides currently is a matter of weeks at such laboratories as those of Ciba and Sandoz. The older work must look uninspired and plodding in comparison. This was not so. The number of people then involved was miniscule and the amount of interest equally miniscule. Especially Helmer and Bumpus and for a time Plentl, worked unceasingly. Now that angiotensin is readily available in bottles and is widely used, most people have forgotten the sweat that went into making it that way. It still is being taken for granted.

Skeggs and Kahn made important contributions showing there were two peptides, a decapeptide with little physiological activity and an octapeptide resulting from splitting off histidylleucine by the action of an enzyme contained in plasma. Elliott and Peart first showed the correct sequence of amino acids in bovine angiotensin I and that from horses was determined by Skeggs *et al.*

A long time ago, Plentl, Davis, and I (*20*) showed by means of ammonium sulfate fractionation and electrophoretic analysis that renin substrate is contained in the alpha-2-globulin fraction of plasma. Skeggs and his group (*24*) recently synthesized a tetradecapeptide which acts as a renin substrate. Renin splits off the decapeptide angiotensin I by hydrolysis between two leucine residues. When the tetradecapeptide is added to plasma, the rate of its hydrolysis by renin is sharply reduced. Skeggs and Kahn's latest contribution is to show that hog renin substrate is separable into three major and two minor forms. They are all glycoproteins with molecular weights of about 57,000 and have similar amino acid compositions. Differences exist in sialic acid, glucosamine,

and neutral hexose content. But they are all hydrolyzed by renin at similar rates and yield the same angiotensin I.

Our synthesis of angiotensin, beginning with the eight naturally occurring amino acids, led through 26 steps to an octapeptide identical with angiotensin II. Schwyzer and his group synthesized valine-5-angiotensin II asparagine amide, while ours was the isoleucine-5-angiotensin II.

Even though we isolated enough pure angiotensin from the original synthesis for proof of structure, the methods needed improving. The impurities in the earlier preparations were so similar to angiotensin that they were difficult to remove. For example, during addition of aspartic acid as a penultimate step in the synthesis, followed by removal of blocking groups, a moderate amount of the beta-aspartyl, rather than the alpha analog of angiotensin, resulted.

To study the relationship between structure and biological activity, peptide analogs must be prepared that are both chemically and optically pure. To accomplish this, another synthetic approach was used. Here, Arakawa and Bumpus employed blocking groups that could be removed simultaneously by catalytic reduction under mild acidic conditions. For optimum yield of optically pure peptide, we showed it best to start with the *C*-terminal amino acid ester, adding one acid at a time.

Acetic acid does not cause the alpha to beta shift of aspartic acid, while even a dilute solution of strong mineral acids such as hydrochloric or sulfuric acid brings it about. Even though the beta-aspartyl analog in terms of pressor activity equals that of angiotensin itself, it is not split by the plasma enzyme angiotensinase A. Use of angiotensin with the beta-aspartyl impurity present could lead to erroneous conclusions, especially in studies on rates of enzymatic destruction.

Structural Requirements for Activity. It is of great importance to know the structural requirements for the activity of so powerful a substance as angiotensin. A summary of the work from our laboratory is contained in Figure 3. The hexapeptide is the minimum chain length for significant activity

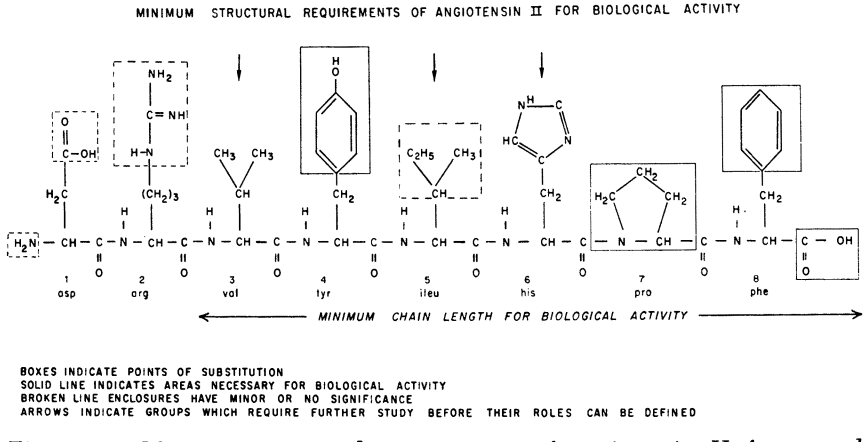

Figure 3. Minimum structural requirements of angiotensin II for smooth muscle contraction

of angiotensin. Boxes indicate the points at which substitutions have so far been made and the solid lines the areas essential for biological activity. Broken line enclosures contain groups of minor, or no, significance. Arrows show groups which need further study. The essence of this problem seems to be that the carboxyl group of phenylalanine[8] needs to be free to bind a receptor site. Alanyl[8] angiotensin is inactive, showing the importance of the phenyl group in this position. Proline also is necessary in position 7.

The activity is not altered by change of valine to isoleucine in position 5. The phenolic hydroxyl and aromatic ring in position 4 are important.

The observation that incubation of angiotensin with urea or arginine decreased its myotrophic action suggested that its secondary structure is important to its activity. Study of optical rotatory dispersion by Smeby *et al.* (*26*) showed the peptide to have definite order which is decreased by urea. Measurements on poly-γ-L-glutamate had indicated that the helical configuration becomes stable when there are eight amino acids in the chain. Further, ultraviolet spectral studies on the phenolic hydroxyl group indicate that it does not interact with any other group in the molecule.

A model consistent with these physical data has been constructed on the assumption that the peptide chain will form an alpha helix in so far as possible. In this model three of the groups essential for biological activity, the *C*-terminal carboxyl group and the two aromatic rings, are all in close juxtaposition. The smallest unit capable of forming an intramolecular hydrogen bond to maintain these groups in close proximity is the hexapeptide and the latter is the smallest unit possessing significant biological activity. All do not agree with the evaluation of the conformation of angiotensin. Paiva, Paiva, and Scheraga (*19*) believe the evidence is much more in favor of random conformation. Only more study can resolve this problem.

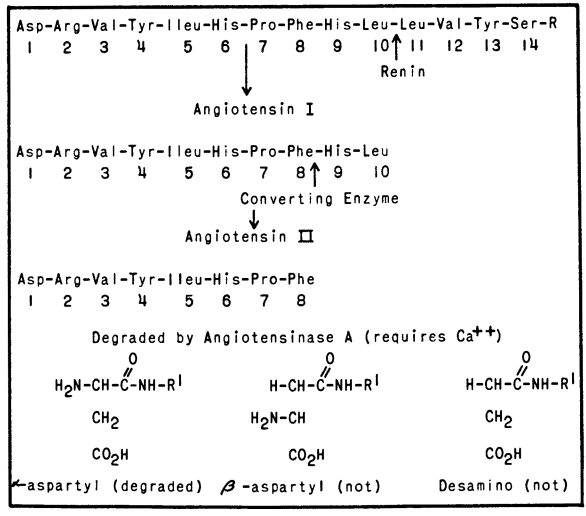

Figure 4. Angiotensin formation and enzymatic destruction

Destruction of Angiotensin. There is now firm evidence that the destruction of angiotensin is enzymatic. Khairallah, Bumpus, Smeby, and I (*10*) have separated a peptidase from plasma and red cells which has a common pH optimum, requires calcium ions, and hydrolyzes both valyl[5] and isoleucyl angiotensin II. The enzyme is specific, because if the terminal aspartic acid is shifted to the beta-position or arginine is substituted for it, or the terminal amino group is removed or poly-*o*-acetylserine substituted, the enzyme is inactive. We have called this enzyme angiotensinase A to distinguish it from other closely related leucine aminopeptidases. One possible source of error in measuring angiotensinase activity could be the fact that the alpha-aspartyl angiotensin is easily converted to the beta-linked one, yet the latter retains full pressor activity.

The reason this problem is currently of especial interest is that several competent investigators have reported contradictory results on the measurement of the ability of plasma to destroy angiotensin, depending on whether the plasma was from hypertensives or normotensives. We still do not know whether the hypertensive destroys angiotensin more rapidly or less rapidly than normal, or just normally.

We have also tritiated angiotension to determine the rapidity of destruction and the distribution of angiotensin in the body. The destruction must be rapid, because within 30 minutes peptide fragments were recoverable from organs of rats infused with it. I have summarized what we know about the information and destruction of angiotensin in Figure 4.

So far, no one has found a true inhibitor of angiotensin, though I have suspected that one might be concerned in the mechanism of renin tachyphylaxis—that is, the phenomenon of failure to respond after repeated administration of renin. Curiously, the response to angiotensin is also lost. I say, curiously, because tachyphylaxis to angiotensin itself is very difficult to elicit. There is also some inconclusive evidence that renin tachyphylaxis can occur before failure of angiotensin to elicit a pressor response. This only suggests the possibility that an inhibitor might be involved and is of interest in connection with the rate of destruction of angiotensin.

Angiotensin as a Physiological Regulator of Blood Pressure. Angiotensin is, on a weight basis, by far the most active pressor substance known. It acts on isolated smooth muscle to cause severe contraction. In unanesthetized dogs the rise in blood pressure from single injections or short infusions is due chiefly to an increase in peripheral resistance while cardiac output either falls or stays about the same. Olmsted and I have given continuous infusions into free-living dogs for 6 weeks and the effects depend on how long and how much has been given. It needs pointing out again and again that pharmacological studies on the circulation, to be complete, must be carried out in animals with implanted flowmeters and catheters for infusion and blood pressure measurements. Anesthetics and abnormal positions of the animals, among other things, greatly alter the response to vasoactive drugs.

I must brush over the problem of the occurrence and release of angiotensin lightly, even though this is one of the "hottest" subjects in the field. Its great importance practically stems from the fact that clinicians would like to know whether the kidney is producing the hypertension in a particular patient. If renin and angiotensin are part of the mechanism of renal hypertension, they

should be measurable in the blood. Many capable people are working on the problem, many methods are currently available, and they mostly disagree with one another.

From the studies of the Hartrofts (5) and of Tobian (27) the evidence is fairly strong that the granules seen in the juxtaglomerular cells around the afferent renal arterioles contain renin. In this case as in the measurement of renin, now is the time to use great caution in the interpretation of the changes that occur in these granules as measured by counting their number in sections of the kidney. Already it is clear from the literature that this is far from a wholly reliable method.

Skinner, McCubbin, and I (25) have asked ourselves what the stimulus is for the release of renin. According to our experiments reduction in perfusion pressure, even a very small one, is a sufficient stimulus. Ischemia or lack of blood is not necessary. In short, it seems to us that the juxtaglomerular cells are baroreceptors—pressure-sensitive stats that respond to physiological changes in renal arterial pressure.

This question of ischemia has plagued the field ever since Goldblatt put on his clamps. We are on the "anti-ischemia" side, because Corcoran and I years ago measured renal blood flow by clearance methods in dogs developing cellophane perinephritis with hypertension. The hypertension appeared well before any significant ischemia. Furthermore, McCormack of the Cleveland Clinic's Department of Pathology has several examples of kidneys removed from our patients which morphologically show no evidence of ischemia, yet the hypertension was relieved by the nephrectomy.

I am more interested in the demonstration of the appearance of angiotensin and renin in the renal vein effluent and in the peripheral blood. The methods used for such demonstrations have been so divergent that there is much skepticism about the results. We, at least, feel we have good physiological and chemical evidence for the appearance of angiotensin. Helmer, Genest, Skeggs, Paladini, Peart, Morris, and others might feel differently.

We believe that our evidence suggests that angiotensin is not just a factor in hypertension but is a normal regulatory hormone helping to maintain normal blood pressure. When pressure falls, renin is secreted and the angiotensin formed raises arterial pressure. When this happens, the secretion is reduced.

The demonstration that angiotensin is the chief regulator of aldosterone secretion, by Genest (*4*), Laragh (*11, 12*), and later Davis (*1*) and several others, created a sensation in cardiovascular and endocrine fields. In retrospect, it is easy to see why this makes sense from the body's point of view.

Aldosterone acts to retain Na^+ powerfully and with the retention of Na^+ a corresponding amount of water is retained, so increasing extracellular fluid and blood volume. This alone tends to raise blood pressure. But retention of Na^+ by the blood vessel wall tends to increase vascular tone. This effect has been recently well studied by the Friedmans (3).

Neural Regulation

Since Koch and Mies had produced hypertension by cutting the buffer nerves in dogs and rabbits, it was clear that the presumption that the nervous system was concerned in the genesis of hypertension was not unfounded. The

various forms of sympathectomy added some support, but the evidence was uncertain and highly emotionally tinged.

There were also, and in fact still are, attempts by psychiatrists to paint the picture of a mental mechanism responsible for hypertension. The trouble is, therer are so many pictures that it is impossible to decide on any one or, indeed, any combination. Furthermore, attempts at psychoanalysis did not help matters, in that it has not appeared to help the patient's blood pressure. Still there is the feeling that the psychological aspect at least plays a supporting role.

Attempts have been made to elicit hypertension in animals by various stressful means such as continual audiogenic stimuli, and by diabolically designed frustrations in anthropoids. I saw such an effort during a visit to the monkey colony in Sukumi on the Black Sea. On the whole the results so far have not been impressive. But the sort of studies now under way under the guidance of Horsley Gant and Cowles Andrus at Johns Hopkins could be rewarding.

The odd fact is that much truly convincing evidence of nervous participation as contrasted with the mental has come from therapeutics. Think of the many drugs now available for the treatment of hypertension. Tranquilizers alone have not been effective, but where this aspect of their actions is combined with others as in reserpine, their partial effectiveness is unquestionable. Reserpine has both a central and peripheral action and seems chiefly involved in the release and storage of catecholamines and serotonin.

The autonomic ganglia have also been favorite points of attack by the therapist. You all recall those dreadful days when the ganglioplegic drugs such as hexamethonium and pentolinium were being used. The difficulty was, of course, that they paralyzed all ganglia in which acetylcholine was the transmitter, which included both sympathetic and parasympathetic. Still, they did lower blood pressure effectively in many cases, if used in great enough doses. Then came drugs that acted at the vasomotor myoneural junctions, such as guanethidine, along with hydralazine, which possibly acted on the smooth muscle itself.

The dosage for all these drugs when used alone was too high to achieve adequate blood pressure control; hence it was a blessing when chlorothiazide and its many derivatives appeared. These natriuretic drugs by virtue of acting in a variety of ways allowed most of the antihypertensive drugs to be much more efficient and hence smaller doses were required. This reduced the intolerable side effects in many cases.

Along with the clinical results there was good evidence from the experimental side as well. We gave guanethidine, as had Maxwell, to dogs with chronic experimental renal hypertension and showed that severe falls in blood pressure could be achieved. Yet most would agree that this form of hypertension has as its chief component a renal mechanism. Clearly it has a neural one as well.

I will end this part of the discussion by telling a little of the work that James McCubbin and I are now doing.

We had made a long and tedious study of the cardiovascular reactivity of normotensive compared to hypertensive dogs, both with and without anesthesia. An important point we found was that tyramine was unusual in showing an in-

creased response in both acute and chronic renal hypertension but much less in the latter. Angiotensin infusion also increased the response to tyramine in normal dogs, but not when the angiotensin infusion was given during the stage of acute hypertension. The upshot is that since tyramine acts by releasing norepinephrine at nerve endings the released norepinephrine seems to act more effectively in the presence of angiotensin.

This phenomenon provides a suggestive bridge between one of the important renal mechanisms and a neural one. There must be many more but you will see in this still another way interdigitation of the mechanisms controlling blood pressure and tissue perfusion occurs. It is not important to memorize all the twisting and turnings of these complicated regulating mechanisms but rather to realize how many different facets there are to this problem, any one of which can importantly influence the mechanisms and levels of arterial blood pressure.

The Resetting Phenomenon

The carotid sinus buffering mechanism is one of the most powerful in the body. When, for example, the buffer nerves are cut, blood pressure rises to markedly hypertensive levels and stays there. If this is so, it might have been confidently anticipated that this mechanism would be called upon as a defense against development of hypertension. Yet clinical observation showed that pressure over the sinus elicited in hypertensive patients as much of a response as in normotensive individuals. In short, it was as active in the hypertensive as in the normotensive.

This seeming lack of interest of the buffer mechanism to help maintain arterial pressures at normal levels became the subject of study by McCubbin, Green, and myself. The electrical impulses coming from the carotid sinus nerve were measured and it could be shown readily, as others had done, that when pressure within the sinus was raised by intravenous injection of norepinephrine, the impulse traffic to the vasomotor center greatly increased, inhibiting the vasomotor center and reducing the outflow of vasoconstrictor impulses. This led to a decrease in pressure. These observations were in normal dogs.

If the same measurements were made in dogs in which experimental renal hypertension was induced, something different happened. At first the sinus fired with vigor to aid in keeping blood pressure down, but after days it seemed to give up the struggle. Now it responded only normally at the higher pressures. In short, the regulator had been reset for higher pressure levels and after this operated to maintain the higher level.

At the moment I am more interested in the principle of this resetting phenomenon than the details of its mechanisms. It illustrates beautifully the great importance of looking at diseases such as arterial hypertension and atherosclerosis as "diseases of regulation." The term "arterial hypertension" is not a good one to describe the cardiovascular disease in which not only is the pressure abnormally elevated but there is accompanying vascular disease. It is obvious that it is the vascular disease which kills the patient.

I believe that drugs will be found which will aid in resetting the regulating mechanisms down as well as up. This will be still another facet where success may be achieved in the treatment of hypertension.

Clinical Renal Hypertension

There are few subjects that have had such a checkered career as clinical renal hypertension. Most historians credit Richard Bright with the recognition of the association of hypertension with the kidney. Probably Volhard did as much as anybody in our time to bring out such a relationship. During short periods the kidneys have received the loving attention of urologists, who removed them if they seemed to offend. Then the kidneys were given the cold shoulder, only again, some years later, to achieve the limelight, but this time mostly in the hands of a new group, the vascular surgeons. As usual, we medical people stand by to pick up the pieces, if and when the whirlwind has passed.

There was much skepticism about whether renal stenotic lesions actually existed, and many pathologists said they did not. It was necessary to persuade physicians that the renal arteriogram was both a safe and a highly useful procedure. Howard (*7*) started the ball rolling as a result of the very careful and perceptive study of a few patients and Poutasse and Dustan (*17*, *21*) kept it going by resolute and skillful use of the arteriograms.

I have often twitted both the cardiologists and the pathologists for so long having overlooked myocardial infarction, which has not increased my popularity. But it is strange how long a thing can be overlooked that is so obvious, once it is called to our attention. It is only a little less puzzling that the renal arteries were so little examined that the bizarre lesions which afflict this artery went unrecognized. Even odder seems to be the fact that other arteries in the body do not seem to participate in the same pathological processes. Or is it that we just have not looked hard enough? Thrombosis and stenosis of the carotid arterial system have only recently been recognized.

McCormack, of the Cleveland Clinic Division of Pathology, has probably seen more of these elsions than most. He classifies them, aside from atherosclerosis, largely on the basis of muscular and fibrous hyperplasia. There are a great variety of lesions ranging from aneurysmal dilatation to tight fibromuscular hyperplastic stenosis.

The problem of whether the stenosis seen on an x-ray film is of sufficient magnitude to produce the hypertension remains unsolved. Dustan and I are convinced that many such lesions can be associated with normal blood pressure, and some others would agree. But opinion is also strong on the other side. All that can be said about this important problem is that neither the arteriogram nor the radioactive renogram can determine positively whether the renal lesion is the cause of the hypertension. Fortunately, in many patients it gives a very good indication of it as judged by the fall in arterial pressure when the lesion is corrected surgically.

One of the reasons so many investigators have been interested in measuring the angiotensin and renin content of renal vein or, even better, peripheral blood, is to use this as a measure of the participation of the kidneys in the production of the hypertension. This is also a reason for being sure that the method is correct; otherwise many patients will be operated upon needlessly. And in view of the fact that many of these renal hypertensives have widespread atherosclerosis, the surgical risk is increased.

We do not hesitate to treat patients medically if we think surgery is either

dangerous or may not help. On the other hand, there are times when operation is recommended not on the basis of lowering blood pressure but to conserve renal parenchyma.

I want to convince you that hypertension is a "disease of regulation" in which the regulation of the perfusion of individual tissues is at stake. I have tried to make it clear that, in my view, diseases of regulation are mosaics in which all the pieces are in dynamic equilibrium. The creation of a steady state within the circulating fluids of the body is essential to their life and well being. To accomplish this, the body uses both the sophisticated integrated nervous system and, as well, the more primitive but no less complicated chemical systems. It is the interplay of these two great systems expressed on the hereditarily conditioned cardiovascular system that finally determines how well blood gets distributed and how long the system will last.

Accelerated Atherogenesis and Hypertension

It has been clear to most clinicians who are familiar with the natural history of hypertension that atherosclerosis occurs relatively early, and severely, in these patients. Elevated arterial pressure is one of the important facets of atherogenesis. The recent careful studies by Kannel *et al.* (9) done in Framingham, Mass., show beyond doubt that the magnitude of the increased risk of coronary heart disease as a result of hypertension is of the order of a 2.6-fold increase in men 40 to 59 years of age and a sixfold increase in women in the same age group. In contrast to the much greater risk factor of hypertension in women is the fact that elevated serum cholesterol levels contributed only slightly to increased risk in them as compared with men. Elevation of cholesterol levels above 245 mg. per 100 ml. was associated with more than a threefold increase in risk among men aged 40 to 59 years. You can readily see why men long ago lost the "battle of the sexes."

Reversal of Hypertension by Long-Continued Treatment

When I first came to work with Van Slyke in 1930, there was no treatment for hypertension worthy of the name. Sympathectomy was probably the first serious attempt to lower blood pressure as a therapeutic measure and potassium thiocyanate the first medical contribution, along with low-salt diets. Then followed a period in which kidney extracts were tried experimentally and this showed beyond a doubt that the occasional reversal of the malignant syndrome seen with sympathectomy occurred and with considerably more regularity. The problem of the use of kidney extracts then divided into two parts, one concerned with the use of pyrogens and the other with the possibility that the kidneys contained a depressor substance. Arthur Grollman and his associates were much involved, as was our group. Much was learned during this period about the natural history of hypertension. We learned, for example, to recognize the pheochromocytoma, the hypertensive diencephalic syndrome, and the importance of pyelonephritis.

Then came the period of rapid growth in drug treatment of hypertension: hydralazine, ganglion blocking agents, reserpine, chlorothiazides, guanethidine, and much more recently, methyldopa and pargyline. I want to keep your

minds on the regulatory nature of this disease. If we could reset these regulators down by lowering blood pressure for long periods of time, the pressure might stay down.

This thesis has been examined by Harriet Dustan and myself. We took our patients who had been under exacting treatment for many years and withdrew all treatment, often substituting placebo. A number of them showed an immediate rise in pressure, but to our delight a number also showed no rise, even after a couple of years. It was as though keeping the pressure down had reset the barostats and the chemostats and now they worked to keep the pressure down instead of up.

When we speak of exacting treatment, we mean just that. In short, lying as well as standing blood pressure must be measured. Several of the recent drugs which are being introduced do little more than elicit orthostatic hypotension. This is not enough. We also insist the patients take his blood pressure himself, regularly morning and evening. He reports to us if his pressure is not being controlled. Usually, we readmit him to the clinic for reevaluation which would ordinarily be done routinely anyway, every 1 to 12 months. It has been said that patients become neurotic when they take their own blood pressures. This is nonsense; anyway, if patients are not a bit neurotic these days they must be illiterate. We do not take blood pressure control lightly and if the patient does not want to cooperate fully we make it clear that we do not want our time, or his, wasted. We are not yet certain which among the various groups of hypertensives respond with a semipermanent fall in pressure.

Along with the fall in pressure that I had seen years ago was the remarkable phenomenon of clearing of the vascular lesions in the eyegrounds of patients with malignant hypertension. This was so impressive that all during the contentious days of sympathectomy and kidney extracts, the one triumph we never doubted was the reversal of these lesions and the reduction in heart size. This has often made me wonder if a form of vascular remodeling does not take place when pressure is lowered. It has been noted recently, for example, by Naeye, that during fetal life the mass of muscle in the pulmonary bed much exceeds that in the systemic, but at birth the mass of systemic muscle continues to increase while that in the pulmonary decreases about 40% in two weeks. It has been known, or at least suspected, for some time that the muscle mass of the heart changes with varying loads. It could well be that part of the improvement we have seen in our hypertensives was due to smooth muscle remodeling.

Conclusions

I fear I have brushed altogether too lightly over many complex problems, but I have done so in the hope that you would keep the main theme of "diseases of regulation" in mind. I know that age is beginning to play tricks on me; that sometimes old concepts get dressed up in new garb. This change is, I think, beautifully expressed in the modern version of wine, women, and song, which has become Metrecal, the same old gal, and sing along with Mitch. It has been 43 years since I first wrote a paper and that one was on the algae of Hyannis Port harbor. Shields Warren was the other member of our team and

he studied the shells. His early acquaintance with the "shell game" has been invaluable in his later negotiations. Algae prepared me far less well.

I hope I have not told too much about the less chemical aspects of hypertension. I wanted to give some inkling of the many facets of blood pressure control that can be influenced by various chemicals. My greatest criticism of chemists at the moment is that they become too preoccupied with drugs that act on much the same mechanism. One of our major needs, currently, is substances that block the action of angiotensin. If you will just provide me with such a substance, all this talk will have been richly rewarding.

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Antihypertensive Therapy

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Effective antihypertensive drug therapy began with the ganglion-blocking agents, followed by reserpine and hydralazine. Later advances included the benzothiadiazine saluretic agents and more recently compounds which specifically inhibit the sympathetic nervous system, such as guanethidine, alpha-methyldopa, and certain amine oxidase inhibitors. Among the antihypertensive drugs, molecular modifications seem to have yielded the greatest clinical benefits in the period immediately following the discovery of an agent with a new type of action. Further improvements have been limited because of inability to circumvent objectionable side effects which were indissolubly linked with the mode of antihypertensive action of a given class of compounds.

It is remarkable that effective antihypertensive agents have been developed entirely during the past 15 years. The thread which ties together this period of therapeutic progress has been based on a continuous interchange of information between the clinic and the chemical laboratory, in which specific therapeutic needs have motivated the direction of chemical development. This paper discusses the manner in which new compounds lead not only to therapeutic advances but also to fresh problems. It also indicates how molecular modifications have been used sometimes successfully, sometimes fruitlessly, to meet the continuing demands of the clinician.

Ganglion-Blocking Agents

The main path of development of antihypertensive agents began with the observation that tetraethylammonium chloride inhibited the transmission of nerve impulses through sympathetic ganglia in both animals and man and transiently lowered blood pressure in hypertensive patients (*12*). Paton and Zaimis (*15*) then proceeded to investigate a series of quaternary ammonium salts in which varying numbers of methylene groups were interposed between

two trimethylammonium cationic heads. The higher members of the series, such as decamethonium, were found to have a curare-like action—that is, they produced paralysis of voluntary muscle. The compounds with fewer methylene groups produced ganglion-blocking action; hexamethonium appeared to be the most effective and was found to be a potent antihypertensive agent. It represented the first clinically practical drug treatment for severe hypertension (*6, 16*). The reversals of malignant hypertension to a more benign phase of the disease were so dramatic that they revolutionized the methods of treating hypertension which, until that time, had been dominated by surgical sympathectomy and low-salt diets.

There were, however, many undesirable features related to hexamethonium treatment. The drug was not very effective or predictable in its action when given by mouth, unless the doses were pushed to high levels and, at these high doses, severe side effects could occur. Paralysis of the bowel was a particularly dangerous reaction when large doses had been given orally, since a large amount of the drug remained in the intestinal tract, prolonging and aggravating the toxic effect. Because of poor absorption, the drug was given by injection in most cases, in the same manner as insulin in a diabetic. An unusual toxic reaction associated with hexamethonium was pneumonic infiltration of the lung, which was not seen with the blocking agents developed later.

Because of potentially severe toxicity and the need for parenteral injections, pharmaceutical research was directed toward developing ganglion-blocking agents which would be less toxic and could be effectively administered orally. Such a drug, another diquaternary ammonium salt, was soon discovered and was given the name of pentolinium tartrate (*7, 20*). There was no doubt that pentolinium, or Ansolysen, represented a considerable improvement over hexamethonium. It was more potent, much more effective by mouth, and somewhat longer acting, and caused no pneumonitis and far less paralysis of the bowel. It quickly replaced hexamethonium as the treatment of choice in severe hypertension.

Pentolinium, however, had certain disadvantages which made it impractical for the treatment of mild or moderate hypertension. The disturbing side effects associated with blockade of the sympathetic and parasympathetic nervous system were still present: constipation, dry mouth, impotence, chilling in a cold environment, and blockade of the reflex adjustment of blood pressure which normally occurs when the patient assumes the erect position. Occasionally, the postural hypotension was so severe that there was insufficient blood flow to the brain and the patient fainted.

Another feature of all ganglion-blocking drugs was that the blood pressure fluctuated considerably from very low to high values. Despite careful adjustment of the dose, this fluctuation could not be done away with entirely. In the attempt to prevent the peaks of pressure the doses were elevated so that the patient at another time might be unable to stand erect because of postural hypotension.

Quaternary ammonium bases are poorly absorbed from the gastrointestinal tract. In the case of hexamethonium and pentolinium less than 10% of the ingested dose is actually absorbed. Further development of effective ganglion-blocking agents, therefore, was directed toward improving absorption. It was hoped that the fluctuations in blood pressure and the severity of the side effects

associated with pentolinium would be overcome if the blocking agent was completely absorbed.

This reasoning led to the development of mecamylamine by Stone and his associates (*19*). Mecamylamine is not a quaternary base but rather a secondary amine which permits complete absorption from the gastrointestinal tract. This modification unfortunately did not strikingly improve therapeutic effectiveness (*8*). Clinical trials soon showed that the fluctuating blood pressure response associated with the ganglion-blocking agents was not due primarily to variable absorption.

Saluretic Agents

This clinical experience with mecamylamine made it clear that the cause of the variable responsiveness to the ganglion-blocking drugs was not poor absorption but rather changes in the responsiveness of the patient's blood pressure to ganglionic blockade. A most important factor was the state of hydration, or amount of salt and water in the body (*5*). When patients became edematous they required larger doses of blocking agents to lower their blood pressures, and the blood pressure was more difficult to control even with the larger dose. Thus, accumulation of salt and water, leading to expansion of extracellular fluid volume, produced increased resistance to the antihypertensive effects of ganglion-blocking drugs. Contrariwise, mercurial diuretics or salt-depleting diets made patients more responsive to these blocking agents. Furthermore, salt-depleting procedures lowered blood pressure moderately in hypertensive patients even in the absence of other drugs. These observations led to the next major development in antihypertensive therapy: the discovery of practical agents for inducing and maintaining a state of moderate salt depletion in hypertensive patients.

This development came about through a series of molecular modifications which began with the basic observation that certain sulfonamide drugs inhibit the enzyme carbonic anhydrase. The diuretic acetazolamide was one practical result of such investigations.

In a systemic program of molecular modification of the benzenedisulfonamides, Novello and Sprague, in collaboration with the pharmacologists Baer and Beyer, observed an unexpected high order of diuretic activity in such compounds as benzene-1,3-disulfonamide (*17*). Their investigation led eventually to cyclic compounds, of which chlorothiazide appeared to be one of the most active. Chlorothiazide was only a weak carbonic anhydrase inhibitor and its mode of action was not the same as that of either the mercurials or acetazolamide. However, chlorothiazide was well absorbed after oral administration and was effective in causing sodium excretion in hypertensive patients. The initial antihypertensive effect of the drug appeared to be accompanied by a reduction of plasma volume associated with the saluretic effect (*5*).

Certain side reactions are associated with long-term treatment with chlorothiazide. The most common side effect is a reduction in serum potassium concentration. There is also an elevation in serum uric acid concentration, occasionally with precipitation of acute attacks of gout. In rare instances, hyperglycemia and diabetes mellitus have occurred. Numerous molecular modifications have since been made, with the hope of producing a compound

that would retain the sodium diuretic effect but not induce potassium loss or uric acid retention. Despite considerable work, these efforts have not succeeded in developing an agent with significantly improved clinical usefulness.

Hydrogenation of the chlorothiazide molecule led to hydrochlorothiazide, which permitted the dose to be reduced tenfold, but the only practical advantage was that the patient took less drug. This, and various other modifications of the basic compound, led to insignificant changes in electrolyte excretion ratios which were of little practical value clinically.

A compound of somewhat related structure, chlorthalidone, is of interest because of its long duration of action and considerable potency (*13*). A convenient single-dose-per-day schedule is sufficient to induce and maintain an adequate natriuresis. At times, some of our patients seem to become tolerant to chlorothiazide and transferring their medication to chlorthalidone again invokes a saluretic effect. However, chlorthalidone induces the same hypokalemia and hyperuricemia that has been so frequently observed during long-term treatment with the benzothiadiazines.

An interesting new development has been the discovery that certain pteridine compounds promote sodium excretion and, at the same time, potassium retention. Clinical studies in hypertensive patients carried out with triamterene indicated that the drug was not in itself sufficiently potent as a natriuretic agent in hypertensive patients to be clinically useful (*10*). When combined with half-strength doses of hydrochlorothiazide, however, its natriuretic effect was comparable to that obtained with full doses of hydrochlorothiazide but without significant urinary loss of potassium or reduction in serum potassium concentration. Thus, triamterene may represent a beginning in the development of new compounds with more specific natriuretic properties.

Adrenergic Blocking Drugs

With the combined use of chlorothiazine and ganglion-blocking agents, the chemotherapeutic method for controlling severe hypertension became firmly established. The disturbing side effects produced by the ganglion-blocking agents, however, remained a definite nuisance. Patients complained not only of side reactions produced by sympathetic blockade, such as weakness and faintness in the erect position due to postural hypotension, but also of parasympathetic blocking effects which included constipation, dryness of the mouth, failure of visual accommodation, difficulty in emptying the urinary bladder, and impotence. Therefore, a search was made for compounds which would block the sympathetic system selectively, leaving parasympathetic functions undisturbed.

As a result of this search, a number of new compounds with interesting properties have been developed. These include bretylium tosylate (*2*), guanethidine (*14*), methyldopa [3-(3,4-dihydroxyphenyl)alanine] (*18*), and pargyline (*11*). All these compounds produce a more selective inhibition of the sympathetic nervous system, although each differs somewhat one from the other, in both primary mode of action and clinical effects. Of these compounds, guanethidine has received the most extensive therapeutic trial and clinical acceptance.

Guanethidine was the outgrowth of certain pharmacological observations

made by Maxwell, Plummer, and coworkers, on compound SU-4029 (*14*). SU-4029 antagonized the pressor effects of amphetamine and ephedrine, blocked the carotid occlusion pressor reflex, and lowered blood pressure in neurogenic and renal hypertensive dogs. The duration of action was remarkably prolonged over a period of several weeks. SU-4029 produced disturbing side reactions in clinical trials but guanethidine did not, thus providing another example of successful molecular modification.

Guanethidine lowers blood pressure in hypertensive patients by reducing both cardiac output and total peripheral resistance (*4*). There also is some redistribution of blood flow, so that less of the cardiac output is distributed to the intestines and liver. When guanethidine is given to patients intravenously, there is a transient period of elevation of cardiac output and rate and blood pressure, suggesting that catecholamines have been released (*4*). The duration of action of the drug in man appears to be about one week, so that doses taken daily tend to be cumulative over that period (*9*). As with the ganglion-blocking drugs, the effective hypotensive dose varies widely from one patient to another, and the saluretic agents enhance the antihypertensive effects of guanethidine. The side effects are considerably less than with the ganglion-blocking agents and the blood pressure seems to fluctuate to a lesser degree.

The newer antihypertensive agents, of which guanethidine is one example, represent an outgrowth of current concepts of catecholamine metabolism. It is now believed that norepinephrine is stored in the form of granules in the sympathetic nerve endings (*3*). Under adequate stimulation, the norepinephrine is released to constrict the smooth muscle of blood vessels or increase the force of contraction of the heart, thereby raising blood pressure. Certain pressor amines such as tyramine and ephedrine are believed to act in this fashion by releasing the norepinephrine stored in the nerve endings. Reserpine, an antihypertensive agent that we have not yet discussed, produces depletion of the norepinephrine stores (*3*). Guanethidine may also act in somewhat the same fashion, although the clinical effects of guanethidine and reserpine are different. Although guanethidine may produce some release of catecholamines on intravenous administration, the exact mechanism of action of this drug has not been clarified, except that it produces some sort of a peripheral block of the sympathetic nervous system (*14*).

Methyldopa was synthesized for the purpose of inhibiting the decarboxylation of dopa to dopamine, which is believed to be the precursor of norepinephrine (*18*). Thus methyldopa should prevent the formation of catecholamine stores. It seems probable that the mode of antihypertensive action of methyldopa is more complex than simple inhibition of norepinephrine synthesis. Clinically, the drug lowers blood pressure with less prominent orthostatic hypotension than guanethidine. However, some patients seem to be resistant to the drug and it is not generally dependable in reducing blood pressure as is guanethidine (Figure 1).

It has been known for some time that monoamine oxidase inhibitors will lower blood pressure in hypertensive patients and produce orthostatic hypotension. Theoretically, by preventing the metabolic degradation of norepinephrine, such compounds should raise blood pressure by increasing catecholamine stores. However, these enzyme inhibitors also have other actions, including a ganglion-blocking effect. Early trials with monoamine oxidase inhibi-

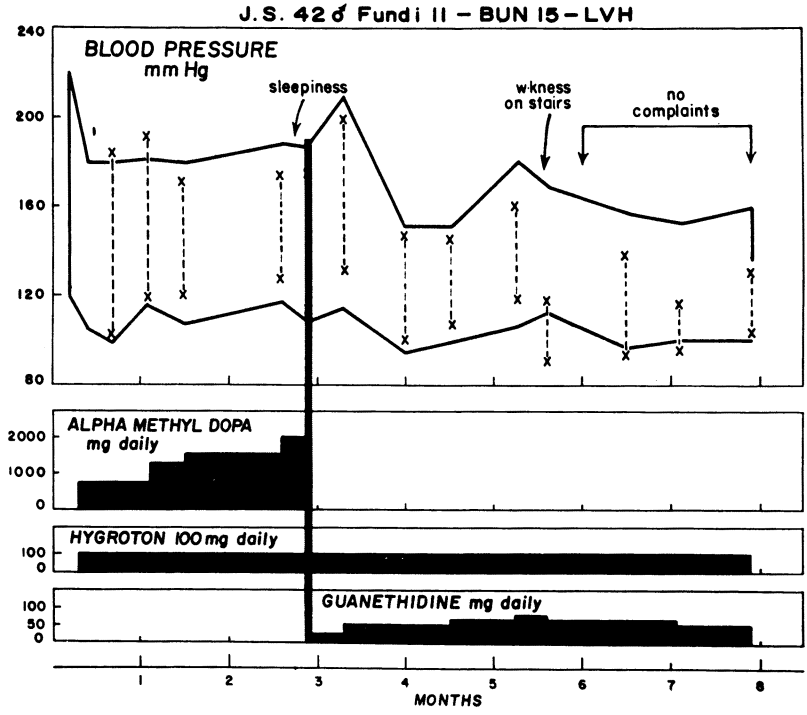

Figure 1. Effect of alpha-methyldopa and chlorthalidone (Hygroton) compared to guanethidine plus chlorthalidone in patient with hypertension resistant to treatment

Vertical dashed lines indicate blood pressure readings taken while patient was standing in erect position. Alpha-methyl-dopa did not control blood pressure in this patient, but did not produce postural hypotension seen with guanethidine

tors in the treatment of hypertension were disappointing because of the development of toxic reactions associated with the hydrazine group. More recently, pargyline, which does not contain a hydrazine, has been shown to exert monoamine oxidase activity and also to be a potent antihypertensive drug (*11*). Clinical experience confirms the active antihypertensive effect, including orthostatic hypotension. Final evaluation of pargyline and alpha-methyldopa as antihypertensive agents must await more extensive clinical trials.

Other Antihypertensive Agents and Combinations

This survey would not be complete without mentioning the Veratrum alkaloids (whose use is limited because of their emetic effect) as well as hydralazine and reserpine. Both of these latter agents still hold a prominent place in the clinical management of patients with hypertension. The placebo-controlled double-blind studies carried out in the Veterans Administration Cooperative Study on Antihypertensive Agents (*1*) indicated that hydralazine

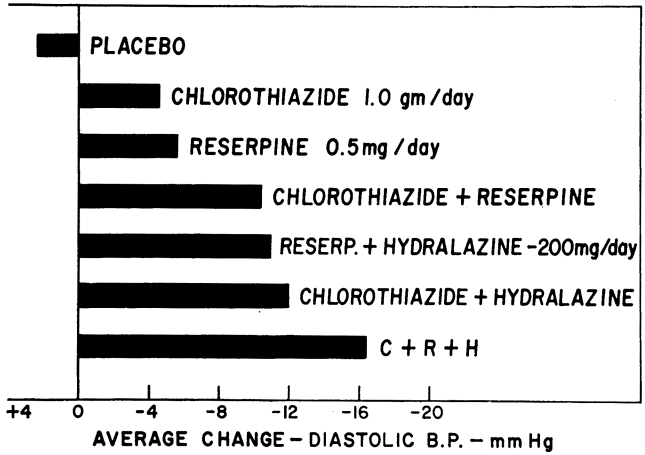

Figure 2. Results of Veterans Administration Cooperative Study on Antihypertensive Agents

Each bar represents average of large group of patients with mild and moderate hypertension, and refers to change in diastolic pressure from pretreatment hospital control values to 3 months posttreatment

and reserpine are most effective when combined with each other or with a saluretic agent.

Whereas there was a slight rise in the average diastolic blood pressure of the group of patients in the VA study receiving only placebos, there was a slight reduction in the groups receiving chlorothiazide alone or reserpine alone (Figure 2). In the patients who received chlorothiazide plus reserpine the reduction was approximately twice that obtained with either drug alone. Similar reductions in blood pressure were obtained with the combination of reserpine and hydralazine, or chlorothiazide and reserpine, while the administration of all three agents resulted in a highly significant blood pressure reduction.

Such results indicate that in the present state of development of antihypertensive drugs any new compound, which in itself may not be sufficiently potent to be relied upon for adequate blood pressure control, may still be clinically useful if its mode of action is different from previously known agents and it does not cause disturbing or dangerous side effects. Such new agents may be useful in combination with other drugs, as shown in the Veterans Administration Cooperative Study.

Conclusions

The most successful programs of molecular modification seem to have included recognition of clinical need and willingness to explore new approaches in answer to therapeutic problems. Among the antihypertensive agents, molecular modification of existing compounds has been most effective in the period immediately following the discovery of an agent with a new type of

action. Improvement beyond a certain point is prevented by inability to overcome clinically undesirable characteristics associated with the basic mode of action of a particular class of compounds. Early recognition of this point of diminishing returns should avoid unnecessary expenditure of time and effort.

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Discussion

C. J. CAVALLITO, presiding

Dr. John Holly (Ottawa): I would like to direct one question to Dr. Page. There is a general feeling, I know, among a number of clinicians, that women can tolerate an elevated blood pressure better than men. Does he believe this is statistically true, and why?

Dr. Page: I think the answer is probably yes, but not on a statistical basis. They do it on the basis that they cut a much better figure than we do as males. You have to face the fact that the battle of the sexes has long since been won by the female. She has a longer life span, she is a healthier person, and she is running things anyway.

So if you think it is a man's world, remember it is in your wife's name.

Dr. Louis Friedman (U. S. Vitamin): Is it due to hormonal differences?

Dr. Page: I suspect it is, but I have not the slightest idea which hormone. You see, there is supposed to be a difference between femininity and being a woman and this is a distinction which I hesitate to bring up with my wife.

Dr. Cavallito: I would like to ask Dr. Freis if it would be fair to conclude from his presentation that although in numerous instances molecular modification has not led to a significant improvement, yet there have been cases of definite improvement. Do you think this is a reasonable assessment of the past ten or fifteen years?

Dr. Freis: Yes. I tried to give some examples of that. The modification of SU-4029, which was not very successful clinically, led to guanethidine which was a most successful modification, whereas a great deal of work went into modifying thiazides and most of this has not led to any great practical advantage. The same could be said for the ganglionic blocking drugs after pentolinium tartrate had been developed.

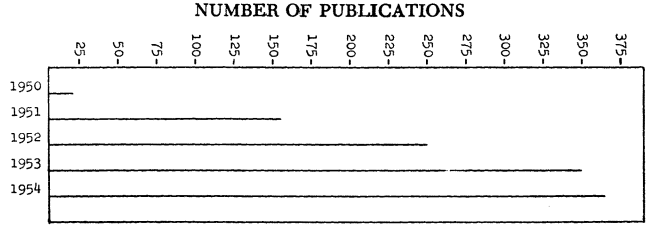
Dr. Cavallito: Would it also be fair to say that one would not know this in advance of implementing the molecular modifications?

Dr. Freis: I do not know about that. I would think one would have guessed after about the first 50 modifications of chlorothiazide and those that hit the market, that further modifications might not be of any great value. That could have been said of the benzothiadiazines, and of the ganglionic blocking agents.

There is a tendency to proceed on one line a little long in pharmaceutical research. This may be profitable; I do not know. But it is not scientifically or therapeutically very profitable. I think there is a time to quit.

Dr. Cavallito: I have one more question, and in leading up to it, I would like to show one illustration.

I recall that ten or fifteen years ago one of the questions was: Is it really useful to lower blood pressure? The consensus at that time seemed to be that you had better leave it alone. For a talk presented in mid-1955, I was interested in getting some reflection of the amount of interest in hypertension and its therapy, and plotted as a reflection of interest the number of publications that appeared per year, covering the chemistry, pharmacology, and clinical aspects of hypertension. Using the same general background of available literature, this chart shows clearly the rapid increase in interest in hypertension and its therapy as reflected by the literature.



I would like to ask Dr. Freis if he feels that therapy or treatment of hypertension is really useful, from the standpoint of the patient's well-being and ultimate life expectancy.

