Antibiotics

BIOLOGY
COLLOQUIUM
1953

OREGON STATE CHAPTER OF PHI KAPPA PHI
OREGON STATE COLLEGE ⋆ CORVALLIS ⋆ 1953

Fourteenth Annual Biology Colloquium
Saturday, April 18, 1953

Antibiotics

ༀ

TABLE OF CONTENTS

FOREWORD

The Biology Colloquium is conducted in a spirit of informal discussion and provides opportunity for participation from the floor. The colloquium is sponsored by the Oregon State Chapter of Phi Kappa Phi with the collaboration of Sigma Xi, Phi Sigma, and Omicron Nu. Sigma Xi assumes special responsibility for the colloquium luncheon. Phi Sigma and Omicron Nu provide afternoon tea. The College Library arranges special displays of the writings of colloquium leaders and notable works on the colloquium theme.

Grateful acknowledgment is made of the cooperation and interest of the several faculties of Oregon State College that are concerned with biology, of those biologists contributing to the program, of Chancellor Charles D. Byrne, President A. L. Strand, and other executives of Oregon State College.

The first Biology Colloquium was held March 4, 1939, with Dr. Charles Atwood Kofoid of the University of California as leader, on the theme "Recent Advances in Biological Science." Leaders and themes of succeeding colloquiums have been: 1940, Dr. Homer LeRoy Shantz, Chief of the Division of Wildlife Management of the United States Forest Service, Theme "Ecology"; 1941, Dr. Cornelis Bernardus van Niel, Professor of Microbiology, Hopkins Marine Station, Stanford University, in collaboration with Dr. Henrik Dam, Biochemical Institute, University of Copenhagen, theme "Growth and Metabolism"; 1942, Dr. William Brodbeck Herms, Professor of Parasitology and Head of the Division of Entomology and Parasitology, University of California, theme "The Biologist in a World at War"; 1943, Dr. August Leroy Strand, Biologist and President of Oregon State College, theme "Contributions of Biological Sciences to Victory"; 1944, Dr. George Wells Beadle, Geneticist and Professor of Biology, Stanford University, theme "Genetics and the Integration of Biological Sciences"; 1945, Colloquium omitted because of wartime travel restrictions; 1946, Dr. Robert C. Miller, Director of the California Academy of Sciences, theme "Aquatic Biology"; 1947, Dr. Ernst Antevs, Research Associate, Carnegie Institution of Washington, theme "Biogeography"; 1948, Dr. Robert R. Williams, Williams-Waterman Foundation, theme "Nutrition"; 1949, Dr. Eugene M. K. Geiling, Head of the Department of Pharmacology, University of Chicago, theme "Radioisotopes in Biology"; 1950, Dr. Wendell M. Stanley, in charge of Virus Laboratory, University of California, theme "Viruses"; 1951, Dr. Curt Stern, Professor of Zoology, University of California, theme "Effects of Atomic Radiations on Living Organisms"; 1952, Dr. Stanley A. Cain, Conservationist, University of Michigan, theme "Conservation."

COLLOQUIUM COMMITTEES

COLLOQUIUM COMMITTEE

K. S. Pilcher
  (chairman)
R. O. Belkengren
E. C. Bubl
V. H. Cheldelin
D. M. Goode
Elmer Hansen

J. A. Harper
R. S. McCutcheon
J. E. Oldfield
Ivan Pratt
J. O. Schnautz
Robert Storm
Clara Storvick

Student members:
Nancy Davies
Sherley Jean Fleming

Flora Jane Schuster
Marie Shepardson

PHI KAPPA PHI EXECUTIVE COMMITTEE

F. A. Gilfillan
  (president)
Kenneth Andresen
  (vice president)
Georgena S. Knapp
  (secretary-treasurer)

Elaine Yunker
  (assistant secretary)
Katherine Hughes
  (journal correspondent)
E. B. Lemon
  (past president)