Dr. Freis: I think that it has been proved that in malignant hypertension you can prolong the lives of many patients and can salvage the lives of many more patients than you could before any hypertensive drug therapy, and that in the mild and moderate grades of hypertension, there is a great deal of circumstantial evidence to indicate that reduction of blood pressure should prolong

lives by decelerating, or slowing down, the process of degenerative vascular diseases. There is a lot of evidence to indicate that elevation of blood pressure is bad for blood vessels, accelerates atherosclerosis and arteriosclerosis, and promotes hypertrophy of the heart, and other bad things.

That being the case, one would expect reduction of blood pressure to be beneficial. It has been shown in experimental animals that animal atherosclerosis can go on at the same rate as in a normotensive animal if you make the animal hypertensive but keep his blood pressure from going up by giving him antihypertensive agents.

In man it is difficult to run a controlled trial. It takes many years to determine whether or not a patient with mild or moderate hypertension is going to develop organic complications. The only kind of studies we have are sort of hindsight studies which are not very valid, but even these suggest that in mild and moderate hypertension, life is prolonged by adequate blood pressure control.

Dr. Page: As Dr. Freis suggested, this is our life blood. It has got to work. The evidence to me is incontrovertible. The first bit that was mentioned by Dr. Freis, the reversal of malignant hypertension, has no question about it; nobody would question it today.

Believe me, in the first days, there was a pretty dismal group to take care of. All you could do was give better nursing, because the outlook was universally fatal.

The second thing to realize is that it is not the height of the blood pressure that really kills the individual. It is the effect of blood pressure and other factors on the smooth muscles of the body. If you plot any measurement which measures the change in the quality of smooth muscle, you will find that antihypertensive drugs do make the curve of deterioration level out.

The third thing—one that Dr. Freis did not mention—is that when there is heart failure, very often antihypertensive reduction of blood pressure will overcome the heart failure without even the use of digitalis.

So if you put all these things together, we need not really go into computer medicine or large scale statistics to prove what clinical experience and physiological thinking will show you really happens.

I would like to add just one more piece of advice—that I think the pharmaceutical industry and the chemists have perhaps restricted their thinking a little too much in the kinds of drugs that they have worked with, as Dr. Freis suggested. Perhaps they overdid the saluretic drugs and have not given enough attention to the many other facets of the problem of blood pressure control which are now apparent to anyone who works in the physiology and chemistry of mechanisms which control blood pressure.

Therefore you have got your work cut out for you, and you do not have to keep beating some of the horses that are fairly well dead in order to accomplish things. I would like to see another striking out anew and approaching some of these other mechanisms which are just crying to be antagonized or to be used in order to lower blood pressure.

Dr. Cavallito: In essence it is evident that the work of the past ten or fifteen years has not been in vain. However, it is also evident that you still have some unresolved problems.

Molecular Modifications among Antihypertensive Agents

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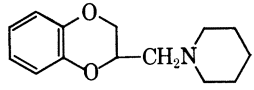
The historical development of antihypertensive agents is reviewed, with particular attention to the part played by molecular modification in evolving useful drugs. During the past 15 years many thousands of new organic compounds have been prepared and tested in order to provide the handful of antihypertensive agents currently in use. Molecular modifications of prototype structures have yielded anywhere from little or no to moderate to significant improvements in antihypertensive drugs. In each instance the ultimate degree of success was unpredictable prior to the synthesis and testing of structural variants. Molecular modifications of drugs and the elucidation of their mechanisms of action are complementary activities, each of which can contribute to the other and to the development of better drugs. However, at our present state of knowledge we will probably need to depend for some time primarily on the continued molecular creations of the medicinal chemists for our progress in drug therapy.

Increasing attention has been given to the cardiovascular diseases and to hypertensive disease in particular. During the past 15 years the development of improved drugs for use in hypertension has been coupled with an increasing medical awareness of the value of drug therapy in hypertensive disease. My purpose now is to outline briefly some of these drug developments and to illustrate associated processes of molecular modifications.

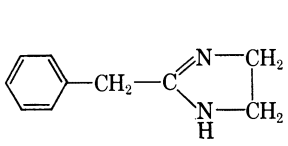
Some of the older antihypertensive agents such as the nitrites, nitrate esters, thiocyanates, and crude plant products are not considered in this discussion of molecular modifications.

One of the principal pharmacological routes followed in the continuing search for useful antihypertensive agents has been selective modulation of the functioning of the autonomic nervous system. Among the earliest synthetic chemical developments in this direction were the sympatholytic or adrenolytic agents. Thirty years ago, Fourneau and Bovet (*12*) described some synthetic benzodioxanes (I) with adrenolytic properties. During the next 20 years, a variety of chemical types of adrenergic blocking agents were prepared and

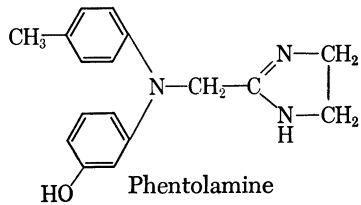


I. Piperoxan

studied, among which the more interesting have been the imidazolines (*15*, *39*)



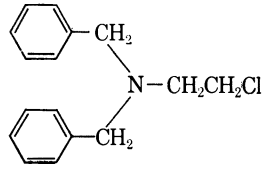
Tolazoline



Phentolamine

II

(II), and the β-haloethylamines (*25*) (III) which are illustrated by prototypes.

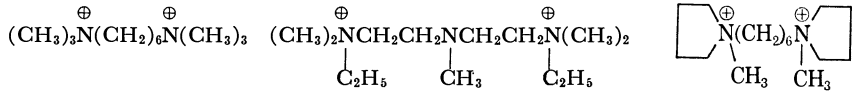


III Dibenamine

The adrenergic blocking agents have had only limited clinical utility in certain forms of peripheral vascular disease and the occasional hypertension from a pheochromocytoma. These drugs generally have limiting side reactions or toxicity and lack specificity and adequate effectiveness for use as antihypertensive agents. The β-haloethylamines, however, have been the subject of interesting studies by medicinal chemists who have learned something of the interrelationships between pharmacological properties and chemical-structural characteristics [details are given in the review by Ullyot and Kerwin (*38*)]. The β-haloethylamines permitted an analysis of the contribution to activity of such factors as ionization, chemical reactivity, lipophilic characteristics, and steric influences. It is in these physical and chemical areas of interpretation and correlation that the medicinal chemist can particularly contribute to the design of a program of molecular modification.

Another chemical approach to modification of the functioning of the autonomic nervous system was the development of compounds whose primary

target is selective blockade of the sympathetic ganglia. In 1946 Acheson and Moe (*1*) suggested that the ganglionic blocking properties of tetraethylammonium might have clinical utility. Its limitations, however, were too great. In 1948 Barlow and Ing (*3*) and Paton and Zaimis (*29*) described the pharmacological characteristics of a homologous series of α,ω-bis(trimethylammonium)-alkanes and pointed out the sympathetic ganglionic-blocking activities of the C_5 and C_6 homologs. Within a short time, these two agents, hexamethonium and pentamethonium, found their way into clinical practice. Their lack of specificity and relatively poor oral efficacy limited their use and stimulated efforts at improvement. Structural modifications of the interquaternary linking chain and of the cationic terminal groups led to the development of two somewhat more effective but still limited drugs, azamethonium (*4*) and pentolinium



Hexamethonium Azamethonium Pentolinium

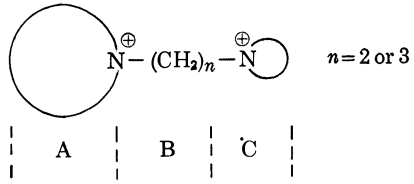
(*20, 42*). Although not ideal drugs, their activity in man sparked a great deal of further work and there probably have been more quaternary ammonium derivatives synthesized than any other chemical class of hypotensive agent.

Among the monoquaternary and symmetrically substituted bisquaternary ganglionic-blocking agents, optimum structural requirements for activity soon were elucidated. Robinson (*32*) and Winbury and colleagues (*43, 44*), for example, described some 90 monoquaternary molecular modifications and established certain configurational relationships with activity. Unfortunately, the most active compound, diethyldiisopropylammonium, was only about 12 times as potent as tetraethylammonium. Among the symmetrically substituted bisquaternaries, the opportunities for improvement in activity also were soon shown to be rather limited, with regard to the dimensions of both the terminal cationic structures and the interconnecting chain.

In our laboratories, we sought to explore the characteristics of unsymmetrically substituted bisquaternary alkanes, and the first few compounds with C_3 linking chains showed activities materially greater than those of the symmetrical bisquaternaries. It soon became evident that these required wholly novel structural characteristics for activity and the earlier guidelines developed from monoquaternaries and symmetric bisquaternaries were of little value in predicting optimum structural requirements. Extensive series of compounds were described in the next few years by L. M. Rice and C. H. Grogan and by our group (A. P. Gray, T. B. O'Dell, C. J. Cavallito) and selected compounds by others [detailed bibliographies are given by Cavallito and Gray (*7*) and Nádor (*24*)].

The literally hundreds of unsymmetric bisquaternaries synthesized resulted in the development or introduction of only a few end products. These may be illustrated by chlorisondamine (*30*), pentacynium (*2*), trimethidinium (*17*), and methindethyrium (*14, 27*). The development of other classes of antihypertensive agents at about this time led to only a modest use of these.

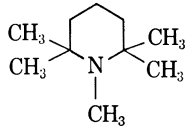
The more active unsymmetric bisquaternaries may be illustrated schematically by IV, which can be considered as comprising three components subject



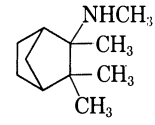
IV

to molecular modification. For maximum intensity and duration of activity, A is preferably a large, lipophilic substituted cationic group of moiety weight between about 150 and 350; B is a C_{2-3} methylene chain; C is a small compact cationic group, usually trimethylammonium. Dimensional modifications of A generally have a greater effect on activity at the lower than the upper end of the moiety weight range given. Increasing length of B may reduce not intensity but rather duration of activity and increase toxicity. There is evidence that these compounds have central as well as peripheral components of action (*8, 14, 27*) which vary relative to one another with molecular modification. The interrelationship of molecular modifications with pharmacological properties among these quaternaries has been extensively considered also from the perspective of attendant changes in their physical-chemical characteristics (*7*).

The limited oral absorption of quaternaries encouraged the investigation of a variety of amines as sympathetic ganglionic-blocking agents. These may be illustrated by perolysen (*19, 34*) and mecamylamine (*35*), which are readily absorbed orally but still demonstrate side reactions associated with nonspecific ganglionic blockade. Optimum activity appears to be associated with branched structures offering partial steric hindrance for the amino nitrogen. Stone *et al.* (*36*) studied a variety of mecamylamine isomers and analogs and by their test methods determined the positions of methyl substitution which favored activity. Here again, relationships were established only after molecular modifications.

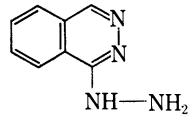


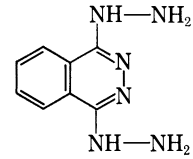
Perolysen



Mecamylamine

One of the more successful types of antihypertensive agents is that of the hydrazinophthalazines, exemplified by hydralazine and dihydralazine. Sum-

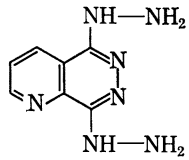




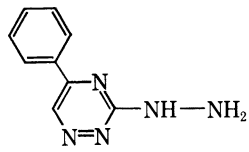
mary discussions of these and related compounds have been published by Schlittler, Druey, and Marxer (*33*) and by Druey and Marxer (*10*). These compounds again well illustrate the unpredictable outcome of molecular modifications among novel classes of compounds. Of the more than 250 compounds

reported (*10*), about six reached clinical trial and two have been successful drugs. Druey and Marxer draw some generalizations from their work and also illustrate some examples of structural specificities.

Among the hydrazinophthalazines substitution with alkyl or aryl substituents on the hydrazino group results in compounds which are essentially inactive. The hydrazones of hydralazine, however, are active as hypotensive agents. This is related to the ability of the organism to convert the hydrazones but not the alkyl or aryl substituted derivatives back to hydralazine. Hydrazino derivatives also have been prepared of heterocyclic systems other than phthalazine and considerable structural specificity is evident. For example, 2-hydrazinoquinoline is inactive, whereas 1-hydrazinoisoquinoline is active. The only other two heterocyclic systems for which appreciable activity was reported are V and VI. As a generalization, the authors conclude that hypotensive properties seem to be associated with structures containing six-membered rings with the

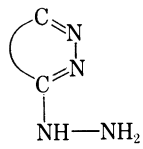


V



VI

sequence shown in VII, except for 1-hydrazinoisoquinoline. These seem to be necessary but not sufficient criteria for hypotensive activity. In the phthalazine series derivatives in which the hydrazino group was replaced by amino,

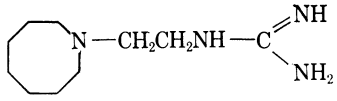


VII

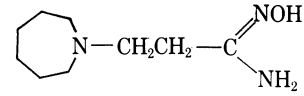
alkylamino, morpholino, piperazino, aminoalkylamino, etc., in some instances showed blood pressure–lowering propensities but of rather short duration. Certain open-chain amidine and hydrazide analogs were not active.

The mechanism for the hypotensive activity of the phthalazines is still not clearly defined and there is evidence of both central and peripheral actions. The investigators have not described any attempts at interpretation of the structural modifications in terms of physical-chemical characteristics, although Fallab and Erlenmeyer (*11*) have observed that the activity within the group does not seem to be related to the chelation propensities of the compounds.

An interesting example of the development of a useful drug by molecular modifications of a related lead is to be found in another chemical class of antihypertensive agents epitomized by guanethidine. Maxwell *et al.* (*21*) relate the development of this drug to the observation of certain pharmacological characteristics of an earlier chemical relative, SU-4029. The preparation and properties of a variety of related compounds have been presented by Mull



Guanethidine

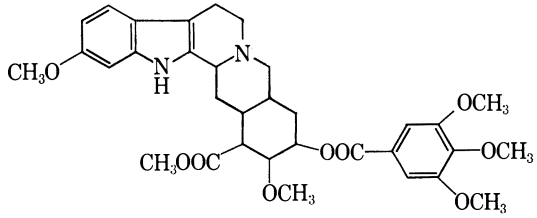


SU-4029

et al. (*22*, *23*). These authors described their investigations of heterocyclic large-membered ring compounds with antihypertensive activity which led to guanethidine. Pyrrolidine and piperidine ring systems contributed only moderate activity with increases evident with the hexahydroazepinyl and maximum activity with the octahydro-azocinyl analogs. Larger rings provided compounds of lower activity. A similar trend was observed in the amidoxime series, except that in these the hexahydroazepine system showed maximum activity. A variety of other hetero ring systems were less active. Acyclic dialkylaminoalkylguanidines also were relatively inactive. The C_2 chain appeared to be optimum for activity. Replacement of guanidine by the other basic groups tested provided less activity. There was no attempt at correlation of these structural variations with physical-chemical characteristics of the derivatives. The pharmacological action of these drugs includes some suppression of sympathetic nervous transmission. Schlittler, Druey, and Marxer (*33*) include a good review of this group of compounds.

Among the guanethidine analogs it again should be emphasized that there would not appear to be any *a priori* rationalization for proceeding directly to the optimum compound other than by chance, and a considerable number of molecular modifications needed to be prepared in order to permit selection of the most interesting derivative.

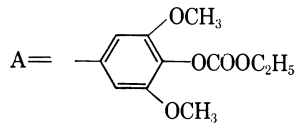
Nature has provided us with antihypertensive alkaloids of some complexity. One of these, reserpine, introduced in the early 1950's, has continued to have extensive use and molecular modifications have not yet yielded compounds of material advantage. Most efforts have been directed at modifying



Reserpine

A

the natural alkaloid in an effort to separate hypotensive from psychopharmacological characteristics. This has included ester group modifications and ring-substituted analogs. Garattini *et al.* (*13*), for example, reported that an ester modification, SU-3188, showed a separation of sedative and hypotensive activities; however, in man the compound has been reported to be much less active than reserpine (*16*). Serpentine, one of the less potent Rauwolfia alkaloids,

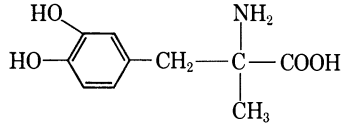


SU 3118

has been converted through its carboxyl group to other ester analogs (*40*), but these modifications have not as yet led to improved drugs.

Among the hypotensive alkaloids which have found a modest use for a long time are those of *Veratrum* species (*viride* and *album*). Preparations containing *Veratrum* and later certain alkaloid fractions have been in use in this country for some 30 years. With the elucidation of the structures of these complex steroid-like, ester alkaloids, efforts have been made to modify their structures in attempts to separate further their tendency to induce nausea or vomiting from the hypotensive activity. Kupchan and colleagues have been particularly active in this field; in recent reports (*18*, *41*) these workers summarize the structure-activity relations evolved to date among synthetic modifications of protoverine esters. The effects of variations in the number and structures of the acid moieties introduced in the esters, the positions of esterification on the polycyclic system, oxidation of alcohol to ketone groups, or acetonide formation were individually evaluated. Some modifications had little effect on activity, others considerable. Prediction of trends prior to implementing and testing the modifications had not been possible. Although in some instances, improved modifications could be made, the requirement of a natural source of pure alkamine poses economic limitations. The *Veratrum* plants are indeed most prolific molecular manipulators and generate a mixture of complex alkaloids with individual differences in hypotensive activities and in tendencies to induce side reactions (*28*).

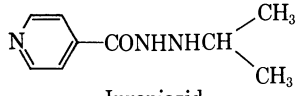
During the past few years, there have been developed new antihypertensive agents that might be considered as specific enzyme inhibitors. One of these, α-methyldopa, is an analog of the amino acid 3,4-dihydroxyphenylalanine. The L-isomer inhibits the decarboxylase enzyme (*31*) which converts



α-Methyldopa

aromatic substituted amino acids into amines, including serotonin, dihydroxyphenylamine, dihydroxyphenethylamine, tryptamine, and tyramine. This reduction in amine biosynthesis and depletion of tissue norepinephrine has been associated with the lowering of blood pressure in hypertensive patients and a transient sedative effect (*26*). However, the antihypertensive and decarboxylase inhibitory activities of this and related compounds are as yet not very well correlated.

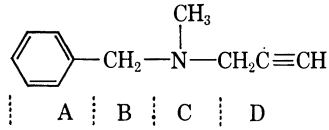
Another mechanistic category of antihypertensive agents comprises the monoamine oxidase (MAO) inhibitors. The developments that have occurred with the MAO inhibitors provide another interesting example of the tortuous and unpredictable course of molecular modification. Iproniazid, originally of interest as an antitubercular drug, was found with experience in practice to



Iproniazid

have possible useful properties as a psychotherapeutic agent in combating depressed states. The compound was shown to be an inhibitor of the enzyme monoamine oxidase. This sparked a great deal of molecular modification in the field of hydrazide and related chemical categories of psychotherapeutic agents. Further use of compounds of this type in clinical practice led to the general observation that therapy with monoamine oxidase inhibitors may produce orthostatic hypotension. With the drugs originally introduced for other purposes, the hypotensive characteristics were a nuisance and side reaction. However, there have been molecular modification efforts at more specifically tailoring these inhibitors for their antihypertensive properties.

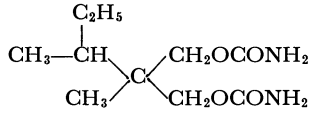
Swett and colleagues (*37*) have described some of the more than 100 molecular modifications that have been prepared of the recently introduced antihypertensive agent, pargyline, which is a nonhydrazide type of MAO inhibitor. In assessing the influence of molecular modifications on MAO inhibitor activity, these authors have considered this molecule from the basis of four component parts. From their modifications, they concluded that portion



Pargyline

A of the molecule is the least specific in its structural requirements for activity; B has little influence on activity in the 1- to 3-carbon chain range but falls off beyond that point; C appears to be relatively critical, requiring the NH or NCH_3 moiety; D again appears to be fairly specific, requiring a triple bond in the position β to the nitrogen. This work again well illustrates how molecular modifications can produce unpredictable results in a new type of compound. However, from a mechanistic standpoint, there is no particular correlation apparent between the quantitative aspect of MAO inhibition and antihypertensive effects of the pargyline-type compounds (*6*).

Compounds which are primarily sedative or central depressant also have found use in treating hypertension. In this category, one might include mebutamate (*5*, *9*), a molecular modification of the carisoprodol and meprobamate family of compounds. These molecular modifications have coursed through the fields of tranquilizers, muscle relaxants, and most recently, antihypertensive drugs. The relatively old drug, phenobarbital, has been used as a central depressant component in a number of antihypertensive combination products.



Mebutamate

The categories of drugs discussed here by no means exhaust the field, particularly with regard to new compound leads that have been described from time to time but which for one reason or another may not have resulted in a marketed product.

Concluding Remarks

From a practical end result, it is evident that molecular modifications of a new compound lead have yielded anywhere from little or no to moderate to distinct improvements in different instances but that, in each case, the usefulness of molecular modification was initially unpredictable.

From the standpoint of random *vs.* rational approaches to molecular modifications, our present state of knowledge requires us to lean on the first before we can exploit the second. New classes of active compounds usually are discovered by random testing or by accident. The exploitation of a lead becomes more rational as more modifications are made. The retrospective correlations made possible after molecular modifications are of only limited value in rationalizing modifications in a different kind of molecule or with the same group of molecules for other biological properties.

To the medicinal chemist, it comes as somewhat of a shock that molecular modification should be criticized. This is his science and his art and difficult to appreciate by the nonchemist who is unaware of the effort that may be required in the synthesis of that which on paper appears to be a minor molecular modification.

If critics of molecular modifications would come forth with supported suggested alternative routes to drug advances, we as medicinal chemists would welcome such suggestions. Important as it is that we learn more of the basic pharmacology and biochemistry of drug actions, our past progress in drug developments would have been puny indeed if we had had to wait for and rely upon the actual accumulation of such knowledge. The medicinal chemist should appreciate proposed biochemical enzymology and pharmacological mechanisms of drug actions but not let such speculations restrictively confine his horizons of exploration. He might profitably guide his molecular modifications with more astute physical-chemical rationalizations of the effect of structural changes on biological properties. The profile of activity of a drug is encompassed by its ability to get to, bond or react with, and leave, sites of action. As a chemist, he can seek to interpret changes in these components of action in terms of the effects that a molecular modification may have on kinds and disposition of bonding structures, ionic properties, lipophilic-hydrophilic characteristics, stability, and steric factors such as planarity, linearity, rigidity or flexibility, hindrance, etc. A consideration of such factors can not only make his work more stimulating, but more quickly delineate the scope of his active compounds and contribute to the mechanism of action at the molecular level. Scientists as individuals will vary in their abilities to visualize and appreciate the significance of these variables. There will thus continue to be considerable differences in the apparent sophistication of implementation of molecular modification but at our present state of knowledge, who is to say which approach will be most fruitful in yielding better drugs?

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Some Results of Molecular Modifications of Diuretics

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The xanthines and the organic mercurials provided the early leads for structural modification among the diuretics. But the sulfonamides have provided the most fruitful series of compounds in this field. The rationale of the early work was based upon the effect of sulfonamides on the function of carbonic anhydrase in renal mechanisms. The parent sulfa drug, sufanilamide, was the prototype for this series. The heterocyclic sulfonamides, the benzene disulfonamides, and the thiazides have provided highly useful, orally effective drugs. The recognition of hormonal control over electrolyte excretion led to the identification of aldosterone as the principal sodium-retaining steroid of the adrenal cortex. Useful antagonists of this hormone have been found among the 17-spirolactones in the steroid series. Pteridine derivatives have been shown to be natriuretic and, like the spirolactones, may have less influence on potassium excretion. Recently, a series of unsaturated acylphenoxyacetic acids has come under exploratory study and certain of these may be the most potent saluretic agents thus far developed.

Historically, diuretics have been defined as any agents that increase urine volume, and the effectiveness and potency of drugs were appraised in terms of their ability to increase the volume of urine output. However, as a more precise and detailed understanding of kidney function developed, emphasis has been placed on electrolyte excretion. By present-day concepts, diuretics primarily promote the elimination of excess electrolyte, principally sodium,

chloride, and bicarbonate, by direct action on the kidney, and the urine volume increases as a secondary consequence of the excretion of the osmotically active electrolytes. Today, chemicals are evaluated as diuretics primarily by measurement of the kind and quantity of electrolyte excreted in the urine.

In many fields of drug development, nature has supplied the first effective agents and the first clues for further structural modification by syntheses. This has been true also in the diuretic field but to a much less extent than in many other areas of medicinal chemistry.

Edema and specific drugs for its treatment have long been problems of the physician and of the chemist interested in medicinal products. Digitalis and the many related cardiac glycosides were the first effective agents for the treatment of dropsy associated with congestive heart failure. However, it was soon recognized that the mobilization of the excess tissue fluid associated with this condition was due to a primary action on the heart with improved cardiovascular hemodynamics and only secondarily to an action upon the kidney.

The naturally occurring xanthines, caffeine, theophylline, and theobromine, were the only natural products with a direct renal action that provided a structural clue for synthetic development. Since these structures were readily amenable to synthesis, they initiated the study of the effect of structural modification on diuretic action. No striking improvements were forthcoming. However, theophylline, and to a lesser extent, theobromine, and some pyrimidine derivatives related to intermediates in the synthesis of the purine ring system were useful but mildly effective agents (*30, 46*). Although xanthines were the only useful renal drugs until the advent of the organic mercurials in 1920, they have been largely displaced by more effective agents with fewer undesirable side effects (*25, 30, 46*).

The organomercurials were the first highly potent, effective diuretics. The lead into this type of agent arose from the incidental observation of diuretic activity in an antisyphilitic agent, merbaphen, a mercurated phenoxyacetic acid. Following this observation, many life-saving mercurials were developed (*28, 29*). For 30 years, from 1920 to the early 1950's, they were the mainstay of all diuretic therapy; there were no other potent, consistently effective agents. They may still be the drugs of choice when a profound diuresis is required. The chief disadvantages of the mercurials lie in their erratic (or lack of) oral effectiveness and irritation by this route that make necessary parenteral administration. The oral mercurials available are less reliable than the parenteral forms.

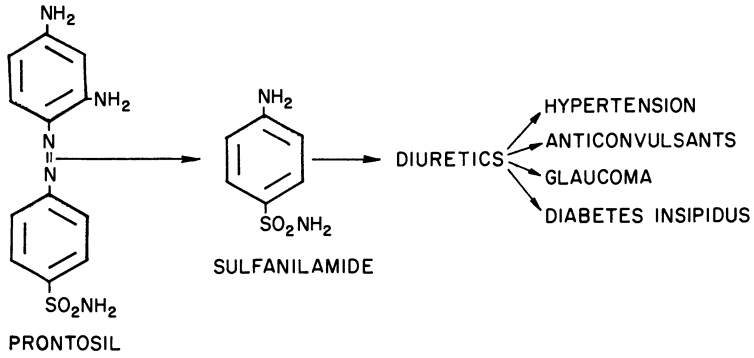
Space does not permit a detailed discussion of these two classes of diuretics and the contribution that they have made to an understanding of structure-activity relationships (*18, 54*).

Sulfonamides

In the last ten years, the sulfonamide type of diuretic has yielded spectacularly to intensive study. The recognition that certain sulfonamides could have a useful influence upon renal electrolyte excretion is scarcely 15 years old The basic observations for this diuretic development arose from the sulfa drug era, from observations on the side effects of the first sulfa drug, sulfanilamide. Tishler (*62*) gives a chart depicting the many new drug fields where valuable

agents evolved from studies, and subsequent modification, of sulfanilamide: the antibacterial drugs, including those for the treatment of leprosy and tuberculosis, the antithyroid drugs, the antidiabetic agents, agents for treatment of gout, and the sulfonamide diuretics. All of these arose by alteration of a common structural beginning, sulfanilamide, which itself was the result of a long line of structural modifications in the azo dye series seeking antibacterial agents.

Here we are concerned only with the diuretic portion of this story.



However, the story does not end with diuretics, the agents that promote renal elimination of excess electrolytes and fluid, but the study of these diuretics led to further agents useful in the treatment of hypertension, of epilepsy (anticonvulsants), of glaucoma, and of diabetes insipidus—i.e., antidiuretics.

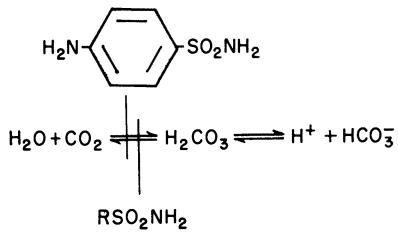
The basic steps in the development of sulfonamide diuretics from the sulfa era are well known, but it is purposeful to summarize them briefly.

The recognition of sulfanilamide as the active moiety of the antibacterial azo dye, prontosil (*63*).

The clinical acidosis and alkaline urine following sulfanilamide administration (*58*).

Discovery of carbonic anhydrase in the kidney (*19*).

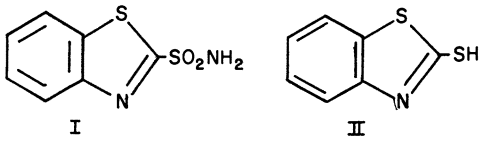
The carbonic anhydrase inhibitory activity of sulfanilamide and other sulfonamides unsubstituted on the nitrogen (*35, 38*).

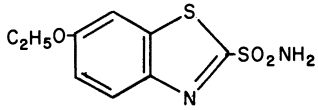


The demonstration that carbonic anhydrase plays an important role in providing hydrogen ion for the kidney and that sulfanilamide depresses this function (*47*).

The demonstration that sulfanilamide in patients in congestive heart failure causes excretion of an alkaline urine containing increased amounts of sodium, bicarbonate, and potassium ions with consequence loss in weight and decrease of the edema (*56*).

Although the shrewd deductions of Schwartz from the accumulated observations were correct, and sulfanilamide proved to be a diuretic, the dose required was large and there were marked side effects. From this beginning, many laboratories set out to find other orally effective sulfonamides with greater potency and fewer unwanted actions. In 1950, Roblin and coworkers (*43*, *48*) of the American Cyanamid Laboratories reported that heterocyclic sulfonamides show a high order of in vitro carbonic anhydrase inhibitory activity. Among the most active compounds was benzothiazole-2-sulfonamide, which had a potency several hundredfold (730 to 2500) that of sulfanilamide.

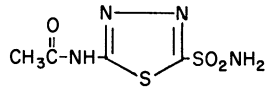




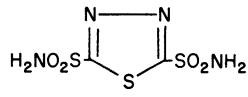
ETHOXZOLAMIDE

However, it proved to be inactive as a diuretic when administered orally to dogs. Later it was shown that the compound was rapidly metabolized to the 2-mercaptobenzothiazole, which was excreted as the glucuronide conjugate (*9*). More recently, the 6-ethoxybenzothiazole-2-sulfonamide (ethoxzolamide), has been found to be active in animals and effective in man (*26*).

Several 1,3,4-thiadiazolesulfonamides (*43*, *48*) have high in vitro activity



ACETAZOLAMIDE



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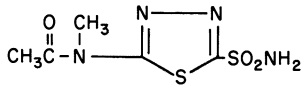

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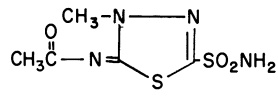


METHAZOLAMIDE

(300 to 900 times that of sulfanilamide) and the 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide is the well-known acetazolamide, the first orally effective, clinically useful sulfonamide diuretic.

The effect of many structural modifications of acetazolamide has been reported. Substitution on the sulfonamide nitrogen destroys in vitro activity, but activity in animals may be retained if the substituent can be removed by metabolism (*39*). The absence of the acetyl from the amino group greatly reduces antienzyme activity. Acyl groups higher than acetyl (*65*) retain in vitro activity and diuretic activity in animals and in man but may exhibit more pronounced side effects (*26*). Methylation (*70*) gives the two isomeric prod-

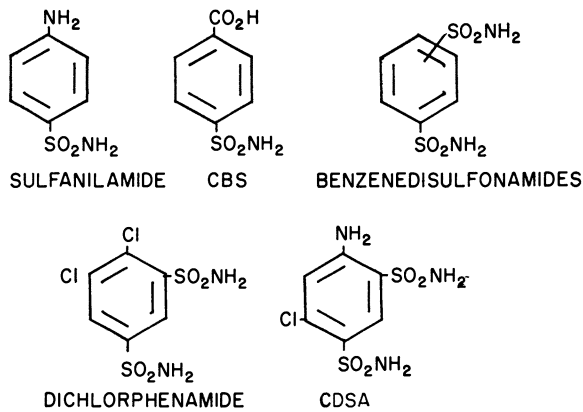
ucts, II and methazolamide. These are slightly more active in vitro and are active diuretics but offer no advantage over acetazolamide. However, methylation blocks the weaker of the two acidic centers (pK_a, 7.5, 9.0) of acetazolamide, and methazolamide (pK_a, 7.3), the more stable of the two isomers, has improved penetration into the brain and the eye (*17*).

Sulfonamide derivatives of many heterocyclic ring systems are reported to exhibit enzyme-inhibitory activity in vitro, but the correlation of this activity with activity in animals is not available.

Acetazolamide and its congeners represent a great stride forward in the search for potent, effective, orally active, nonmercurial diuretics. They effectively facilitate excretion of electrolyte and an osmotically equivalent amount of fluid. Aside from effects attributable to electrolyte balance, they show a low order of toxicity. The major increase in electrolyte excretion is found in the sodium, bicarbonate, and potassium ions; there is little or no increase of chloride ion excretion. As a consequence of this electrolyte excretion pattern that leads to loss of sodium bicarbonate, a state of general acidosis develops. This gives rise to a state of refractoriness when the drug is given continually and an intermittent schedule of dosage may be required. Acetazolamide, and many other subsequent, highly active carbonic anhydrase inhibitors, therefore, have certain shortcomings as the ideal diuretic (*27*). They promote excretion of bicarbonate as the main anion and of little chloride ion; acidosis and drug refractoriness develop; the increased potassium excretion is also an undesirable attribute.

However, in the study of these compounds, other applications were acquired. The occurrence of carbonic anhydrase, as a functionally important enzyme in the eye and in the central nervous system, led to the use of the inhibitors to treat glaucoma and epilepsy. Methazolamide has certain advantages due to greater penetration and intracellular distribution (*17*); its principal use is in the treatment of glaucoma.

In the search for more desirable agents with a more favorable electrolyte excretion pattern, our investigation (*59*) turned to the aromatic sulfonamides more closely related to sulfanilamide. Emphasis was placed on animal (dog) excretion data rather than in vitro enzyme inhibition results (*4*). One analog of sulfanilamide, *p*-carboxybenzenesulfonamide (CBS), a relatively weak car-



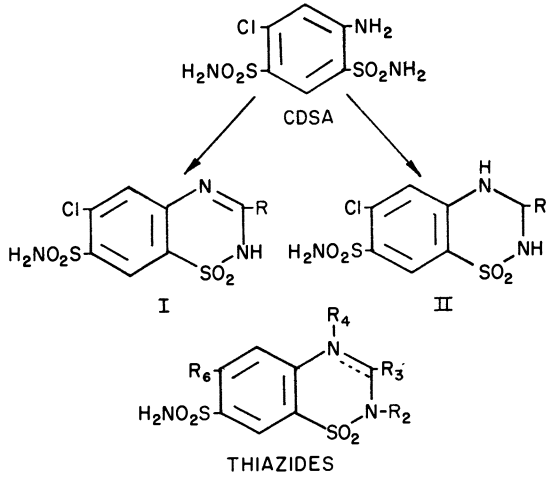
bonic anhydrase inhibitor (3 to 4 times sulfanilamide), showed an appreciable increase in sodium and bicarbonate excretion in the dog but was accompanied by a significant increase in chloride ion (*5*). This response was obtained in man also, but the compound has a low order of activity and the oral absorption is poor and erratic (*37*, *42*).

Benzenedisulfonamides, and especially the 1,3-disulfonamides, show a high order of sodium-excreting (natriuretic) activity in the dog, and many are active carbonic anhydrase inhibitors. Further substitution into the ring not only enhances activity in terms of lowering the required dose but, more importantly, alters the spectrum of electrolyte excretion (*5*). The dichloro derivative, dichlorphenamide, has an in vitro enzyme inhibitory activity equaling acetazolamide. In parallel tests, the activity of acetazolamide was 180 times and dichlorophenamide was 175 times that of sulfanilamide (*5*). In contrast to acetazolamide, dichlorphenamide definitely increases chloride excretion in dog (*5*) and in man (*14*). However, the sodium is still accompanied by considerable bicarbonate, as indicated by a rise in pH of the urine and, like acetazolamide, this drug too finds its main use in the treatment of glaucoma.

The introduction of chlorine and an amino group to give 5-chloro-2,4-disulfamoylaniline (CDSA) brings about marked changes in the charter of the biological activity. The anticarbonic anhydrase activity is reduced drastically to that corresponding to CBS (3 times sulfanilamide) (*5*). However, the electrolyte excretion pattern is shifted to favor chloride as the major anion (*5*). There is little bicarbonate excretion, as shown by the lack of a rise in urine pH. Although in the dog CDSA is less active than chlorothiazide in terms of dose required, it has comparable activity in man. Sodium and chloride are excreted in approximately equivalent amounts, thus differing from the pattern of the potent carbonic anhydrase inhibitors and resembling more closely the action of the organomercurials.

Thiazides

The chlorodisulfamoylaniline (CDSA) became the key intermediate in the preparation of the family of diuretics that is generically known as the "thia-



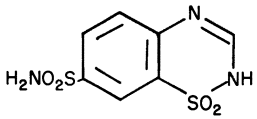
zides." Reaction of CDSA with acylating reagents leads to cyclization and to 1,2,4-benzothiadiazine-1,1-dioxides (I), of which chlorothiazide (R = H) is the prototype, while the dihydro derivatives, or hydrothiazides (II), are obtained by the reaction of aldehydes (or of ketones).

The thiazides retain the electrolyte excretion spectrum first seen with CDSA itself—namely, equivalence of sodium and chloride ion—and, therefore, have been referred to as saluretic agents. Since most of the clinically effective thiazides are exceedingly weak carbonic anhydrase inhibitors as measured in vitro, it appears that the saluretic activity of this series has been disassociated, at least grossly, from enzyme inhibitory activity. Nevertheless, no highly active, useful derivative has been reported that does not have a free, unsubstituted sulfamoyl group in the 7- position, unless the substituent is of such character as to permit metabolic removal. The unsubstituted sulfamoyl group conforms to the long accepted requirement for carbonic anhydrase inhibitory activity. However, Maren (*40*) has recently presented evidence that N^7-acetyl derivatives of both chlorothiazide and hydrochlorothiazide (I and II, R = H) exhibit weak saluretic activity with no detectable cleavage of the acetyl group and are essentially inactive in vitro as enzyme inhibitors.

Many hundreds of thiazides and related structures have been reported in the literature, and the influence on activity of the saturation of the thiadiazine ring and of substituents in positions 2, 3, 4, and 6 has been examined in some detail. Substitutions at positions 5 and 8 in the benzenoid ring are less favorable to activity and fewer representatives are available.

In the following structures, the effect of structural changes upon activity is presented, using as examples mainly drugs that have had clinical application. The numerals under each structure give the approximate relative activity in man compared to chlorothiazide. These activities are based only upon the relative amounts of drug required to produce a given electrolyte response. These values bear an inverse relation to the recommended daily dose of each drug.

In both the benzothiadiazine series and the dihydro series, the lack of a halogen or halogen-like group at position 6 (I) gives substantially inactive



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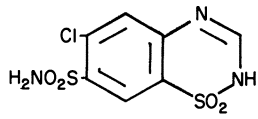
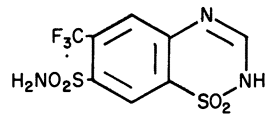
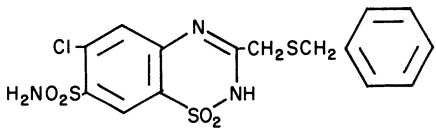


CHLOROTHIAZIDE
1.0



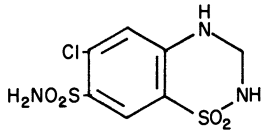
FLUMETHIAZIDE
1.0



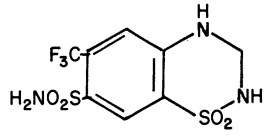
BENZTHIAZIDE
10

compounds, at least in the dog (*5*, *6*, *59*). Chlorine and trifluoromethyl are equally effective as enhancing groups. The importance of substitution at position 3 is shown in benzthiazide, where a benzylthiomethyl increases activity tenfold (*21*, *45*).

Saturation of the heterocyclic ring also increases activity and further de-

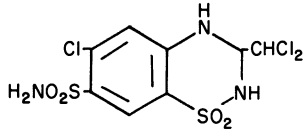


HYDROCHLOROTHIAZIDE
10

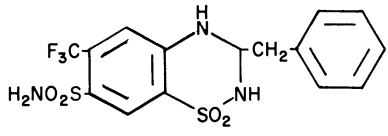


HYDROFLUMETHIAZIDE
10

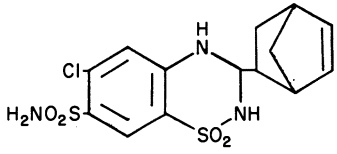
presses the residual anticarbonic anhydrase activity. In the two examples shown, hydrochlorothiazide and hydroflumethiazide, there is a tenfold increase in activity (*5*, *31*). However, with benzthiazide, which has already ten times the activity of chlorothiazide, saturation of this thiadiazine ring raises activity by only two- to threefold (*23*). Because of the marked increase of activity in the hydrothiazide series, extended structural changes have been studied. The pronounced influence of the 3-substituent is shown where there is an increase



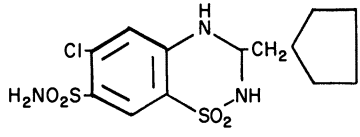
TRICHLORMETHIAZIDE
100-200



BENDROFLUMETHIAZIDE
100-200



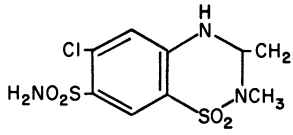
CYCLOTHIAZIDE
250



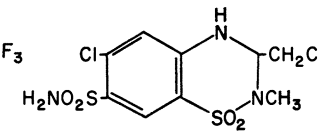
CYCLOPENTHIAZIDE
1000-2000

of 10- to 200-fold compared with the unsubstituted analogs. The great influence of the nature of the 3-substituent is illustrated further in compounds lacking an activating group (halogen, trifluoromethyl, etc.) at the 6 position. Where the 3-substituent is appropriately selected, such as dichloromethyl or cyclopentylmethyl, these compounds without a 6-substituent approach the activity of chlorothiazide.

In the thiazide series, substitution on either of the nitrogens in position 2 or in position 4 (of the tautomeric form) decreases activity in animals and labilizes the ring to hydrolytic opening (*44*). In the hydrothiazide series, however, certain 2-substituted derivatives are highly active, and are claimed to have increased duration of action. Two 2-methyl derivatives with substitution also at position 3 are shown.

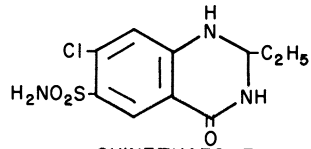


POLYTHIAZIDE
500

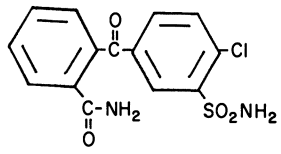


METHYCLOTHIAZIDE
200

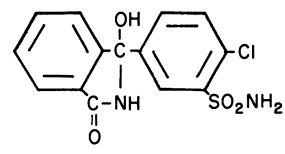
A number of more radical changes in the benzothiadiazine ring system of the thiazide structure have been reported. A pyridine ring (*13*, *69*) in place of the benzenoid ring gave unpromising compounds; replacement of the heterocyclic thiadiazine ring by other heterocycles, such as isothiazole or isoindoline (*12*), has yielded active compounds but few data are available. However, the benzoquinazolinone analog (*10*, *24*, *33*, *57*), quinethazone, has recently been introduced and has an activity comparable to hydrochlorothiazide. Here too,



QUINETHAZONE
10



I



CHLORTHALIDONE
10

the dihydro series is more active than the unsaturated quinazolones.

An interesting sulfonamide diuretic that has little resemblance structurally to the thiazides is chlorthalidone, a benzophenone derivative. On a dose basis, it has an activity comparable to hydrochlorothiazide but is a much stronger carbonic anhydrase inhibitor than the thiazides, approximately 40 times sulfanilamide. Physical data indicate this compound to have the isoindoline structure rather than the benzophenone structure, I. The outstanding feature of this drug is its long duration of action, up to 72 hours (*22*, *32*, *66*).

Biological Activity

Thus far, we have referred to the order of activity of the thiazides in terms of the dose required to give a certain electrolyte response. Since, by this scale, the activities spread over a 1000- to 2000-fold range, one might expect the highly active compounds to be able to produce a greater electrolyte response than the weaker compounds. However, this has not proved to be so. All of the thiazides appear to have the same limitations or ceiling on the amount of electrolyte that can be excreted regardless of the dose. This is graphically portrayed in the sodium dose response curves in man for thiazides covering a 200-

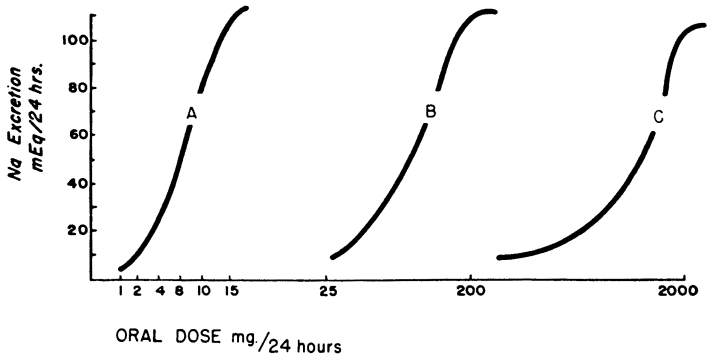
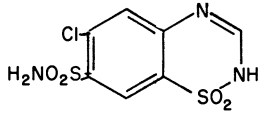


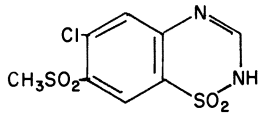
Figure 1. Dose response of thiazides

fold difference in effective dose level (Figure 1). In each case, they approach the same plateau value; only the dose required to produce this value differs from drug to drug. This figure is from the publication of Fuchs *et al.* (*31*), reporting results obtained in nonedematous patients. Similar results have been obtained with cyclopenthiazide, where the dose range ratio is 1000 to 2000:1 (*64*).

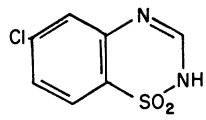
Not only have the thiazides proved to be highly effective for the mobilization of electrolytes and fluid in such conditions as congestive failure and cirrhosis, but they are valuable hypotensive agents. In the treatment of hypertension, the thiazides have become the basic therapy on which other antihypertensive agents are superimposed as needed. How the thiazides exert this hypotensive action is an open question. Considerable attention has been given to attempts to disassociate the structural features responsible for the antihypertensive and the saluretic activity. The earliest compound studied was the methylsulfonyl analog of chlorothiazide (I). This compound, lacking the



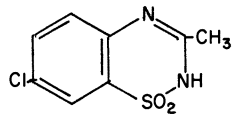
CHLOROTHIAZIDE



I



II



DIAZOXIDE

sulfamoyl group, is devoid (or nearly so) of electrolyte activity in animals. However, the methylsulfonyl group is spatially and electronically very similar to the sulfamoyl group. Consequently, this compound exhibits many of the

physical and chemical properties and some of the biological properties of chlorothiazide (*44, 60*). Nevertheless, when it was substituted for chlorothiazide in hypertensive patients, it was ineffective in maintaining control of blood pressure (*68*). Subsequent results in the Schering Laboratories have shown that complete removal of the sulfamoyl group (II) does not destroy antihypertensive activity. Diazoxide has had extensive study in man and is a very effective hypotensive agent. However, without the characteristic sulfamoyl group, the compound now causes electrolyte and fluid retention and edema. Thus, saluretic and hypotensive action have been disassociated by structural changes, but further modifications are obviously required to produce a generally useful antihypertensive agent (*51, 52, 53, 61*).

There are few definitive ideas at the chemical level regarding the mechanism of action of these diuretics. It is not certain whether carbonic anhydrase inhibition is involved, although the requirement of a free sulfamoyl group may suggest that it is, but Maren's recent claims do not support this idea (*40*). Why there is such a large spread in the required dose for a common ceiling on the electrolyte excretion is unanswered. To begin to approach these answers, more precise and definitive chemical, physical, and biological information is required. However, the mercurial diuretics have been used for 40 years and there is, even today, little agreement regarding their mechanism.

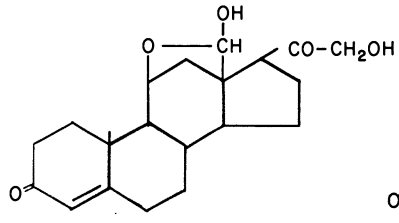
Although the sulfonamide diuretics have contributed greatly to closing the gap between therapy attainable with the mercurials or their predecessors and the ideal therapy, there are troublesome, if not serious, shortcomings in these new agents. They all cause potassium loss which may require administration of potassium salts; they may increase uric acid blood levels and, in the predisposed patient, may precipitate attacks of gout. There are an increasing number of reports that they may raise blood glucose levels and, again in the predisposed patient, may cause a diabetes-like state. There are conditions where neither the sulfonamides nor the mercurials, nor combinations, are effective.

Other Structural Types

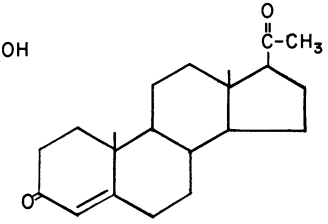
Although the seriousness or incidence of the shortcomings is not great, they provide the impetus for further research. So the search for different and better drugs continues.

The knowledge that salt and fluid retention by the kidney is, in part, due to sodium-retaining adrenal corticosteroids led to attempts to antagonize this effect. Progesterone was shown to antagonize the sodium-retaining action of deoxycorticosterone (*11*). The discovery and structure determination of aldosterone as the important salt-retaining factor of the adrenal cortex stimulated interest in antagonists of this substance. Investigators in the Searle Laboratories developed a series of steroid 17-spirolactones as competitive inhibitors of aldosterone action (*8, 34*). Compounds I and II lack oral activity, but the 7-acetylthio derivative, spironolactone, is orally effective. By the very nature of their mode of action, the spirolactones are effective only in those conditions where there is aldosterone secretion. Since aldosterone causes sodium retention and potassium excretion, antagonists of this action result in sodium excretion and potassium retention. These lactones counteract the potassium loss of

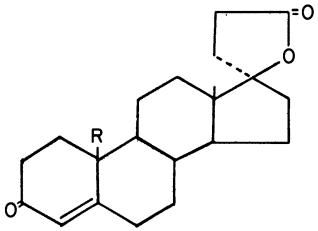
the thiazides and are most useful in combination therapy. They are only mildly effective when used alone.



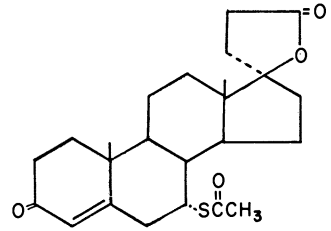
ALDOSTERONE



PROGESTERONE

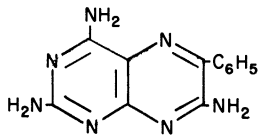


I R=H
II R=CH$_3$

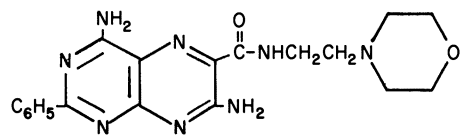


SPIRONOLACTONE

Recently, pteridine derivatives have been shown to be natriuretic agents. Triamterene, developed in the Smith Kline and French Laboratories, pro-



TRIAMTERENE



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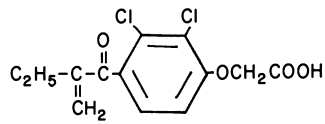
motes sodium excretion without increase of potassium excretion. It antagonizes sodium-retaining steroids but it is also active in adrenalectomized animals, thus indicating that the action of the compound is more direct than aldosterone antagonism. Triamterene potentiates the action of thiazides and reduces the potassium loss (*1, 14, 15, 16, 36, 67*). No information has appeared relating structure-activity influences in this series. However, a second related pteridine, Wy-3654, has been reported from the Wyeth Laboratories to be active orally in animals and in man, but appears to differ from triamterene in some properties. Although it causes minimum potassium loss, it does not exhibit the antikaluretic activity of triamterene (*49, 50*).

Recently, a high order of diuretic activity in dogs and in man has been found in a series of unsaturated acylphenoxyacetic acids (*55*). Of this series, ethacrynic acid has been most extensively studied. The effective daily clinical



ETHACRYNIC ACID

dose is comparable to hydrochlorothiazide when administered orally. The electrolyte excretion is mainly sodium and chloride, but the ceiling response is manyfold that attainable with the thiazides and, in this respect, resembles parenteral mercurials. There is potassium loss but, considering the large sodium excretion, the sodium-potassium ratio is favorable (*2, 3, 7, 20, 41*).

For the general consideration of structural modifications and the value of the results for future drug design, ethacrynic acid structure has several points of interest. In its structure are incorporated features of other series of drugs.

The phenoxyacetic acid structure had been found to be a good carrying moiety in organic mercury diuretics.

The α,β-unsaturated acyl group has a selective reactivity for sulfhydryl groups that resembles the mercurials.

The enhancing effect of the chlorine ortho to the electron-withdrawing acyl substituent is reminiscent of the enhancing activity of halogen ortho to the sulfamoyl group in the thiazide series.

The utility of a potent drug of this type is yet to be proved. However, ethacrynic acid has already proved life-saving in a number of patients that had become resistant to both mercurial and thiazide drugs (*7, 20, 41*).

The present classes of diuretic drugs have contributed much useful information to the chemist and to the biologist. From the mercurial diuretics, on the biological side, some facts have been learned about their site of action, if not the exact mechanism of action, and they have been helpful in the study of the site of the transport of various ions by the kidney. The importance of sulfhydryl enzyme systems has been demonstrated, although the specific enzyme has not been identified. On the chemical side, the chemist has learned about the structural requirements for useful activity, the nature of the carbon-mercury attachment, and the organic structures most useful as carrying moieties for mercury.

The sulfonamide carbonic anhydrase inhibitors, such as sulfanilamide, acetazolamide, and their congeners, have contributed significantly to the knowledge of renal function—e.g., the site and origin of the hydrogen ion excretion and the site of potassium ion excretion.

The mercurials and the carbonic anhydrase inhibitors thus have contributed knowledge concerning two apparently independent renal mechanisms concerned with electrolyte excretion.

The thiazide diuretics and their spectrum of activity point to still a third mechanism for renal electrolyte transport.

The spirolactones and other mineralocorticoid antagonists have led to useful facts concerning the hormonal control of kidney function. The novel action of the pteridines and ethacrynic acid will, no doubt, make their own unique contributions to a clearer understanding of renal mechanisms.

The progress in the development of useful diuretics, just as in other fields of drug development, has occurred through the continued combined effort of biologists and chemists. The result has not only been clinically improved drugs, but the design and synthesis of active structures by the chemist have provided the pharmacologist and the physiologist with useful tools for the study of fundamental facts of renal function. The results help evaluate current concepts and give rise to new concepts or hypotheses of renal function and diuretic action. Using these ideas, the chemists have been able to devise a more

systemic approach that complements the traditional empirical approach to drug design. Improved agents have resulted that are, in turn, more potent tools for fundamental biological investigations. This dual or parallel progress has yielded clinically useful drugs and a better, although incomplete, understanding of their mode of action and of their clinical applications.

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Discussion

C. J. CAVALLITO, presiding

Dr. Cavallito: There is one aspect of molecular modification that has not been touched on this afternoon, and that is one of particular concern to those medicinal chemists in industry. In many ways, we are caught between the horns of a peculiar dilemma in philosophy as reflected by our friends at the Food and Drug Administration and our friends at the Patent Office. Those in academic circles have less occasion to be exposed to these differences in philosophy, but very often we get the feeling that everything we do, no matter how minor, is considered to be potentially of major impact on something or other by FDA, but to be an insignificant variance from the old by the Patent Office.

9

Sulfonylureas, Science and Serendipity

F. GILBERT McMAHON

The Upjohn Co., Kalmazoo, Mich.

Serendipity and structural modification of sulfanilamides and sulfonylureas have advanced our abiilty to treat diabetes mellitus. Attempts to develop an agent clinically superior to tolbutamide by chemical modification have been remarkably difficult in spite of animal and human assay. Over 6000 compounds have been screened for hypoglycemic potency, and some structure-activity generalizations are possible. Chemicals are made into therapeutic products only in man. Animal screens are often based on a standard marketed product and may lead only to "me too" drugs. Many major therapeutic advances have been made by serendipitous observations in man, rather than animal screening. Human screening for a spectrum of activities deserves consideration, so that time-consuming development programs are done on compounds known to possess human activity.

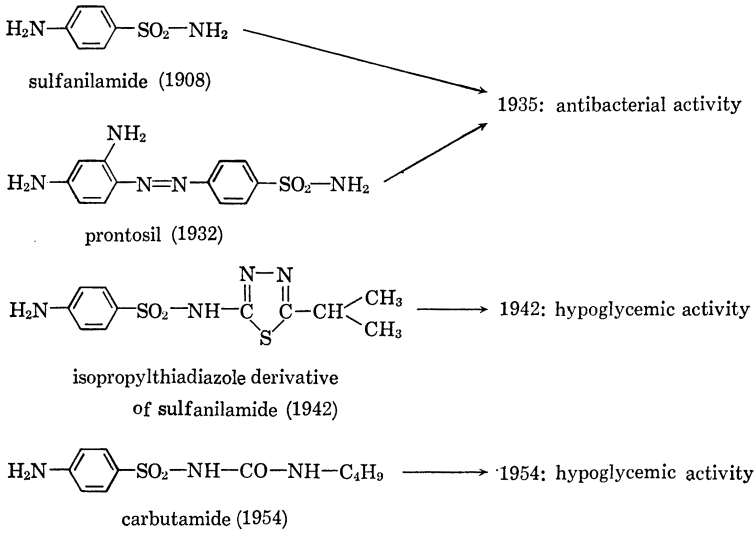
In 1908 Gelmo discovered sulfanilamide while working with azo dyes. Other workers subsequently found that related sulfa compounds combined tenaciously with the proteins of wool and silk. This suggested the possibility that they might also react with bacterial protoplasm. A quarter of a century after Gelmo's synthesis, Domagk observed that mice with various bacterial infections could be protected by sulfonamides, an observation for which he was awarded a Nobel Prize in 1938.

Of the 2,100,000 diagnosed diabetics in the United States today, 45% are on oral drugs, 33% are on insulin, and 22% are on diet alone. Approximately one million Americans take either tolbutamide or chlorpropamide every day as primary therapy for their diabetes mellitus. The sulfonylureas represent a very significant contribution to medical therapy. That guanidine bases depress blood sugar was first reported in 1918 by Watanabe (27). The real story of the antidiabetic sulfonamides began in 1942.

In 1941 and 1942 sulfonamide derivatives were being studied extensively

for superior antibacterial properties. One of these, an isopropyl-thiadiazole derivative of sulfanilamide, was undergoing evaluation in patients with typhoid fever in France (*17*). When the clinician, Janbon, noted the symptoms of hypoglycemia occurring in some of his patients receiving this drug, he consulted Loubatières (*18*); and these two men initiated interest which has continued intensively to this time and which has produced effective antidiabetic therapeutic agents. Thus, the sequence of events indicates that molecular manipulation of the azo dyes produced sulfanilamide and prontosil and subsequent modification resulted in the sulfonylureas (Table I).

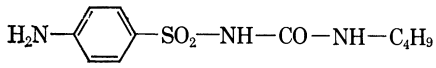
Table I. Sulfonylureas



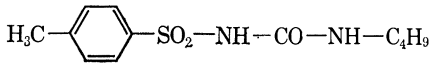
Loubatières studied several analogs of sulfanilamidoisopropylthiadiazole and found that the butyl, isobutyl, and amyl derivatives were more potent than the original isopropyl. Chen (*6*), in 1946, reported that the cyclopropyl derivative was hypoglycemic in normal rabbits, although it was also goitrogenic. In 1954 a group of German investigators (*1*, *4*, *12*) studied the efficacy, in diabetics, of the first sulfonylureas as such, carbutamide (or BZ-55) and, later that same year, tolbutamide (Table II).

Carbutamide (BZ-55) was studied in several thousand diabetics in Europe, the United States, and Canada and found to be an effective agent in the mild, maturity-onset type of patient. Its half life is 36 hours, and the usual maintenance dose is 250 to 1000 mg. once a day. Carbutamide produced toxic reactions in about 5% of patients, including serious liver, kidney, and bone marrow toxicity. Its clinical studies were therefore discontinued in 1957 in the United States, although this drug is still on the market and popular in many foreign countries. European clinicians generally use smaller doses and encounter less toxicity. I would agree with West's (*28*) observation that had the original American trials with carbutamide been conducted with 50- or 100-mg. tablets instead of 500-mg., it might well be on our market today. Incidentally, car-

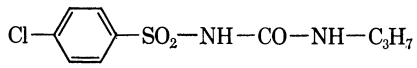
Table II. Sulfonylurea Derivatives



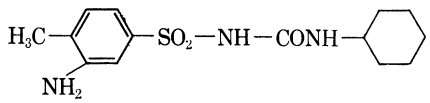
Carbutamide (BZ-55)



Tolbutamide (Orinase)



Chloropropamide (Diabinese)



Methahexamide

butamide, in contrast to the other sulfonylureas, has both Gram-positive and Gram-negative antibacterial activity.

Metahexamide was studied intensively in 1958 and 1959 in the United States and found to be effective in most maturity-onset diabetics at a dose of 100 to 300 mg. per day. It is about three or four times as potent as tolbutamide and has a biological half life of 22 hours. Twenty-six cases of jaundice occurred among approximately 4800 diabetics treated. Liver toxicity appeared even with small doses, and clinical studies with metahexamide were abandoned in this country in 1959.

Since 1956 The Upjohn Co. has tested over 6000 chemical compounds in animals for their hypoglycemic potency, and I am reporting on 23 sulfonylureas which have been given to man (*19*). This paper discusses briefly our methods of testing new agents, reviews some of our results, and attempts to correlate structure with potency, duration, and toxicity in animals and man. Some general observations regarding other serendipitous drug discoveries are also made.

Methods

Our animal screening method essentially consists in the oral administration of the test drug to glucose-primed intact and adrenalectomized rats and the estimation of potency from dose-response curves compared with tolbutamide as the standard (*9*).

To estimate human potency, healthy male volunteers were used to compare the blood sugar–lowering effects of test drugs, placebo, and tolbutamide as standard. Following an overnight fast, the drugs were given as single doses, immediately after a zero-hour blood sample was obtained. With the subjects fasting throughout the study, blood glucose levels were determined at 1, 2, 4, 6,

8, and 10 hours after treatment. Blood sugars were determined on the Auto-Analyzer (*16*). At least 30 subjects were utilized in each test, and from three to ten studies were run with each compound. The data from blood sugar curves for various compounds were compiled and analyzed statistically. The "acute potency" of a compound was estimated by comparing the dose needed to produce a hypoglycemic effect at 4 hours, equivalent to that produced by 1.0 gram of tolbutamide. From these 4-hour blood sugar values, dose-response curves were obtained.

"Duration of hypoglycemic effect" was estimated by employing a triple crossover randomized, three-component design. Each of 33 normal subjects received placebo, test drug, or standard drug (1.0 gram of tolbutamide) on the evening before each of the three test periods. While continuously fasting, blood glucose was determined at 12, 14, 16, 18, and 20 hours. Sixteen ounces of milk were given at 6 A.M. and 12 noon—i.e., at 10 and 16 hours following ingestion of the drugs. With each person serving as his own control, and receiving both standard and test drug, blood glucose curves were constructed and the data analyzed statistically.

Chemical or radioisotopic estimation of the biological half life of several of the new compounds was performed by methods developed by Forist (*11*).

Animal Toxicity. The LD_{50} was determined in both rats (p.o.) and mice (i.p.) (*10*). Then large single doses ("safety testing") were given to dogs before the cautious administration of small single doses of sulfonylurea derivatives to healthy male subjects. Prior to any prolonged administration to diabetics, test of subacute toxicity to two animal species was first performed. Chronic toxicity was tested with all drugs which underwent extensive trials in diabetic subjects.

Results

Table III lists the 23 sulfonylureas included in this study and gives their chemical structures. The sulfonylureas are grouped into four classes on the basis of their chemical configurations. Tolbutamide (Orinase), carbutamide (BZ-55), chlorpropamide (Diabinese), and metahexamide are included for purposes of reference standards because of the extensive clinical data available.

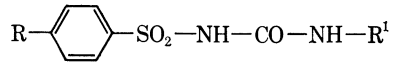
The response-time plots for various doses of tolbutamide are shown in Figure 1. Figure 2 shows a typical acute blood sugar curve obtained when two different doses of U-12,504 and a single dose of U-17,835 are employed. At the 4-year levels, U-12,504 has two to four times and U-17,835 has seven times tolbutamide's potency. Furthermore, there is a suggestion that the hypoglycemic effect of U-12,504 persists longer than for the other drugs from the sixth to the tenth hour.

By using the triple crossover technique described under "Methods," a slight but significant ($P < 0.05$) "duration" effect can be demonstrated for U-12,504 during the 12th through the 20th hours (Figure 3).

Table IV summarizes the acute potencies and durations of hypoglycemic effect of sulfonylurea compounds in man, together with the biological half lives. The acute potencies in diabetic patients (not shown here) were virtually identical with those for normal subjects.

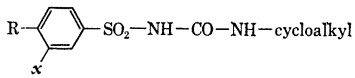
Table III. Structural Formulas

CLASS I COMPOUNDS



1.	Tolbutamide (U-2043)	CH_3—		—C_4H_9
2.	Sodium tolbutamide (U-7064)	CH_3—	—$\overset{\ominus}{N}$— $Na+$	—C_4H_9
3.	Carbutamide (U-7354)	NH_2—		—C_4H_9
4.	Chlorpropamide (U-9818)	Cl—		—C_3H_9
5.	U-13,835	Br—		—C_4H_9

CLASS II COMPOUNDS



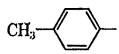
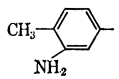
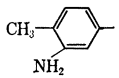
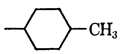
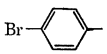
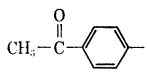
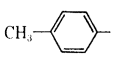
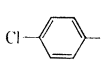
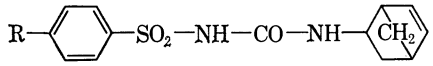
1.	U-19,803		
2.	Metahexamide (U-9970)		
3.	U-18,399		
4.	U-19,359		
5.	U-14,812		
6.	Cycloheptolamide (U-14,462)		
7.	U-14,827		

Table IV. Human Acute Hypoglycemic Potencies and

Compound	*Normals* — *Acute potency times tolbutamide*	*Normals* — *Duration*	*Half Life, Hours*
	CLASS I COMPOUNDS		
Tolbutamide	1	1	5.7
Sodium tolbutamide	1–2+	>1	5.7
Carbutamide	0.5	—	36
Chlorpropamide	1	>1	33
U-13,835	1	—	—
	CLASS II COMPOUNDS		
U-19,803	3	—	—
Metahexamide	3	—	22
U-18,399	4	—	—
U-19,359	4	—	—
U-14,812	2.2	1	1.3
Cycloheptolamide	4	1	4.7
U-14,827	2	—	—

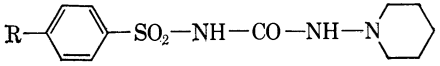
of the Various Sulfonylureas

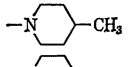
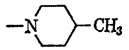
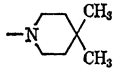
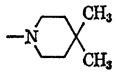
Class III Compounds



1.	U-10,549	CH_3—
2.	U-17,547	Cl—

Class IV Compounds



1.	U-14,184	CH_3—	
2.	U-14,262	CH_3—	
3.	U-13,398	Cl—	
4.	U-17,073	CH_3—	
5.	U-16,444	Cl—	
6.	U-14,378	CH_3—	
7.	U-16,002	Cl—	
8.	U-17,835	CH_3—	
9.	U-12,504	Cl—	

Duration of Effect for Sulfonylureas

Compound	*Normals* — *Acute potency times tolbutamide*	*Normals* — *Duration*	*Half Life, Hours*
	Class III Compounds		
U-10,549	2	—	—
U-17,547	3	—	—
	Class IV Compounds		
U-14,184	5	1	9.7
U-14,262	7	1	8.1
U-13,398	5	—	—
U-17,073	7+	1	6.4
U-16,444	5	—	—
U-14,378	5	1	6.7
U-16,002	10	>1	—
U-17,835	7	1	7.2
U-12,504	2–4	>1	16–18

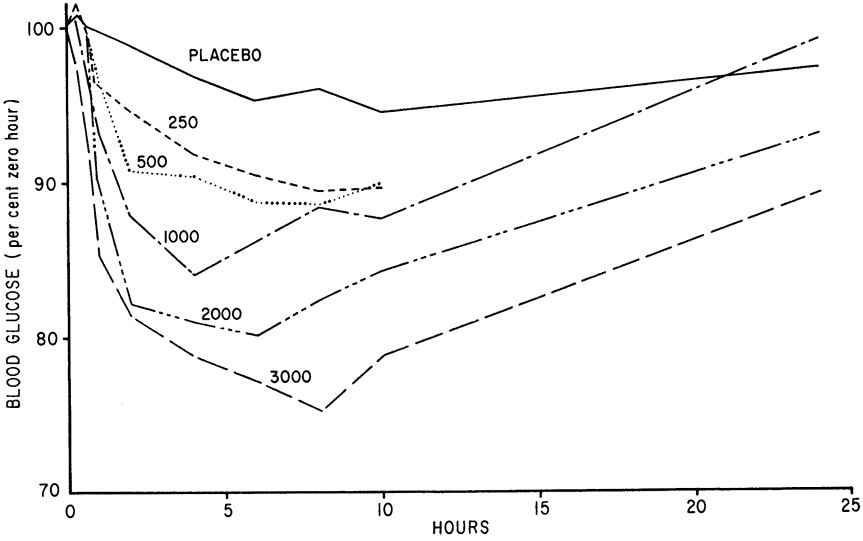


Figure 1. Response-time plot for various doses of tolbutamide

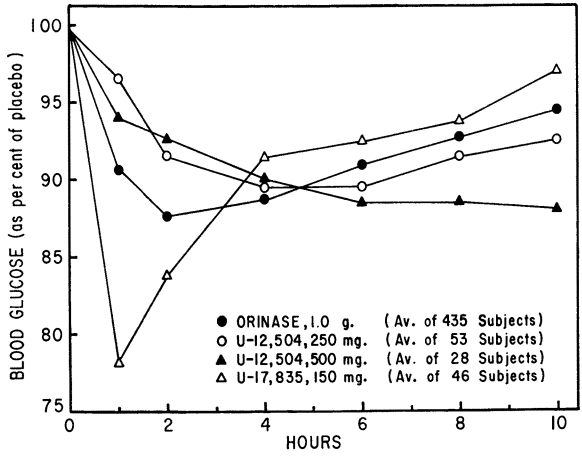


Figure 2. Single-dose potencies of tolbutamide

Table V gives the results of clinical experience with nine different sulfonylureas which have been given chronically to diabetic patients. The clinical data shown for U-14,812 (acetohexamide) are taken from the literature (3).

Discussion

Correlation of Structure, Potency, and Duration. From the "acute potency" data shown, it will be noted that in Class IV sulfonylureas—i.e., in the semicarbazide derivaties—potency was far greater than the other three classes. In this group of compounds, the presence of an amino or chloro group in the

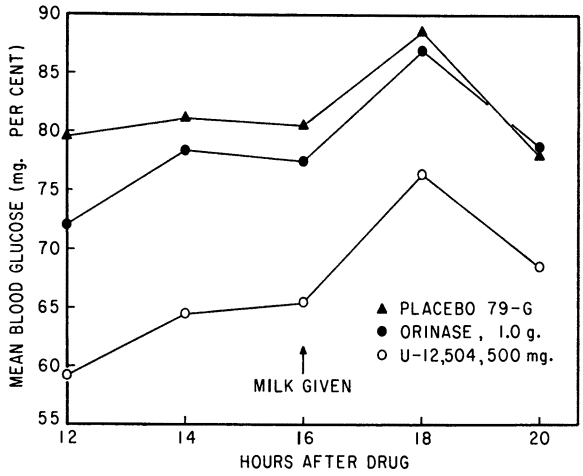


Figure 3. Duration of hydroglycemic effect

Table V. Summary of Clinical Experience with Various Sulfonylureas

	Experience in Diabetics				
Compound	*No. pts. treated*	*Pt. months*	*Potency*	*Jaundice*	*Incidence of side effects, %*
Tolbutamide	>1,000,000	—	1	2?	1.5–3.2
Carbutamide	> 1,000	—	3–4	+	5 or +
Chlorpropamide	> 100,000	—	4–7	4/1000	?
Metahexamide	> 4,800	—	10	26/4800	1–11
Cycloheptolamide	> 980	>8000	3.7	0	4.3
U-12,504	> 2,500	>5000	5–10	3–8/2500	<5
U-17,835	> 2,000	>5000	5–10	0	<5
U-14,184	30	50	7	0	<1
U-14,812	27	68	1–4	0	Very low

aryl ring (at least in the para or meta position) prolongs the half life and duration of hypoglycemic effect. It may also contribute to hepatotoxicity.

By employing the triple crossover technique, in which each subject serves as his own control, a slight but statistically significant "duration" advantage was shown for the para-chloro analogs, U-12,504, U-16,002, and chloropropamide.

It is very likely that the solubility and rates of absorption of sulfonylureas influence the contours of their hypoglycemic curves (*20, 28*). It is also possible that differences in metabolism affect their acute potencies. Nevertheless, it is likely that the known sulfonylureas act via the same mechanism—i.e., via β cell stimulation with release of insulin (*5*). The spectrum of activity of all the current sulfonylureas seems to be virtually identical—i.e., their effectiveness as sole hypoglycemic therapy is limited to the maturity-onset type of diabetic.

Attempts to correlate animal toxicity data with human toxicity are very disappointing.

The most hepatotoxic sulfonylurea in the dog, for which we also have human data, is cycloheptolamide (Orabeta), in which doses as small as 12.5 mg. per kg. produced mild degenerative changes in the dog livers. However, in over 8000 patient-months' experience with cycloheptolamide, not a single

case of jaundice occurred and the drug was very well tolerated. On the other hand, chlorpropamide at doses of 100 or 150 mg. per kg. produced no liver toxicity in the dog, yet its human incidence of liver toxicity is generally given at four cases per 1000 (*21*). Tolbutamide, for which there are only two cases (one confirmed and one possible) of documented drug-induced jaundice among more than one million treated diabetics, is far more toxic to the dog liver than chlorpropamide and about the same as metahexamide. This latter sulfonylurea produced 26 cases of jaundice in 4800 patients receiving the drug. So the correlations of animal to human toxicity with the sulfonylureas are poor.

Simplicity, rapidity, and reliability are essential in the orderly screening of hundreds of drugs for hypoglycemic effect. In the clinical investigation of these drugs, the single-dose testing of the healthy normal human subject has provided a means of estimating ultimate clinical potency and duration of hypoglycemic effect. By these methods, the proper tablet size and dosage schedule can be estimated before extensive clinical trials are undertaken.

Although in these studies blood sugars are used as our end point, it is recognized that diabetes mellitus is a complicated metabolic disease and probably involves more than insulin lack. Such things as insulin antagonists, pituitary factors, genetic factors, and insulin binding must be kept in mind when searching for new agents designed to treat the disease. However, at present the regulation of hyperglycemia is the major consideration in the practical management of the diabetic patient. Blood sugar also affords us a convenient objective tool for rapidly evaluating the effect of new compounds.

Future Outlook

Why have we put so much effort into the sulfonylureas? Because we are convinced that available therapy can and will be improved. The problem of treating diabetes mellitus seemed once before to have been solved by the discovery and production of crystalline insulin in the early 1920's. But the orally active sulfonylureas introduced 30 years later, though accepted initially with a great deal of caution and doubt, have now firmly entrenched themselves in the therapy of this disease.

Yet treatment of diabetes today is far from complete. In spite of insulin, cardiovascular, renal, eye, and nervous system complications still occur. In spite of sulfonylureas, many primary and secondary failures occur; and there is yet no oral insulin substitute for the juvenile or labile diabetic.

Aside from diabetes therapy, sulfonylurease could have other uses. Table VI depicts 23 nondiabetic uses for which tolbutamide has been investigated and reported in the medical literature during the past five to six years. Tolbutamide has not been established as efficacious in most of these conditions. But it is possible that some analog of tolbutamide, perhaps one that does not lower blood sugar, could be effective in some nondiabetic condition. It should be no less thinkable today than it was in 1935 to state that a chemical dye could be effective in treating bacterial infections or in 1942 that an antibacterial drug could be effective in treating diabetes mellitus.

The hypoglycemic effect of the sulfonylureas was tested initially in human patients, not in animals. Once bioactivity was established in man, then Loubatières and German investigators went back to animals, and worked on

Table VI. Tolbutamide in Nondiabetic Conditions

Schizophrenia	Arterial insufficiency
Epilepsy	Diagnosis of insulinoma
Multiple sclerosis	Diagnosis of diabetes mellitus
Parkinsonism	Malabsorption syndromes
Tuberculosis	Underweight
Psoriasis	Dandruff
Acne	Pemphigus
Anogenital eczema	Pityriasis
Cirrhosis	Eczema
Angina pectoris	Thromboangiitis obliterans
Hepatitis	Necrobiosis lipoidica
Hypercholesterolemia	

analogs and on mechanism of action, and ultimately a product for human use was obtained.

This is not an unusual sequence of events in the development of major pharmaceutical advances. It happened with cortisone (the anti-inflammatory effect) (*15*) and dimenhydrinate (Dramamine) (the antimotion sickness and the antiemetic effect) (*13*); and these later led to other antihistamines, including chlorpromazine (Thorazine), a promethazine derivative, being tested in animals and then in man as antiemetics. The diuretic effect of organic mercurials (*24*, *26*) and the ataractic effect of rauwolfia (*14*) were first noted in man. The CNS stimulatory effect of isoniazid and iproniazid (Marsilid) (*23*) was first observed in tuberculous patients; and this led to several hydrazine analeptics and amine oxidase inhibitors (Marplan, Nardil, Parnate, and Eutonyl). Imipramine (Tofranil) (*2*), found to sedate animals, was studied in man as a tranquilizer and found, indeed, to be an antidepressant. Southworth's observation in man in 1937 that sulfanilamide lowered plasma CO_2-combining power ultimately led to the carbonic anhydrase inhibitors, acetazolamide (Diamox) and ethoxzolamide (Cardrase) (*25*). Novello's and Sprague's synthesis of the benzothiadiazine, chlorthiazide (Diuril), evolved from this earlier observation in man, although, of course, only after monumental chemical and biological work.

The chemical explosive, pentaerythritol tetranitrate (Peritrate), was found by French pharmacologists to lower blood pressure of animals. When given man, it proved ineffective, but serendipity again intervened and it apparently alleviated angina pectoris (*7*). The antinarcoleptic effect of amphetamine (Benzedrine) was first observed in man by Prinzmetal and Bloomberg in 1935 (*22*), and the anorexigenic activity of this same drug was first noted in man by the astute observations of Davidoff and Reifenstein in 1937 while treating narcoleptic patients (*8*).

All of these important observations were first made in man, by serendipity, and not in the animal. The implications of looking for a spectrum of activities with each new drug investigated in man, and for astute clinical pharmacologists who must be alert for the unexpected, are apparent. I do not wish to imply that this is the universal pathway for major drug discoveries; but, certainly, many major drug activities have originated by serendipitous human observations rather than through the classical animal screening techniques.

Mechanism of action was not of primary importance. The fact that carbutamide and tolbutamide were effective in reducing blood sugar in man at

doses which appeared to be nontoxic was the significant thing. After these facts were established, only then did working out the mechanism of action become important. We do not yet fully understand the mechanism of action of insulin, cortisone, or aspirin. If pharmaceutical discoveries are to be made, ordinarily the first important question is, "Does it work in man at safe doses?" I think that many academic pharmacologists and even some biologists and biochemists in the pharmaceutical industry are too concerned about how a compound works before they even know whether or not it has bioactivity in man at doses that are tolerated.

Conclusions

Molecular modification of the azo dyes has led to the antibacterial sulfanilamide drugs. Further modification has led to the antidiabetic sulfonylureas.

Further modification of sulfonylureas may lead to superior antidiabetic therapy, or even to nondiabetic therapeutic agents.

The sulfonylureas' effectiveness as hypoglycemic agents resulted from a serendipitous observation made in man, not from a "rational" animal research program designed to discover such agents. Many major therapeutic breakthroughs have been uncovered by this approach.

Acknowledgment

The data on dog subacute toxicity and human incidence of jaundice for chlorpropamide are based on product information from Chas. Pfizer & Co., Inc. (*21*).

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Discussion

C. J. CAVALLITO, presiding

Dr. Oscar Keller (Hoffmann-La Roche): Has insulin itself been used?

Dr. McMahon: You have to ask the people involved. We have not standardized the insulin in man as a reference point. The main reason is that DBI does not work in a normal man; insulin does. But we also do this screening in diabetic man with nonsulfonylureas.

Dr. Johnson (Chas. Pfizer): You mentioned earlier some interesting antibacterial activity against Gram-positives and Gram-negatives. Are you talking about in vitro or in animal protection?

Dr. McMahon: I mentioned that in reference to sulfanilamide. I do not know whether it was extended in vitro.

Dr. Louis Friedman (U. S. Vitamin): In reference to Dr. McMahon's report on the molecular modification in sulfonylureas, I think mention should be made of similar work in modification of biguanide, which, as you know, forms another clinical compound of utility in the treatment of diabetes. It is interesting to note, in commenting on the question of toxicity in man and animals, that Taubman and his associates in 1919 found that lower alkyl biguanides were too toxic in animals and recommended that they not be used in man.

However, we picked up this work 29 years later and found that some of the derivatives, particularly the aracyl compounds, were nontoxic to man and have now formed the basis of some very interesting products in the clinical use and treatment of diabetes.

Dr. Love (Smith, Kline & French): How well does the animal screening correlate with results of human testing?

Dr. McMahon: The rat, as far as efficacy goes, serves very well. There are some exceptions, but in general it has served us very well, and even in duration of effect.

10

Some Rationales for the Development of Antidepressant Drugs

JOHN H. BIEL

Research Division, Aldrich Chemical Co., Milwaukee, Wis.

Discovery of drugs for the treatment of mental disease is a direct outgrowth of the continuing search by the medicinal chemist for improved therapeutic agents in a diversity of somatic disease areas. The technique of molecular modification proves particularly applicable to the development of the antidepressant drugs, since mental depression is rarely a "pure" disease phenomenon and hence requires specifically tailored molecules for successful therapy. An attempt is made to correlate the structure of the antidepressant drugs with their activity on certain neurohormones, their pharmacologic effects, and their scope of clinical efficacy for a given depressive disorder. Chemotherapy of mental depression must aim at re-establishing central chemical homeostasis by controlling the biogenesis, physiologic action, and metabolism of certain neurohormones presumably implicated in the control of the emotional state.

Drugs for the treatment of mental disorders were developed by the systematic molecular modification of agents originally designed for the therapy of such somatic diseases as:

Cardiovascular ailments
Allergic manifestations
Peptic ulcer and gastrointestinal disorders
Pain

In the quest for more effective and selectively acting drugs for these disturbances, the medicinal chemist set out to produce a myriad of structural alterations. This intense effort by many industrial and academic institutions gave birth to the breakthrough in the chemotherapy of mental illness. Until 1952 the major therapeutic tools available were insulin or electroshock therapy and psychotherapy.

This particular aspect of the symposium deals with the chemotherapy of mental depression—a disease which has yielded to drug treatment only during the past six years.

Mental depression is a highly complex disease syndrome. Its multifacetness requires the production of specifically tailored molecules to deal with the various phases of this crippling psychiatric illness. Molecular modification has been an invaluable technique in achieving such specificity.

The medicinal chemist confronted with the task of designing new drug entities in this area must be cognizant of the problems inherent in this disease process and the therapeutic qualifications expected of the new agent he has on his drawing board. While depression may at times be a "pure" disease, more often it is the overt expression of an underlying pathologic process which requires other than the mere symptomatic treatment of surface phenomena.

Mental depression may be the result of an underlying schizophrenic disorder, and "pure" antidepressant therapy may merely unleash a full-blown psychotic attack with which an available drug may be powerless to cope. A useful agent must, therfore, have both antidepressant and antipsychotic properties.

In the case of manic-depressive illness, the "stimulant" type of antidepressant drug may "push" the patient into the manic phase and make him a dangerous security risk. Hence, drug design must aim for a tranquilizing antidepressant. A fine differentiation must be made here between tranquilization and central depression. Obviously, central depressant properties would exacerbate the depressive phase of the disease.

Deep-seated anxiety will often accompany mental depression. A drug which affects only the depressive component of the illness will often increase anxiety symptoms. Efforts are now being directed to the development of antianxiety-depression drugs.

A rapid onset of action is of paramount importance in the treatment of depression because of the high degree of suicidal tendencies of these patients. Present available therapy is inadequate in this respect. Molecular modification can assist in obtaining drugs which can act within 24 to 48 hours.

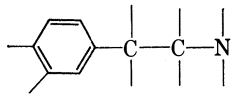
Structural design must also take into account the severity and degree of chronicity of the disease. There are drugs particularly suited to deal with temporary emotional fatigue states. Reactive depressions may be more responsive to the "energizing" type of antidepressant drug, while endogenous depressions may yield more successfully to the antipsychotic antidepressant agent.

The antidepressant drugs were derived via the process of molecular modification from the sympathomimetic agents, antihistamines, antispasmodics (anticholinergics), tranquilizers, and tuberculostatic agents.

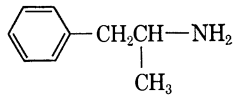
From Sympathomimetic to Antidepressant Drugs

The classical studies of Barger and Dale (*12*) on the molecular modification of the epinephrine molecule demonstrated very clearly that minor alterations in structure of an endogenous hormone can evoke major qualitative and quantitative physiologic changes in the properties of the resultant derivatives.

They established the critical importance of the β-phenethylamine skeleton



in the production of the characteristic sympathomimetic effects. But it remained for Alles (*6*) to discover the potent central stimulant properties of amphetamine:

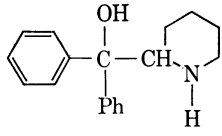


which served as a starting point for some of the newer antidepressant drugs.

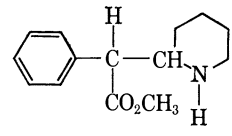
While amphetamine had important antifatigue and moderate antidepressant properties, its therapeutic usefulness was severely limited because its stimulant action was followed by a depressive phase, tolerance to the drug developed, it depressed appetite, it tended to cause anxiety and jitteriness, and it had fairly potent effects on the cardiovascular system.

Molecular modification attempted to overcome these shortcomings and the following classes of drugs evolved from this effort.

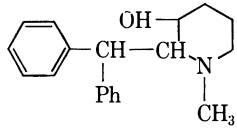
Mild Antidepressant, Antifatigue Agents, useful in the treatment of mild reactive depressions arising from emotional exhaustion and mental fatigue. Drugs such as pipradol (*7, 28, 87*) (Meratran), methylphenidate (Ritalin) (*74, 80, 112*), Sch 5472 (*77, 97*), and W-1206 (*51, 55*) (Reactivan) are representative of this class. They produce their antidepressant effect principally through psychomotor stimulation.



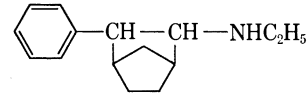
Meratran, Pipradol



Ritalin, Methylphenidate



Sch 5472



Reactivan, W-1207

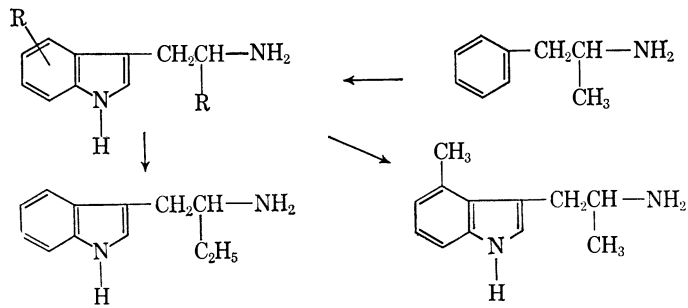
Pipradol and Ritalin may be used in uncomplicated, reactive depressions. They produce less anorexia, insomnia, and euphoria than amphetamine. The cardiovascular effects are also considerably reduced by this structural change. Unlike amphetamine, these drugs do not elicit a postdepressive phase nor cause tolerance. The activity spectrum of Sch 5472 is qualitatively similar to that of methylphenidate, except that the drug is 10 to 20 times as potent as amphetamine or methylphenidate as a psychomotor stimulant. Doses as low as 0.25 to 0.75 mg. per day have been found effective in combating exhaustion and fatigue. Unlike pipradol and methylphenidate, Sch 5472 in-

creases learning behavior in rats. Compared to amphetamine, the dosage ratio for producing disorganized behavior *vs.* increased wakefulness is considerably greater with Sch 5472. Hence, from the standpoint of behavioral side effects, Sch 5472 would be distinctly superior to amphetamine. Jitteriness and apprehension were also absent.

W-1206 (Reactivan) has also been claimed to produce significant antifatigue effects in man at doses of 10 to 20 mg. Its duration of action is shorter than that of amphetamine. It is said to increase both work capacity and performance without the concomitant amphetamine-type side effects.

Thus it may be concluded that in this group of compounds molecular modification of the amphetamine structure achieved a distinct selectivity of action with regard to psychomotor stimulation and a considerably lessened incidence of the usual amphetamine side effects such as anorexia, jitteriness, apprehension, cardiovascular effects, insomnia, tolerance, and postdrug depression.

Moderately Potent Antidepressant Agents. Replacement of the phenyl by an indole ring in amphetamine resulted in the development of a group of α-alkyltryptamines:

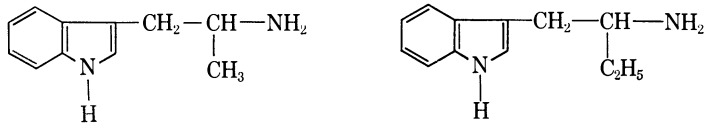


Etryptamine (Monase) MP-809

Etryptamine (*88*) and MP-809 (*10*) are particularly effective in the treatment of neurotic depressions. Etryptamine has since been removed from the market because of a few instances of blood dyscrasias which were thought to be related to the drug (*102*). Compound MP-809 produced a significant antidepressant action in 74% of neurotic depressions and in 52% of all types of mental depression (*10*). While etryptamine exhibits definite MAO inhibitory effects in the laboratory, its action in man is rather weak in this respect (*68*, *88*, *99*). Furthermore, MP-809, which displayed rather potent antidepressant effects in man, is only a weak MAO inhibitor in the laboratory (*10*). Hence, the mode of action of this group of drugs does not appear to be a consequence of either MAO inhibition or psychomotor stimulation.

Conclusion. Structural alteration of the amphetamine molecule by replacing the phenyl ring with an indole nucleus brought about some rather major qualitative changes in both the pharmacologic and clinical activity spectrum of these derivatives—the most important one being the production of a significant antidepressant effect with the virtual elimination of the potent psychomotor stimulant effects seen with amphetamine.