W. E. Milne
  (president elect)

SIGMA XI EXECUTIVE COMMITTEE

W. D. Wilkinson
  (president)
V. H. Cheldelin
  (vice president)

A. W. Marsh
  (secretary)
C. L. Clark
  (treasurer)

WAYNE W. UMBREIT, Ph.D.
Leader of Fourteenth Annual Biology Colloquium

Fourteenth Annual Biology Colloquium

Theme: ANTIBIOTICS

Leader: WAYNE W. UMBREIT, Head of Department of Enzyme Chemistry,
Merck Institute for Therapeutic Research

DISCUSSION LEADERS:

WAYNE W. UMBREIT, Ph.D.
Head of Department of Enzyme Chemistry, Merck Institute for Therapeutic Research.

JAMES C. LEWIS, Ph.D.
Biochemist in Charge of Biochemistry Section, Vegetable Processing Division, Western Regional Research Laboratory.

ROBERTSON PRATT, Ph.D.
Professor of Pharmacognosy and Plant Physiology, University of California College of Pharmacy.

NORMAN A. DAVID, M.D.
Professor of Pharmacology, University of Oregon Medical School.

JAMES McGINNIS, Ph.D.
Professor of Poultry Husbandry, The State College of Washington.

CHAIRMEN OF SESSIONS:

HUGO M. KRUEGER, Ph.D.
Professor of Physiology, Oregon State College.

RALPH W. MACY, Ph.D.
Professor of Biology, Reed College.

WILLIAM D. WILKINSON, Ph.D.
Professor of Geology, Oregon State College.

F. A. GILFILLAN, Ph.D.
Dean of the School of Science, Oregon State College.

Opening of the Colloquium

DR. KRUEGER: The president of any institution is always a busy man, particularly when he is president of a large educational institution such as Oregon State College. Therefore, we are very happy whenever Dr. Strand can be with us. It is a pleasure for me to introduce our able president, Dr. A. L. Strand, who will give us a word of welcome.

DR. STRAND: Mr. Chairman, Dr. Umbreit, and Participants in the Fourteenth Annual Biology Colloquium: One way to keep on our time schedule would be to do away with my remarks, but they can be brief. We welcome you to the 1953 Colloquium. We welcome you as participants who are free to join in the discussions. Our meeting is sponsored by Phi Kappa Phi with the collaboration of Sigma Xi, and we have several outstanding scientists in the general area of antibiotics present. I am sure the meeting will be worth while to everyone.

Those who are new here usually hesitate at the word "colloquium." I know I did when I first came to Oregon State College. Yet, if we have something unique at this institution, I believe it is this Colloquium. The word means a discussion, and what makes the meeting unique is the variety of professions and interests represented. In any colloquium audience you will find teachers from all levels in our educational system and persons from engineering, biology, chemistry, economics, physics, geology, and often other fields of study. Everyone present is free to join in the discussions and, when you have something to say, to interrupt the speakers.

Analogous to our colloquium here, that is as a discussion, is the dialectic materialism of communist Russia. That involves a discussion too, but the difference between the two is that in the Russian dialectics the conclusions are fixed at the start. It may be that our conclusions are somewhat fixed too, because the leaders know more about the subject than we do, but we will contribute new ideas and information which may affect the findings of the meeting.

I have here Professor Zirkel's book, *Death of a Science in Russia,* which is an account of the astounding dialectics over genetics which took place in Moscow in August of the year 1948. That month marked the climax in the rise of Lysenko and his Michurinist theories having to do with the inheritance of acquired characters. Of course

in that discussion, or colloquium, Lysenko had an "ace in the hole." He concluded his paper by saying that it had the approval of the Central Committee. That meant that science in Russia had to conform to approved political theory and, accordingly, all the geneticists who had accepted the work of Mendel, Weismann, and Morgan, or "western bourgeoise" scientists, had to recant publicly if they wanted to live.