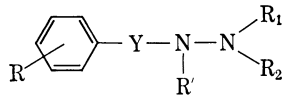
Important differences in behavioral and autonomic effects between α-methyltryptamine and α-ethyltryptamine were noted by Murphree *et al.* (*76*) in human volunteers:



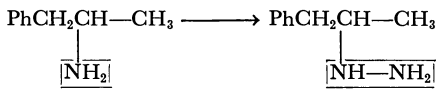
These authors report that feelings of exhilaration were experienced with α-ethyltryptamine, while the α-methyl compound produced feelings of tenseness, restlessness, and generalized malaise. α-Methyltryptamine was likened to LSD by nine of the 12 subjects at a dose of 20 mg., while α-ethyltryptamine, at a dose of 150 mg., was likened to LSD by only two subjects. The action of the α-methyl compound was delayed, while the α-ethyl homolog produced an immediate effect. With respect to the cardiovascular effects, the α-methyl compound produced a rise in systolic and diastolic blood pressure; the α-ethyl derivative caused a slowing of the heart with little change in blood pressure. Hence, drugs differing in structure by only one methylene group may, nevertheless, be very dissimilar in their actions.

Potent Antidepressant Agents. Aralkylhydrazines. In the search for sympathomimetic agents with a greater intensity and duration of action, Biel and coworkers (*19, 22*) investigated a large number of phenylalkylhydrazines which were patterned structurally after the sympathomimetic amines. In essence, the hydrazine moiety either replaced an amino radical or served to produce a nitrogen isostere of a sympathomimetic amine:

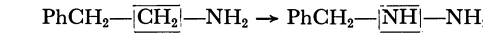
Sympathomimetic Hydrazines



Replacement of NH_2 by $NH—NH_2$

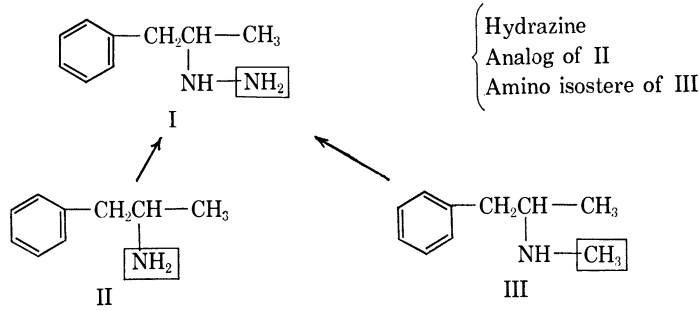


Isosteric replacement of CH_2 by NH



Replacement of the amino by a hydrazine moiety preserved some of the sympathomimetic effects of the parent amines such as psychomotor stimulation and rise in the dog blood pressure. The hydrazine isosteres, on the other hand, were essentially devoid of the direct CNS stimulant and potent blood pressure effects of the sympathomimetic amines (*19, 22, 52*).

Of much greater importance, however, are the differences in the biochemical, pharmacologic, and clinical properties, as compared to the structurally closely related sympathomimetic amines. For instance, α-methylphenethylhydrazine (Catron) has 1000 times the in vitro monoamine oxidase inhibitory ac-



tivity of α-methylphenethylamine (*53*, *100*) and is effective in vivo (*100*).

Clinically, the differences between the amines (II and III) and the hydrazine derivative (I) are even more pronounced. While amphetamine produces a short central stimulant and euphoric effect, which is of little consequence in the treatment of mental depression, its hydrazine congener (I) is a potent antidepressant drug capable of producing lasting remissions in both moderate and severe depressions (*5*). The central stimulant effect of amphetamine (II) (*11*) and methamphetamine (III) is quick in onset and lasts only for a few hours. Repeated administration of the amines results in tolerance and addiction liability. On the other hand, α-methylphenethylhydrazine (I) is slow in its onset of action (5 to 10 days), but its effects may last from 7 to 14 days following discontinuance of therapy. Neither tolerance nor addiction liability develops with continued use of the drug for a period of months. While the hydrazine drug produces some initial motor excitement, this action does not seem to be related to its therapeutic effect in depressions, inasmuch as related aralkylhydrazines such as phenethylhydrazine (Nardil) (*96*) which are essentially devoid of psychomotor stimulation exhibit similar antidepressant properties and are effective in this regard. While amphetamine and methamphetamine produce a rise in blood pressure and increase in heart rate which diminish on repeated administration, the hydrazine analog (Catron) produces a gradual and sustained blood pressure lowering in moderate and severe essential hypertension (*100*, *101*). Amphetamine is a potent anorexigenic agent; Catron actually increases appetite.

	Comparative Activities	
	$PhCH_2CH(CH_3)NH_2$	$PhCH_2CH(CH_3)NHNH_2$
MAO inhibition	Weak	Potent
Antidepressant	Mild	Potent
Postdrug depression	Yes	No
Tolerance	Yes	No
Drug dependency	Yes	No
Antihypertensive	[a]	Potent
Angina pectoris	[a]	Potent, pain relief
Appetite	Decreased	Increased

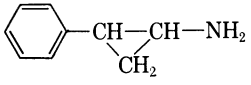
[a] Contraindicated in these conditions.

The above table summarizes both the qualitative and quantitative differ-

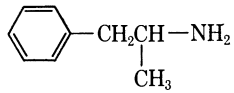
ences in the biochemical and clinical activities between the amine and its corresponding hydrazine analog. Most striking is the fact that this molecular change in the parent structure endowed the derivative compound with novel therapeutic properties, rendering it effective in the treatment of moderate to severe mental depression, essential hypertension, and angina pectoris (as a pain reliever) (*45, 69*). Single instances of irreversible liver necrosis for Catron (α-methylphenethylhydrazine) have been reported (*14, 37, 44*) which limit the therapeutic usefulness of this interesting drug. Further molecular modifications have overcome this particular toxicity problem and are discussed below.

Phenethylhydrazine (Nardil) appears to act more specifically as an antidepressant (*8*) inasmuch as no antihypertensive or antianginal effects have been reported. Instances of orthostatic hypotension have been described, but these effects appear to be more variable and less intense than with α-methylphenethylhydrazine.

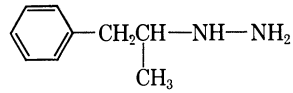
Nonhydrazine MAO Inhibitors. Phenylcyclopropylamine. Interest in cyclized sympathomimetic amines prompted Burger and Yost (*30*) to prepare 2-phenylcyclopropylamine (IV):



IV



V



VI

Phenylcyclopropylamine resembles both amphetamine (V) and α-methylphenethylhydrazine (VI) in its actions. Like amphetamine it produces a pressor effect (*70*); as a central stimulant it has one seventh the activity of amphetamine (*70*). In blocking selectively the conditioned response in the rat, it has about twice the potency of amphetamine. It is approximately equal to amphetamine as a reserpine antagonist (*111*). As an MAO inhibitor it appears to be somewhat more potent than α-methylphenethylhydrazine (*109, 110*), and 5000 times as potent as amphetamine. Clinically, tranylcypromine (Parnate) (IV) is an effective antidepressant in the same dosage range as the hydrazine derivative (VI). However, its onset of action is much more rapid (24 to 72 hours instead of 5 to 10 days) (*93*). The most "striking" effects are achieved in chronic neurotic patients who have not responded well to previous therapy, including EST. The benefit is sustained as long as the drug is used. Good results are also obtained in pseudoneurotic depressions, a schizophrenic condition marked by neurotic manifestations. Tranylcypromine shows little efficacy in agitated and manic-depressive depressions, as well as in the treatment of chronic withdrawn schizophrenics. This is in contrast to the aralkylhydrazines which effected definite improvements in psychotic depressions (*11, 43*). Hence, the outstanding differences between phenylcyclopropylamine and the phenylalkylhydrazines are (1) a more rapid onset of action, (2) a lack of cumulative effects due to a shorter duration of action, (3) a lesser intensity of action restricting its use mainly to the nonpsychotic depressions, and (4) greater safety with respect to hepatotoxicity and visual disturbances (*93*).

From Table I it may be concluded that cyclization of the amphetamine side chain brought about a striking change in the biochemical and clinical

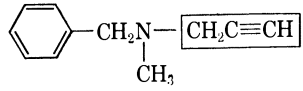
Table I. Comparative Activities

	$PhCH_2CHNH_2$ | CH_3	$PhCH(CH_2)NH_2$	$PhCH_2CHNH{-}NH_2$ | CH_3
Activity		*Pharmacology*	
Pressor	Yes	Yes	Yes
CNS stim.	Potent	Moderate	Moderate
C.A.R.[a]	Blocks	Blocks	?
MAO inhibition	1	5000	1000
Tolerance	Yes	No	No
		Clinical Activity	
Antidepressant			
Neurotic	Mild	Potent	Potent
Psychotic	Inact.	Weak	Moderate
Onset	Fast	Fast	Slow
Duration	Brief	Moderate	Long
Postdrug depression	Yes	No	No
Antihypertension	No[b]	Variable	Potent
Angina pectoris	No[b]	No[b]	Striking pain relief
Appetite	Decreased	?	Increased
Liver toxicity	No	No	Yes

[a] Conditioned avoidance response.
[b] Contraindicated.

activity spectrum of the derivative drug (tranylcypromine) which is intermediate between the phenylalkylamine and phenylalkylhydrazine series.

Acetylenic Amines. Further modification of the phenylalkylamine side chain to include acetylenic moieties resulted in the discovery of a highly potent MAO inhibitor (*107, 108*), pargyline (Eutonyl):

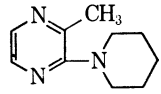


As an MAO inhibitor, this drug approaches the phenylalkylhydrazines in biochemical and clinical potency (*54*). From the standpoint of hepatotoxicity, however, it appears to be a much safer drug. Thus far, no instances of liver toxicity have been reported. Its clinical activity profile resembles that of α-methylphenethylhydrazine with respect to its antidepressant (*29*) and hypertensive activity (*71, 106*), although on a dosage basis it is only one fifth to one seventh as potent. Because of its consistent efficacy in lowering blood pressure in moderate and severe essential hypertension, it has become one of the more valuable antihypertensive agents now being marketed (Eutonyl). In its therapeutic efficacy, it appears to compare favorably with guanethidine and α-methyl-3,4-dihydroxyphenylalanine (α-methyldopa). As with the latter two drugs, blood pressure lowering is achieved primarily via orthostatic hypotension, although some decrease in blood pressure is seen in the supine position (*71, 106*). Symptoms of diarrhea often seen with guanethidine sulfate (*32*) are not encountered with pargyline. Unlike α-methyldopa, pargyline produces a stimulant rather than a sedative effect (*32*) on the central nervous system.

Its concomitant antidepressant activity (*29*) would appear to render it particularly useful in the treatment of the depressed hypertensive patient.

In conclusion, the incorporation of an acetylenic moiety into a benzylamine structure conferred completely novel biochemical and therapeutic properties on an otherwise inert phenylalkylamine. Outstanding in this respect are the potent MAO-inhibitory, antihypertensive, and antidepressant effects of this new drug. The potent autonomic side effects normally encountered with the ganglionic blocking type antihypertensive agents are much less pronounced with this drug and a definite advance has been scored toward achieving selectivity of action.

Pyrazine Derivatives. Another compound which has recently been described by a number of investigators (*33*, *35*, *38*, *46*) under the code number W-3207 is 2-methyl-3-piperidinopyrazine:



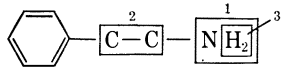
Originally developed as antianginal agent, this drug elicits powerful MAO-inhibitory and antidepressant effects. It appears to be more potent than phenelzine (phenethylhydrazine) in this respect.

The presence of a 2-alkyl and a 3-*N*,*N*-disubstituted amino group is highly critical to a retention of the MAO-inhibitory properties.

As with phenylcyclopropylamine (tranylcypromine, Parnate), the onset of its antidepressant action is rapid (48 to 72 hours). According to Dunlop (*35*), W-3207 is qualitatively different from other MAO inhibitors, "in that there appears to be much less tendency to produce 'excitation,' 'jitteriness,' or 'overstimulation,' although headache and insomnia are not uncommonly found in patients treated with W-3207B." Hence, the need for concomitant tranquilizing medication in anxious patients would seem to be much reduced with the use of this agent. The incidence of orthostatic hypotension was also less (1 in 21) with W-3207 in these preliminary studies. The drug is particularly effective in neurotic depressions, including anxiety depressions, but less useful in psychotic depressions. In manic-depressive and schizophrenic states, some of the patients may tend to become maniacal. In its therapeutic spectrum, the drug seems to take its place between the stimulant type of MAO inhibitors and imipramine, the tranquilizing type of antidepressant agent. However, it would be premature at this time to predict the ultimate therapeutic scope of W-3207 in antidepressant therapy. If its lack of cardiovascular and sympathomimetic side effects and its antidepressant efficacy can be confirmed in larger scale studies, the development of the aminopyrazines as a new class of antidepressant drugs would mark another advance in the selective treatment of mental depression.

Summary. The molecular modification of the sympathomimetic amines has produced an array of highly significant biochemical, pharmacologic, and clinical changes from the original properties of the parent structures. From a therapeutic standpoint, major advances have been scored in the successful treatment of various types of mental depression and cardiovascular disease. Furthermore, these structural alterations have achieved a significant selectivity of drug action, resulting in a sharp decrease of both toxic and annoying side effects.

Structural departures from the original β-phenethylamine skeleton:



included:

Substitution of an amino group by a hydrazine moiety
Cyclization of the isopropyl side chain to a cyclopropane ring
Incorporation of an *N*-propinyl substituent on the amino group
Replacement of a phenyl by a pyrazine or indole ring

With respect to the role of the brain monoamines in mental depression, it may be stated that all of the antidepressant drugs described in this section are capable of increasing brain levels of norepinephrine, Serotonin, and dopamine. Recent work by Spector (*103*) would seem to indicate that norepinephrine may be the critical neurohormone which mediates the effects of the MAO inhibitors, inasmuch as pargyline was incapable of counteracting the depressant effects of reserpine (even in the presence of significant amounts of Serotonin and dopamine) until there was a small but definite rise in norepinephrine levels. This work was done in rabbits which had previously been depleted of brain monoamines by the administration of reserpine.

According to Axelrod (*60*) the MAO inhibitors block both the spontaneous release of norepinephrine and the metabolism of the more firmly bound and relatively "unavailable" norepinephrine.

Molecular changes in the structure of the MAO inhibitors will have a profound influence on their ability to penetrate the blood brain barrier, their relative affinity toward certain tissue sites, their relative susceptibility toward metabolic degradation, and their preferential action on certain enzyme systems. Although it is conceivable that these drugs may have a common underlying mechanism of action, their individual structural make-up will endow them also with distinct therapeutic properties which will make them invaluable in the selective treatment of a specific disease process.

The Hydrazides

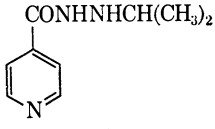
The early pioneering work by Zeller *et al.* (*115*) on the potent MAO inhibitory effect of iproniazid—a structural modification of the tuberculostat Isoniazid—and his realization of the physiologic consequences that might arise from such a profound alteration in catecholamine metabolism, the actual confirmation by Brodie, Pletscher, and Shore (*27*) of the rise in brain monoamine levels following the administration of iproniazid and JB-516 (α-methylphenethylhydrazine), and the early euphoric effects noted by Selikoff, Robitzek, and Ornstein (*96*) in tuberculosis patients on iproniazid therapy led Kline and his associates (*67*) to investigate the possible application of iproniazid in the treatment of mental depression. It was their conclusion that MAO inhibition and antidepressant effect had a causal relationship and that a new approach for the treatment of mental depression had been uncovered. The subject of the MAO inhibitors has been reviewed extensively up to 1960 by Pletscher, Gey, and Zeller (*84*) and by Biel, Horita, and Drukker (*21*) to 1963, in comprehensive reviews of the chemistry, biochemistry, pharmacology, clinical application, and structure-activity relationships of the MAO inhibitors.

The significance of molecular modification in this series may be summarized as follows:

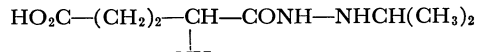
Masking of the free hydrazine with an acyl radical lowered the toxic properties of isopropyl and benzylhydrazine. It also protected a highly reactive hydrazine group against undesired interaction with nontarget organ systems. Once the target organ was reached, the acyl hydrazine was cleaved to the "active" free hydrazine, which then exerted its characteristic action.

$$RCONH—NH—R' \rightarrow RCO_2H + R'NH—NH_2$$

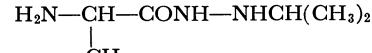
The choice of the acyl group was, therefore, important with regard to tissue selectivity and ease of cleavage to the active moiety. Thus Pletscher (*82*) showed that the L-glutamyl derivative of isopropylhydrazine had a greater affinity for brain tissue than the corresponding isonicotinoyl derivative (iproniazid). On the other hand, the D-glutamyl and β-alanyl derivatives had only low grade activity, presumably because the D-glutamic acid has little affinity for brain tissue and the unnatural amino acid hydrazide is not subject to the same degree of metabolic cleavage necessary for eliciting activity. 1-Pivaloyl-2-benzylhydrazine produces a greater inhibition of cardiac MAO than of brain MAO and this compound is, therefore, marketed as an antianginal drug (*31*) (Tersavid).



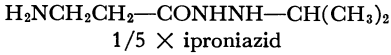
Iproniazid



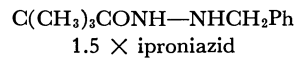
2.5 × iproniazid



2 × iproniazid

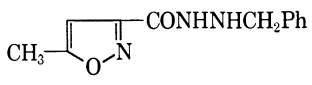


1/5 × iproniazid

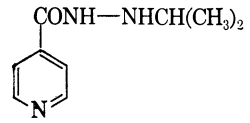


1.5 × iproniazid

The work of Zeller *et al.* (*116*) on the amino acid hydrazides thus demonstrated several interesting points: the need for considering susceptibility to drug metabolism in the design and evaluation of a new agent; the stereospecificity of drug action; and the importance of incorporating transporting moieties into the design of new drugs, in order to achieve selectivity of drug action and a reduction of toxicity and side effects. Obviously, it was the authors' goal to produce an MAO inhibitor antidepressant with the positive therapeutic attributes of iproniazid, but lacking in its hepatotoxic properties. The antidepressant agent which emerged from these investigations is isocarboxazid (Marplan):



Isocarboxazid
(3 × IPN)

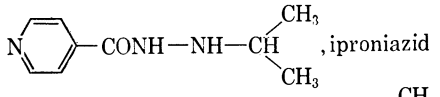
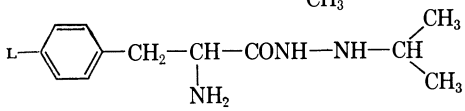
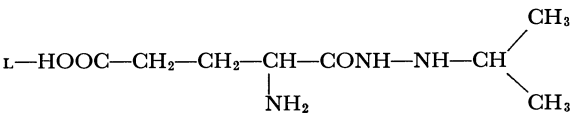
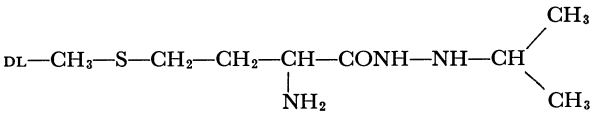
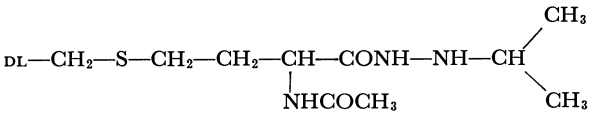
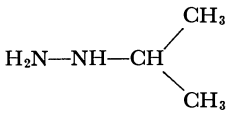


Iproniazid

This drug has three times the potency of iproniazid and apparently greatly reduced hepatotoxicity. Tables II and III, which are taken from Zeller's

work (*116*), illustrate the dependency of these hydrazides on metabolic degradation for the unmasking of their activity properties, and the influence of the acyl moiety on selective transport.

Table II. Inhibition of Monoamine Oxidase of Rat Brain in Vitro and in Vivo by Amino Acid Isopropylhydrazides as Compared to Iproniazid[a]

	In Vitro Inhibition of Monoamine Oxidase[b] Relative to Marsilid Concentration of Inhibitors, 3×10^{-5} Moles/Liter	*In Vivo Increase of 5-HT in Rat Brain 16 Hours after i.p. Application Equimolar Dose to 100 Mg./Kg. Iproniazid*
 , iproniazid	100 ± 5	100 ± 6
	44 ± 5	171 ± 3
	59 ± 8	227 ± 21
	57 ± 6	147 ± 4
	29 ± 8	143 ± 13
	>200	200 ± 13

[a] (*83, 116*).
[b] Tyramine oxidation by brain mitochondria.

Table III.[a] Effect of Acyl Group on MAO Inhibitory Activity

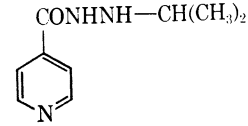
	% Increase of Monoamines		
Isopropylhydrazide of	*Heart catecholamines*	*Brain 5-HT*	*Ratio*
Isonicotinic acid (Marsilid)	100	100	1.0
Glutamic acid (α-derivative)	75	250	3.3
Palmitic acid	145	60	0.4

[a] (*83, 116*).

It may be seen that the in vitro activity of the aminohydrazides is low when compared with that of iproniazid. Once they are administered to the intact animal, however, they approach the intrinsic activity of the free isopropylhydrazine.

Another effort toward reducing the toxicity properties of iproniazid is the work of Bloom and Carnahan (*25*) on isonicotinoylhydrazide of β-(*N*-benzylcarboxamido)ethylhydrazine (nialamide):

Nialamide
(3 to 12 × IPN)

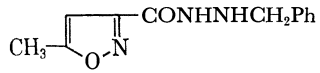
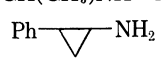


Iproniazid

In various animal tests, this drug had 3 to 12 times the MAO-inhibitory potency of iproniazid (*89*). As a clinical antidepressant (Niamid) the drug is somewhat less effective than iproniazid; however, it is devoid of the hepatotoxic properties of the latter agent.

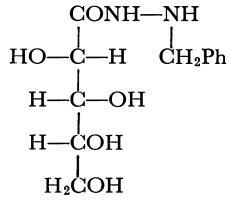
The relative activities of iproniazid, nialamide, isocarboxazid, phenelzine, pheniprazine, and tranylcypromine are shown in Table IV, which is taken from the work of Maxwell, Gray, and Taylor (*72*). The method employed was the in vitro and in vivo potentiation of tryptamine.

Table IV. Comparative Activities of Some Clinically Effective MAO Inhibitors[a]

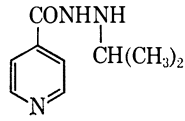
Drug	*Relative Activity*	*Structure*
Iproniazid	1.0	
Nialamide	1.8	
Isocarboxazid	3.1	
Phenelzine	18	$PhC_2H_4NH—NH_2$
Pheniprazine	31	$PhCH_2CH(CH_3)NH—NH_2$
Tranylcypromine	45	

[a] Based on tryptamine potentiation test (*72*).

More recently, Gardner, Wenis, and Lee (*41*) reported on a series of sugar acyl, 4-hydroxybutyryl, and pantoyl derivatives of benzylhydrazine, α-methylphenethylhydrazine, and 4-dimethylaminobenzylhydrazine. Significant detoxification was obtained without corresponding loss in activity. For example, 1-benzyl-2-(D-ribonoyl)hydrazine:



MAO—I = IPN
LD_{50} < 1/10 IPN



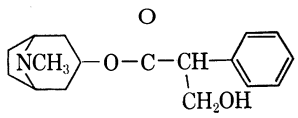
Iproniazid

was comparable to iproniazid in activity and tissue distribution; however, the LD_{50} could not be reached with doses up to 4000 mg. per kg. (interperitoneal mice) or ten times the LD_{50} of iproniazid. Hence, the "metabolic hooks" provided by the acyl portion afforded a marked decrease in the toxicity of the aralkylhydrazines.

Conclusions. Major molecular modifications in the acyl portion of the potent antidepressant agent iproniazid resulted in a marked decrease or abolition of its hepatotoxic properties and an increase in the therapeutic potential of the resultant derivatives. The choice of the acyl groups was determined on the basis of their ability to transport the "active" alkyl or aralkylhydrazine to a specific organ tissue and their susceptibility toward metabolic cleavage to release the active component (the free alkyl or aralkyl hydrazine) at the desired target site. Thus, certain natural amino and sugar acyl moieties had a specific affinity for brain tissue, while certain fatty acid hydrazides proved more potent in inhibiting cardiac MAO. The acyl groups also prevented premature interaction of the "free" hydrazines with nontarget organ and enzyme systems, thereby lowering their toxic properties and incidence of undesirable side effects.

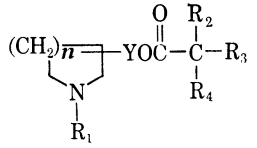
The Anticholinergic Drugs

The search for more potent and selectively acting atropine substitutes for the treatment of peptic ulcer, ulcerative colitis, and other gastrointestinal disturbances led to a systematic structural modification of the atropine molecule by Biel and his associates (*18, 20, 24*). Atropine consists of the following structurally modifiable entities:



1. Pyrrolidine ring
2. Piperidine ring
3. *N*-Alkyl group
4. Ring-bound OH group
5. Phenylhydroxyacyl group

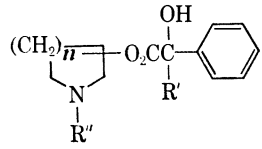
Each of these moieties was subjected to structural modification as represented by the following generic formula:



$n = 1,2$
Y = chemical bond, alkylene chain
R_1 = H, alkyl, aralkyl, alkenyl
R_2 = phenyl, substituted phenyl
R_3 = phenyl, cycloalkyl
R_4 = H, OH, hydroxyalkyl

From this work emerged several drugs which were superior to atropine with respect to: anticholinergic potency, antisecretory effects, selectivity of action for certain sections of the G.I. tract, and reduced incidence of side effects.

In addition, these investigations yielded a group of compounds distinguished by virtue of their potent CNS stimulant and anticholinergic properties. In man, Abood *et al.* (*2, 3, 4*) found these drugs to be potent and long-lasting (12 to 36 hours) hallucinogenic and psychotomimetic agents. In general, these compounds corresponded to the following generic formula:



$n = 1,2$
R′ = phenyl, cycloalkyl
R″ = lower alkyl group

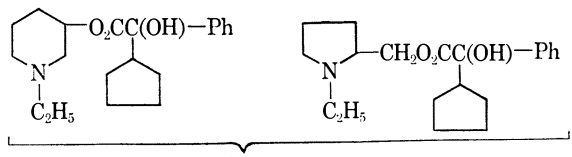
Maximum potency was provided by:

A pyrrolidine or piperidine ring.
A hydroxyl group bonded directly to either ring in the 3- or 4- position.
An unsubstituted phenylglycolate ester moiety, where R′ was cycloalkyl. The presence of the OH group in the acyl portion was absolutely essential.
A lower *N*-alkyl group.

The structure-activity relationships were reviewed in detail by Biel *et al.* (*23*) and the following conclusions drawn:

Only piperidyl and pyrrolidyl glycolate esters that were also potent anticholinergics exerted a profound stimulant effect on the CNS.
Both central and autonomic effects could be reversed by a cholinesterase inhibitor, 9-amino-1,2,3,4-tetrahydroaminoacridine.
These drugs exerted little effect on any other known enzyme system.
The greatest concentration of these agents in the brain occurred in the hypothalamus and caudate nucleus, from which they disappeared only slowly.

However, a more important aspect of this work was the discovery by Abood and Meduna (*2*) of the potent antidepressant properties of one of the compounds, Ditran, particularly in psychoneurotic depressions. Ditran is a 30:70 mixture of *N*-ethyl-3-piperidyl and *N*-ethyl-2-pyrrolidylmethyl phenylcyclopentyl glycolate:



Ditran

Remissions occurred in about 70% of all types of depressions treated, as confirmed by subsequent investigators (*2, 16, 39*) in over 1000 patients. Usually, one to three administrations of 15 mg. of Ditran spaced 5 days apart were able to produce a prolonged or lasting remission of the depression.

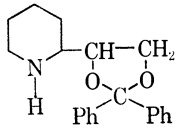
Two questions need to be answered:

Is potent central anticholinergic activity a pharmacologic requirement for the central stimulant and antidepressant properties of these compounds, or is it merely coincidental?

Is the initial psychotic episode a necessary prerequisite for the subsequent remission of mental depressions?

The fact that many available drugs used in the treatment of the depressed patient are given in dosages that produce either potent anticholinergic side effects (mydriasis, dry mouth, or constipation) or a rise in brain norepinephrine levels would implicate both acetylcholine and norepinephrine as two mutually antagonistic neurohormones in the control of brain function. Hence, the first question must be answered in the affirmative at this time. Our own structure-activity studies have failed to uncover a piperidyl or pyrrolidyl ester in the above series with weak anticholinergic and potent CNS properties, although the reverse has been found to be true in many instances.

As to the second question—i.e., the necessity for eliciting an initial psychotomimetic response prior to the alleviation of the mental depression—we feel that this is not an essential prerequisite and that further structural modification might produce an effective antidepressant agent in this series which is devoid of potent psychotogenic properties. Some recent work by Hidalgo *et al.* (*49, 50*) indicates that certain related piperidyl dioxolanes:



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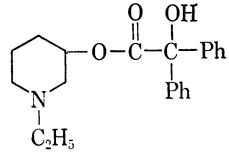


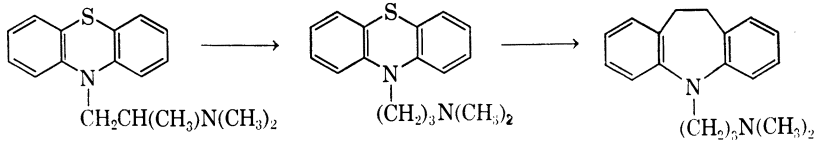
JB-318

exhibit antidepressant properties in animals with little, if any, hallucinogenic properties. Clinical confirmation of the potential antidepressant activity of this novel series will be required before further conclusions can be drawn from these investigations.

Conclusion. Central cholinergic blockade may be another approach to the treatment of mental depression in the same way that central adrenergic blockade has been a pharmacologic approach to the therapy of combative and hostile behavior and the control of severe anxiety reactions. Further molecular modification will be required to divorce the concomitant psychotomimetic side effects from the desired antidepressant action which is produced by this series of anticholinergic esters.

Antidepressants Derived from Antihistamines and Tranquilizers

Imipramine (Tofranil). The development of Pheneragan, and *N*-amino-alkylphenothiazine, as an effective antihistaminic agent set the stage for a vast effort in molecular modification of both the polycyclic ring structure and the side chain. One of these modifications synthesized by Häfliger and Schindler (*47, 95*) represented the isosteric replacement of the sulfur bridge in Promazine by an ethylene bridge:



Promethazine (Phenergan)	Promazine	Imipramine (Tofranil)
(Potent antihistamine)	Weak antihistamine	Weak antihistaminic
Sedative	Tranquilizer	Mild anticholinergic
		Sedative in normals

to yield an iminodibenzyl derivative, imipramine (Tofranil). Similar to Promazine, its antihistaminic activity was weak. Its anticholinergic action was more pronounced and approximately one hundredth that of atropine on the isolated guinea pig ileum. In normal humans it produced a sedative effect. Largely through the pioneering work of Kuhn (*63*, *64*), imipramine became a major milestone in the therapy of mental depression. Kuhn reported his work on 500 patients of various diagnostic categories. Best therapeutic results with full social recovery were obtained in endogenous depressions showing the characteristic symptoms of motor and mental retardation. The onset of action was highly variable in different patients, ranging from 3 days to 6 weeks, with an average onset of 18 days. Kuhn's findings are all the more remarkable in view of the misleading psychopharmacologic spectrum of activity established in animals (*36*, *98*) which would have classified the drug as a weak promazine-like tranquilizer.

Many subsequent investigators confirmed Kuhn's original observations. Thus, Kiloh and Ball (*57*) showed that after 6 months the remission rate obtained with ECT equaled that of imipramine. Improvement began after 5 days to 6 weeks in endogenous depressions. Maximum improvement occurred after 2 to 12 weeks. In reactive depressions, onset was slower, with a mean of 18 days. Maximum improvement ranged from 10 days to 24 weeks. Side effects occurred as follows:

Dry mouth	44/81
Sweating	26/81
Constipation	19/81
Faintness (orthostatic hypotension)	16/81
Nausea	11/81
Tremors	7/81

Orthostatic hypotension was noted as a side effect, particularly in hypertensive patients (*66*, *75*), the incidence being about 24%.

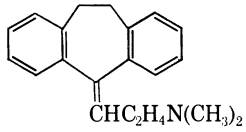
Sargant's (*91*, *92*) studies with impramine showed that the drug was not too effective in the reactive or neurotic type of depression, but was of definite value in endogenous depressions. The reverse was found to be true for the MAO inhibitors.

Imipramine thus represented the first antidepressant drug with a selectivity of action for endogenous depressions. Unlike the MAO inhibitors, it proved efficacious also in the treatment of manic-depressive disease. Although

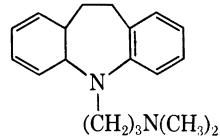
a close structural analog (isostere) of promazine, its clinical activity spectrum in the treatment of mental disease was altogether different.

Several undesirable properties of imipramine which forecast the need for further molecular modification were its slow and variable onset of action, its anticholinergic side effects, the relatively high incidence of orthostatic hypotension in elderly and hypertensive patients, and exacerbation of symptoms in schizophrenic patients, including catatonic stupor and hallucinations (*61*).

Amitriptyline (Elavil). The first molecular variant of imipramine which became of clinical importance represented a departure from the usual diamines of the promazine and imipramine type to a monoamine, where an $N-CH_2$ group was replaced by a $C=CH$ moiety:



Amitriptyline
(Elavil)



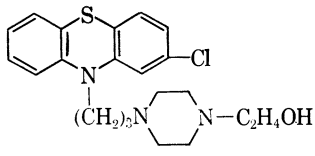
Imipramine
(Tofranil)

Amitriptyline resembled imipramine in its efficacy in the treatment of endogenous depression and its relative ineffectiveness in patients with neurotic (reactive) depressive symptoms (*9*). Some patients, who were unreactive to imipramine, responded to amitriptyline (*9*). Side effects were usually less severe (*9, 34, 114*) but there was a high incidence (18%) of somnolence (*114*). Some of the disadvantages inherent in imipramine were overcome, at least to some extent, by this structural variant:

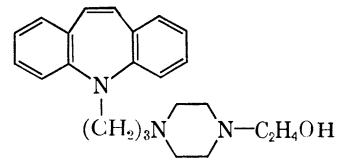
The onset of action was somewhat faster.
The anticholinergic side effects were less pronounced.
There was a lesser incidence of orthostatic hypotension (*34, 113*).
Amitriptyline did not exacerbate psychotic symptoms and hence appeared to be a less troublesome drug to use in the depressed schizophrenic patient (*13*).
Because of its sedative action amitriptyline could be used successfully in anxiety-tension states (*40*).

Thus, in its activity spectrum, amitriptyline appears to be intermediate between chlorpromazine and imipramine and, hence, must be considered a "tranquilizing" type of antidepressant, particularly useful in depressions accompanied by anxiety, hostility, agitation, and hallucinations.

Opipramol (Ensidon). In an effort to impart antianxiety properties to the imipramine molecule, Schindler and Blattner (*94*) incorporated the perphenazine side chain (piperazinoethanol) into the unsaturated form (iminostilbene) of imipramine:



Perphenazine
(Trilafon)

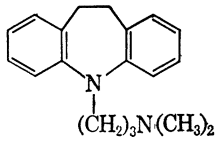


Opipramol
(Ensidon)

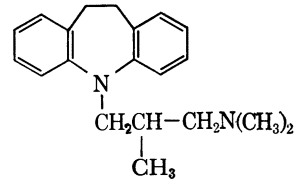
Perphenazine is a highly potent tranquilizer with less pronounced CNS depressant properties.

The effect of this structural hybrid formation was an antidepressant drug with tranquilizing and antianxiety properties (*59*). The drug was most effective in neurotic depressions and of little value in psychotic depressive reactions (*59, 85*). Its activity range would thus appear to be between imipramine and meprobamate (*85*). Side effects were minimal with this drug and its onset of action occurred within 1 to 2 days (*81*). Hence, the drug is particularly useful in the treatment of ambulatory patients.

Surmontil (RP-7162). This agent differs from imipramine only in having a β-methyl group in the propyl side chain:



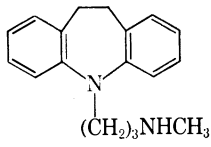
Imipramine



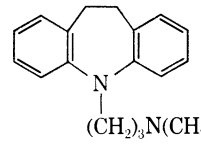
RP-7162

Reports on the drug are preliminary. The acute and chronic toxicities of RP-7162 are said to be of the same order of chlorpromazine. When used in dosages of 150 to 500 mg. per day in 100 hospitalized patients, this agent was claimed to be three times as potent as imipramine as an antidepressant (*65*). In addition, it has a tranquilizing action. The L-isomer was more potent in this respect. All of the stimulant effects appear to reside in the D-isomer. Remissions in atypical melancholia and neurotic depressions occurred usually within 2 to 3 weeks (*65*). The ultimate place of this drug in antidepressant therapy has yet to be established.

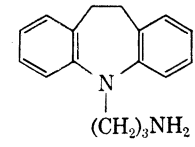
Desipramine (Norpramin, Pertofran). The slow and variable onset of action of imipramine and the paradoxical behavior displayed by the compound in animal *vs.* human pharmacology prompted this author and his associates to embark on a program of structural changes of the imipramine molecule which would bring out more nearly its therapeutic potential. While in animals imipramine had all of the pharmacologic earmarks of a mild tranquilizer of the "weak chlorpromazine" variety, in depressed patients it elicited powerful antidepressant effects (*62*) and was devoid of the tranquilizing and antianxiety properties of chlorpromazine. Its action was also delayed in onset. It was reasoned that the central depressant component of imipramine was due to its tertiary amine group and that a more favorable antidepressant action might be elicited by a secondary or primary amine radical.



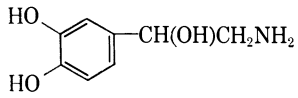
Desipramine



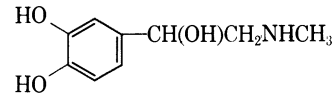
Imipramine



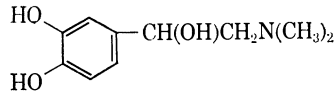
There was good precedent for this type of reasoning in the sympathomimetic amine series. Progressive methylation of the amino group of norepinephrine changed this molecule from a vascular excitant to a vascular depressant.



Pressor

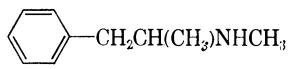


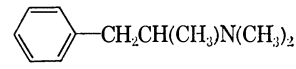
Pressor—depressor



Depressor

Methylation of the potent CNS stimulant, methamphetamine, to *N,N*-dimethylamphetamine





abolished most of the central stimulatory properties of the parent compound.

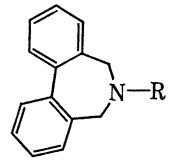
The normethyl derivative of imipramine displayed different pharmacologic and clinical properties from its fully methylated parent structure. In rats it increased spontaneous motor activity (*73*) and did not potentiate the action of reserpine in animals but rather antagonized it (*26*, *105*). In both these respects, it thereby differed from imipramine. Its clinical onset of action was more rapid than with imipramine (*73*, *105*), although efficacy did not appear to be increased. Side effects were less severe and their nature also different (*58*, *78*).

Table V. Comparative Incidence of Side Effects of Two Antidepressants

Side Effects	% *Imipramine* (*57*)	% *Normethylimipramine* (*78*)
Dry mouth	55	4
Drowsiness	—	10
Dizziness	20	6
Agitation	—	2
Perspiration	33	12 (mild)
Nausea	12	—
Tremors	10	—
Blood pressure	Depressor (*75*)	No effect

As in the antispasmodic series, a tertiary amino group will generally elicit a greater cholinergic blockade than a secondary or primary amino moiety and, hence, produce a greater number of side effects associated with cholinolytic activity. Similarly, adrenolytic properties are shown mainly by tertiary amines rather than their secondary or primary amine precursors.

In the dibenzazepine series, the secondary amine (R = H) produced no



Dibenzazepine

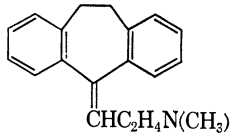
effect on the dog's blood pressure, whereas the *N*-alkyl or *N*-allyl derivatives (R = alkyl, allyl) were potent blood pressure depressants (*56*). Hence, the lesser intensity of the side effects of normethylimipramine (desipramine) is presumably a consequence of weaker cholinergic and adrenergic blocking properties which are normally more pronounced with tertiary than with secondary or primary amines. Concurrent with our own work, Herrmann and Pulver (*48*), Sulser, Watts, and Brodie (*105*), and Gillette (*42*) isolated a metabolite of imipramine which they found to be identical with the normethyl derivative (desipramine). Sulser (*105*) and Brodie *et al.* (*26*) concluded, on the basis of pharmacologic and clinical evidence, that the antidepressant activity of imipramine was due to its metabolite and that the delayed onset of action was a consequence of the slow biotransformation to the demethylated form (*26*).

Table VI. Results of Treatment with Nortriptyline in Affective Disorders (and Comparison with Other Related Drugs)

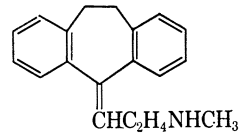
	Nortriptyline				*Imipramine*			
	Total				*Total*			
	No.	*A*	*B*	*%A*	*No.*	*A*	*B*	*%A*
Psychoneurotic depressive reaction (mixed, 3)	21	18	3	86	22	15	7	68
Manic depressive reaction	16	14	2	87	34	23	11	68
Involutional reaction	9	4	5	44	9	5	4	56
Psychotic depressive reaction	2	2	0	100	14	13	1	93
Others	2	2	0	100	1	1	0	100
Senile with depression (1)								
Alcoholic with depression (1)								
Totals	50	40	10	80	80	57	23	71

	Amitriptyline				*Desmethylimipramine*			
	Total				*Total*			
	No.	*A*	*B*	*%A*	*No.*	*A*	*B*	*%A*
Psychoneutotic depressive reaction (mixed, 3)	17	13	4	76	9	8	1	89
Manic depressive reaction	16	11	5	69	26	18	8	69
Involutional reaction	9	6	3	67	8	4	4	50
Psychotic depressive reaction	5	4	1	80	6	5	1	83
Others	3	2	1	67	1	0	1	0
Senile with depression (1)								
Alcoholic with depression (1)								
Totals	50	36	14	72	50	35	15	70

Nortriptyline (Aventil). A similar approach was pursued with amitriptyline (Elavil) to produce nortriptyline (Aventil):



Amitriptyline



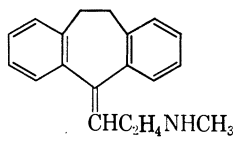
Nortriptyline

This drug produced a more consistent and prolonged response rate to reinforcement stimuli than amitriptyline or imipramine (*15*). Nortriptyline had only one half the anticholinergic activity of amitriptyline on the guinea pig ileum and suppression of salivation (*86*).

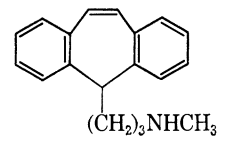
Table VI, which is taken from the work of Oltman and Friedman (*79*), illustrates the comparative efficacy of nortriptyline, amitriptyline, imipramine, and desmethylimipramine.

Nortriptyline appeared to be somewhat more consistently effective than the other agents, except in involutional melancholia. Particularly impressive was the improvement rate in the manic depressive group (87% *vs.* 69% for the others). Side effects were generally mild, although dryness of the mouth occurred in 52% of the patients. Somnolence occurred in only 8% of the patients and, as with desipramine, the secondary amine congener appeared to be less of a central depressant. Onset of action was fairly prompt. Initial improvement occurred after 7 to 10 days.

Protriptyline. A more radical change in the activity spectrum of amitriptyline was brought about by further changes in the nortriptyline structure (*104*).



Nortriptyline

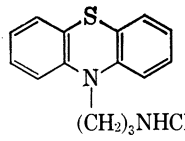


Protriptyline

Clinically, protriptyline is a "purer" type of antidepressant agent, in that it lacks the sedative properties of amitriptyline and nortriptyline. It is said to be the more potent of the three agents and useful also in the treatment of anxiety depressions. However, until further clinical work is published, it will be difficult to assess the therapeutic spectrum of this most recent agent.

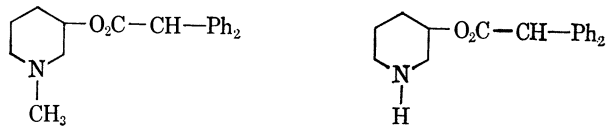
The original speculation by Biel, that secondary amines would provide more selectively acting antidepressants, appears to have been borne out on the basis of the clinical properties displayed by desmethylimipramine, desmethylamitriptyline, and the modified form of desmethylamitriptyline (protriptyline).

Further confirmation of the above generalization is the work of Bickel, Sulser, and Brodie (*17*) with the desmethyl form of promazine which acted like desmethylimipramine in reversing reserpine stupor; here again the loss of one *N*-methyl group from the tertiary amine side chain caused a reversal of the tranquilizing properties.



Desmethylpromazine

In a different series, Abood and Biel (*1*) found that the tertiary amine *N*-methyl-3-piperidyl diphenyl acetate was an anticholinergic agent which was essentially devoid of psychopharmacologic activity. Removal of the *N*-methyl group produced a compound with potent CNS stimulant and muscle-relaxant properties:



It was this finding which then stimulated the investigations on the desmethylimipramine compound.

Hence, minor structural changes in the imipramine, amitriptyline, and promazine group of psychotropic agents can bring about very marked alterations in the psychopharmacologic properties of the derivative compounds which have endowed these compounds with a greater selectivity of antidepressant action and, in some instances, shortened the latent period of onset.

Summary

We have traced the origin of the antidepressant drugs from structures originally designed for the treatment of a wide variety of somatic diseases. Drugs which displayed such a diversity of effects as sympathomimetic, antianginal, antitubercular, anticholinergic, antihistaminic, and tranquilizing activity were converted to highly effective antidepressant agents via the process of molecular modification. This technique not only made possible the discovery of a chemical approach to the treatment of mental depression, but proved invaluable in the development of selectively acting drugs, specifically tailored to cope with the many facets of a highly complex disease syndrome.

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11

Molecular Modification in the Development of Phenothiazine Drugs

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In the phenothiazine area, it has been amply demonstrated that structural changes, in addition to producing expected quantitative changes in tranquilizing activity, also produce unexpected qualitative changes in biological activity. As a result antipruritic, antispasmodic, anticonvulsant, antibacterial, antiemetic, antimotion sickness, anthelmintic, and antidepressant compounds have been developed by molecular modification of the phenothiazines.

The discovery of the phenothiazine tranquilizing drugs provides an outstanding example of the totally unpredictable, but often useful, results which may be obtained by structural modification of prototype drug molecules. The ancestry of these agents may be traced back to the original antihistamines of the benzodioxane type discovered by Bovet and collaborators in 1937 (*7*, *34*). With the benzodioxanes as a starting point, a long series of molecular modifications was carried out in various laboratories over the next decade in a search for other types of antihistaminic agents. This slow, gradual process eventually led to the synthesis of phenothiazine derivatives and the discovery of their therapeutic utility. In turn, the phenothiazines have served as structural prototypes for further studies in molecular modification. Even small changes in the structure of a substituted phenothiazine can lead to marked changes in potency, toxicity, or degree and types of side effects, or even to new and useful medicinal agents that are qualitatively different from the prototypes.

The steps which led to the phenothiazine drugs are outlined in Figure 1. Variations of the benzodioxane structures (I) led to ethers of the ethanolamine type (II), which were further developed in two directions: first, toward the benzhydryl ethers of the diphenylhydramine type (III), and second, toward the ethylenediamine types (IV). The ethylenediamine types, in turn, were evolved further in two directions: first, to the pyribenzamine type (V) (tripelennamine), and second, via the diphenylamine types to phenothiazine derivatives such as the anti-Parkinson agent diethazine (VI) (*15*, *17*) and the antihistamine promethazine (VII) (*6*, *23*, *25*).

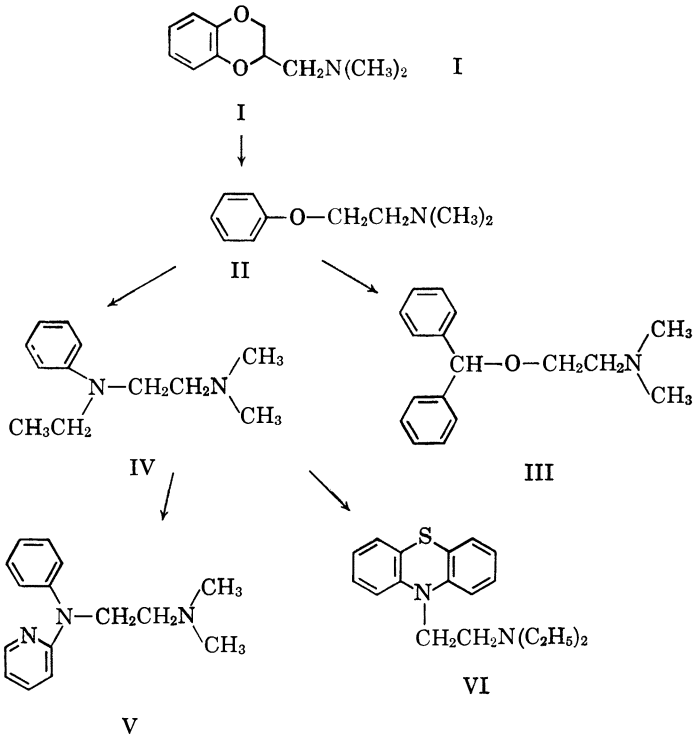
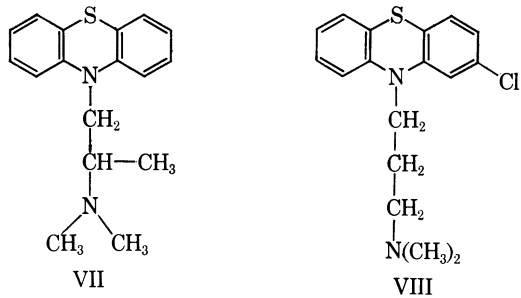


Figure 1. Evolution of the phenothiazines

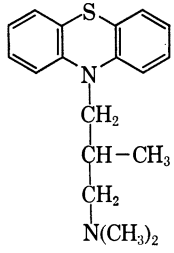
Promethazine is a potent antihistaminic drug. In the course of its clinical use, sedation was noted as a prominent side effect. In an effort to enhance this sedative effect, the Rhone-Poulenc research group studied many modifications of promethazine, which led to chlorpromazine (VIII). Chlorpromazine, in marked contrast to promethazine, has diminished antihistaminic activity, and



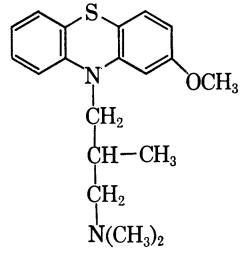
has pronounced sedative and antipsychotic activity. Therefore, relatively minor molecular modifications of a sedative antihistamine resulted in a major tranquilizer which helped spark the revolution in the therapy of the mentally ill.

Phenothiazines vary in degree of tranquilizing activity, but possess other useful biological properties. For purposes of this discussion, the index of

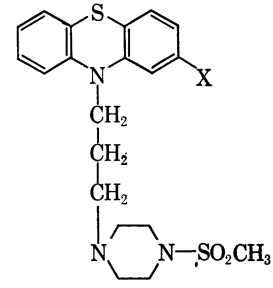
tranquilizing activity is blockade of the conditioned avoidance response in the rat, which is blocked selectively by tranquilizers such as chlorpromazine (*8*, *9*, *10*). In general, although there are some conspicuous exceptions, blockade of the conditioned avoidance response in rats correlates well with antipsychotic activity in man. Relative to chlorpromazine, trimeprazine (IX) (*32*) has greatly reduced conditioned response blocking activity and greatly enhanced antihistaminic and antipruritic activities; it has found wide clinical use as an antipruritic and antihistaminic agent.



IX



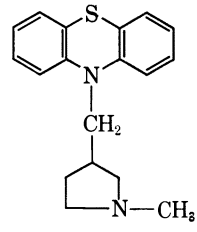
X



XI

The levo isomer of the 2-methoxy derivative of trimeprazine, known as levomepromazine (X) (*11*, *16*), has not only conditioned response blocking and tranquilizing activity comparable to chlorpromazine, but also pronounced antihistaminic activity. Levomepromazine is active in the hot-plate analgetic test, but not in the tail-pinch method in the rat, and is reported to be an analgetic agent in man (*26*). There are some reservations about the possible analgetic activity of levomepromazine, inasmuch as it is reported to have analgetic activity by the parenteral route, but not by the oral route. Yet, drowsiness is seen after oral administration, so the drug must be absorbed when given orally. Additional experimentation in man will be necessary to resolve these discrepancies. The methylsulfonylpiperazinopropylphenothiazine derivative (XI) is also claimed to have analgetic activity (*33*).

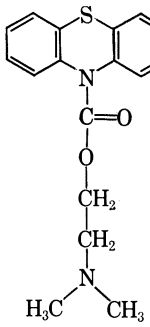
Replacement of the dimethylaminopropyl side chain of promazine by an *N*-methyl-3-pyrrolidinomethyl side chain gives methdilazine (XII), a compound with a low degree of conditioned avoidance response-blocking activity, but with antipruritic activity (*27*, *29*).



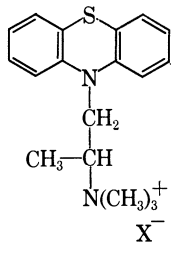
XII

Replacement of the 10-aminoalkyl chain by $-COOCH_2CH_2N(CH_3)_2$ gives a compound with antispasmodic activity (XIII) (*13*), which is devoid of con-

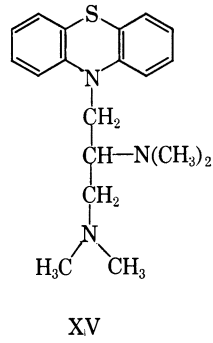
ditioned response blocking activity. Similarly, antispasmodic activity is observed for the quaternary salt of promethazine (XIV, Multergan) (*1*, *24*). Antispasmodic activity is also seen where the 2-carbon atom of the propyl side chain in promazine is replaced by a dimethylamino group as in aminopromazine (XV) (*35*). This compound is devoid of tranquilizing and conditioned escape response-blocking activity.



XIII

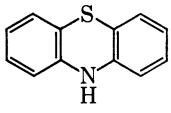


XIV

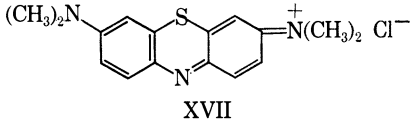


XV

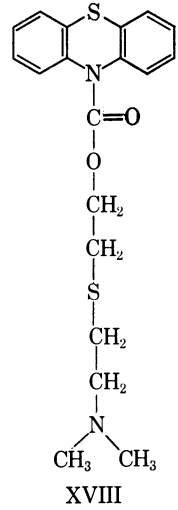
Unsubstituted phenothiazine (XVI) is, of course, well known as an anthelmintic agent, and is devoid of tranquilizing properties (*12*). Another unalkylated phenothiazine, methylene blue (XVII) (*21*), is known to have antibacterial activity and to be devoid of tranquilizing properties. Antibacterial activity is also claimed for XVIII (*28*). Compound XIX with a dimethylsul-



XVI



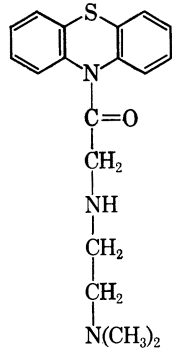
XVII



XVIII

fonamido group in the 2-position, shows potent tranquilizing properties, but also has relatively greater antiemetic activity. This property is shared by many of the piperazinopropylphenothiazines (*20*).

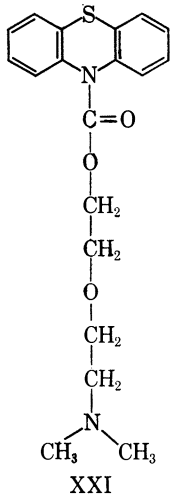
XIX



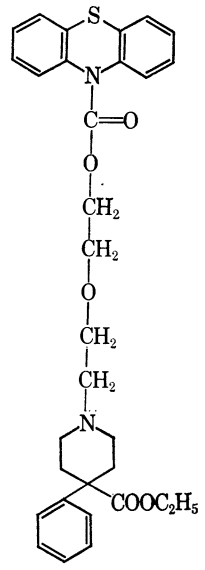
XX

Compound XX has antimotion sickness properties and some stimulant effects, but is relatively devoid of tranquilizing properties (*18*).

A phenothiazine with an aminoalkoxyalkyl carbamate side chain (XXI) has antitussive activity (dimethoxanate), but it is devoid of tranquilizing and conditioned escape response-blocking activity. Compound XXII similarly has antitussive activity, but is not stated to have analgetic and tranquilizing activities (*14*).



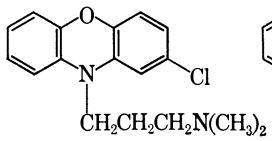
XXI



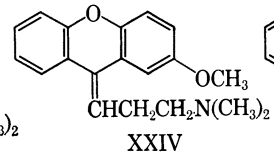
XXII

Therefore, changes in the ring substituents and in the side chain produce marked qualitative and quantitative changes in biological activity. Isosteres of these phenothiazine drugs also vary greatly in biological activity relative to that of chlorpromazine. For example, if the sulfur of chlorpromazine is replaced by oxygen to give a phenoxazine (XXIII), the resulting compound is only one tenth as active as chlorpromazine in reducing the motor activity of mice (*5*). The xanthene drug, dimeprazan (XXIV), also is less active than

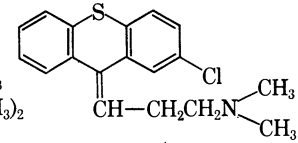
chlorpromazine (*4*). On the other hand, replacing the nitrogen of chlorpromazine by carbon leads to a thiaxanthene derivative, chlorprothixene (XXV), which is approximately equal to chlorpromazine in the conditioned escape response test and in tranquilizing activity in man (*30*).



XXIII

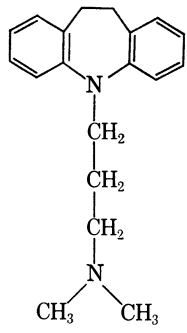


XXIV

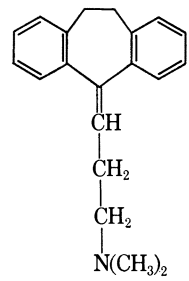


XXV

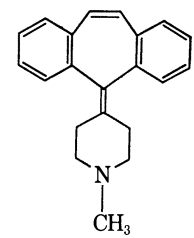
Replacement of the sulfur atom in promazine by two methylene groups leads to imipramine (XXVI) which is not a tranquilizer, but is instead a clinically useful antidepressant agent (*22*, *25*). Similarly, the dibenzocycloheptadiene analog, amitriptylene (XXVII) (*2*), is devoid of tranquilizing activity and is a useful antidepressant like imipramine.



XXVI



XXVII

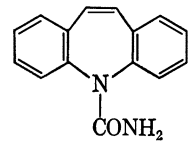


XXVIII

Cyproheptadine (XXVIII) (*36*), an analog of amitriptylene, is devoid of antidepressant and tranquilizing activities, but possesses potent antiserotonin and antihistaminic activities.

Antipruritic compounds have been obtained by appropriate molecular modification of both the phenothiazine tranquilizers and the dibenzocycloheptadiene antidepressants (compare IX and XXVIII).

Replacement of the side chain of imipramine by a carbamoyl group as in compound XXIX (G 32883, Tegretol, Geigy) gives a compound without tranquilizing or antidepressant activity, which is claimed to have anticonvulsant activity (*3*).



XXIX

The phenothiazine tranquilizers presumably act by interaction with cellular receptor sites in the central nervous system, although the mechanisms involved and the nature of the drug receptors are unknown. However, for the

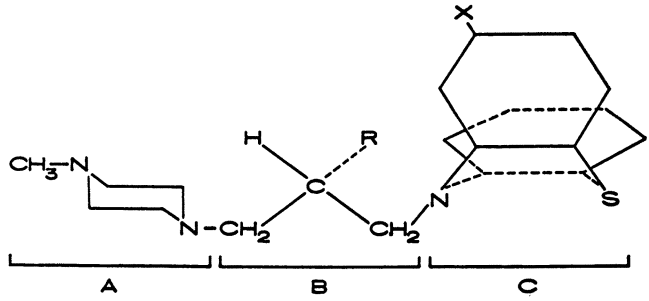
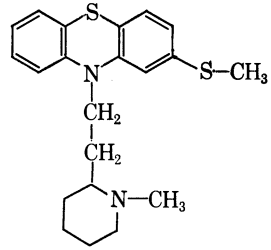


Figure 2. Possible conformation of prochlorperazine molecule interacting with receptor surface

XXX

sake of a brief discussion of structure-activity relationships in the phenothiazine series, we refer to structure XXX, which depicts schematically one of perhaps many possible conformations that a prochlorperazine molecule might assume in interacting with a receptor surface. Replacement of the chlorine in the 2-position (Figure 2, *C*) by hydrogen results in a decrease in activity, whereas replacement by a trifluoromethyl or dimethylsulfonamido group results in an increase in activity. Placement of the ring substituent in position 1, 3, or 4 results in a marked loss in tranquilizing activity, as does simultaneous substitution in both aromatic rings. As far as tranquilizing activity is concerned, the three-carbon side chain seems to be optimal (Figure 2, *B*). A derivative with a substituent at the 2-carbon atom of the side chain is optically active, and the levo isomer is by far the more active isomer of the enantiomorphic pair. According to a hypothesis of Pfeiffer (*31*), the amount of structural specificity at any given point in a biologically active molecule is in proportion to the ratio of activities of the optical isomers. In keeping with this hypothesis, one would expect a great deal of structural specificity at the 2-position, and this is indeed the case. For example, replacement at the 2-carbon by phenyl, dimethylamino, or hydroxyl results in an almost complete loss of tranquilizing activity. On the other hand, substitution by a small group—e.g., hydrogen or methyl—results in retention of activity. If the 2-carbon is incorporated into a ring as in methdilazine (XII), there is a marked loss in tranquilizing activity. On the other hand, if only the terminal carbon is involved in a ring, as in thioridazine (XXXI), activity is retained in man, although the conditioned escape response-blocking activity is much reduced in animals. The receptor into which the molecule fits



XXXI

seems to be long and narrow, since replacement of the methyl group on the nitrogen (Figure 2, *A*) of the piperazine ring by such a bulky group as the *p*-aminophenethyl can result in no loss of activity (*19*).

In Figure 2, *A*, the piperazino group usually leads to more active compounds than the dimethylamino group. On the terminal nitrogen of the piperazine the *N*-β-hydroxyethyl group leads to higher activity than the *N*-methyl.

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12

Role of Synthetic Drugs in Therapy of Mental Illness

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The general role and need for various synthetic agents in the treatment of mental tension states, insomnia, and psychotic behavior are outlined. The sedatives, tranquilizers, and antidepressant agents were considered in their chemical, pharmacological, and clinical aspects. The relative usefulness, mode of action, and toxic or adverse actions are discussed and suggestions advanced for possible improvement in molecular structure. Advantages resulting from molecular modification in these series of agents are presented. A theory is proposed that allergic reactions, at least in certain classes of drugs, are reduced as potency is increased and less foreign substance is introduced into the body. An interesting hypothesis on the role of catechol amines in mentally depressed states is explained, and some preliminary findings are presented.

Even before the dawn of recorded history, studies of primitive civilization show that man had need for drugs that relieve mental tension (*11*). This is not surprising when we realize that man has also continually sought to relieve physical unpleasantness. The ancient development of the lever and wheel, and more recently, of machines to relieve him of strenuous labor and the fatigue such brings, gives eloquent testimony to man's success in this endeavor. It is, therefore, not necessary to postulate that drugs for mental tension states are in demand because of abnormal or pathological emotional states, any more than to consider a society developing labor-saving devices as abnormal or physically weak. Unfortunately, however, we are still prone to consider the need for agents to relieve mental tension as being close to, if not actually pathological in nature or at least a sign of weakness. Unquestionably, the development of an effective, nontoxic, inexpensive agent capable of relieving individuals from excessive, useless, mentally fatiguing, emotional stress will be a most worthy contribution to human welfare.

Society has made great strides in organizing and developing machines and techniques to relieve physical discomfort. The flood of labor-saving devices has vastly changed the physical aspects of human existence. However, except for a few natural products, it was less than 10 years ago when the first synthetic agent appeared which exerted an effect on emotional tension. In 1836, Justus von Liebig synthesized chloral hydrate, but it was not until 1869 that Oscar Liebreich introduced it into medicine as a useful sedative. Although Frobenius prepared ether in 1730, and von Liebig chloroform in 1836, neither was used to any extent until the last half of the 19th century. A quick perusal of the literature at the time these highly useful anesthetics were introduced readily shows that the misgivings, apprehension, and opposition to their use are not too dissimilar to the present-day reaction to the tranquilizing drugs. Apparently man is eager and willing to accept any relief from physical stress and strain but is apprehensive about making much of his psychological tension.

Many ask, "Is there a real need for agents to relieve mental tension except in individuals where such serious psychological disturbances seriously impair their adjustment to society?" Obviously, there is a serious need for agents to revert depressed states, break up schizophrenia, and control pathological behavior. There is also an increasing need for more satisfactory agents to aid so-called normal individuals in their adjustment to the complexities of our society. Increased emotional strain created by herding of millions from rural or semirural areas into densely populated cities, pressures to produce so much per hour of work as determined by efficiency experts and their techniques, a weakening of home life, parental, and school discipline, and the mental strain created by a highly industrialized society, plus local, national, and world tensions have all combined to make modern-day living highly stressful for a large segment of society. This tension is readily reflected in the widespread and often excessive use of tobacco, alcohol, and sedative drugs of all types.

Although a good beginning has been made in the past decade, we are still far from our desired goal and research must be pushed to find new molecules and improve by molecular variations the present promising compounds. There are now available many agents which act on the central nervous system. Those affecting emotional states can for clinical purposes be divided into three large categories: sedatives, tranquilizers, and antidepressants.

Sedatives

Agents belonging to the sedative group are among the most widely used in medical practice. Although the bromides discovered by A. J. Balard and introduced in medicine by François Magendie in 1821 were the first sedatives, they were weak in action and proved to be of only limited value. They were widely used in the 19th century and are still employed today, in spite of their not inconsiderable toxicity as ingredients in some proprietary sedative preparations. They have been retained in the United States Pharmacopeia as useful agents in the management of convulsive states in pediatric practice. However, their use is extremely limited, as they have been replaced by far more effective and less toxic preparations.

Chloral hydrate, first employed as a sedative by Liebreich in 1869, immediately proved effective. It remains one of the least toxic and most useful seda-

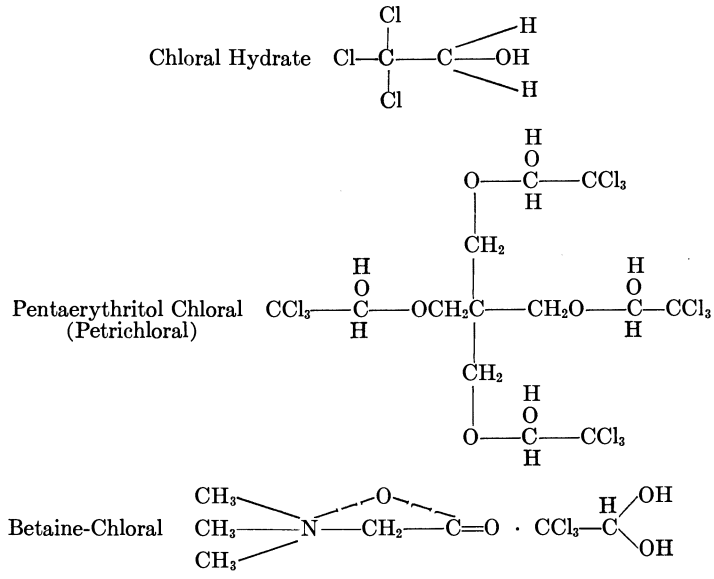


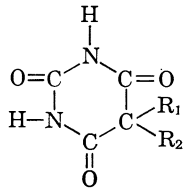
Figure 1. Chloral hydrate

tives available. It is cheap and promptly effective, causes few allergic reactions, and does not produce a hangover reaction the next day. Its chief drawback is its physical state, necessitating that it be given as a liquid or as a thick sirup in capsules, and its tendency to cause stomach irritation (Figure 1).

In recent years, some improvement has been made in the use of this molecule. Pentaerythaerythritol chloral has been synthesized, resulting in a more stable substance, effective, perhaps less irritating, and certainly easier to handle. Recently, chloral betaine has been released for use. Although it had been known for many years that chloral was a highly reactive molecule which formed many complexes, apparently this property was never employed until recently to improve the molecule for clinical use. Chloral betaine is far easier to handle physically than chloral hydrate, is less irritating to the mucous membranes, and in studies made so far, seems to be a highly effective sedative. Further improvements of this old and highly useful drug should be possible.

Chloral hydrate is quickly absorbed even from the rectum. In the body, most of it is converted to trichloroethanol, also a potent hypnotic. Some is oxidized to trichloroacetic acid. Trichloroethanol accounts for the hypnotic effect. It, in turn, is in varying amounts combined, mainly by the liver, with glycuronic acid to form urochloralic acid, an inactive preparation, which is excreted by the kidneys (*15*). Thus individuals with an enzymatic defect precluding their ability to elaborate glycuronic acid or utilize it as a detoxifying substance because of inability to conjugate it with trichloroethanol could be seriously poisoned by chloral hydrate. Since the fetus and premature infants have greatly impaired ability to utilize this detoxifying mechanism, chloral hydrate should be used with caution in the pregnant female and new-born infant (*3*). Although there have been no reports of toxicity, this may be

Barbituric Acid



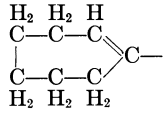
Drug	R_1	R_2	Action
Amobarbital	C_2H_5—	CH_3(CH$_3$)CH—CH_2—CH_2—	Intermediate
Barbital	C_2H_5—	C_2H_5—	Long
Butabarbital	C_2H_5—	CH_3—CH_2—CH(CH_3)—	Intermediate
Heptabarbital	C_2H_5—		Intermediate
Pentobarbital	C_2H_5—	CH_3—CH_2—CH_2—CH(CH_3)—	Intermediate
Phenobarbital	C_2H_5—		Long
Secobarbital	CH_2=CH—CH_2—	CH_3—CH_2—CH_2—CH(CH_3)—	Short

Figure 2. Barbiturates

because there have been no careful observations for untoward effects (*4*).

Since the synthesis of diethyl barbiturate, literally hundreds of barbiturates, and more recently nonbarbiturate sedatives, have been prepared. Most have been abandoned as not of sufficient merit to use clinically. About a dozen barbiturates are still used and half as many nonbarbiturate sedatives are described in the New and Non-Official Drugs or the United States Pharmacopeia (Figure 2). Most of these are used to some extent clinically. There is very little merit in retaining for use any more than these, since they represent the best agents for use in medicine.

With such a plethora of agents to select from, it is necessary to set up certain criteria in order to secure the best drug for special needs. For practical purposes, these criteria can be considered under six categories.

1. Speed of action—i.e., time to take effect on the patient once administered
2. Length of action—ultrashort, short, intermediate, long
3. Route of administration
4. Metabolism and route of elimination
5. Toxicity, untoward pharmacological reactions and allergy
6. Cost

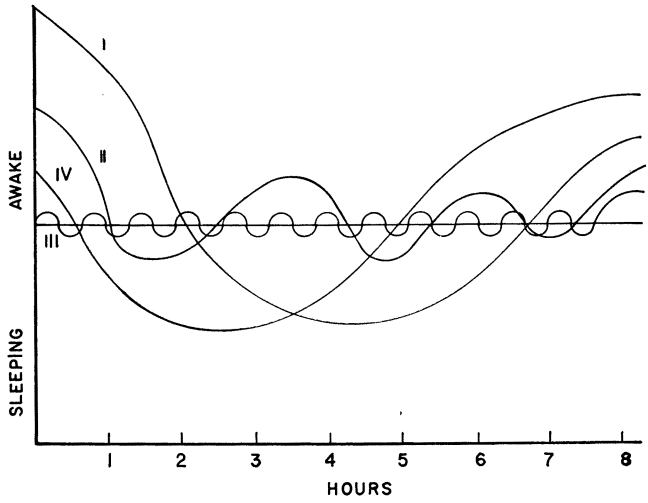


Figure 3. Types of insomnia

To select a sedative wisely, the physician should first consider the particular need and then select the agent best suited to give the optimum effect.

Sedatives are used clinically to relieve emotional tension, allay anxiety, reduce overactivity, as supplements to analgesic and anesthetic drugs, as anticonvulsants, and most importantly to sedate or induce sleep.

Insomnia. Their use as sedatives to induce sleep unquestionably constitutes their most important role in clinical medicine. It is therefore of interest to examine the problem of insomnia briefly, in order to formulate an intelligent basis for its drug therapy. In general, insomnia may be divided roughly into four types (5) (Figure 3). Individuals exhibiting Type I are tense, agitated, and overactive, and have great difficulty in relaxing sufficiently to go to sleep. It may take them 2 hours or more to get to sleep. It is obvious that they need a very rapid-acting, quickly metabolized agent such as ethinamate, heptabarbital, secobarbital, or chloral hydrate.

Type II represents individuals who go to sleep readily but awaken in 1 or 2 hours, then go back to sleep, only to awaken again, so that they swing in and out of sleep two or three times a night. Often there are anxious, tense individuals. Frequently, they have some organic disease producing a low grade fever such as occurs in tuberculosis or other infectious or malignant diseases. Vasomotor instability can also be a source of difficulty. As the vasomotor system relaxes with sleep, blood vessels in skin dilate and the skin heats up. This leads to sweating or in the case of the menopausal woman, hot flashes. The patient is awakened by the discomfort. In patients with arthritis, a painful joint may cause awakening. The joint is splinted by muscles while awake and thus protected from pain, but as the muscles relax in sleep, stress is brought to bear on the joint and pain results. On awakening, these patients soon become comfortable and go back to sleep, only to have the episode recur. Obviously, they need a longer acting sedative such as chloral hydrate, amobarbital, phenobarbi-

tal, pentobarbital, or butabarbital. The newer, nonbarbiturate sedatives, glutethimide, and methyprylon are useful in this type of insomnia, but have no advantage over the older drugs. Frequently, an analgesic drug given alone or with the sedative proves very useful.

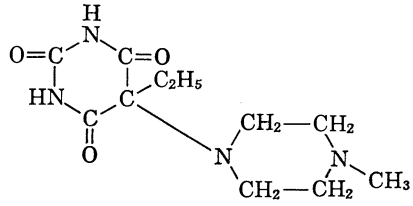
The most puzzling and perhaps most anxious and disturbed patients fall into Type III, where there are hundreds of fluctuations between waking and sleeping during the night. These patients are positive they have had no sleep for weeks or months. They hear everything that goes on during the night, including the clock striking all the hours. Even the slightest disturbance is noted. Since they know that no one can live without sleep, they are exceedingly worried over their plight. As seen by the physician, these individuals do not appear to be any the worse physically and it is obvious that they have not been awake as much as they believe. A glance at the chart will show at once that most of these individuals get at least 50% and more likely 75% of a night's sleep, but since they can remember only what happens when they are awake they, of course, feel that they have had no sleep. A promptly effective intermediate acting agent is useful in these patients. Chloral hydrate, butabarbital, amobarbital, pentobarbital, and phenobarbital are excellent. Some prefer glutethimide, methyprylon, or ethchlorvynol, but these agents have no distinct advantage and are more expensive.

Finally, Type IV represents those who tend to turn night into day. Frequently, these are elderly patients who become increasingly fatigued as the day wears on and by 8 P.M. are ready for bed. They often fall asleep in their chairs after the evening meal or while looking at television. Chronically ill patients with debilitating diseases also tend to be in this group. Once in bed, they promptly sink into sound sleep, only to awaken at 3 or 4 in the morning. They go to bed with the birds and get up with the birds. Once awake, they are restless and usually must get up and prowl around, frequently getting some food. They then may be able to resume sleep, but are more likely to find that they are now thoroughly awake and ready to start their day. Occasionally, this type of insomnia is associated with arthritis, poor vascular circulation, or other diseases which lead to somatic discomfort as the night wears on.

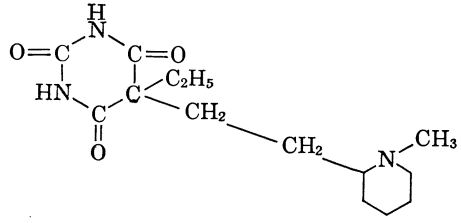
Frequently, nothing need be done for this type of insomnia, but at times there is a definite need for help. The best agents are the intermediate to long-acting drugs such as butabarbital, pentobarbital, chloral hydrate, and occasionally glutethimide, methyprylon, or ethchlovynol. Phenobarbital, because of its strong cerebral depressant action, should usually be avoided, especially if the patient is elderly, since the cerebral depression caused by it may lead to confusion, agitation, excitement, and physical activities which can do serious harm. Tranquilizing drugs such as prochlorperazine or chlordiazeporade are useful in this type of insomnia, but are capable of causing confusion. Occasionally, this type of insomnia is best treated by a quick-acting, quickly dissipated drug such as ethinamate.

There is a definite need for a good, long-acting sedative which will have no cumulative properties—perhaps a long-acting chloral hydrate or an agent with slow onset of action but which is rapidly eliminated.

Barbiturates. It has been a considerable time now since a new effective sedative of the barbiturate series has been developed. The profound changes made by simple substitutions in the first and fifth position of the barbituric acid



Ethylpiperazine Barbituric Acid



Ethyl-*N*-methyl-2-piperidyl Ethyl Barbituric Acid

Figure 4. Barbituric acid

ring have produced a variety of potent sedatives. Diethyl barbituric acid, introduced as a hypnotic by Fischer and von Mering in 1903, was followed by the phenyl ethyl preparation, phenobarbital, in 1911. Since then, a large series of these agents has been studied. Certain conclusions concerning the effect of site of substitution and type of group substituted on the probable action of a new member of the series can now be made.

There is a need for chemists to take a new look at the barbituric acid molecule. Although barbituric acid *per se* is inactive, it is the carrier for many synthetic active preparations. Because of this unique property, it might be utilized to make more effective use of other agents such as some of the antihistamine or tranquilizer preparations. The simple adding of a piperazine ring in the carbon side chain to the chlorpromazine molecule resulted in a whole series of new phenothiazines and should be seriously considered for the barbituric acid molecule. More thought should be given to linking of barbituric acid ring with other groups that have useful effects on the central nervous system (Figure 4).

Animal studies show that the barbiturates are metabolized in the liver, more especially in the smooth surfaced, lightest density microsomes. The enzyme systems involved require both reduced triphosphopyridine nucleotide and oxygen (*14*). The kidneys also metabolize certain other barbiturates, enzymetrically to a limited extent, but their role in this respect is far less than the liver. The brain, muscle, and other tissues may also to a very limited extent metabolize these agents.

Barbiturate metabolism apparently follows four different routes:

Oxidation of substituents in position 5 of the ring to a ketone, alcohol, or acid
Loss of alkyl groups attached to nitrogen
Desulfuration in the case of the thiobarbiturates
Hydrolytic splitting of the barbiturate ring

The loss of an alkyl group at position 5 of the ring and the addition of a methyl group to a nitrogen in the ring may also be routes (*14*).

The rate of metabolism is influenced by many factors. On the whole, it seems to be very slow in man. It probably can be accelerated by administering drugs which are able to stimulate metabolism, as has been shown for several groups of agents (*1*). By judicious use of this phenomenon, it may be possible to convert long-acting barbiturates into intermediate or even short-acting agents. On the other hand, by use of agents which themselves are inactive but prolong the rate of enzymatic destruction, it may be possible to convert short-acting agents into intermediate or long-acting drugs. Such an agent would be useful in enabling an intermediate-acting drug to be of greater use in Type IV insomnia.

Toxicity. On the whole, drugs in this category are relatively free from seriously toxic properties. Skin rashes which clear promptly are the most commonly observed reaction. Abuse of these agents can lead to serious effects. Addiction of a pernicious type develops when approximately four times the usual dose is given daily for 3 or 4 months. The individual deteriorates and finally becomes physically and mentally handicapped. Withdrawal of the drug must be cautiously done over a period of several days if excitement, agitation, or convulsions are to be avoided.

Tranquilizers

This group, of which there are approximately 50 agents now available, has been developed in the past 10 years. The speed with which they were accepted by physicians and their patients has been most amazing. They are now among the most popular drugs and few emotionally disturbed patients have not been exposed to one or more of them. Rauwolfia and its derivatives, used for centuries as a mild tranquilizing agent, have been almost completely replaced by the more effective and less toxic newer synthetic preparations. The phenothiazines still furnish the bulk of the effective tranquilizers.

Although chlorpromazine is still very widely used, it has strong competition from newer members of the series. Substitutions of hydrogen, methyl, ethyl, thiomethyl, thioethyl, trifluoride, acetyl, and many other groups in the 2 position of the molecule have resulted in a whole series of new compounds, some weaker, others more potent than the chlorine-substituted compounds. Furthermore, changes in the side chain made by substituting branched chains, piperazine, and piperidyl groups followed by substitution of various groups on these also led to a new series of agents. Various combinations of the ring and side chain substitution have resulted in still another series. The end is far from sight; however, it is now becoming evident that most of these new preparations have little to offer in the way of improved effects over the better, older agents. Definite advantages have been gained, in that the newer and more potent agents which are given in much smaller doses (usually one fifth to one tenth) result in much less liver and bone marrow toxicity and perhaps less skin sensitivity. This phenomenon of reduced adverse reactions as potency of the molecule is improved, as observed so clearly in the phenothiazine series of agents, has led me to propose this theory (*7*):

The less amount of a foreign molecule introduced into the body, the less likelihood there is of a sensitivity-like reaction occurring. Furthermore, the less amount of a foreign molecule to be metabolized, detoxified, and eliminated, the less possibility there is of a toxic effect occurring.

According to this theory, if an active molecule gives a certain percentage of allergic or toxic effects, improving the potency of the molecule by molecular modification should result in a reduced incidence of adverse reaction. The incidence of adverse effects from prochlorperazine, perphenazine, and fluphenazine which are given in a greatly reduced dose is considerably less than that of chlorpromazine, promazine, and perazine, agents which require introducing into the body five to ten times as many phenothiazine molecules. This principle has been found true for other substances. For example, von Pirquet and Shick years ago showed that if the usual dose of horse serum is given for passive immunity to tetanus, approximately 10% develop a serum sickness as an allergic adverse reaction, but if the dose is gradually increased and a sufficient amount is given, approximately 85 to 100% of the human race get an adverse reaction. Hunt and Gerlough have shown that the relation between the amount of diphtheria antitoxin and incidence of serum sickness is dependent on the amount given (*8, 10*). This seems to be true in the case of the sulfonamides, where the incidence of adverse reactions is approximately 5% when the usual full sulfonamide dose is given but is greatly reduced when the dose is reduced to 1 or 2 grams daily, as in the treatment of urinary tract infections.

A close watch is also being kept on the thiazide diuretics, where through molecular modification vastly more potent diuretics have been created. So far, in my experience, and from what has appeared in the literature, there seems to be a lessening incidence of serious adverse, allergic, and other toxic effects other than those inherent in the drug's action, as the potency is increased and the dose decreased. Since allergy and other toxic effects, except those inherent in the molecule's pharmacological action, are so small in the case of the steroid hormones, no definite trend can be seen in that series of compounds as yet.

The phenothiazines exert many effects in the body. These effects may be considered in three categories:

Action on the central nervous system and behavior
Circulatory effects
Action on endocrine, skeletal, skin, and hematopoietic systems

The effect on the central nervous system is profound. They calm agitation, reduce motor activity, relieve anxiety, sedate, dull emotional distress, promote a tendency to forget or poorly remember emotionally charged material, and finally tend to break up schizophrenia behavior. They also exert a powerful effect on the chemoreceptor trigger area and to a much less extent on the emetic center, thus making the phenothiazines highly useful antiemetic agents. They exert definite effects on the electroencephalogram pattern which resembles that seen with slight drowsiness. There appears to be some evidence from the electroencephalogram that there is a mild depressant action on the reticular activating system, as shown by the increased synchronization. There is also ample evidence that the phenothiazines potentiate the effect of many centrally acting drugs. Alcohol, barbiturates, narcotics, analgesics, and anesthetics are all potentiated. The effect is variable and is related to the dose.

The action of the phenothiazines on the extrapyramidal system is now well recognized and a source of concern and at times alarm when individuals develop bizarre extrapyramidal motor effects. Fortunately, these effects soon wear off with cessation of the drug.

The cardiovascular effects, although at times alarming, are usually of little clinical significance. Body temperature falls and orthostatic hypotension, at times of severe degree, may appear. Vasodilation may appear in the extremities. The effect of norepinephrine is reduced and the hypertensive effect of epinephrine is blocked, but not the hyperglycemic action.

The endocrine effects are interesting but not of much clinical importance. Menses may be suppressed; breasts may lactate in the female or become swollen in the male. Gonadotropin, estrone, estradiol, pregnandiol, and 17-ketosteroids may be reduced in the urine. The depression of gonadotropin may lead to false negative pregnancy tests. There appears to be some central suppression of the antidiuretic hormone (*6*).

The mechanism of action of the phenothiazines is still not definitely known. They tend to block important effector substances such as acetylcholine, epinephrine, and histamine. The phenothiazines produce uncoupling of phosphorylation from oxidation. They appear to act at all steps along the electron transport chain. Cytochrome oxidase, succinoxidase, and adenosine triphosphatase are inhibited. Some data indicate that the phenothiazines may decrease the permeability of storage granules for brain amines.

Many other interesting observations may ultimately prove to have some bearing on the mode of action of these substances. As yet, none of these many observations have given definitive evidence of the exact mode of action. It may be that multiple factors are involved.

The metabolism and elimination of the phenothiazines are most complex and, even yet, very little is understood. A portion of chlorpromazine goes to the sulfoxide, a small amount is excreted in the urine unchanged, and considerable is conjugated with glucuronic acid, and excreted as the complex. As many as 15 different metabolites have been detected for chlorpromazine. Excretion may continue for many weeks or months after cessation of chronic dosage (*2*).

Toxic Effects. All the phenothiazines are capable in varying degrees of toxic effects. Obstructive jaundice is more common in the older members of the series but most have produced this complication. Blood dyscrasias are not uncommon (*17*). Skin rashes, photosensitivity, pruritus, and other varieties of dermatitis have been observed. A host of other effects have been observed which probably are more inherent in the drug's action than a true toxicity. These include dry mouth, tachycardia, sedation, lethargy, hypothermia, lactation, hypotension, convulsions, and extrapyramidal reactions.

No serious physical dependence has been observed. Occasional individuals may present indications of increased agitation, nausea, vomiting, and insomnia on withdrawal, but these are not common and the reaction is not serious.

The extrapyramidal reaction may be very bizarre and at times frightening to the patient's family and physician, but with care, no serious trouble ensues and the reaction promptly subsides once the phenothiazine is discontinued.

There is little to be gained in preparing more similar-acting phenothiazines. More attention should be focused on preparing, altering, or combining phenothiazines with other agents to produce long-acting preparations. A

phenothiazine that could be given in one injection to cover a 2- or 3-week period would prove most useful. There is some work indicating that such a preparation is possible. One is under study at this time in the hope it will prove effective. Such an agent would prove most helpful in the nausea and vomiting of uremia, cancer, and other chronic emetic states. There is also need to combine phenothiazines with antidepressant drugs in order to prevent their depressant effect. An antidepressant drug and a phenothiazine in combination have given results in treating depressed patients with agitation.

Chlordiazepoxide and Meprobamate. In addition to the phenothiazines, at least two additional tranquilizers are widely used. Chlordiazepoxide is a unique substance which is utilized largely to suppress agitation, anxiety, fear, and excitement. Its mode of action is not known. It has proved to be a useful agent in suppressing the excitement and agitation of alcohol and barbiturate withdrawal. Extremely agitated patients can be controlled; individuals with agitated depressive states also respond well to its action. Ataxia and sedation are common side effects and recently blood dyscrasias have been observed (*17*). Addiction with withdrawal effects can be seen if large doses are given for long periods of time, but with ordinarily used doses there is no problem. The drug needs further controlled clinical studies, since it seems to be a useful addition to the tranquilizers.

Meprobamate is widely used. Its action in man is altogether similar to that of a weak sedative. In this respect, it behaves like the barbiturates. It can be substituted for the barbiturates with modest success. Although many claims have been made for unusual effects of the drug, and animal data are interesting, so far the experienced clinician has not been convinced that this drug has any unusual merits (*13*). Certainly, physical dependence develops and blood dyscrasias have been observed (*17*).

There are several more interesting tranquilizing agents but, as yet, there are insufficient controlled data to establish their role in medicine. Unquestionably, some of these have definite merit.

Antidepressant Agents

The monamine oxidase inhibitor, iproniazid, was the first useful antidepressant drug. Unfortunately, it proved too toxic for clinical use. Since its effect was observed, many monamine oxidase inhibitors have been prepared. Most have shown an antidepressant effect, but unfortunately, many of the newer agents also exhibited serious toxic effects. At present, the most commonly employed monoamine oxidase inhibitors are isocarboxazid, phenelzine, and nialamide. These agents exert a highly useful effect in the depressed patient.

Their exact mode of action in depressed states is unknown. It is doubtful if monoamine oxidase inhibition is the only basis of their antidepressant effect. Certainly, potent antidepressant agents are available which exhibit no effect on monoamine oxidase. It is, of course, possible and probable that there are several means of relieving depression, of which monoamine oxidase inhibition is one. Although the agents in use now are much less toxic than the older preparations, they must be given with caution, since hepatitis, severe orthostatic hypotension,

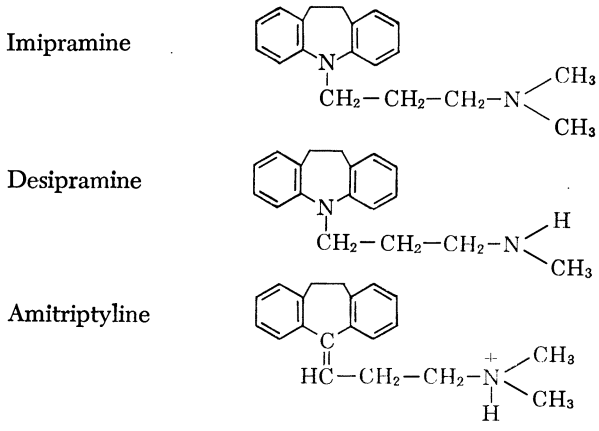


Figure 5. Imipramine, desipramine, and amitriptyline

and other less toxic effects have been observed. Although useful, these agents are effective in less than half the patients exhibiting depression. Frequently, they prove effective in the first episode of depression, only to fail in subsequent attacks. There is need for more controlled data in the use of these drugs, in spite of the difficulties inherent in such a study.

Imipramine, a substance with a structure similar to the phenothiazines (Figure 5) but varying in that the ring is a dibenzazepine rather than a phenothiazine, was the first active antidepressant of the nonmomoamine oxidase inhibitor series of agents. It, like the monoamine oxidase inhibitors, is effective in less than half the patients treated. Its mode of action is not clearly understood, but there is increasing evidence that it too exerts an effect on catechol amine metabolism (*19*). Although serious toxic effects have been uncommon, excitement, jaundice, and blood dyscrasias have occurred (*17*).