Recently, Dr. Robert Hutchins emerged from a two-year period of aestivation in southern California and broke out in a speech in which he said that a good university is recognized by the controversy and discussion which it is able to generate. I think he means real, heated discussions. Our discussions here may not produce so much heat, but they come close to fulfilling one of the cardinal purposes of a university.

This is a new building and this is one of the first meetings to be held in its auditorium. I hope no one entering will slip and fall in the aisles, because the rubber coverings have not arrived. I was a little disturbed also that this room would be too big. You remember how we used to crowd into Memorial Union 105. I trust the additional room will add to your comfort, however, and not dampen your discussions. I think we can depend on Dr. Umbreit and the other leaders to take care of that. Thank you.

Dr. Pilcher: It's interesting to recall that as recently as 12 years ago the word "antibiotic" had hardly been introduced into the English language. In the rather short period of time since then, accelerated by the more or less urgent needs created by the second World War, there has been developed a large new branch of industry. The therapy of many infectious diseases has been revolutionized to a considerable extent. More recently other important applications have developed in the field of nutrition, the exact extent of which can not be fully realized as yet.

The theoretical aspects of antibiotics, both in regard to the manner in which microorganisms and other living organisms are able to produce these agents and to the mode of action of antibiotics, are both fascinating and challenging subjects. The development of antibiotics, of course, has its roots in biology and chiefly in microbiology, but the development of the subject could not have reached its present state without the participation of a great many other sciences. These have included biochemistry, organic chemistry, physical chemistry, pharmacology, chemical engineering, nutrition, and others.

In short, this field of biological science, which in terms of age is hardly dry behind the ears, has grown to such proportions that it is not possible within the space of one day's discussions to cover the whole subject or even attempt it. Nevertheless, I think we are fortunate today in having with us a group of men who have had a great deal of personal experience in some one or more phases of antibiotic research, and they are going to present some of the more interesting aspects of the subject.

Contemporary Viewpoints on Antibiotics

Wayne W. Umbreit

The discovery, the development, and the practical use of antibiotics are an area of knowledge relatively recent, sufficiently important, and reasonably consolidated so that a contemporary survey—a consideration of achievements to date and present problems—is not as pretentious a matter as it may sound. Certainly, twenty years ago, knowledge of this field was very meager. Ten years ago, indeed, it had achieved its first major success. Today, knowledge is so widely distributed that we tend to take antibiotics for granted. Yet, essentially all of us were reared in an era in which antibiotics were not known and we have lived at least a portion of our adult lives at the time in which knowledge of the antibiotics has become available and in which this knowledge has profoundly influenced scientific thought, medical practice, and various other areas, not to mention the improvement in our own chances of survival. It is indeed proper that a colloquium should devote its attention to antibiotics.

Familiarity with antibiotics should not obscure the remarkable nature of the process. Indeed, if we did not know that a substance such as penicillin really existed and if we did not know that it has its array of properties, we with the generations before us would believe that such a substance was impossible. Antibiotics have been with us always, but knowledge of antibiotics is so recent that we are still caught in the confusion of claim and counterclaim without benefit of the test of time.

So vast are the observations, so unexpected some of the phenomena, so undigested the many and varied experiments that reference in detail generates confusion. I propose, therefore, to compress a great deal of experimental work into some general viewpoints which I assume to be relatively contemporary. Naturally, we do not as yet know whether these viewpoints may not be altered in the future, but at the moment they serve as a condensation of experience into broad and experimentally sound principles.

The first of these principles is that in Nature there exist organisms which, during their growth, produce chemical substances which are toxic to other forms of life. These substances are called "antagonistic substances" and are definite chemical entities rather than alterations in the physical environment (such as pH, etc.) of the organism antagonized. These substances are called antibiotics—i.e., an antibiotic is a substance produced by one living organism which inhibits or kills another. This definition, as most definitions, is both too narrow and too wide. With modifying adjectives or clauses we shall use it, however, to designate specific types of materials more precisely. Under these circumstances, the definition will suffice.