Desipramine is closely related to imipramine. It differs in that one methyl on the amine group in the side chain is replaced by hydrogen. As yet, there is no controlled evidence that it is in any way different from imipramine in its action and effect.

Amitriptyline differs from imipramine in having a dibenzocycloheptadiene ring. Its action is similar to imipramine, to which it is related chemically and pharmacologically. It may exert some tranquilizing action in addition to its antidepressant action. In studies done so far, it seems to be about as effective as imipramine in relieving depression in the depressed phase of manic-depressive psychosis and involutional melancholia. It is also useful, like imipramine, in depressive phases of anxiety and in some patients with neurotic depressive reactions with obsessive ruminative tendencies. On the whole, less favorable results are obtained with the usual neurotic depressive reactions. The tranquilizing effect of amitriptyline is useful and perhaps some lessened agitation is seen when it is used. However, it can cause drowsiness, tachycardia, skin rash, and the other types of reactions seen with imipramine. There is evidence that it acts promptly when given intravenously.

These agents should not be given in conjunction with or soon after stopping

the monoamine oxidase inhibitors, since there are a number of reports of serious reactions, including collapse and death, as a result of their combined action. They can, however, be given with the phenothiazines when indicated. Frequently, it is wise to give a phenothiazine in reduced dosage along with imipramine or amitriptyline in order to reduce the agitation and excitement which so frequently develop. In the case of amitriptyline, the dose of the phenothiazine should be one half or less of the usual dose, or some patients will become so sedated that the therapy must be discontinued.

Unfortunately, our knowledge of the mode of action of these substances is so limited that it is difficult to decide what chemical approaches would prove beneficial. In controlled studies with depressed patients, the feeding of catechol amine precursors such as α-dihydroxyphenylalanine alone or with imipramine has not clinically altered the depressed state in patients who have failed to respond to imipramine or monamine oxidase inhibitors alone (*12*). These studies have raised further problems concerning the role of the catechol amines in depressed states.

Recent studies designed to elucidate the mechanism of action of α-methyldihydroxyphenylalanine have again focused attention on the catechols in mental depressed states. This agent, like reserpine, is capable of causing mental depression. We know of one patient who has developed serious mental depression on α-methyldihydroxyphenylalanine and heard of another who has been studied in England. This agent, like reserpine, leads to loss of peripheral catechol amines (*9, 16*). It also penetrates into the central nervous system and, like reserpine, releases central catechol amines. On the other hand, guanethidine, a potent peripheral catechol amine releaser, does not penetrate the blood brain barrier and even after long and extensive use has never been found to produce mental depression.

Recent studies on the mechanism of action of α-methyldihydroxyphenylalanine point to the possibility that it is decarboxylated to produce α-methyldihydroxydopamine and this substance by beta oxidation is converted into α-methyldihydroxynorepinephrine. These agents in turn are thought to bring about release of norepinephrine from storage sites (*18, 19*).

This interesting theory led us to consider the possibility that α-methylated catechol amines might under certain situations be present in tissue and in certain metabolic situations could lead to catechol amine loss and mental depression. Although methylation of carbon is biochemically a difficult problem, the idea was intriguing enough to secure some preliminary data.

A chromatographic technique was devised to separate α-methylnorepinephrine from human urine after pure α-methylnorepinephrine had been added. The chromatogram of the control solution and the urinary extracted solution showed excellent separation from norepinephrine and similar R_f values between the control solution and urinary extracted solution on the paper strip. A 24-hour urine specimen from a depressed patient on no drugs was next chromatogrammed against a control urine to which α-methylnorepinephrine had been added. There was excellent separation and a spot appeared in the patient's chromatogram at the same R_f as the control α-methylnorepinephrine specimen. These very preliminary data have sparked considerable interest and we are now extending this work.

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Discussion

SYDNEY ARCHER, presiding

Alfred Burger (University of Virginia): I would like to ask a factual question of Dr. Friend. That fascinating hypothesis proposed that alpha-methylation would take place and that this may have something to do with the condition of the abnormally metabolized patient, if you assume that the patient himself introduces a methyl group in the alpha position. This is a very difficult thing to visualize on chemical grounds. Even if you form an anion at the position that carries the amino and the carboxyl group, it is almost impossible to visualize by what chemical mechanism the methyl group might be introduced.

Dr. Archer: Dr. Burger has a really good point, and I think the most disturbing thing about the theory is that there are transmethylations on oxygen and nitrogen, but on carbon it is a unique experience, and I think you are going to cause a lot of interest in certain quarters.

Dr. Friend: This is a very important question. It is very difficult to say. We certainly have found alpha-methylnorepinephrine on our chromatographs. The spotting is the same as in controls in a depressed person's urine. I do not know just exactly how this metabolic activity may take place.

We are now getting urines from people who are fed Aldomet to check further on what happens to metabolism along this line. We may be all wrong in this, but so far it looks exceedingly exciting. Remember, there are many enzyme systems we do not know much about in the human organism as yet.

13

Narcotic Antagonists as Analgesics

Laboratory Aspects

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The usual rodent analgesic assays carried out on morphine, meperidine, methadone, and their congeners correlate well with clinical analgesic potency and ability to support addiction. Nalorphine, an analgesic antagonist, which is inactive in these tests, is a strong analgesic in man but does not support addiction. In an attempt to find a clinically useful analgesic comparable to morphine but without addicting properties, a series of benzomorphans was synthesized and evaluated for analgesic antagonist activity in rats. Compounds ranging in potency from about 1/70 to 10 times nalorphine were obtained. Several of these were studied more intensively before being submitted for clinical evaluation.

A renewal of efforts within the past decade has led to the synthesis of many strong analgesics, including some which are several hundred times as potent as morphine, but the fundamental problem of divorcing strong analgesia from addiction liability remained unsolved. In 1956, Schaumann (*17*), pioneer in the development of synthetic analgesics, stated, "It is therefore not correct to say that the depression of respiration and the constipating effect of the analgesics are side effects. They are inseparable from their analgesic action. This is unfortunately also true for the liability to cause addiction. It will therefore not be possible to find morphine-like analgesics without this undesirable addition and in fact all efforts in this direction have been unsuccessful."

Two of the more reliable laboratory methods for evaluating strong analgesics are the Bass-VanderBrook (*1*) modification of the D'Amour-Smith (*3*) rat tail-flick method and the Eddy-Leimbach (*6*) variant of the mouse hot-plate technique. The tail-flick assay seems to be more specific for morphine-like drugs, since compounds which are positive in this test are also active in the

mouse test, but the reverse is not always true. The vast majority of drugs which are active in both rodent tests, when studied in man, were found to be analgesics.

In Table I the rank-order of the following parameters is listed for seven well-known analgesics: activity in the Eddy-Leimbach test, activity in the VanderBrook-Bass rat test, milligram potency in man, and ability to suppress abstinence signs in addicts. All but the rat values are based on data in a review by Eddy, Halbach, and Braenden (*5*). The rank-order in rats was established on the basis of data obtained in our laboratory (*10*).

Table I. Rank-Orders of Seven Analgesics Parameters

Compound	*Mouse*	*Rat*	*Clinical Potency*	*Support of Addiction Liability*
Methadone	1	2	2	1
Isomethadone	4	3	4	4
Meperidine	5	5	5	5
Ketobemidone	2	1	1	2
Codeine	7	7	6	6
Morphine	3	4	3	3
D-Propoxyphene	6	6	7	7

The Spearman rank-order correlation coefficients for the various parameters are:

Mouse *vs.* rat	0.93
Mouse *vs.* clinical potency	0.93
Mouse *vs.* support of addiction liability	0.99
Rat *vs.* clinical potency	0.93
Rat *vs.* support of addiction liability	0.89

The interesting point emerges that the rodent assays correlate equally well with clinical analgesic potency and the ability to support addiction liability. Thus, the major virtue of these screening techniques, their high degree of predictiveness of clinical efficacy, is at the same time their major disadvantage, their equally high degree of foretelling addiction liability in man. On the basis of this and similar analyses it was clear that some other laboratory method had to be found that was applicable to the problem of separating clinical analgesia from addiction liability.

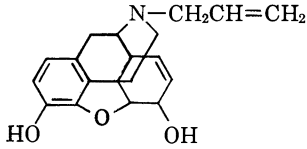
The case of nalorphine attracted our attention as an approach worth pursuing. This drug, which is capable of antagonizing the pharmacodynamic effect of morphine in man and animals, when given alone is analgesic in man (*14*) equivalent in milligram potency to morphine (*13*). Yet in the standard laboratory tests for analgesia this drug is inactive. Of equally great significance was the fact that Isbell (*11*) was unable to induce significant physical dependence with the drug in postaddicts. Although clinical analgesia and addiction liability were finally separated in the case of nalorphine, the high incidence of psychotomimetic side effects precluded the use of this drug as a morphine substitute.

Since it was now possible to dissociate strong analgesia from addiction liability, it seemed that the diminution of the undesirable side effects of nalorphine ought to be within the realm of attainment also (*4*). Keats (*19*) pur-

sued this approach clinically and studied a heterogeneous group of analgesic antagonists. Although there were marked differences in properties among the group, none of the compounds was considered to be an acceptable drug.

When we included nalorphine in our rank-order correlations described above, it turned out that the rodent tests actually correlated better with the parameter of addiction liability than clinical analgesic effectiveness. Accordingly, at the outset of the present studies it was decided that compounds which were positive in the D'Amour-Smith assay would be discarded, since it was highly probable that such drugs would eventually cause addiction in man. The antagonism of meperidine analgesia in rats was used as a primary screen for the evaluation of analgesic antagonist potency. It was our hope to find a broad spectrum of activity and then after appropriate pharmacological and toxicological studies, to choose carefully selected members of the group, of differing biological profiles, for evaluation clinically as analgesics.

Nalorphine, the prototype of the analgesic antagonists, is *N*-allylnormorphine, 1, a pentacyclic compound (*20*). The tetracyclic analog, levallorphan, 2, is also an antagonist (*18*), but the bicyclic meperidine derivative, 3, is an analgesic, not an antagonist (*2*).



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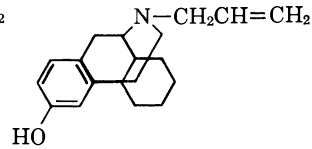
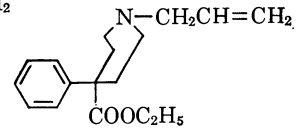


2



3

Another structural feature present in 1 and 2 but absent from 3 is a phenethylamine fragment. The absence from this set of a tricyclic derivative coupled with the high clinical interest shown in phenazocine (*16*) prompted us to turn our attention to the synthesis and pharmacological evaluation of a series of antagonists derived from the benzomorphan nucleus. May and his associates (*7*, *15*) prepared the *N*-methyl derivatives, 5 and 6, according to the sequence outlined in Figure 1. Demethylation followed by realkylation furnished the desired compounds in both the α (*cis*) and β (*trans*) series.

The relative analgesic antagonist activities, AD_{50}, of a representative group of benzomorphans which were prepared by direct alkylation of the nor-base, 7, are given in Table II together with the values for the standard drugs.

Compound 9 (R = CH_3; R′ = $CH_2CH = CH_2$) was prepared independently by Gordon *et al.* (*9*) and is known as SKF-10047.

It is clear that in the α series of benzomorphans, high antagonist activity is present. All these compounds were inactive in the D'Amour-Smith test and were relatively weak on the inclined screen test, a procedure which is indicative of muscle relaxant activity in this series of compounds.

A group of cycloalkylmethyl derivatives, of which 10 was the prototype, was prepared as indicated in Figure 1. Compound 10 was a potent meperidine antagonist comparable to 9 (R = CH_3; R′ = $CH_2C = CH_2$). In contrast to the alkenyl-substituted benzomorphans, the members of the cycloalkylmethyl-substituted series were potent on the inclined screen and showed some degree

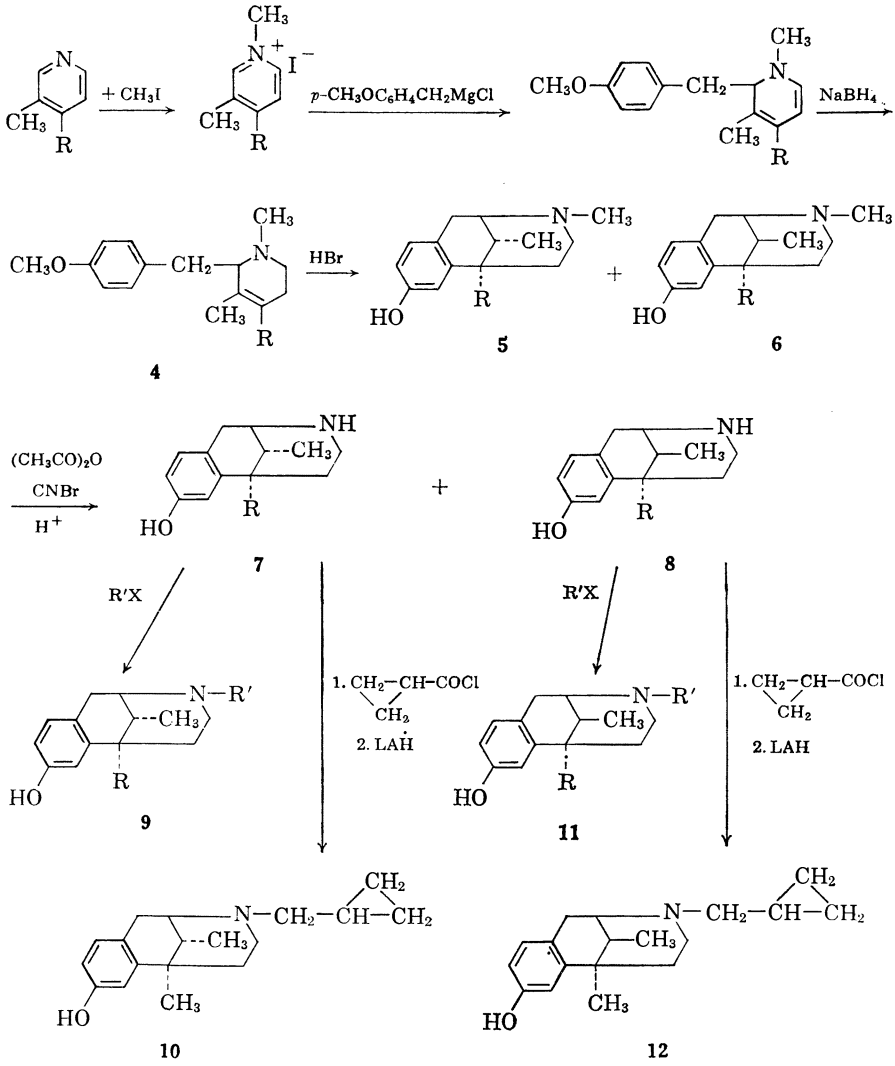


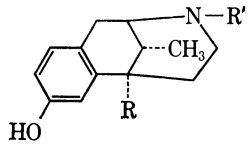
Figure 1. Synthesis of the benzomorphans

of positivity in the D'Amour-Smith test. The results are summarized in Table III.

The benzomorphans thus far studied were all racemic. In view of the diverse pharmacological properties of the cycloalkylmethyl derivatives it was desirable that the compounds be resolved to ascertain wherein each action resided. Fortunately, for comparison with the morphine and morphinan nuclei we had available from Gates (*8*) the corresponding cyclopropylmethyl derivatives, 13 and 14. Table IV compares the analgesic antagonist activity of a group of cyclopropylmethyl derivatives with the resolved optically active dimethylallylbenzomorphan, 9.

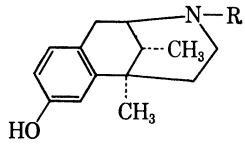
As in the case of the allyl compounds, the cyclopropylmethyl compound,

Table II. Analgesic Antagonist Activity (AD_{50}) of Benzomorphan Antagonists



R =	R' =	AD_{50} *vs. Meperidine, Mg./Kg.*
CH_3	$-CH_2CH{=}CH_2$	0.047
C_2H_5	$-CH_2CH{=}CH_2$	0.049
CH_3	$-CH_2CH{=}CHCl$ (*cis*)	0.018
CH_3	$-CH_2CH{=}C(CH_3)_2$	3.9
C_2H_5	$-CH_2CH{=}C(CH_3)_2$	10.9
Nalorphine		0.13
Levallorphan		0.052

Table III. Cycloalkylmethylbenzomorphans



R' =	AD_{50} *vs. Meperidine*	*D'Amour-Smith (Rat Tail-Flick)*	*Inclined Screen* ED_{50}, *Mg./Kg.*
CH_2–△	0.019	25% at 120 mg.	2.8
CH_2–□	0.37	64 mg./kg. (ED_{50})	5.5
CH_2–⬠	0.28	Inactive	32.5
CH_2–⬡	14.5	Inactive	Inactive

10, is more active than the corresponding morphine, 13, and morphinan, 14, derivatives. Another interesting but unexplainable result is that the levo isomer is four times rather than twice as active as the racemic compound. The other pharmacological activities as well as the analgesic antagonist activities were predominant in the levo isomer. To see whether this difference between isomers was confined to strong antagonists, the dimethylallyl congener, 9, was resolved. Again the levo isomer was four times as active as the racemic one.

As indicated in Figure 1, cyclization of the tetrahydropyridine, 4, furnishes a mixture of cis, 5 ($R = CH_3$), and trans, 6 ($R = CH_3$), isomers, the former predominating (*15*). It had been reported that 6, the trans isomer, was seven times as active as an analgesic in mice as 5. Since we had a moderate supply of two trans isomers 6 ($R = CH_3$ and $R = C_2H_5$), a few derivatives were prepared. The effects of this structural variation are shown in Table V.

Table IV. Analgesic Antagonist Activity of Some Optically Active Compounds

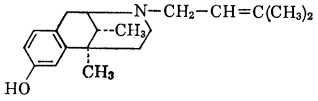
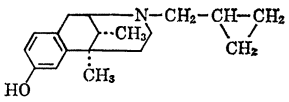
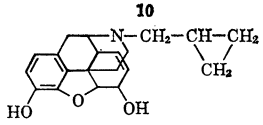
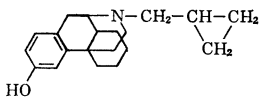
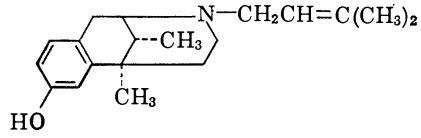
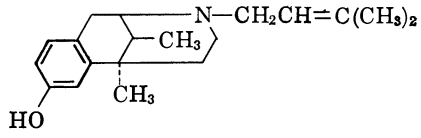
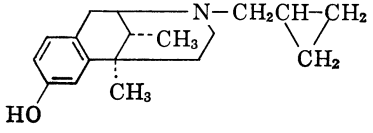
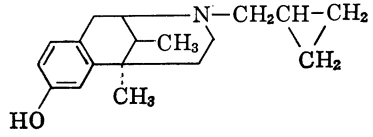
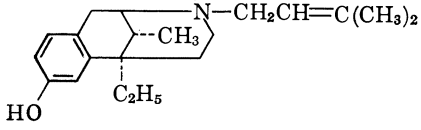
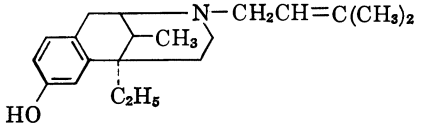
	AD_{50}, Mg./Kg.
	(±) 3.9 (—) 1.1 (+) 18
9 	(±) 0.019 (—) 0.005 (+) 2.5
10 	(—) 0.046
13 	(—) 0.034
14	

Table V. Comparison of cis and trans Isomers

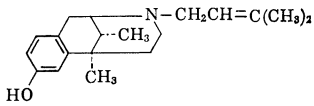
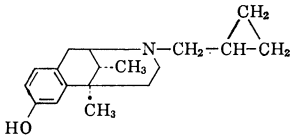
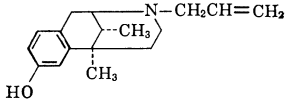
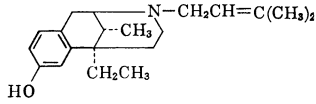
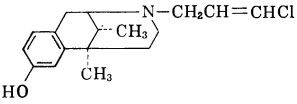
cis Isomers	*trans Isomers*
	
AD_{50} = 3.9 mg./kg.	AD_{50} = 3.3 mg./kg.
	
AD_{50} = 0.018 mg./kg. ED_{50} = 2.75 mg./kg. (inclined screen)	AD_{50} = 0.014 mg./kg. ED_{50} = 0.27 mg./kg.
	
AD_{50} = 10.9 mg./kg. ED_{50} = inactive (D'Amour-Smith)	AD_{50} = 0.087 mg./kg. ED_{50} = 1.4 mg./kg.

The most striking result of this chemical manipulation is exemplified by the last pair of benzomorphans. The cis isomer is a weak antagonist that is negative in the D'Amour-Smith test. The trans isomer on the other hand is the more potent antagonist. The exact figure was difficult to determine, but an AD_{50} as low as 0.087 mg. per kg. *vs.* meperidine was obtained. Equally surprising was the observation that the trans isomer was about three times as active as morphine in the D'Amour-Smith test.

The trans isomer of the cyclopropylmethyl pair, which was approximately equal to the cis isomer as an analgesic antagonist, was ten times as active in the inclined screen test.

Although a great deal of biological information about the benzomorphan antagonists was obtained in the laboratory, the key question—whether analgesic activity unaccompanied by psychotomimetic effects was present—could only be answered clinically. For this reason a group of compounds were selected for further pharmacological and toxicological study prior to submission for clinical evaluation. The profiles are summarized in Table VI.

Table VI. Profiles of Benzomorphans

Compound	*AD_{50} vs. Meperidine, Mg./Kg. S.C.*	*Reversal of Respiratory Depression*	*D'Amour-Smith Rat Tail-Flick*	*Inclined Screen ED_{50}, Mg./Kg. S.C.*
	3.9	Weak	Inactive	33
	0.019	Active at 0.015–0.030 mg./kg.	25% effect at 120 mg./kg.	2.7
	0.049	Active at 0.030–1.0 mg./kg.	Inactive	37
	10.9	Weak	Inactive	38
	0.018	Active at 0.030–1.0 mg./kg.	Inactive	23

The clinical experience with these drugs and SKF-10047, 9 (R = CH_3; R′ = CH_2—CH = CH_2), is discussed by Keats (*12*).

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14

Narcotic Antagonists as Analgesics

Clinical Aspects

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The demonstration that nalorphine is a potent nonaddicting analgesic in man stimulated a search among other narcotic antagonists for potent analgesics without the psychotomimetic effects of nalorphine. Most recently a series of benzomorphan derivatives capable of antagonizing meperidine analgesia in animals was surveyed in man for analgesic activity and psychotomimetic effects. Of the six derivatives studied, two were shown to have the desired characteristics of potent analgesia without psychotomimetic effects. To date, one of these has also been shown to be without addiction liability of the narcotic type in man. The study of these benzomorphan derivatives permitted correlations to be drawn among analgesia, narcotic antagonism, psychotomimetic effects, respiratory depression, and addiction liability.

In 1954, Lasagna and Beecher (*8*) demonstrated that nalorphine, which at that time was known to be a potent antagonist of many of the actions of narcotics, was in itself an analgesic in man. This demonstration was of considerable significance, first, because the analgesic activity of nalorphine could not be demonstrated by several techniques in laboratory animals and, second, because nalorphine possessed no morphine-like addiction liability when studied in post-addicts (*4*). These observations suggested that despite general skepticism a potent nonaddicting analgesic had in fact been identified in nalorphine.

Early studies in our laboratory confirmed the analgesic activity of nalorphine in man and we estimated that nalorphine was approximately as potent an analgesic as morphine on a milligram basis (*5*). We also confirmed that nalorphine produced other effects remarkably similar to those of morphine, including sedation, nausea, vomiting, mental clouding, and respiratory depres-

sion. As others had (8), we found that nalorphine, unlike morphine, also produced psychotomimetic effects in approximately 20% of patients (6). The most disturbing of these were hallucinations, disorientation, and other depersonalization phenomena. This high incidence precluded the routine clinical use of nalorphine as an analgesic, since the side action liability of morphine is low when used clinically

Following this lead, however, we postulated that a clinically useful nonaddicting potent analgesic could more likely be found among the narcotic antagonists than among compounds which showed classical morphine-like characteristics by animal screening techniques. We therefore investigated a number of compounds which were available to us in sufficient quantity for clinical testing and which demonstrated morphine antagonistic activity in animal studies. Most of these compounds, which were derived from the morphine and morphinan nucleus, did not show analgesic activity in animal assays and screening for analgesia was of necessity carried out in man. Among these narcotic antagonists, some were found to produce potent or moderate analgesia, but in each instance the analgesia was associated with a certain incidence of psychotomimetic effects similar to those which followed nalorphine (11).

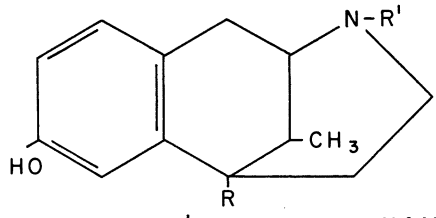
Following the synthesis of benzomorphans by May and Fry (10), Archer and his associates (1) prepared a number of benzomorphan derivatives showing a wide range of potency as antagonists of meperidine analgesia in animals. These compounds were made available to us. They were screened in man primarily for analgesic and psychotomimetic activity, since these were limiting characteristics in the search for a potent nonaddicting analgesic. When analgesia in the morphine range was obtained without psychotomimetic activity, other parameters of drug action were investigated. To date, we have studied five of these benzomorphan antagonists. A sixth has been studied by Lasagna and DeKornfeld (9), whose preliminary results have been included. This study suggests that compounds with potent antimorphine activity in animals associated with potent analgesia in man are also associated with psychotomimetic effects in man. Conversely compounds with low antimorphine potency in animals associated with potent analgesia in man are without psychotomimetic activity. The study of two of these compounds in postaddicts (2, 3) also suggests that compounds possessing antimorphine activity in animals, possess little or no addiction liability. At least one of these compounds promises to be a clinically useful, nonaddicting potent analgesic which may represent an important contribution to therapeutics (7).

Methods

The methods used in these assays were presented in detail in a recent publication (11). For orientation, the following brief description is included. The drugs studied, their chemical relationships, and the designations by which they are known in this and other publications are presented in Table I. All drugs were given intramuscularly and all studies were double blind.

Analgesic potency was determined in postoperative patients following major surgical procedures. The drug to be studied and a placebo (saline) were alternated in individual patients as often as every hour as necessary to obtain relief from pain. The degree of analgesia produced by each injection was

Table I. Benzomorphan Derivatives Studied in Man



R	R'	NAME
CH_3	$-CH_2CH_2C_6H_5$	PHENAZOCINE
CH_3	$-CH_2CH=CH_2$	SKF 10047
C_2H_5	$-CH_2CH=CH_2$	WIN 19362
CH_3	$-CH_2CH=CHCl$(CIS)	WIN 29 M
CH_3	$-CH_2-\Delta$	WIN 20740
CH_3	$-CH_2CH=C(CH_3)_2$	WIN 20228
C_2H_5	$-CH_2CH=C(CH_3)_2$	WIN 20264

estimated by technicians who interviewed patients before and after drug administration. One dose of drug and one of placebo were considered a pair. The frequency of analgesia in 20 to 30 pairs of doses (usually 10 to 15 patients) was compared and expressed as analgesic potency of the drug relative to the placebo. Several dose levels of each drug were studied in successive groups of patients in order to obtain a dose effect relationship. From such data, it is difficult to estimate the dose of the drug which produced analgesia equivalent to that of morphine. Morphine was not used as the reference standard, since the compounds with which it would be alternated in the same patient possessed antimorphine activity. The question of antagonism of morphine analgesia would always be present. Therefore estimates of analgesic potency relative to morphine were crude and limited to estimates of doses which achieved the range of potency expected of morphine when studied in this fashion with a placebo.

Postoperative patients were observed during analgesic assays for the appearance of gross psychotomimetic activity such as hallucinations, disorientation, uncontrollable excitement, and profound sedation. In addition, preoperative patients who were receiving no medications and were awaiting elective surgery were given these drugs at several dose levels. They were interviewed before and at three periods after injection for a variety of subjective effects which were characteristic of both morphine-like and nalorphine-like activity.

Respiratory depression was estimated in healthy young subjects in whom a respiratory stimulus response curve was determined in response to inhalation of three mixtures of carbon dioxide in oxygen. Simultaneous measurements were made of alveolar ventilation and alveolar CO_2 tension at each gas mixture. These provided the data for the curves. A control and two postdrug curves were obtained. The degree of displacement of these curves to the right represented respiratory depression.

Results

The results of these assays are summarized in Table II, which also includes data on morphine, nalorphine, and phenazocine for comparison purposes. The estimates of relative analgesic potency and relative psychotomimetic activity were derived from data collected in our laboratory for all compounds, except WIN 20,740. This compound was studied by Lasagna and DeKornfeld (*9*). Relative analgesic potency is expressed as the number of milligrams of each compound estimated to produce analgesia equivalent to 10 mg. per 70 kg. of morphine in man. For two compounds, SKF 10,047 and WIN 29M, both of which produced definite analgesia, the dose equivalent to morphine was not achieved. Studies with these compounds were discontinued at the doses indicated because of the severity of psychotomimetic effects which appeared. Relative psychotomimetic potency could not be expressed in milligrams because of the lack of a scale for quantitating such activity. Instead, compounds were ranked according to the intensity of psychotomimetic effects observed at any dose studied. The relative potency estimates of these compounds as narcotic antagonists were derived from the milligram potency data of Archer *et al.* (*1*), utilizing antagonism of meperidine analgesia as the test object. Three grades of activity (0, +, ++) were arbitrarily used.

Table II. Comparison of Pharmacological Effects of Benzomorphan Antagonists Studied in Man

Drug	*Analgesic Dose, Mg./70 Kg.*	*Psychotomimetic Effects*	*Respiratory Depression*	*Potency as Narcotic Antagonist (Animal)*
Morphine	10	0	+	0
Nalorphine	10	++	+	++
Phenazocine	3.5	0	++	0
SKF 10,047	15 +	++	?	++
WIN 19,362	5	++	+	++
WIN 29M	2 +	++	?	++
WIN 20,740	0.25	+	+	++
WIN 20,228	20	0	+	+
WIN 20,264	30	0	+	+

It is immediately apparent from Table II that all compounds studied produced some degree of analgesia, ranging in milligram potency from 0.25 mg. for WIN 20,740 to 30 mg. for WIN 20,264. Potency as a narcotic antagonist was not strongly related to analgesia in man, since WIN 20,740 and SKF 10,047 were equally effective in antagonizing meperidine analgesia in the animal, but WIN 20,740 was at least 60 times as potent as an analgesic in man. Strong antagonist activity in the animal was consistently associated with psychotomimetic effects in man. The only compounds which did not produce bizarre mental effects were relatively inactive as meperidine antagonists (WIN 20,228 and WIN 20,264). WIN 20,228 when administered intramuscularly to almost 200 patients in doses ranging from 0.14 to 0.56 mg. per kg. failed to produce definite psychotomimetic effects. Four patients received 3.0 to 6.0 mg. per kg. of WIN 20,228 intravenously without psychotomimetic effects (*7*). Comparable studies with large doses of WIN 20,264 and WIN 20,740 have not been carried out.

WIN 20,740 occupies an intermediate position. Analgesic studies were initiated at doses of 2 mg. (9) (doses were not related to body weight). At this dose, both marked analgesia and some psychotomimetic effects were observed. In subsequent groups of patients the dose was reduced. It was found that doses as low as 0.25 mg. produced analgesia equivalent to morphine, and psychotomimetic effects were not observed in any patient given less than 1.0 mg. In contrast to WIN 29M, WIN 20,740 produced strong analgesia in low dose, psychotomimetic effects in high dose; the opposite was true of WIN 29M.

From this comparison, it would seem that potent narcotic antagonism in the animal and potent analgesia in man are associated with some psychotomimetic effects in man, whereas potent analgesia without potent narcotic antagonism is relatively free of psychotomimetic effects, at least in the dose ranges studied. An interesting contrast to support this formulation is provided by *N*-methallylnormorphine, a compound we studied previously in doses up to 20 mg. per kg. (*11*). This compound produced neither analgesia nor psychotomimetic effects in man, but was a potent narcotic antagonist in both animals and man. It would seem then that potent narcotic antagonism in animals is not necessarily associated with either analgesia or psychotomimetic effects in man, suggesting again that both potent antagonism in animals and potent analgesia in man are necessary for psychotomimetic activity. To date all compounds shown to be strong narcotic antagonists in animals have proved to be strong narcotic antagonists in man.

For interest, we have included in Table II the results of our studies of the respiratory depressant activity of these compounds in man. We have not studied SKF 10,047 or WIN 29M for this parameter, since analgesia equivalent to morphine was not obtained. The other compounds were studied at doses approximately equal to morphine in analgesia. The data on WIN 20,740 were obtained by Lasagna *et al.* (9). All compounds produced respiratory depression equal to that produced by 10 mg. per kg. of morphine and this respiratory depressant activity was independent of the potency of the compound as a narcotic antagonist or its psychotomimetic activity. It was related to analgesia. Despite the similarity to morphine, some preliminary observations from large intravenous doses of narcotic antagonists indicate that the slope of the dose effect curve for respiratory depression by narcotic antagonists is much less than that for morphine. Possibly the maximal respiratory effect of narcotic antagonists may not be respiratory arrest, as is true with morphine-like compounds. The parent compound, phenazocine, produced more respiratory depression than morphine in doses which produce equal analgesia.

Further Studies with WIN 20,228. The subjective effects produced by WIN 20,228 were studied in preoperative symptom-free patients at 10, 20, and 40 mg. per 70 kg. (7). These effects were strikingly similar to those of morphine and included dizziness, grogginess, difficulty in concentration, sleepiness, nausea, and vomiting. The incidence and severity of the effects produced by 20 mg. per 70 kg. of WIN 20,228 were almost identical to those of 10 mg. per 70 kg. of morphine. Those of 40 mg. per 70 kg. of WIN 20,228 were somewhat more marked than morphine. The only suggestion of any psychotomimetic effects from WIN 20,228 was the single report of one patient that she experienced "crazy thoughts" after the 20 mg. per 70 kg. dose.

We attempted to antagonize the respiratory depression of morphine by WIN 20,228. We were able to demonstrate slight antagonism in seven subjects. It was more significant, however, that WIN 20,228 did not add to the respiratory depression of morphine.

To study the circulatory effects of WIN 20,228, we administered total doses of 3 to 6 mg. per kg. of WIN 20,228 to three patients in small intravenous increments and compared this to the effects of 1 mg. per kg. of morphine administered under identical circumstances in other patients. Progressive respiratory depression was observed at these large doses, manifested primarily by an increase in the alveolar pCO_2. However, in contrast to morphine, tachycardia and hypertension were produced when the dose of WIN 20,228 exceeded 2 mg. per kg. As noted above, these large doses produced psychic effects indistinguishable from morphine.

Two patients who received 2.5 and 3.1 mg. per kg. of WIN 20,228 intravenously in small increments were then given nalorphine or levallorphan. In neither patient was the respiratory depression of WIN 20,228 antagonized by these potent narcotic antagonists.

Addiction Liability. The addiction liability of WIN 20,228 was studied by Fraser and Rosenberg (*3*) at the Addiction Research Center at Lexington, Ky. The compound was administered both subcutaneously and intravenously to nontolerant postaddicts, who reported that the subjective effects were not morphine-like. When substituted for morphine in morphine-dependent subjects, WIN 20,228 did not suppress the abstinence syndrome of morphine. Chronic administration of WIN 20,228 was disliked by postaddicts. One subject who continued taking the compound for 25 days exhibited a mild abstinence syndrome, but no physical dependence could be demonstrated when nalorphine was administered. These effects were considerably different from those associated with morphine and it was concluded that WIN 20,228 had minimal addiction liability.

These same investigators studied WIN 20,740 in a similar manner and concluded that it too had little or no addiction liability (*2*). In nontolerant postaddicts, WIN 20,740 produced less euphoria than morphine. It precipitated an abstinence syndrome in morphine-dependent subjects and acute withdrawal after 25 days' administration of WIN 20,740 resulted in a mild atypical abstinence syndrome in four subjects.

The inability to demonstrate a narcotic-type addiction liability for WIN 20,228 and WIN 20,740, and the previously reported failure to demonstrate this for nalorphine (*4*), strongly suggest that compounds shown to produce narcotic antagonism in animals will prove to be nonaddicting in studies in man. All three of these compounds are therefore potent nonaddicting analgesics.

Discussion

If the results reported here can be confirmed by others, a clinically useful nonaddicting potent analgesic will have been attained. More significantly, the means by which other similar compounds may be identified in this and other chemical groups will now be apparent. Having thus far dissociated addiction liability and psychotomimetic activity from potent analgesia, it may now be

possible in the continued search to extend this dissociation of potent analgesia from nausea and vomiting, constipation, or respiratory depression. On the other hand, radical new departures may be required for this further dissociation.

The achievement of a nonaddicting potent analgesic and possibly a morphine substitute does not have great significance for the general problem of narcotic addiction. It would contribute something, however. The availability of such a compound would eliminate the small number of "medical addicts" whose initial addiction results from the chronic treatment of a painful disease with narcotics and the small amount of narcotics for medical use which finds its way into illicit channels by theft or deceit. Much more importantly, it would permit physicians to treat pain adequately and eliminate the terrible decisions of whether to risk addiction in relieving the pain of a patient with chronic disease or to prolong suffering. On the other hand, the compounds studied to date do not represent a panacea. They still possess their other unpleasant side actions, as do morphine and other potent narcotics.

The availability of this type of drug would also eliminate the tedious and expensive accounting of all supplies and administrations of narcotics, now mandatory in physicians' offices and hospitals, and by distributors, manufacturers, and the Federal Government.

Finally and probably most important can be the contribution made by such drugs in further pharmacological research. Contrasting compounds such as morphine and WIN 20,228, which are so similar except for their addiction liability, may provide another tool as valuable as nalorphine for further exploration of the problems of analgesia, physical dependence, tolerance, and the relationship between molecular modification and drug action.

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Clinical Control of Fertility

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Combinations of progestins with estrogenic steroids have been used extensively in clinical and field trials, and remarkable contraceptive efficiency has been demonstrated for seven commercially available preparations. A careful comparative clinical study of the effects of three preparations (Enovid, Orthonovum, and Ovulen) has shown remarkable similarity in a number of physiological phenomena and even in reported "reactions" in the women using the drugs. Contraceptive doses of Enovid in women using the drug over a number of years have caused no significant deviations from normal in blood pressure, blood cholesterol, and radioiodine collection by the thyroid; post-treatment fertility seems to increase somewhat. The frequency of varicosities tends to be reduced, as do mean clotting time and the occurrence of suspicious Papanicolaou smears.

The control of fertility by chemical agents is, as Babcock has shown (*1*), a practice known to endocrinologists for many years. The first application of this knowledge for purposes of contraception is now in its tenth anniversary. In 1953 Chang and I demonstrated the possibility of ovulation inhibition in rabbits by oral progesterone (*17*) and shortly thereafter Rock and I (*11*) found similar inhibition in women when progesterone by mouth was administered during the period of the menstrual cycle when ovulation might occur (from the fifth to the 25th day). The high oral dosages necessary for ovulation inhibition and the imperfect control of menstruation with progesterone led us to seek more effective ovulation inhibitors and menstrual cycle regulators. A screening in animals led to the finding of the highly potent progestational 19-norsteroids (*18*, *19*), and their development as oral ovulation inhibitors in clinical (*11*, *20*, *25*, *26*) and field trials (*21*, *23*) followed in reasonably rapid sequence. The development thereafter of a number of orally active contraceptives is a process to be expected and still continuing.

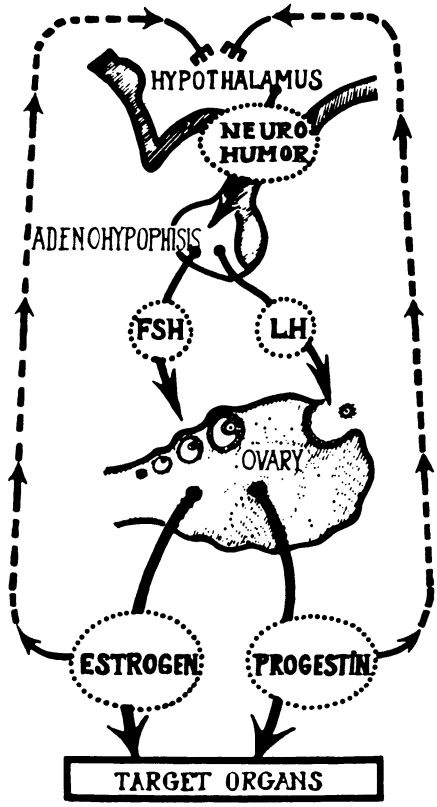

Figure 1. Ovulation-producing process

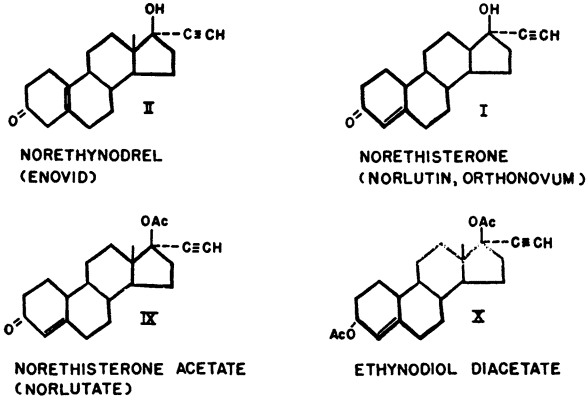

Figure 2. Progestins tested as oral contraceptives in field trials

Although there are many vulnerable steps in the sequence of events leading to reproduction in mammals (*10, 14*), all of the present-day oral contraceptives are steroidal ovulation inhibitors. Their primary mode of action is demonstrable in the ovulation-producing process set forth diagrammatically in Figure 1. Ovulation is initiated by a neurohumor carried to the anterior lobe of the pituitary gland from the hypothalamus. This neurohumor stimulates the increased release of pituitary luteinizing hormone (LH) which is primarily responsible for the swelling and bursting of large follicles and the release of their ova. The ovaries also secrete steroidal hormones (estrogen and progestin) which act upon the neurohumor-producing hypothalamic tissues. The relatively elevated level of such steroid hormone production at and shortly after ovulation and particularly the marked postovulatory increase in progestin secretion lead to inhibition of the secretion of the LH-stimulating hypothalamic neurohumor and therefore of ovulation. All of the currently used oral contraceptives depend primarily on their effect as inhibitors of this hypothalamic pituitary ovulation mechanism. They all embody, furthermore, the original pattern found essential in our clinical studies—namely, a combination of estrogen and progestin (*20, 24, 25*)—since this not only inhibits ovulation but also assures adequate control of bleeding from the uterus. In the day 5 to 25 regimen of use menstruation usually occurs 2 to 5 days following the taking of the last tablet and a succession of very regular menstrual cycles is assured to the faithful user (*15*).

The most recent chemical developments in this field, therefore, have been concerned with the primary ingredient of oral contraceptives, the progestin. With the possible exception of one nonsteroidal ovulation inhibitor whose mode of action is not yet clear (*2*), all of these have been steroidal, and two major types of orally active progestins have emerged as effective ovulation inhibitors. The first of these, already mentioned, are the 19-norsteroids, examples of which are presented in Figure 2. The second are derivatives of 17-acetoxyprogesterone; some of these have not yet been fully tested as contraceptives in human subjects, but their relative potency as ovulation inhibitors in the rabbit is given in Figure 3.

Efficacy of Oral Contraception

Before discussing the clinical application of these steroidal agents it is well to keep in mind the extraordinary efficacy of this mode of contraception. In Table I we present the data assembled by Venning (*31*) on the relative efficacy of various contraceptive methods. All of those studied in various experimental trials effectively reduced fertility, but the oral contraceptive most extensively studied (the norethynodrel-estrogen combination) is much the most effective. Indeed, the pregnancy rate listed appears to be attributable to failure to use the method properly rather than to a rare physiological exception to the action of the drug.

The efficacy of oral contraception in practice was recently emphasized in a symposium held by the International Planned Parenthood Federation in Singapore. The reports made were summarized by Tyler (*28*) and his assemblages of the data are given in Tables II and III, which include the investigator, the name (or number) used by the manufacturer, the dosage of the

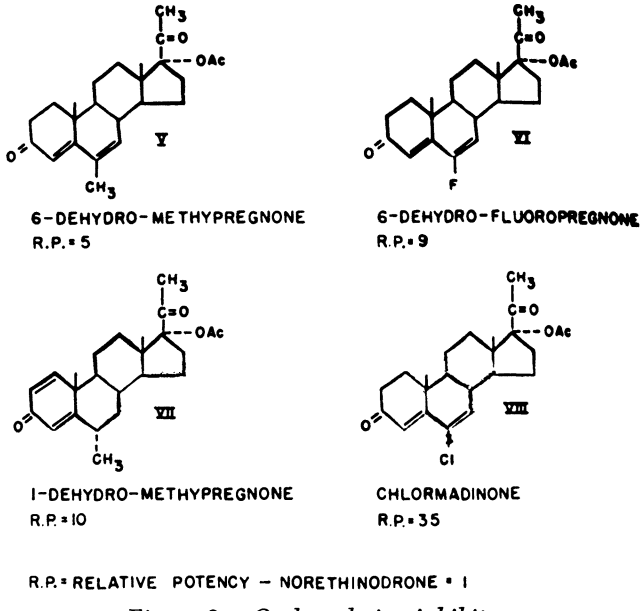

Figure 3. Oral ovulation inhibitors

Table I. Effects of Method of Conception Control

Method	*Average Pregnancies per 100 Woman-Years*
No contraception	115
Douche	31
Safe period (rhythm)	24
Jelly alone	20
Withdrawal	18
Condom	14
Diaphragm (with or without jelly)	12
Norethynodrel + estrogen	1.1

progestin component in milligrams and of the estrogen component in micrograms, the number of patients in the study, the number of cycles of use of the drug, and a resumé of reported results in terms of patients leaving the study (dropouts), alleged side effects, and the occurrence of pregnancies (PN). The frequency of occurrence of premature menstruation (BTB) is noted in one instance (with Provest, a combination of 6α-methyl-17-acetoxyprogesterone and ethinyl estradiol, in Table III). The estrogen usually employed is the 3-methyl ether of 17α-ethinyl-17β-estradiol (EE3ME) but often ethinyl estradiol (EE) is the estrogenic component. These tables are presented chiefly to indicate that a number of ovulation-inhibiting orally active drugs are in active experimental trial. The progestational components of the newer preparations (Table III) include not only 19-norsteroids—i.e., Lyndiol, Norlestrin, and SC-11800—but also 17-acetoxyprogesterone derivatives—i.e., Megestrol, Provest, and Chlormadinone. A detailed account of the studies referred to in Table III is given in the Proceedings of the Seventh International Conference on Planned Parenthood (*6*).

Table II Medical Session Reports (Singapore) on Norethindrone and Norethynodrel Combinations

Investigator	*Product*		*No. Pts.*	*Cycles*	*Results*
K. Menon, Madras	Enovid	2.5/150	75	692	Large % side effects, heavy dropouts
S. Chinnatamby	Enovid	2.5/100	100	1,626	1 early failure ? 1 failure, mod. side effects, generally successful
	Conovid		300		
E. Mears *et al.*, London	Conovid	5/75	216	3,267	About 20% dropout, no method failure
		2.5/100	262	2,718	
	Anovlar	4/50 EE	196	2,093	
J. Goldzieher (Texas), Rice-Wray (Mexico), Moses *et al.* (Ohio)	Ortho-N	10/60	210	8,000	<1% Rx dropout, no PN
		2/100	104	1,100	
Satterthwaite and Gamble Puerto Rico	Enovid	10/150 5/75 2.5/100	838	15,000	17.5% Rx dropout, 13 incorrect use PN
E. T. Tyler *et al.*, California	Ortho-N	10/60	567	16,000	
		5/75	172	832	About 5% total dropout, 1? PN
		2/100	412	4,700	
	Enovid	5/75	334	10,000	About 10% total dropout. No PN
		2.5/150	114	700	About 70% total dropout. No PN
	Gestop	5/50 EE	169	3,088	About 5% total dropout, no PN
		2.5/50 EE	141	571	10% dropout, no PN

Table III. Medical Session Reports (Singapore) on New Combinations, Progestins, or Dosage Schedules

Investigator	*Preparation and Dose*	*No. Pts.*	*Cycles*	*Results*
E. Mears *et al.*, London	Megestrol (2/0.05 EE to 5/0.1 EE) Lyndiol, SC-11800, Norlestrin, and Chlormadinone sequential	243	996	Generally good
Goldzieher (Texas), Moses (Ohio), Martinez *et al.* (Mexico)	Chlormadinone (2 mg.)	467	4793	Generally good, no PN
	Chlormadinone sequential	938	6314	Good acceptance, 3 PN
E. T. Tyler *et al.*, California	SC-11800 (1/0.1 EE3ME)	163	1500	About 5% total dropout, no PN
	Provest (10/0.1 EE, 10/0.05 EE, 2.5/.025 EE)	173	1900	BTB excessive, 25% dropout, no PN
	Chlormadinone sequential (2/80)	40	700	About 4% total dropout, 1? PN
	Ortho-Novum sequential (2/100)	30	600	About 2% total dropout, no PN

In this list the outstanding fact is the almost perfect record of contraception for each. For purposes of contraception, therefore, there appears to be no differentiation between them despite divergences in molecular structure. The lowest daily dose listed is that of 1 mg. for SC-11800, which is compound X of Figure 2. But the sequential administration of Chlormadinone (compound VIII of Figure 3) and of Orthonovum (compound I of Figure 2) involves

administration of the estrogenic component alone for 15 days and then of the progestin-estrogen combination for 5 days to give the 20-day medication period. Therefore, 10 mg. of the progestin per cycle is used. On the other hand, Chinnatamby (3) finds 0.5 mg. of X plus 0.1 mg. of EE3ME in the day 5 to 25 regime (or a total of 10 mg. of progestin per cycle) to be just as effective as the 1 mg. per day dose listed in Table III. In short, relative contraceptive potency in the human of these various preparations is not yet effectively defined.

If we cannot be certain of relative contraceptive potencies in relation to molecular structure, we can attempt to compare other measurable phenomena in women using oral contraceptives. Such an attempt has been made in a comparative study of three of these drugs by Satterthwaite in Humacao, Puerto Rico (4). In Table IV we list the three drugs studied, the dosage of the progestin contained in each, the number of patients enlisted in the use of each, and the rate of dropout of users in the course of 11 medication cycles. The reasons for discontinuance are often irrelevant to the acceptability of the medication—e.g., moving to a distant city, widowhood, desire to have another child, etc.—but those reasons which involve rejection of the medication—e.g., alleged side effects such as nausea, headache, requests for other forms of contraception, etc.—have been consolidated to give the data of the last column of Table IV. Considering the numbers involved, the rejection rate is statistically identical for the three drugs.

Table IV. Statistics of Comparative Study

Dose, Mg.	*Total No. of Patients Admitted*	*No. Discontinuing in 11 Cycles*	*% Discontinuing*	*% Rejecting Method as Such*
Enovid, 2.5	209	76	36	16
Orthonovum, 2	209	78	37	13
Ovulen, 1[a]	182	56	31	13

[a] Ethynodiol diacetate (compound X, Figure 2) plus 0.1 mg. of 17α-ethenylestradiol-3-methyl ether.

Similarity of effects is suggested when we consider other phenomena reported by the volunteer subjects. Thus in Table V are listed their information on the nature of the menstrual flow and the incidence of dysmenorrhea at menstruation; the data are statistically identical. Each patient reported for each cycle the duration of the menstrual flow in days, and the mean values are summarized in Table VI. The average durations reported for Orthonovum and Ovulen are probably significantly different from the mean for Enovid users, but the difference is not very great. In a number of other parameters measured—e.g., cycle lengths, weight changes, hematocrit and hemoglobin values, etc.—no obvious differences among the three groups have emerged. Even the complaints voiced by the users of these three preparations have been remarkably similar (Table VII). During this study 28 to 32% of the contraceptive users complained of symptoms which they attributed to the medication. Approximately 70% of the women had no complaints, although each was questioned every month for any possible symptoms. When these symptoms are classified as in Table VII into gastrointestinal phenomena, such as nausea and gastralgia, subjective symptoms such as nervousness and tension headaches,

and so on, it is obvious that little difference exists on any basis in the three groups. The use of each of these drugs, therefore, leads to a similar frequency of occurrence of "side effects." As we have previously pointed out on the basis of a double-blind study (*16*), practically all of these side effects appear to be psychogenic, which probably accounts for the similarity of the data of Table VII in the three groups.

Table V. Comparative Study of Menstrual Phenomena

	% Reporting					
	Amount of Flow			Pain and Distress		
Compound	Light	Average	Heavy	None	Slight	Moderate to severe
Enovid	14	80	6	69	19	12
Orthonovum	18	78	4	68	20	12
Ovulen	14	81	5	71	19	10

Table VI. Comparative Study of Menstrual Phenomena

	Mean Duration of Flow, Days		
Cycle No.	Enovid	Orthonovum	Ovulen
1	4.4	4.2	3.8
2	4.2	3.7	4.0
3	4.0	3.6	3.8
4	4.0	3.7	3.7
5–8	3.8	3.6	3.4
9–12	4.0	3.5	3.4
13–17	3.9	3.3	...
All cycles	4.0	3.6	3.7

Table VII. Comparative Study of Various Symptoms

	% Complaining		
Symptoms	Enovid	Orthonovum	Ovulen
Gastrointestinal	13	10	10
Subjective	20	19	14
Vaginal and lower abdominal distress	6	7	6
Breakthrough bleeding	6	1	2
All symptoms	28	32	28

Some Physiological Effects of Oral Contraceptives

There are, indeed, physiological phenomena of considerable interest involved in the use of contraceptive progestin-estrogen combinations, because as hormonally active compounds they have specific effects similar to those of the natural ovarian steroids. In the use of these preparations for ovulation inhibition we in fact imitate the ovulation-inhibiting action of the ovarian steroids evident in the luteal phase of the menstrual cycle and in pregnancy. It is as if the luteal phase is initiated on the day pill taking is initiated—i.e., approximately 9 days before the usual time. Again since progestational steroid is acting for an extra 9 days, it is as if we have a hormonal milieu toward the end of our medication period equivalent to the first 9 days of pregnancy. The appearance of the uterine endometrium, a prime target for estrogen and

progestin, suggests this (*20, 25*). Since ovulation is inhibited and a mild pseudopregnant state is induced in every month of use, the question has been asked if this might not affect fertility following discontinuance. After many months of use of Enovid women discontinuing it appear to have an enhanced fertility (*16, 21*). A similar more rapid than usual rate of conception has been reported following discontinuance of Orthonovum (*5*). In experimental animals receiving large doses of norethynodrel the ovulation following discontinuance yields more than the expected number of ova (*7*). Therefore it is perhaps not surprising to find Satterthwaite and collaborators (*27*) presenting data on enhanced conception rates in users (Table VIII). It is conceivable that the regular monthly use of a balanced estrogen-progestin combination produces an oviducal environment and uterine tone rather uniformly favorable to fertilization and ovum implantation. This possibility, coupled with a pituitary-ovary relationship favorable to prompt ovulation, may perhaps explain the apparently enhanced posttreatment fertility.

Table VIII. Fertility of Enovid Users in Humacao

	No. of Subjects	*Pregnancies per 100 Years of Exposure*
Before use	838	117
During use	838	1
After withdrawal	239	233
Those withdrawing after 1 to 4 years of use	101	277

One of the physiological states clearly conditioned by circulating progestin and estrogen is lactation. A special substudy of the effects of Enovid on lactation in women has been made by Satterthwaite, who has initiated the administration of this preparation in women at 3 to 5 weeks postpartum. Her data are summarized in Table IX. It is clear that at a dose of 20 mg. per day a majority of the subjects (72%) reported some reduction in lactation and at 2.5 mg. per day the majority (70%) found no change in lactation rate. At intermediate doses the data suggest some reduction of lactation in a minority (38 to 45%). Since the dose of 2.5 mg. per day is just as effective as the higher doses in inhibiting ovulation (*12, 13*), it is clear that lactation may be scarcely affected at efficient contraceptive doses. This may have special significance for countries where breast feeding of infants is an accepted culture pattern.

Table IX. Effects of Enovid on Lactating Women in Humacao

Dosage, Mg./Day	*Total No. Followed Up*	*% Lactating*		
		Less than previously	*Same as previously*	*More than previously*
20	22	77	18	5
10	37	38	57	5
5	84	45	45	10
2.5	34	15	70	15

We have previously reported on variations in a number of physiological functions in women using oral contraceptives. These have varied from measurements of liver function to blood counts to blood cholesterol determinations.

Generally no significant variations have been found associated with either the daily dosage of the drug used or the number of years of use. We have recently measured the blood pressures of women using Enovid and of women in the same areas who have not used the drug. Table X illustrates the striking similarity in these variables in two dosage groups and in nonusers.

Table X. Mean Blood Pressures of Enovid Users and Controls

Examined in Haiti and Puerto Rico—December 1963

Dose of Enovid, Mg.	*No. of Patients*	*Systolic*	*Diastolic*
None (controls)	64	108.6 ± 2	68.3 ± 1
2.5	60	108.2 ± 2	68.1 ± 1
5.0	117	110.6 ± 1	70.9 ± 1

Of some concern recently has been the possibility of an enhancement of the occurrence of thromboembolism in users of progestin-estrogen combinations. Among users of Enovid there is a significant decrease in mean blood clotting time in either short-term or long-term users (Table XI). However, at a conference of experts (*32*) this degree of change in clotting time was considered to lack significance for thromboembolism induction. At this same conference the frequency of known cases of thromboembolism in Enovid users appeared to be not significantly greater than for women generally (Table XII). More recently a committee of experts has examined all cases of death from thromboembolism reported for Enovid users and has compared the incidence rate with similar data from mortality statistics in a large number of the United States death registration areas (*29*). No significant difference in the mortality rates between the two groups has been found. An initial statistical misstatement about older women has been corrected (*30*).

Table XI. Bleeding and Clotting Times, Capillary Method

Years of Use	*No.*	*Mean Bleeding Time, Sec.*	*No.*	*Mean Clotting Time, Sec.*
None (controls)	38	111 ± 8	42	295 ± 9
0–2	127	103 ± 4	127	246 ± 7
2–4	102	116 ± 4	107	255 ± 8
4–6	17	148 ± 14	17	271 ± 12
Withdrawn	11	101 ± 18	11	276 ± 28

Table XII. Incidence of Pulmonary Embolism in North America

	Cases per Million per Year
General population, all ages, both sexes	100
Male population, all ages	99
Female population	
All ages	103
Nonpregnant of childbearing age	60–230
Nonpregnant of childbearing age, hospitalized	265+
Nonpregnant using Enovid	59

Our concern about peripheral thromboembolism led us to determine the occurrence of varicosities in a large sample of our volunteer subjects and in a group of controls. The data (Table XIII) indicate a reduced frequency of

this condition in Enovid users at each of two dose levels. Since reduction is highly significant with long-term users (4 to 6 years) of Enovid, we have deduced that the absence of pregnancy may be the major factor in the lowered frequency.

Table XIII. Varicosities of Enovid Users and Controls Examined in Puerto Rico and Haiti in December 1962

Dose of Enovid, Mg.	*No. of Patients*	*% with Varices*
None (controls)	63	49.2 ± 6.3
2.5	58	29.5 ± 6.0
5.0	106	28.3 ± 4.5
Years of Use		
Controls	63	49.2 ± 6.2
0.2	23	26.1 ± 9.2
2–4	79	31.7 ± 5.2
4–6	62	25.8 ± 5.5

One significant effect of pregnancy or of the elevation in circulating steroid characteristic of pregnancy is an increase in the concentration of protein-bound iodine (PBI) in the blood (*9*). We have observed a significant rise in PBI in Enovid users at each of the contraceptive doses employed (Table XIV). Our data indicate also that patients ceasing to take Enovid (withdrawn in Table XIV) show reversion to control subjects' PBI values. This may be interpreted either as a stimulation of thyroid activity or as an increase in thyroid hormone-binding protein in the blood. To resolve these possibilities we have recently measured the thyroid uptake of radioiodine by women in Puerto Rico. Our data (Table XV) show no significant difference in this uptake at two dosage levels of Enovid and no significant differences among nonusers, short-term ($1^1/_2$ to $2^1/_2$ years) users, and long-term ($2^1/_2$ to 7 years) users. Here again we see the expected hormonal effect of the administered steroids, not an unexpected action upon the thyroid as a target organ. We have also studied radioiodine uptake in women just before and at 3 to 4 months after the initiation of use of Enovid and Ovulen. The data (Table XVI) confirm the findings of Table XV, despite a marginally significant reduction in uptake in one group of Ovulen users.

To inquire further into the previously reported unexpectedly low frequency of suspicious vaginal smears and of cervical malignancy in volunteer subjects taking Enovid (*13*), we have in the past two years obtained cervical and vaginal pool smears from approximately 10,000 women. A summary of the data on the Papanicolaou smears is presented in Table XVII. The "control" subjects are women coming to our clinics in Puerto Rico and Haiti, who have never previously used contraceptives. The prevalence of suspicious smears among them is high—i.e., 3.6% of these women have abnormal cell types in the exfoliative cells. A similar high prevalence rate for women in Puerto Rico has been noted by Lee, Melnick, and Walsh (*8*). Among the users of intravaginal contraceptive foams observed in the course of one year the frequency is somewhat lower, 19.7 per 1000 women users. Users of Enovid or of other oral progestin-estrogen combinations exhibit lower frequencies, which on the basis

Table XIV. Blood Chemistry

Subjects	Cholesterol		Protein-Bound Iodine		Total Iodine	
	No.	Mg. %	No.	Mg. %	No.	Mg. %
Controls	12	233	13	4.8	13	5.7
Withdrawn	26	224	13	4.6	15	4.7
10 mg.	3	227	2	8.0	2	8.8
5 mg.	229	235	74	6.7	72	7.7
2.5 mg.	57	251	24	7.1	23	8.4

Table XV. Radioiodine Uptake

Group	Dose, Mg.	Years	No.	Mean ± S.E.	P
Control	...	...	40	15.565 ± 1.382	...
	2.5	$1^1/_2$–$2^1/_2$	19	22.147 ± 8.218	>0.4
Enovid		$2^1/_2$–7	37	19.476 ± 9.915	<0.5
	5.0	$2^1/_2$–7	51	18.994 ± 7.676	<0.5

Table XVI. Radioiodine Uptake

Group	Dose, Mg.	No.	Mean		Mean of Differences	P
			Before	After		
Enovid	2.5–5.0	36	19.4138	17.6750	—1.5333	<0.2
Ovulen	1.0	20	17.7900	14.9700	—2.6700	<0.025
	2.0	16	21.6687	19.8000	—1.4812	>0.2

of the numbers of years of use of the contraceptive may be deduced as incidences $^1/_5$ to $^1/_4$ that found for the foam users. The occurrence of definitive malignancies in these patients is still too infrequent to permit meaningful comparisons.

Table XVII. Prevalence of Class III to V Papanicolaou Smears

	Total Patients	Class III to V	Prevalence per 1000	Average Years of Use
Controls	2786	101	36.2	...
Enovid users	2850	47	13.0	3.2
All other pills	3403	31	9.4	2.1
Foams	657	13	19.7	1.0

We hope that proper study of true annual incidences over a period of years will afford statistically valid data on the effect, if any, of the use of these types of oral contraceptives upon cancer of the reproductive tract and other normone-sensitive organs such as the breasts. Thus far all investigators using these preparations have had no indications of an increase in malignancies (27, 28). The data of Table XVII suggest a reduction in the occurrence of abnormal tissue growth in the tissues contributing to the vaginal and cervical smears. How general an effect this may be remains to be determined. Both norethynodrel and norethinodrone have been found to cause objective remissions in some cases of advanced breast cancer in women, whereas progesterone itself has had no such effect (22). Perhaps the most sensible deduction from available data is that the regularly repeated hormonal milieu created by the cyclic administration of a fixed estrogen-progestin combination minimizes

marked fluctuations in growth stimulation to target tissues, thereby reducing the probability of abnormal growth responses.

Conclusions

In reviewing the effects of ovulation-inhibiting progestin-estrogen combinations, we have not learned much about the possibility that molecular variations in the steroids used contribute to physiological variations. In large measure this is due to the absence of adequate comparative data. With the advent of a significant number of drugs of this type the occurrence of deviations in effect correlated with structural variation may become more evident. On the other hand, in the area of their primary effect a remarkable similarity in efficiency has thus far been demonstrated. Perhaps the future will give us more information about relative potencies of different drugs in human subjects, but this will yield quantitative, not qualitative, differentiation. Besides this primary contraceptive effect, these drugs have a number of physiological effects. There are two outstanding features to these effects: Thus far none has been pathological and all are accountable to the intrinsic hormonal properties of the constituent compounds. In brief, they act in the same manner as endogenous progestin and estrogen, differing only in the time relations and uniformity of their actions. The long-run effects of the regular regimen of use remain to be ascertained, but thus far no significant differences between long-term and short-term users have emerged. We undoubtedly have at hand the most effective contraceptive method yet devised. Its implications for health and well being must eventually emerge, because its world-wide use is now a fact.

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16

Synthetic Progestational Agents

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For unraveling the complex structure of the steroid nucleus, the Nobel Prize in chemistry was awarded jointly to Wieland and Windaus in 1928. Steroid chemistry then became a highly competitive area of drug research and development. Through molecular manipulation, important improvements followed each other to provide today a versatile selection among effective and specialized agents, most of them only 5 or 10 years out of the laboratory. Resisting until recently the joint attack by chemist, biochemist, biologist, and clinical investigator are the "sex hormones" known as progestins. Combining characteristics of either anabolic-androgenic agents or anti-inflammatory corticoids, progestins have opened new horizons in medical thinking. From maintenance of pregnancy to contraception, significant molecular modifications have made progestins better medicine.

In tracing the impact of molecular modification on the evolution of steroid research and the development of markedly improved therapeutic agents, we hope to judge whether molecular modification, among other research techniques, is still a valid tool for drug development. Although anti-inflammatory steroids provide excellent illustrations of the power of molecular modification, recent symposia have adequately covered this area of steroid research, and selected topics in the field of sex hormones are reviewed here instead.

We define molecular modification as "the synthesis of compounds which bear a close structural similarity to known drugs or to compounds of known biological activity, in search of related drugs significantly better than the parent substance." The parent substance may be a useful and approved drug or a biologically interesting chemical (a "lead") which for various reasons either has not yet been used successfully or cannot be used as a drug.

Molecular modification then may be undertaken to increase potency or decrease side effects or inherent toxicity; to alter the spectrum of activity where

an agent produces several effects; to produce a more rapid effect, a delayed effect, a sustained effect; or to provide critical blood levels or cross an important tissue barrier. Molecular modification may alter the rate or extent of gastric or intestinal absorption, provide an oral form of an injectable drug or a long-acting form of a short-acting drug, or yield a drug which will reach one target but not another. In approaching these problems, the chemist-biologist–clinical pharmacologist team often turns therefore to molecular modification as one of a number of possible approaches designed to provide better medicine. It is rare that a drug achieves wide clinical use without emerging from a sizable research program of intensive molecular modification. It is also rare, because undue preoccupation over effects in animals is increasingly replacing the cautious, well-controlled early trial in man, to find drugs which cannot ultimately be improved by molecular modification.

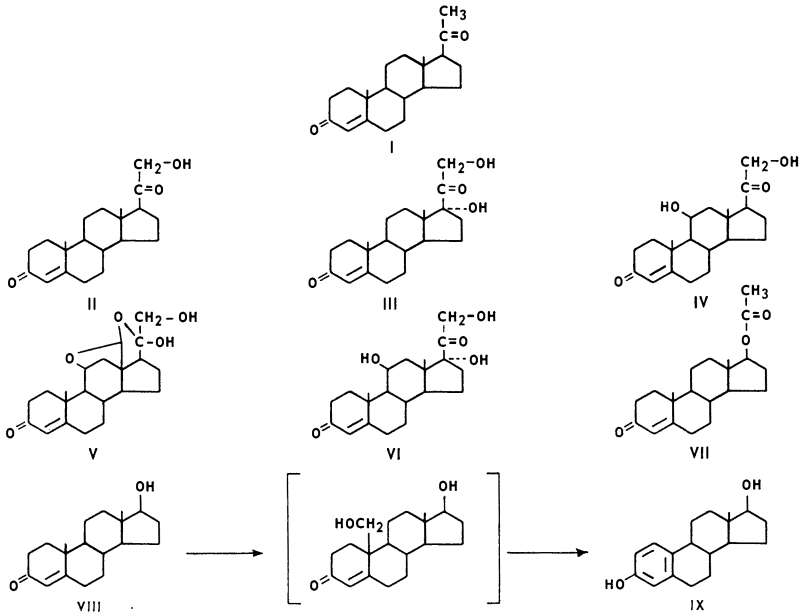
Other approaches to drug development, of course, are important. These include broad screening of random chemicals for biological activity, an essential but costly process, a prime illustration of which is the extensive screening program at the National Cancer Institute (CCNSC). But the leads from such broad screens are generally studied and expanded by molecular modification before a particular agent is selected for extensive biological or clinical studies.

Another approach is the detailed and painstaking investigation of natural processes and the natural products which influence them, often taking years of devoted effort, even lifetimes. Such studies have often led, and will continue to lead, both to useful drugs and to important breakthroughs in understanding. But here again, molecular manipulation can be expected to improve the best that nature has yet provided.

Progesterone and Natural Modifications

This was the case with progesterone, one of the steroids produced in the corpus luteum or yellow body which develops at the point of follicle rupture after release of the ovum approximately every 28 days in the normally functioning human ovary. Removal of this tissue from the ovary of the rabbit sometime after mating prevents nidation or terminates pregnancy (*34, 104*). In 1928, Corner and Allen showed that secretory changes in the endometrium normally seen in animals after ovulation could be blocked by removal of the corpus luteum and restored by administration of corpus luteum extracts (*18*). The same year, the Nobel Prize in chemistry was awarded to Wieland and Windaus for their classic work in unraveling most of the essential features of the steroid nucleus. In the exciting 6 years that followed, certain errors in assignment of the steroid structure were corrected and, stimulated by the knowledge that progesterone was essential in the rabbit for the maintenance of pregnancy, investigators isolated and purified the principal active product in the crude extract of corpus luteum tissue from sow ovaries, identified it as a steroid, and correctly deduced its complete structure. Almost simultaneously, the partial synthesis of progesterone in low yield from available sterol sources was announced (*34*). Today, 30 years later, progesterone and its precursor, pregnenolone, are recognized as major building blocks not only for the steroid chemist and pharmaceutical manufacturer but also for the endocrine glands themselves.

Contrary to popular opinion, as an essential tool in drug development, molecular modification is not the creation of the synthetic chemist, but rather a natural process essential to life itself. For it is through simple molecular modification that drugs and hormones are "detoxified" in the body and excreted, that active agents are made from inert precursors, and that agents with one property are converted to closely related materials of very different activity.



In the adrenal gland, it is through molecular modification that progesterone (I) affords deoxycorticosterone (II), an agent with only slight progestational activity in animals but with important and marked sodium-retaining properties (a molecular modification of progesterone—and yet progesterone is a potent antagonist of the salt-retaining properties of deoxycorticosterone) (*64*).

It is also through molecular modification, introduction of two hydroxyl groups, that progesterone is transformed in the adrenal gland to Compound S or cortexolone (III) (an intriguing but highly insoluble substance for which biological importance has so far been elusive but which is found in excessive amounts in patients suffering from congenital adrenal hyperplasia with hypertension) (*29*), on the one hand, and to Compound B or corticosterone (IV), the principal glucocorticoid in the rodent, on the other. Through molecular modification, aldosterone (V), the extremely potent salt-regulating hormone recently identified and synthesized so elegantly in the laboratory (*6, 48, 99*), is formed in one zone of the adrenal gland while hydrocortisone or cortisol (VI), the principal glucocorticoid in man and parent substance for all the more potent, more selective synthetic steroids that have provided partial relief for the symptoms of rheumatoid arthritis, is formed in another.

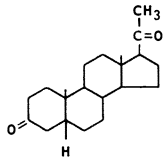
It is through molecular modification that progesterone (generally considered to be one of the two basic "female" hormones) is converted by microorganisms (and perhaps in part by man as well) to testosterone acetate (VII)

(*24*, *37*) and thence, through hydrolysis, to the male hormone, testosterone (VIII), which is responsible for the development of the male secondary sex characteristics. Similarly, by a sequence of simple modifications, the principal male hormone is degraded (hydroxylation at C_{18}, loss of formaldehyde, and dehydrogenation) to the principal female hormone, estradiol (IX), which, in concert with progesterone, is necessary for development of female sex characteristics.

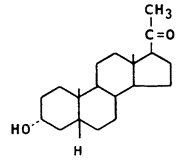
Thus, through minor changes, beautiful in their simplicity yet dramatic in their effect, nature has excelled in molecular modification and man has learned from it. The living cell has transformed progesterone into a host of different modifications essential to its over-all economy, and through molecular modification, the chemist has done likewise.

Biologic Effect and Metabolic Modification

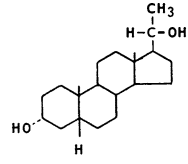
When progesterone is administered intravenously to man, it is rapidly cleared from the blood, but metabolites appear in the urine only after considerable delay. Some evidence indicates that progesterone is rapidly extracted into fat or lipides (*80*, *81*); other evidence suggests that it is tightly bound to protein (*9*, *80*, *89*, *100*). While both may be true, there is no doubt that it is readily metabolized to other agents, some of which are important hormones, others not so well characterized biologically, and still others believed to be waste products. These metabolites include 5β-pregnanedione (X), in which the double bond is reduced, pregnanolone (XI), in which the 3-ketone is reduced as well, and pregnane-3α,20α-diol (XII), the completely reduced end product usually found in the urine as a glucuronide, levels of which provide a diagnostic index of whether ovulation (and hence corpus luteum formation) has occurred.



X



XI



XII

These "metabolic waste products" are not devoid of biological activity and may account for some of the properties attributed to progesterone.

Pregnanedione, a potent depressant of the central nervous system, has been called a "steroid anesthetic" (*94*, *95*). Given to mice, it produces a deep sleep which continues until the material is dissipated. Molecular modification of this chemical afforded hydroxydione sodium succinate, a clinically useful, short-acting steroid anesthetic with muscle-relaxant properties (*66*, *76*). When large doses of progesterone are given clinically, depression, drowsiness, and even sleep have similarly been induced (*73*).

Pregnanedione and pregnanolone produce a pronounced but temporary fever when given intramuscularly or intravenously to man, an effect so far not produced in experimental animals (*56*, *57*, *75*). Likewise, progesterone elevates basal body temperature in man (*36*, *52*).

When given orally, however, these agents produce no fever (*57*). At

reasonable doses, oral progesterone similarly produces little biological activity. It is believed that lack of activity by this route is largely the result of rapid metabolism and conjugation by the liver.

Pregnanedione (5β, but not 5α) is known to block both conductivity and concerted contraction in muscle strip from rabbit myometrium in vitro (*97*). In this respect, it mimics progesterone, which is believed to maintain pregnancy similarly by blocking conductivity and concerted contraction of the uterine musculature in vivo (*19*).

It has been suggested that pregnane-3α,20α-diol, produced endogenously through metabolism of increasing levels of progesterone, may provoke an auto-immune reaction responsible in part for periodic bouts of dysmenorrhea (*47*).

Recently it has been shown that pregnane-3α,20β-diol, found occasionally in mothers' milk, is responsible for persistent jaundice sometimes seen in their newborn children (*41*).

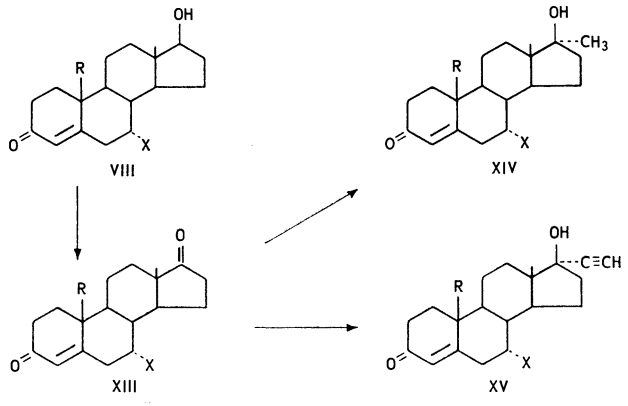
In short, the biological spectrum of progesterone is the sum total of the events initiated by progesterone itself as well as by its metabolic products and is influenced by its physical form, route of administration, site of metabolism, blood and tissue levels, type of binding, etc. Among the biologic events attributed to progesterone and its metabolites are:

Preparation of uterine endometrium for implantation of the fertilized ovum.
Maintenance of the uterus during pregnancy.
Modification of the myometrium to block spontaneous concerted contractions.
Participation in breast development.
Inhibition of gonadotropin release or production.
Inhibition of spermatogenesis and ovulation.
Elevation of basal temperature or production of fever.
CNS-depression, sleep, or anesthesia.
Lowering of blood pressure.
Auto-immune sensitivity.
Inhibition of aldosterone- or desoxycorticosterone-induced sodium retention.
Occasional production of jaundice; and a variety of other associated effects.

It, therefore, seems clear that the molecular modification of progesterone, altering its solubility, distribution ratio, polarity, protein- binding characteristics, steric requirements, crystalline structure, and rate of metabolism (as well as the nature of the metabolic products themselves), would alter some of the properties of progesterone, eliminate some, and introduce new and unexpected properties, many of which may not be evident even after extensive animal studies.

Synthetic Modifications

One of the earliest and most important observations was the result of a molecular modification study which produced a totally unexpected result. In 1938, Inhoffen found that 17-ethinylestradiol was a more effective oral estrogen than estradiol itself (51). When this modification was applied to testosterone (VIII, R = CH_3, X = H), in an effort to prepare an orally active androgen, the 17-ethinyltestosterone (XV) so produced (*58*, *88*) was found instead to be a potent oral progestogen, and is still used clinically in this field.



Attention was directed to the 19-norsteroid class (R = H) when a mixture of products obtained by Ehrenstein from strophanthidin, and believed to contain 19-norprogesterone, was found to be five to eight times as potent as progesterone (*4, 21, 31*). Actually, the principal ingredient was later found to possess unnatural configuration at C_{14} and C_{17}. Other progestins with unnatural configuration and unusual properties include the so-called "retro-progesterones" (isomeric at C_9 and C_{10}), of which dydrogesterone has found clinical use and is reported to produce endometrial effects without inhibiting ovulation (*3, 84, 92*).

Authentic 19-norprogesterone was prepared by Djerassi and coworkers at Syntex (*22*), who also prepared the 19-nor derivatives of 17-methyltestosterone (XIV) and 17-ethinyltestosterone (norethindrone, XV) (*23*). Both agents proved to be highly potent progestins and the latter has been incorporated with estrogens into a variety of contraceptive formulations (*78*). Acylation affords a further increase in potency, and norethindrone acetate has also been combined with estrogens to produce an effective contraceptive (*32, 72, 78*). The $\Delta^{5(10)}$ isomer of XV, norethynodrel, prepared by Colton *et al.* at Searle (*15, 17, 29, 91*) was, however, the first agent to receive wide acceptance as an antifertility preparation (Enovid), and to this group, along with Gregory Pincus and John Rock, must go the principal credit for pioneering a physiologic approach to contraception—a new use for improved agents which would have been less successful, if not impossible, prior to the application of molecular modification techniques to progestational steroids.

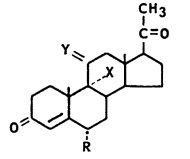
Although norethindrone, norethindrone acetate, and norethynodrel differ slightly in biological properties in animal experiments, all three agents are known to be converted, at least in part, to 17-ethinyl-19-nortestosterone in vivo (*8, 25, 26, 68*).

For over a decade, hormonal activity of any consequence was thought to be limited to steroids containing a Δ^4-3-keto group. A recent development of much interest, therefore, is the removal of the 3-ketone from VIII (R = X = H) via thioketal formation and reduction with sodium in liquid ammonia. Subsequent oxidation and ethinylation afforded the 3-deoxy derivatives of XIII and XV (*20*). The latter, lynestrenol, possesses marked progestational properties. High potency was also found when the ketone (XV, R = X = H) was reduced

to the 3β-alcohol, whose 3,17-diacetate (ethynodiol diacetate) (*16*) has also been incorporated into a contraceptive formulation (*78, 79*). Other modifications investigated include the 2-fluoro-, 6-fluoro-, 6-chloro-, 6-methyl-, and 4-methyl- analogs of 17-ethinyltestosterone (*1, 7, 13, 30, 50, 60, 98*) and the 1-methyl-, 4-chloro-, Δ^6-, 6-chloro-Δ^6, and 21-methyl-, fluoro-, and chloro-derivatives of 19-nor-17-ethinyltestosterone (*38, 60, 70, 87*). Unfortunately, clinical use of a number of 17-alkyl steroids has been associated both with occasional reversible impairment of liver function and with occasional occurrence of fetal virilization (*2, 10, 25, 35, 45, 49, 53, 54, 61, 62, 63, 71, 74, 91, 101, 102*). While these properties have not deterred wide clinical use, they require caution and careful evaluation when new molecular modifications of this class are studied.

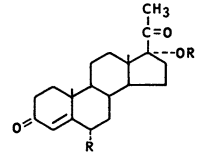
A recent development of considerable interest is the very substantial increase in potency found for compounds VIII, XIII, XIV, and XV when the angular methyl group (R) is transferred diametrically across ring B to the α-side at C_7 to produce 7α-methyl-19-nor steroids such as XIV and XV (R = H, X = CH_3), rather than conventional 19-norsteroids (R = X = H) (*14, 67, 93*).

Another approach to molecular modification of progesterone became possible with the discovery that progesterone could be transformed in high yield to oxygenated products with microorganisms (*33, 77*). The 11α-hydroxy derivative of progesterone was found to lack progestational activity but had instead antiestrogenic properties (*12*). The 11β-hydroxy epimer, on the other hand, retained weak progestational properties and exhibited some weak glucocorticoid activity (*11*). In an effort to prepare 11β-OH steroids from available 11α-hydroxy precursors, in what must be regarded as one of the most significant developments in steroid chemistry, Fried and coworkers at Squibb obtained a $\Delta^{9(11)}$ olefin, and from it a series of 9-halo-11β-hydroxy and 11-keto derivatives (*39, 40*). The 9-bromo-11-keto compound (XVIa) exhibited oral progestational activity and was studied widely as a possible agent for the treatment of breast cancer (*42*). A 9-fluoro derivative (XVIb) exhibited progestational, corticoid, and salt-retaining properties (*39*). Introduction of a 6α-methyl substituent (XVIc) produced a remarkable increase in progestational and anti-inflammatory properties while decreasing sodium-retaining side effects (*96*).



XVI

a. R=H, X=Br, Y=O
b. R=H, X=F, Y=OH
   |---H
c. R=CH_3, X=F, Y=OH
   |---H



XVII

a. R=R=H
b. R=H, R=$C_6H_{13}CO$
c. R=H, R=CH_3CO
d. R=CH_3, R=CH_3CO

Among the many naturally occurring processes leading to molecular modifications of progesterone and its precursor, pregnenolone, is the formation of the 17α-hydroxy derivative. Although 17α-hydroxyprogesterone

(XVIIa) was essentially inactive as a progestin when administered exogenously, it was discovered by Salhanick to be 60 times as potent as progesterone when administered directly onto the rabbit endometrium in the Hooker-Forbes assay (*90, 103*). Junkmann, at German Schering, in an extensive study of derivatives of 17α-hydroxyprogesterone, found that long-chain fatty acid esters, such as the caproate ester (XVIIb), were not only active progestins on injection but exhibited a prolonged duration of activity (*55*). Clinical trial then confirmed activity in man as well as freedom from pain on injection previously associated with injectable progesterone. The 17-acetate was studied but rejected because of its low solubility in oil.

Independent studies in the Upjohn laboratories revealed, however, that 17α-acetoxyprogesterone (XVIIc), prepared earlier by several groups, was a highly active oral progestin in animals and man, and it was subsequently made available for clinical use. Molecular modification of this material has since afforded a variety of similar and highly potent progestins. Of these, the 6α-methyl derivative (medroxyprogesterone acetate, XVIId) has found wide clinical use. Unlike the 19-nortestosterone derivatives described earlier, medroxyprogesterone acetate has not been associated in clinical use with virilization of the human fetus (in contrast to its effects in several lower animals) nor with hepatic aberration (*43, 44, 83, 85*). Instead, it has provided for the first time a progestin which can be used both orally and parenterally and which is outstanding for its safety, potency, and effectiveness. Provest, a combination with a low dose of estrogen, affords a highly effective contraceptive formulation (*82*). Later modifications of 17α-acetoxyprogesterone at C_6 and C_{16} continue to afford progestins of interest, including chlormadinone (the 6-chloro-Δ^6-derivative) (*86*), megestrol acetate (6-methyl-Δ^6-derivative), and melengestrol acetate (16-methylene-6-methyl-Δ^6-derivative) as well as other 6,16-disubstituted compounds (*28, 46, 59*).

New Applications

With newer, more powerful, and more selective agents, new and imaginative approaches to medical and veterinary problems become possible which were scarcely considered 10 and 20 years ago. Recent reports show promise for prolonged but reversible suppression of spermatogenesis (*69*) and temporary elimination of the inconvenience of menstruation (*5*). Another new use is found in the delay or inhibition of premature puberty (*62, 65*). Some progestins are under investigation for their ability to produce regressions of hormonally dependent tumors. Many are being studied in veterinary fields for the synchronization of estrus in entire herds of farm animals to permit wider use of artificial insemination and selective breeding (*105*).

Space does not permit enumeration of the entire range of molecular modifications of progestins nor of the scope of their potential usefulness in a host of new applications made possible through molecular modification. Certainly new agents will be found which will provide better medicine, and these agents in turn will give way to newer and still better drugs. This is progress. And if, during biological evaluation, new and troublesome side effects are encountered, molecular modification will provide a method of choice for their elimination.

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Discussion

JOHN C. BABCOCK, presiding

Dr. Babcock: To get the ball rolling, Dr. Pincus, I would like to ask a simple question that has puzzled me for some time. Can an estrogen or progestin alone do the job that combinations are doing so successfully now? Since we have had estrogens and progestins available for 30 years, is it possible that they could have been used as successfully as the newer molecular modifications of these compounds now appear to be?

Dr. Pincus: Both will inhibit ovulation when given to appropriate test animals and the human female. However, there are disadvantages in the use of each alone, and the main disadvantage is that the very regular succession of menstrual cycles, that one gets by this 20-day use and then discontinuance of the medication, cannot be had normally if you use just progestin alone or estrogen alone.

For example, in the use of progestin alone, which Dr. Rock and I attempted a number of years ago, we get with increasing use more and more of the short menstrual cycles, indicating that there is a true physiological escape. With the estrogens alone, many investigators reported not only escape but, when with-

drawal is practiced, very often rather heavy menstrual flow compared to the light menstrual flow that we get with these combinations.

The other feature which involves the use of these compounds—the natural hormones, for example—is that progestin as such is a very poorly active substance when taken by mouth. Dr. Rock and I had to go up to 300 mg. per day, and we did not get complete control with that kind of dose.

Progestin by injection is fairly effective, but even there you need fairly high doses and you get menstrual escape.

The estrogens are more effective by mouth, and again by injections, as I told you.

Actually everybody agrees that the combination gives you much better control.

You may have noticed that I talked about sequential treatment, particularly with the chlormadinone, but there are others. This involves giving the estrogen for perhaps 15 days and then giving a combination of the estrogen and progestin for another 15 days. This has been fairly successful.

But you always have to have some progestin in order to get adequate control of the menstrual phenomenon. If women could agree to have very irregular menstrual cycles, you might be able to get along with progestin alone and depot injections, but we have not been able to persuade women, if they fail to menstruate on the dot, that they are not pregnant.

Question: I read recently that women over 35 have a greater incidence of thrombophlebitis due to the use of these oral contraceptives. Would you care to comment on that?

Dr. Pincus: I looked over the data of the panel. I think there is a statistical error in this calculation. You will find an account of it—it has already appeared, I believe, in one publication—indicating that there is an error in the statistics on the thromboembolic effects in women over 35. Actually, the probabilities of development in older women are not significantly different from those observed in the population in general. This is an error which I am sure will be officially corrected very soon, but there is no doubt that this is purely a statistical error and not a statistical fact.

Question: Dr. Pincus, have you analyzed your data on the effects of these agents on the menopause?

Dr. Pincus: This is a very good question, and I wish I knew the answer. We are in the process of studying this, because a number of the women whom we began studying eight years ago are still continuing on intermenopausal years, and we will see what happens.

Our present impression, which is not fact but just impression, is that women will go into the menopause on schedule despite the use of these drugs.

Question: Is there any difference in the type of estrogen that can be used?

Dr. Pincus: Dr. Garcia, Dr. Rock, and I studied three estrogens originally: the 3-methyl ether of ethinylestradiol, another synthetic estrogen called Vallestril (allenolic acid is the trivial name), and the third, I think it was estradiol, which is a natural estrogen, and we found that the 3-methyl ether of ethinylestradiol seemed to give the best control of menstrual bleeding.

Subsequently, a number of investigators have used other estrogens. By and large, most of them shy off of diethylstilbestrol as the usable estrogen, for

reasons which are not very clearly validated to me, but the use of the 3-methyl ether of ethinylestradiol seems to be effective. In fact, a number of the preparations I described include ethinylestradiol. I think one of the reasons is that these can be used in extremely low dose for good control of menstrual flow and menstrual bleeding.

Question: Do you have any evidence regarding whether both the FSH and prolactin are equally reduced?

Dr. Pincus: The man who has most evidence is here in the audience. I do not know whether he wants to talk about it or not.

Dr. Nelson?

Dr. Warren Nelson: I do not think I can add to what you said, Gregory.

We have known for a long time that both estrogen and progestin will reduce the production of milk. This probably can be shown with appropriate experiments. It is due to a reduction in the release of prolactin, but also to a direct effect on the mammary tissue. In other words, the ovarian hormones are growth stimulators, and consequently they tend to promote proliferation of tissue, and it is only when the ovarian hormones drop, as they do at the end of pregnancy, that growth stops and lactation is initiated.

So it is quite reasonable to expect that adequate levels of any ovarian hormone would tend to reduce lactation.

Dr. Babcock: I would like to ask Dr. Pincus another question. We have talked in the past, frequently, and there has been a statement of policy by the Department of State about this government's attitude toward the assistance of countries with lower per capita resources to improve their lives, where the need is due in part to the explosive growth of population. Some studies with contraceptives have been directed in ways which could be naturally useful in these populations, and others have perhaps not been.

I would like to ask Dr. Pincus whether he feels that among those agents currently under advanced study there are substances which might prove ideal for widespread use, and if we still have work to be done, whether he feels that molecular modification will be an important tool in further studies.

Dr. Pincus: If I can answer this one, I will claim $64,000. However, I will make an attempt.

If you actually were to inquire in many of these countries which have a population problem what is the economic cost to them of an unwanted and, shall we say, unneeded birth, each of the compounds described today could be used economically and could save each of these countries money. That is the best answer I can give.

The second part, which I really should attempt to discuss, is much more difficult, and that is the extent to which they will be accepted in these countries.

As you noticed, in our own figures there are a number of reasons why after about a year roughly 30% of the women starting the use of these medications drop out. In later years this figure goes down, but nevertheless there is a steady loss of women to the use of any oral medication that has been shown thus far.

If this is due to disillusionment with the method per se, and this appears to be true in the first year in at least a third, or a half, of the women that drop out, there will always be a part of the population that will not use one of the drugs presented.

On the other hand, if you present to the population not one, but seven or eight or even more, and if the woman is not happy with compound A, she might be very happy with compound B. As a matter of fact, when the color of Enovid was changed by the manufacturer from pink to a lovely ivory white, many women said, "Your pill is absolutely marvelous."

The problem of acceptance is the basic problem. I think we have contrived the most effective means of contraception. I do not know if we have accomplished the most practical means, in terms of acceptability.

Many people have said, "Taking a pill a day is so troublesome. Why don't you make it once a month?" This may be a direction in which to go. I rather doubt it.

Actually the lowest classes of women—illiterate, uneducated—were the most faithful users of these pills, and taking one a day became, after a while, second nature.

So I think the problem does not lie in the use of pills per se. It lies in the ability of these countries to persuade their inhabitants to use this type of medication.

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Androgenic-Anabolic Steroids

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Boys with precocious puberty ("Infant Hercules") are striking examples of the androgenic and anabolic effects of the natural androgenic steroids. Large muscles from nitrogen retention (anabolic) appear *pari passu* with genital growth (androgenic). The introduction of the levator ani assay by Eisenberg and Gordan gave the biologists a tool with which to screen steroids for their relative androgenic-anabolic potencies. The chemists responded with a flood of compounds to screen. Preferentially myotrophic compounds have been found from a wide variety of structural changes in androgens. Many have been shown to be anabolic in man and more useful than natural agents because of their lesser propensity for virilization. Some new androgenic-anabolic steroids are playing an important role in cancer therapy.

Androgenic and anabolic steroidal hormones present a complex problem of assessment. Their biology, their clinical applications, and the synthetic routes to the best known agents are considered here.

Androgens are those hormones normally produced by the testes which are responsible for growth of the accessory sexual organs and development of secondary sexual characteristics. These hormones also produce the anabolic effects that make men, in general, larger than women and their musculature heavier. Removal of the testes causes atrophy of the accessory sexual organs, although in many animals, including man, this does not lead to regression of secondary sexual characteristics, such as growth of beard, once they are established. The compounds that restore the size of the sexual accessories in such castrated animals are referred to as androgens, whereas those that produce the larger muscles in males have generally become known as anabolic agents. The natural steroids have both properties. In a stricter sense, anabolic agents are those compounds

which are capable of causing retention of nitrogen and formation of new protoplasm.

Many attempts have been made to find orally active steroids which will also have substantially preferential anabolic or androgenic activity. Such compounds would have much greater clinical applicability than the natural steroids.

The classical experiments of Kochakian and coworkers (*28*, *29*) demonstrated the ability of urinary extracts to produce retention of nitrogen in castrated dogs. Subsequent demonstration that the levator ani muscle of the rat is particularly sensitive to the effects of castration and the administration of androgenic steroids offered a ready means of screening compounds for anabolic (here, myotrophic) activity. These compounds could then be examined further for their effects on nitrogen balance. Eisenberg and Gordan (*19*) developed this new tool for biologic screening, which was shortened and made more suitable for use as a primary screen by the modification of Hershberger, Shipley, and Meyer (*26*). The availability of this biologic tool led to the discovery of the first of the compounds which appear to be proportionately more anabolic than androgenic (*26*), 19-nortestosterone, and it initiated the broad search for such agents which is one of the major subjects of this paper.

Successful application of this screen and introduction of a series of compounds which formed the basis for the present improved anabolic agents resulted in a veritable flood of new compounds, tests, and clinically ever more useful agents. Many active agents with enhanced anabolic or androgenic activity have been found.

Assessment of Androgenic Potency

Measurement of androgenic potency will be considered before the much more complex assessment of anabolic activity. I should like to start with the four-legged laboratory rodents and progress upward through laboratory primates to man. As would be expected, each of these steps creates difficulties of transition which are in part inherent in the differences of the animals, the ability to measure the end points, and the absorption and handling of the steroidal agents by the different species.

Androgenic activity is most commonly assessed in castrated, immature male rats. For hormonal assay some investigators have preferred to use random bred animals rather than homozygous animals. Some years ago Emmens (*20*) made an excellent case for use of random bred mice for estrogen assays. Despite this, I prefer to use homozygous animals whenever possible. This animal variable can be standardized, and our own experience with homozygous animals bred in our laboratory has been much more salutary than our experience with the random bred animals that we have purchased.

One can approach the assay from several aspects. After castrating adult animals, one can compare the ability of two hormones to maintain the sexual accessories in their normal size and function. On the other hand, after castration one can wait for the sexual accessories to atrophy and then test the ability of a given agent to restore them. However, the approach most widely used is to castrate immature animals and administer the hormone immediately, and then gage the ability of the hormone to produce growth of the sexual accesso-

ries. Even in immature animals, some atrophy occurs upon castration, so that this is not a pure study of continued growth. This type of assay, however, is readily standardized and requires a minimum of animal "holding time" and relatively little hormone. Adequate comparison of literature citations of relative potency is generally impossible, since they are frequently given as a note in a chemical paper and lack sufficient biologic facts upon which to base a judgment. If testosterone, the principal testicular androgen, is employed as a reference material, the weight of the ventral prostate of the immature castrate male rat will be found to be the most sensitive end point, whereas the seminal vesicle requires a considerably higher dose (circa 10-fold) for significant response (37).

The levator ani muscle generally requires another logarithmic increase in dose before definitely responding. This extremely useful assay is adaptable for comparison of materials, whether given parenterally or orally (37).

There are many requirements for adequate assay. The reference material should have the same type of absorption phenomenon as the experimental material. In other words, it is not usually productive of parallel and therefore comparable dosage response curves if an esterified, slowly absorbed preparation is compared with a rapidly absorbed compound. All tests must be applied with intelligence and care.

The laboratory mouse can also be used for this purpose, except that its prostate is extremely small and comparatively large doses must be used for response, whereas, in contrast to the rat, its seminal vesicle appears to be somewhat more sensitive and is more generally employed as an androgenic end point. Here the weight of the kidney rather than the levator ani muscle is an index of anabolic activity (27).

Chickens show dramatic sexual dimorphism. The head furnishings of the rooster are an impressive contrast to those of the hen. Many early androgen assays were therefore performed on capons given hormonal extracts parenterally or by direct application to the comb. This has an advantage over the rodent assays, in that one can see what is happening as the assay progresses; the capon can be used repeatedly; and a photographic record can be made of the response. Unfortunately, it has the disadvantage of having to maintain a large colony of capons.

To simplify the assay, use of newly hatched, single-comb White Leghorn chicks was introduced as a highly sensitive and reproducible androgen assay (*16, 23*). Chicks are readily available in large numbers and comparatively little androgen is required for the assay. Androgens given parenterally or by direct application on the comb can be assessed by comparing the weight response of the comb to that produced by administration of standard reference steroids. It is possible to obtain excellent dosage response curves and to carry out rather extensive comparative assays with a minimum amount of steroid.

Assessment of androgenicity in primates is extremely difficult. I am not aware of any carefully sustained, statistically valid comparisons. The cost and numbers of laboratory primates which would be required for valid assays make the study prohibitive.

Studies for androgenicity in man are equally plagued by a difficulty of assessing end points and of obtaining adequate numbers of suitable patients

and materials. In human beings attempts to study comparative androgenicity have involved administration of androgens to eunuchoids, to prepubertal boys, and to women. The best assessments of which I am aware are double blind studies such as those which have been carried out in women receiving androgens for mammary cancer (*24*). This enables the investigator to make a judgment before breaking the double blind and, if the sample is large enough, takes into account the variation from patient to patient. However, it still does not overcome some problems of evaluation for androgenicity in man. At least, visible androgenic end points in women always increase with the increased duration of therapy. The tremendous variation among women is illustrated by the fact that we have seen women who have received as much as 100 mg. of testosterone propionate three times a week for as long as one year without any real virilization, whereas other women receiving this dosage have clitoral enlargement, deepening of the voice, facial hirsutism, and acne within four or five weeks. Thus, attempts must be made to improve evaluation of androgenicity in man.

The problem of assessment of androgenicity is further complicated by the fact that now androgenic steroids appear to have a preference for one or another type of end point. For example, fluoxymesterone (*36*) has been reported as being considerably more potent in the production of clitoral enlargement in women and penile growth in men than in the production of deepening of the voice, facial hirsutism, and acne. Thus, one's assessment of comparative androgenicity may depend upon what one selects as an end point.

Assessment of Anabolic Potency

Adequate assessment of anabolic properties is beset by even more difficulties. Although the myotrophic effect, particularly for the rat levator ani, is capable of standardization, the levator ani assay was originally proposed only as a screening method, which is its real role (*19*). Despite this, many agents have been clinically tested solely on the basis of their preferential effect on the levator ani muscle. There are other end points which produce more striking effects than the levator ani muscle in the rat, but they are more difficult to apply and need more material and more time. These include the temporal muscle of the guinea pig, which shows the greatest response of any of the guinea pig muscles (*30*). One may also use the renotrophic effect of anabolic agents in castrated mice (*27*).

The next stage in the well-ordered anabolic assay should involve the effects on weight gain in female rodents or castrated male rodents, but this introduces a major problem in assessment of anabolic effects which runs through all studies of this property—namely, that the level of nitrogen intake has an important effect upon the response. In other words, one cannot produce nitrogen retention on a diet deficient in nitrogen. Therefore, the diet must contain a little more than is required to replace wear and tear for proteins, yet avoid a surfeit of proteins, since the greatest anabolic effect is obtained by superfeeding of high quality proteins. Thus, it is necessary to restrict proteins to avoid measuring merely the effect upon appetite, well-being and, consequently, greater intake of food. Unfortunately, many assessments are still done with free feeding, so that comparisons are not really valid.

Anabolic agents have been assessed in man under complete external balance conditions. The same conditions apply, although the state of health and the selection of an individual are also important. Much evidence exists that stimulation of nitrogen retention in man, within the limits of metabolic study, seems to be an "all or none" phenomenon (2). In other words, there does not appear to be a dosage response curve. If sufficient material is given, nitrogen retention occurs. Increasing the dosage increases neither the amount of nitrogen retained nor its duration, provided the diet is kept constant.

Probably the greatest need for anabolic agents in clinical medicine today is to combat the antianabolic or catabolic effects of the widely used corticoids. Not only are these nitrogen-wasting but they can arrest growth as abruptly as la belle guillotine can end life. Thus, it is only natural that a major method of assaying anabolic agents is for their ability to counteract the nitrogen-wasting, growth-arresting effects of corticoids given to laboratory animals. Here again the methods vary widely and there is no general agreement regarding whether it is better to use small animals and measure growth arrest, whether it is better to use adult animals and measure nitrogen wastage or, indeed, whether the levator ani muscle may not be the best end point for screening.

Nevertheless, animal experiments with at least some of the compounds now available have shown an ability to counteract at least partially the growth-suppressing properties of a variety of corticoids and to counteract or prevent the nitrogen-wasting effects of such steroids.

An outstanding, desirable clinical property of the androgenic-anabolic agents, and one which has greatly interested me, is their ability to induce objective remission and prolong life in women with advancing carcinoma of the breast. Many biologic systems have been devised for assessment of this property and, indeed, some of the effective clinical agents have been found by this means. However, there seems to be relatively little agreement con-

Table I. Androgens and

Generic Name	*Trivial Name*
Dromostanolone	2α-Methyl-dihydrotestosterone propionate
Fluoxymesterone	9α-Fluoro-11β-hydroxy-17α-methyltestosterone
Methandrostenolone Methenolone	Δ^1-17α-Methyltestosterone
Methylandrostenediol	
Methyltestosterone	Methyltestosterone
Myagen	7α,17α-Dimethyltestosterone
Nandrolone phenpropionate	19-Nortestosteronephenylpropionate
Norethandrolone	17α-Ethyl-19-nortestosterone
Oxandrolone	
Oxymetholone	
Stanozolol	
Testosterone	Testosterone

cerning an animal tumor system which can be used most readily for such screening, because correlations are not complete and the agreement among various workers using the same methods is limited. Indeed, several of the tests I have mentioned may be as important or more important in the selection of agents for clinical trial in this area as antitumor tests, and they will be considered in the discussion of clinical application.

Synthesis of Materials for Clinical Use

In order to obtain a clearer understanding of the compounds we are discussing and before considering some specific biologic and clinical comparisons, I would like to change pace for a moment and discuss the syntheses of the materials currently available for clinical use. It seems ironic that androsterone, the first androgen to be isolated, identified, and synthesized, may finally take its place in clinical medicine as an agent to adjust abnormal cholesterol metabolism rather than as a useful androgenic-anabolic agent. The classical synthesis of androsterone is tedious, yielding a prohibitively expensive product. The newer syntheses which are available will afford ample supplies of reasonably priced materials if this agent should finally prove to have wide clinical applicability (*15*).

In Table I are listed the various names for the most widely available androgenic-anabolic agents. Testosterone itself was synthesized before it was known to be the major hormone produced by the testis. The myriads of synthetic routes to this material have been reviewed frequently, and the agent itself and its esters are so abundantly available at reasonable prices that the synthesis will not be considered here.

The oral activity of testosterone is limited, although its usefulness both as an androgen and as an anabolic agent by parenteral administration has been

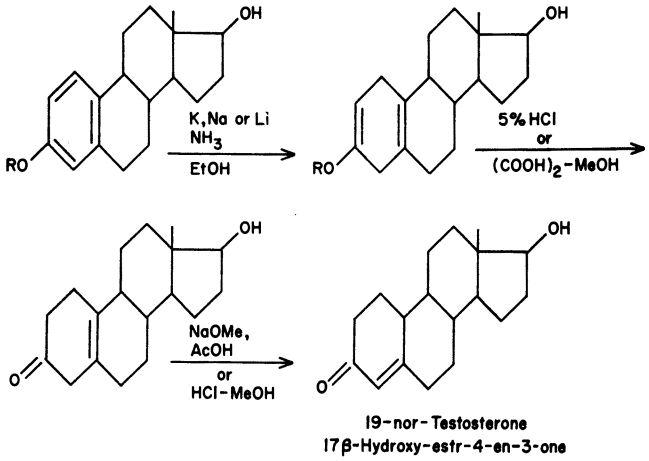
Anabolic Steroids

Proprietary Name	*Chemical Name*
Drolban	2α-Methyl-17β-hydroxy-5α-androstan-3-one, 17-propionate
Halotestin	9α-Fluoro-11β,17β-dihydroxy-17-methyl-androst-4-en-3-one
Ora-Testryl	
Ultandren	
Dianabol	17β-Hydroxy-17-methyl-androsta-1,4-dien-3-one
Primobolan	17β-Hydroxy-1-methyl-5α-androst-1-en-3-one acetate
Neostene	17-Methylandrost-5-en-3β,17β-diol
Stenediol	
Metandren	17β-Hydroxy-17-methyl-androst-4-en-3-one
Neo-Hombreol-M	
Oreton-Methyl	
	17β-Hydroxy-7α,17-dimethyl-4-androsten-3-one
Durabolin	17β-Hydroxyestr-4-en-3-one β-phenylpropionate
Nilevar	17β-Hydroxy-17-ethyl-estr-4-en-3-one
	17β-Hydroxy-17-methyl-2-oxa-5α-androstan-3-one
Adroyd	17β-Hydroxy-17-methyl-2-(hydroxymethylene)-5α-androstan-3-one
Anadrol	
Winstrol	17β-Hydroxy-17-methyl-5α-androstano[3,2-c] pyrazole
Various	17β-Hydroxyandrost-4-en-3-one

steadily increasing through the years by the development of better esters. This permits administration in adequate amounts at fairly widely spaced intervals, thereby removing part of the inconvenience caused by frequent injections.

The need for an orally effective androgenic-anabolic agent for prolonged administration in hypogonadal states was urgent. This was met by the development of 17α-methyltestosterone. The 17-methyl group is introduced into Δ^4-androstenedione smoothly by the Grignard reaction, with the resultant metabolically stable 17α-methyl-17β-hydroxy configuration which is not altered by passage through the liver and thus retains its androgenicity even when given orally.

This yielded an orally active agent, but it remained for Wilds and Nelson at the University of Wisconsin (*40*) to separate the anabolic and androgenic activities. They prepared 19-nortestosterone by a new Birch (*4*) reduction

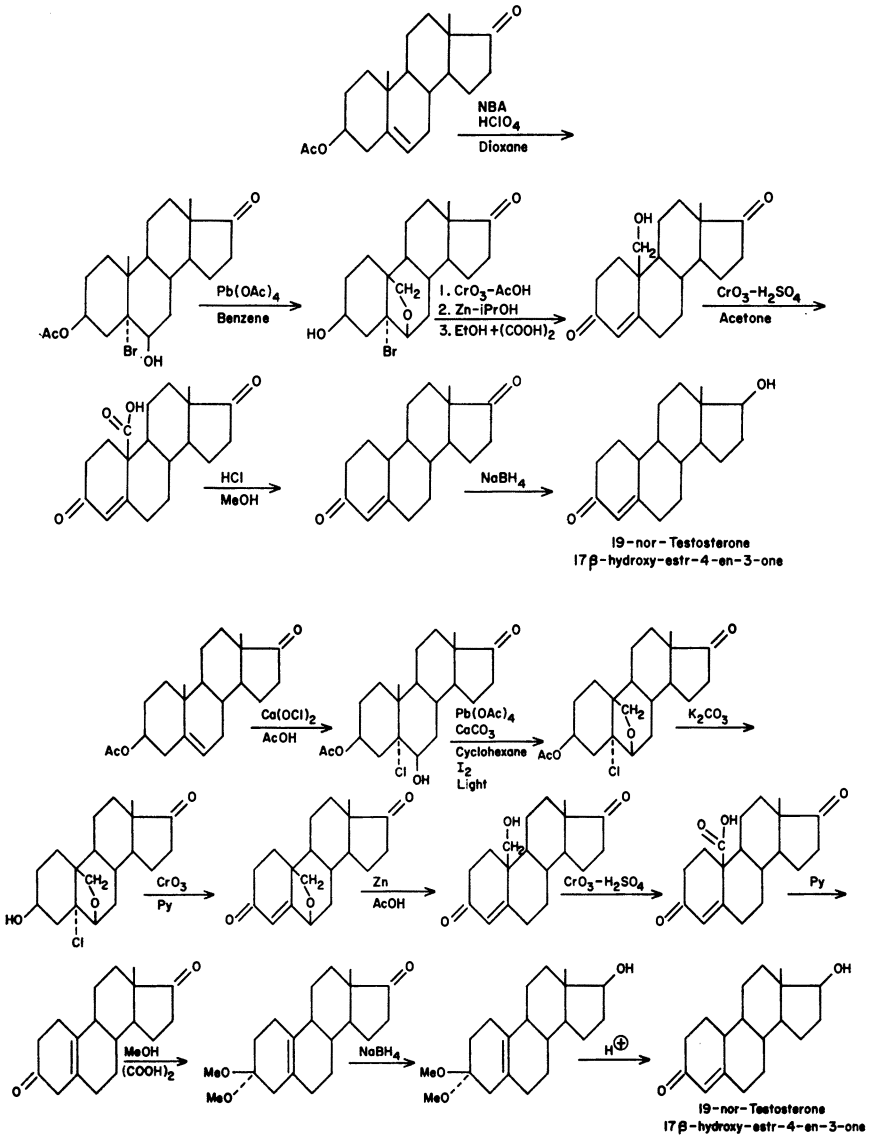


19-nor-Testosterone
17β-Hydroxy-estr-4-en-3-one

of the 3-methyl ether of estradiol. This compound, when assessed by the Hershberger, McShann, and Meyer modification (*26*) of the Eisenberg-Gordan (*19*) levator ani test in the castrated immature rat, was the first to show substantial differentiation in the ability to increase the levator ani muscle as opposed to its androgenicity, and paved the way for the important group of 19-norsteroids as preferential anabolic agents.

19-Norsteroids synthesized by the Birch reduction have been notoriously difficult to free of their estrogen contamination and are unfortunately expensive. The elegant synthesis recently reported by Bowers and coworkers (*5*) offers a direct route to 19-nortestosterone without the necessity of proceeding through the estrogens, and therefore does not require the difficult separation of the 19-nor compound from estrogenic contamination. Ueberwasser and colleagues (*38*) reported a somewhat different non-Birch reduction route to 19-nortestosterone.

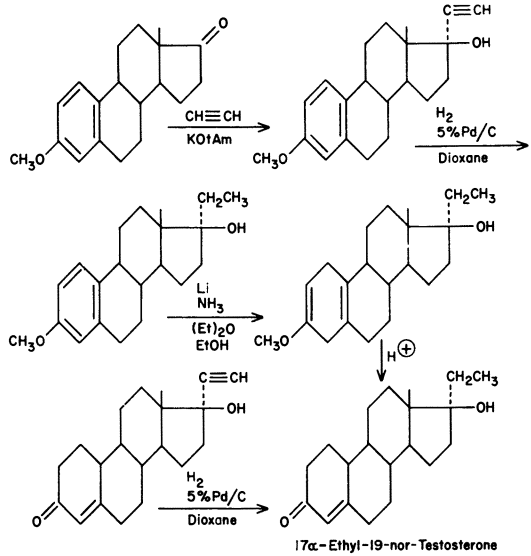
Like testosterone, 19-nortestosterone is relatively ineffective when given by mouth and has to be given parenterally. More recently long-acting esters requiring relatively infrequent injections have been developed. To draw the analogy with testosterone further, it was found that when the 17α-methyl group was introduced to stabilize the 17β-hydroxyl against hepatic inactivation, com-



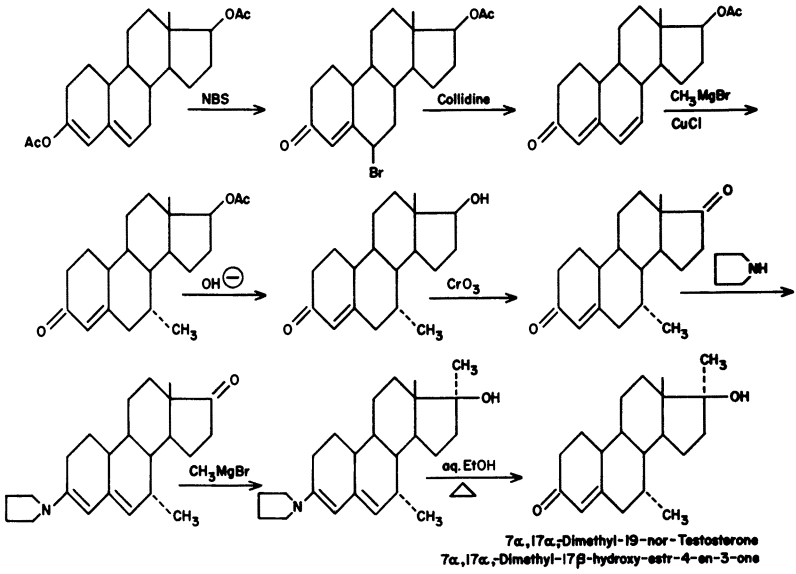
pounds of substantial oral potency were derived. Unfortunately, the combination of the 19-nor and 17α-methyl grouping seems to have increased the propensity of the 17α-alkyl group for the production of hepatic damage in this compound (*10*). 19-Normethyltestosterone is not generally available in this country.

Drill and coworkers (*18*), investigating the properties of a large number of 17α-alkyl-19-nortestosterones, showed that the 17α-ethyl compound possessed the most favorable combination of relatively low androgenicity and relatively

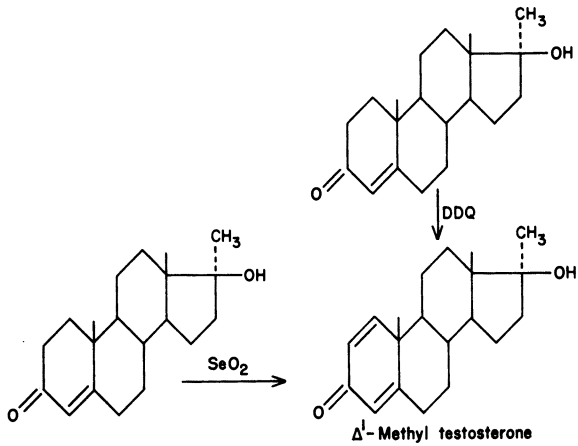
high anabolic effect on oral administration. Colton and coworkers (*12*) reported alternative routes to this compound.



The most recent step in the study of 19-nortestosterone has been introduction of the series of 19-nor-7α-methylsteroids (*31*), which have substantially increased androgenic and anabolic properties. Introduction of the 7α-methyl group in the 19-nor series entailed some difficulties, but these were overcome in the synthesis as outlined, and this has made available an entire series of these compounds (*9*).

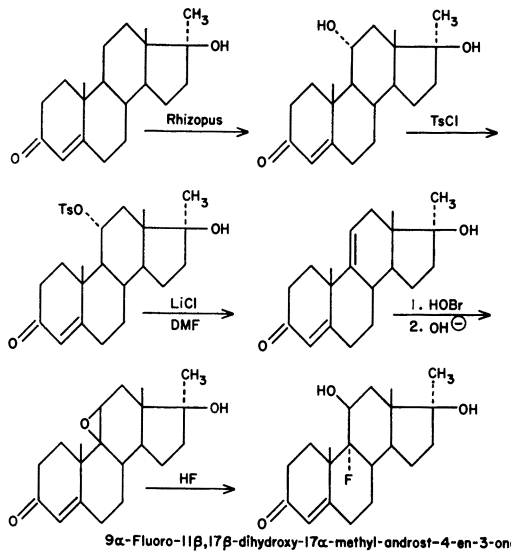


To return to the 17α-methyltestosterones, a series of developments has increased their usefulness. The first of these was introduction of Δ^1-methyltestosterone as a preferentially anabolic agent. The first reported preparation of this steroid employed fungi of the genus Didymella (*39*) but, as can be seen,



it is possible to prepare this compound from methyltestosterone also by dehydrogenation with selenium dioxide or by use of DDQ (*6*, *32*).

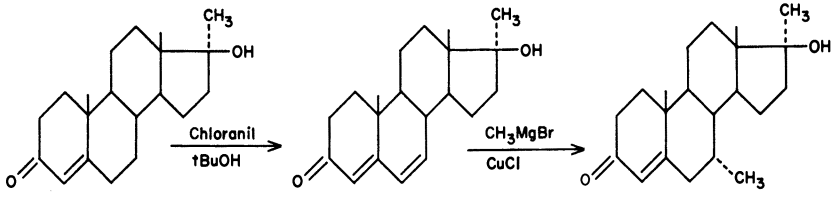
The next improvement in the methyltestosterone series came with development of a rather unique steroid, 9α-fluoro-11β-hydroxymethyltestosterone (*3*),



which probably acts at least as an androgen through some as yet unknown metabolite, since it is inactive when applied locally to the chick comb but highly potent in laboratory rodents and in the chick when given orally or parenterally. This agent has a favorable myotrophic-androgenic ratio. It ap-

pears, however, that this is one of the few available androgenic agents in which there is a real differential in responsiveness to various end organs. Indeed, in man this has been said to be essentially a phallotrophic androgen (*36*).

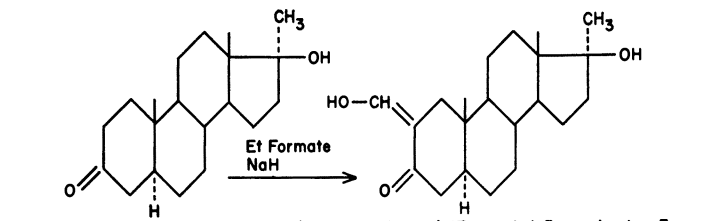
The most recent development in the investigation of the 17-methyl series, which appears to be more favorable in man than in animals, has been introduction of the 7α-methyl group (*8*). Utilization has been made both here



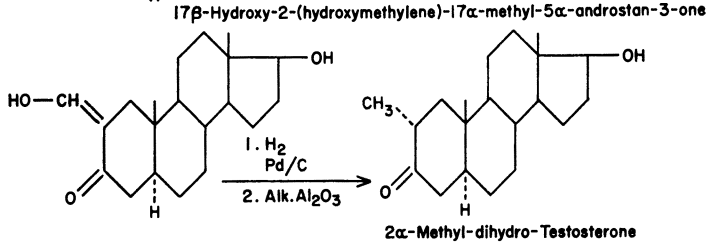
7α,17α-Dimethyl-Testosterone
7α,17α-Dimethyl-17β-hydroxy-androst-4-en-3-one

and in the testosterone series of the ability of chloranil to introduce a double bond in the 6,7 position of many steroids. If one makes methyltestosterone react with chloranil under the proper conditions and then takes the Δ^6-methyltestosterone and makes it react with methylmagnesium bromide, a mixture of 7α- and 7β-methyl-17α-methyltestosterone is produced, which is extremely difficult to separate. If the reaction mixture again reacts with chloranil, however, the 7β component is dehydrogenated, whereas the 7α-methyl component is not dehydrogenated, and then these two components are readily separated to yield essentially pure 7α,17α-dimethyltestosterone. This is an interesting compound with substantial anabolic effect in man.

Ringold and coworkers (*35*) introduced 2-hydroxymethylene-17α-methyl-



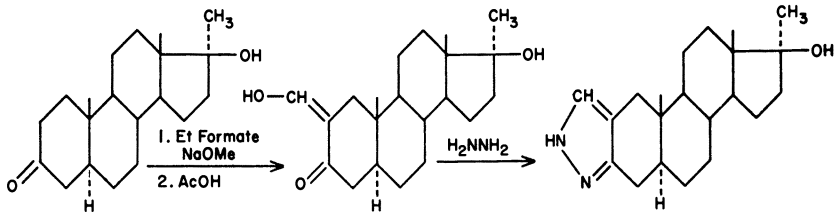
17β-Hydroxy-2-(hydroxymethylene)-17α-methyl-5α-androstan-3-one



2α-Methyl-dihydro-Testosterone
17β-Hydroxy-2α-methyl-5α-androstan-3-one

dihydrotestosterone as a preferentially anabolic agent. They then converted this into the 2α-methyldihydrotestosterone compound, which has lesser androgenicity than testosterone but appears on careful study to have retained testosterone's antitumor effect. This compound is enjoying increasing use as an androgen of choice in the treatment of advancing cancer of the breast.

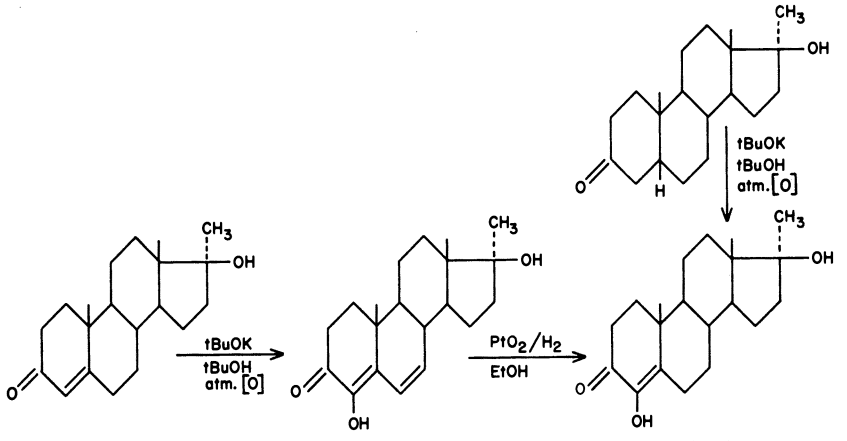
Clinton and associates (*11*) utilized the 2-hydroxymethylenedihydrotestosterones as starting materials for steroids with a pyrazole ring added on to ring A. These compounds have interesting properties. The 17α-methyl-5α-dihydro



17α-Methyl-17β-hydroxy-androstano-[3,2-c] pyrazole

compound has the greatest reported ratio in animals of anabolic over androgenic activity. Clinton and associates (*11*) have also developed new routes to the 2-hydroxymethylene steroids and prepared a series of substituted pyrazoles from the starting materials. Actually, as can be seen, one can start with 5α-dihydromethyltestosterone which reacts smoothly albeit slowly to form the 2-hydroxymethylene derivative, and then make this compound react with hydrazine to produce the pyrazole. Although it was originally thought that two symmetrical isomers should be produced in this reaction, this has not proved to be the case and the reaction goes smoothly with good yield of the indicated pyrazole.

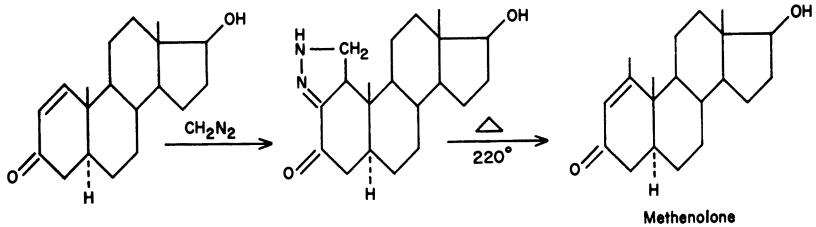
Camerino and associates (*7*) offered alternative routes for the preparation of 4-hydroxymethyltestosterone. This interesting compound is said to be orally



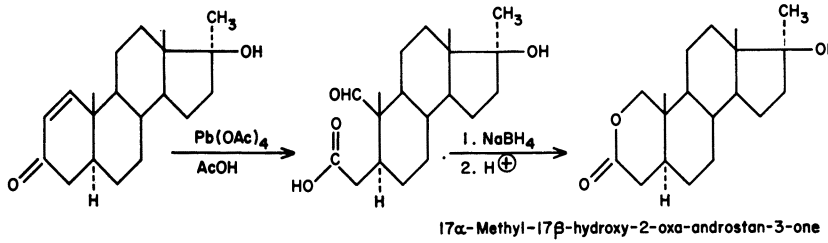
4-hydroxy-Methyl testosterone
17α-Methyl-4,17β-dihydroxy-androst-4-en-3-one

active and preferentially anabolic and has enjoyed much wider use abroad than here.

Popper and Wiechert (*34*) prepared the novel compound methenolone, which with its esters is said to be an effective anabolic agent. These are not yet generally available in this country.



Finally, Pappo and Jung (33) prepared a ring A lactone of dihydromethyltestosterone which has been reported to show distinctly favorable anabolic



activity. This material is not yet widely available and only a modicum of data is available about its clinical activity.

Clinical Application

One of the best efforts to compare the anabolic activity of many steroids which have been discussed is that of Albanese and coworkers (*1*), who devised a careful means of comparing a wide series of steroids. They employed nitrogen balance studies in appropriate patients. They then expressed the steroid protein activity index, the SPAI, on a comparative basis. The antianabolic or catabolic adrenal cortical steroids all have negative values by this assay. Table II shows comparative SPAI's of seven of the steroids which have been discussed. It can be seen by this index that testosterone propionate is the least active and stanozolol the most active. Further reports from Albanese and coworkers will be anticipated with interest.

TABLE II. Steroid Protein Activity Index (SPAI) of Anabolic Steroids in Patients(1)

Steroid	*Number of Assays*	*Dosage Range, Mg./Day*	*Average SPAI*
Testosterone propionate	12	10–25	+6
Norethandrolone	10	30–60	+8
19-Nortestosterone	14	25–75	+9
4-Hydroxymethyltestosterone	14	15–45	+11
Methandrostenolone	16	5–30	+16
Oxandrolone	10	10–30	+17
Stanozolol	10	6–12	+24

To replace androgenic effects in hypogonadal males, testosterone and its esters and methyltestosterone are still the most widely used agents. This use

of androgens is so well documented in all standard textbooks that it requires no further discussion here.

Induction of growth with anabolic steroids is an important clinical application of these agents. All of these agents are capable of increasing the rate of growth in undersized children. Many authors disagree regarding whether these children ultimately increase in height. There is wide variation from individual to individual in the degree of growth stimulation attained and the rate at which skeletal maturation is attained. Most physicians are convinced that in suitable patients below the norm for height, with a disproportionately more greatly delayed bone age, there is a reasonable chance that ultimate height may be increased by cautious use of anabolic agents, provided skeletal maturation is not permitted to proceed beyond that expected for the chronological age. Indeed, under these circumstances, many believe that the ultimate height may be increased or at least attained at an earlier age.

An additional aspect of the problem of growth in children is that many children have illnesses, particularly severe asthma, which require adrenocortical hormones to maintain them in optimal physical condition. Unfortunately, in general the amount of adrenocortical hormone required to keep these children in optimal physical condition usually also arrests their growth (*22*). Falliers and coworkers (*21*) in a study utilizing a single anabolic agent, stanozolol, were able to reverse the growth arrest in a series of such children. We must await further studies in this field, since the anabolic agent also reduced the amount of corticosteroid required for control of the asthma and this may also have contributed to the increased rate of growth with the combined therapy.

Much has been written about the favorable effects of androgenic-anabolic agents on calcium balance, for which they have been widely used. A recent report combined both calcium balance and radioactive calcium turnover studies (*25*). It again showed the favorable effects of the steroids, which these authors attributed to a decrease in resorption of bone.

It has long been thought that anabolic agents should prove beneficial in the treatment of muscular dystrophy. However, studies have generally been disappointing until a strain of mice became available with a hereditary pattern closely resembling human muscular dystrophy. Administration of combinations of certain anabolic steroids and cardiac glycosides to such mice significantly prolonged their life span. Thirty-seven patients with muscular dystrophy who had showed continuous progression of weakness were treated with an anabolic agent, methenolone, and digitoxin for periods of 5 to 19 months. Eight of the patients showed an undoubted increase in muscle strength during the first 4 or 5 months of treatment (*17*). Thus, this would appear to be an important potential area of usefulness for anabolic steroids.

The ability of anabolic steroids to produce gain in weight and to increase appetite in patients with wasting illnesses and in geriatric patients is too widely recognized to require substantiation. The newer agents seem to be able to accomplish these favorable results without undue masculinization of women.

17α-Alkyl steroids do have a tendency, when given in excessive dosage for long periods of time, to affect hepatic function, but this is reversible when use of the drug is discontinued. This is a major reason for interest in the most recently developed non-17-alkyl steroids. The particular hepatotoxicity of the compound 17α-methyl-19-nortestosterone has been mentioned (*10*).

Finally, these agents are particularly beneficial in the management of advancing metastatic carcinoma of the breast, from the point of view of both the patient's well-being (*13*) and the production of objective regression of the metastatic disease. Moreover, the patients who obtain subjective and objective improvement from these agents also live longer (*14*).

In conclusion, gradual, small, chemical modifications of the basic androgen molecule, testosterone, have yielded a series of compounds with biologic properties not found in natural androgens, which are opening up new and useful vistas in clinical medicine.

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Discussion

J. C. BABCOCK, presiding

Dr. Babcock: I would like to ask Dr. Segaloff if he feels there is still room for molecular modification. I know what his answer is going to be, because he is doing it.

I mentioned earlier that a veritable flood of analogs of testosterone has appeared on the scene since testosterone was introduced. I would judge that perhaps 500 to 1000 analogs have been prepared and assayed in animals, and a small percentage of these, maybe 20 or 30, have been evaluated in man. Dr. Segaloff is continuing to make analogs of this agent, so to ask him whether he still thinks it is worthwhile is a foolish question. I imagine he does not feel that they are coming fast enough, or we are not making the right ones, but if his crystal ball is not too cloudy at this time of day, I would like to ask him what lines the future might develop in this field.

Dr. Segaloff: Am I supposed now to lay out my proposed research program?

Actually, it is my own personal feeling, and I think some of the compounds which we have mentioned today bear this out, that there are two important areas to be exploited in the androgenic-anabolic field, as well as in other steroids. One is the introduction of noncarbon atoms into the rings—we have mentioned the use of lactones today—and the other is the use of nonoxygen atoms, such as sulfur or my own pet, selenium, as functional groups rather than oxygen, and the introduction of these in other places in the molecule.

There is evidence beyond what has been mentioned today, that some of these compounds have interesting properties. There are reports in the literature of the fact that you can introduce sulfur instead of oxygen and still have highly active compounds.

Of course, we had to leave out in this entirely all too brief discourse the fact that clinically we are interested in these compounds not only for their androgenic or anabolic properties, but also for their abilities to counteract androgenic properties or anabolic properties. These properties are also, I believe, importantly altered by the same modifications I am suggesting, which as we already know, change the androgenic-anabolic properties.

Dr. Pincus: The testololactone situation is too unreasonable, and therefore I would like to ask you if this might not just be a dosage effect? We know that the introduction of the delta-1,2 double bond just increases potency, and maybe you are not using large enough doses in the analogs.

Number two, you made a statement which I hope is true. If testololactone is inactive, and you get no regressions, what happens if you combine it with the active compound? Will you inhibit the effect of the active compound?

Dr. Segaloff: To answer your last question first, we have not tried it. It is possible that this may be a dosage effect. It has not as yet been tried. Testololactone has not been tried in substantial amount, nor the analogs.

Some of the analogs are hard to get. There are other things about the properties that are different. The delta-1-testololactone is the only one, I believe, of the lactones we have studied extensively, that appears to be completely inert in animals. As you know, testololactone itself was one of the first compounds reported by Eisenberg and Gordan as having a preferential effect on the levator ani, and they have published balance studies, in which they have shown that it is anabolic. While I don't know about the anabolic activity, at least there is some androgenicity in some of the other lactones that have been studied, so I am inclined to think that it is not just a dosage response effect.

One of the problems in clinical assessment is that when you come so far down the line, it is very hard, both to convince oneself and to convince one's colleagues, in the face of a negative result, to go ahead and push still harder with a compound that does not work and does not seem to fit, just to find out whether or not there may be some other property.

Obviously, Dr. Pincus, from the number of patients you are able to discuss, and maybe because of the area in which you are working, you are more persuasive than I am with some of my colleagues.

Perspectives in Drug Therapy

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The technique of "molecular modification" in drug design and development is analogous to the technique of variation as employed in other sciences and the arts. Indeed, it is through the technique of variation applied to prototypes that nature as well as mankind operates toward the full realization of potentialities in various areas of evolution. Molecular modification followed by pharmacologic testing and selection is thus seen to be analogous to biologic development via cross fertilization or mutation followed by survival-selection on the one hand, and dress design through variation and survival-selection in the world of taste basic to the garment industry.

The layman is keenly aware of the salient roles that variation and selection play in the realization of useful varieties in animal husbandry and agriculture. The richness of life forms of the animal and plant world, for food, labor, and esthetic appreciation are all primarily the result of the processes of variation and selection whether applied consciously or unconsciously by man and nature. The artist is keenly aware of the technique of variation and selection as in the development of themes into symphonies in music, in the plastic and pictorial arts, and in architecture. City planning, cooking in the home, adaptation of natural resources such as the harnessing of river systems, and children at play, all share methodologically a common bond with molecular modification and pharmacologic selection.

Those who would degrade the technique of molecular modification by reference to it as mere "manipulation" capable of achieving only "me too" products in the pharmaceutical industry, thus show not only a profound ignorance of the concretely demonstrable results of modern therapeutic research but also their ignorance of a profound method that is everywhere one of the most powerful creative resources in both man and nature.

The present symposium has demonstrated, by outstanding presentations, the force of the above arguments by examples drawn from some of the major medical advances of modern times. Through presentations by some of the foremost chemists and physicians in America today, we have seen, in bold relief, advances in the areas of: infectious disease; cardiovascular disease; control of pain; mental disease; diabetes; and the control of fertility. Moreover, it is clear that these advances are the direct result of systematic modifications and pharmacologic testing such that advances in several of the above-named six areas of human suffering are bound immediately to the method of molecular modification through variation in a given series of chemically related products.

By way of example, we have seen how the discovery of the antibacterial

properties of sulfanilamide has, through molecular modification, yielded key drugs in the treatment of congestive heart failure, essential hypertension, and diabetes. Systematic molecular modification of the early antihistamine drugs has led to major breakthroughs in the control of mental illness and a revolution of formerly prison-like mental institutions to modern hospitals, at the same time freeing the community of enormous expense in the care of thousands of patients who can now pursue their lives in a normal, or nearly normal, way as valuable members of the community.

With products already available through the method of molecular modification of the sex hormones, we now have agents highly potent in the control of fertility but devoid of serious effects referential to secondary sexual characteristics. Their actual employment in the amelioration of what many consider a most serious problem facing all of mankind—overpopulation—is, of course, a decision to be made in the social context of the various centers of overpopulation.

Perhaps even more important for the future are the new science and technology that have grown immediately out of the accumulating information concerning the relationship between the chemical constitution of molecules and their pharmacologic effects. It is through the latter that chemists are now able to predict with a remarkable degree of certainty in given areas of drug design, that substances not yet synthesized will have given qualitative effects and often quantitatively estimate with surprising reliability their potency relative to already known drugs. It is the development of such predictive theoretic methods that marks the coming of age of any science. It is also this coming of age as a predictive science that can be seized upon by concerns which would easily develop "me too" drugs with minimal effort. While such mean employment of the science is possible, it should not in any sense reflect a deprecatory note upon molecular modification as a method. Indeed, that such use of the predictive capacity of a science is possible is actually a measure of the power of that science! Criticism should be leveled rather at those who employ the method for mean ends rather than at the method per se.

In further future perspective, a most serious problem yet to be satisfactorily resolved is the systematic centralization, organization, and retrieval of information, resulting from the method of molecular modification and pharmacologic testing. Here again, the difficulty is not due to the method, for the machines and devices for informative processing, retrieval, and correlation already exist. The difficulty is the human one of making the critical decision to use the machines and devices for the benefit of all while safeguarding rightful ownership. Some years ago the organization of a chemical-biological coordination center represented a step toward the centralization and dissemination of information in this area on a national level. It is unfortunate that this program was discontinued, apparently because of lack of funds.

Over-all, the criticisms leveled at molecular modification, a technique that has yielded a new science, have been quite erroneously aimed at the value of the subject when they should have been directed at human manipulators. The case for molecular modification is clearly summarized in the fact that it has yielded, in the last 25 years, more potent and more useful drug agents in various areas of therapeutics than have been reported in all previous history.

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