There are many thousands of different kinds of organisms which produce discrete substances inhibiting or lethal to other organisms. Not only microbe against microbe, but mold, yeast, actinomyces, algae, higher plants, and animals themselves produce antibiotics toward micro-organisms, molds, yeasts, etc.—with, to my knowledge, almost every possible kind of cross reaction.

The second general conclusion derivable from a large mass of data is that from among these thousands of antagonistic substances it has so far been possible to isolate and to determine the chemical structure of perhaps a hundred of them. At least, a sufficient number are known in detail so that it is apparent that there is no chemical structure common to all or even a majority of them, and essentially every major class of chemical compound is represented. It is a necessary property of all of the substances that they possess a degree of specificity, inhibiting one organism and not another. Sometimes this specificity is very broad; sometimes very narrow. These are not "general cell poisons," whatever that may mean, but have to some degree the virtue of specificity. In the long run it may well be that this specificity will turn out to be the most fundamental scientific problem, just as it has already been the most important factor in their use.

The third principle is that among these antagonists there are some, perhaps of the order of twenty, which possess a degree of specificity so great that it enables them to be used within the animal or human body. There they exert their effect upon micro-organisms of various sorts without harm or at least anything approaching comparable harm to the host. Five have this property
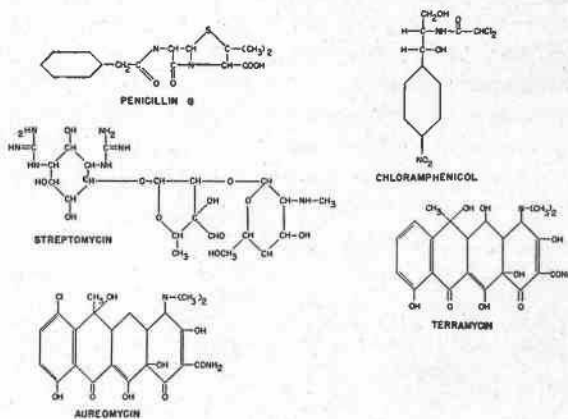

Figure 1. Chemical structures of widely used antibiotics.

to such an important extent that they are effective agents in the treatment of certain infectious diseases. These are, as illustrated in Figure 1, penicillin, streptomycin, chloramphenicol, aureomycin, and terramycin, all of which widespread actual clinical experience under a great variety of circumstances over a fairly long period of time has shown to be effective drugs. Many of us here today owe our lives to them. There are, in addition, a further array of substances—viomycin, neomycin, candicidin, erythromycin, etc.—whose number it is impossible to specify at this point but which seem to hold some promise, if only in specific cases, but are either too recent or too difficult in practice so that experience with them in the actual battle with disease in the community at large is still small. There are, further, several agents—bacitracin, gramicidin, etc.—whose toxicity to the body is too high for internal use but which do find a use in practice under restricted circumstances. They, therefore, have medical importance.

The fourth general principle is not as self-evident as the first three. It is, to the best of my knowledge, a reasonable inference from available data, namely, that the chemical structure of the molecule possessing antibiotic properties is specific in the sense that alteration in chemical structure to any but a very minor extent will destroy

the antibiotic properties. This principle holds for streptomycin, chloramphenicol, aureomycin, and terramycin, but holds for penicillin only if one regards the substance as a conjugated structure in which the "R" portion, as shown in Figure 2, may be varied within limits without fundamentally altering the effect of the "R" group upon the properties of the remainder of the molecule. The case for penicillin may be illustrated in Figure 2, where several of the possible alterations showing activity are listed. While the general properties of these substitutions are similar, their specificity has been altered. The case for streptomycin is illustrated in Figure 3 in which any alterations other than those shown result in essentially complete loss of antibiotic activity.
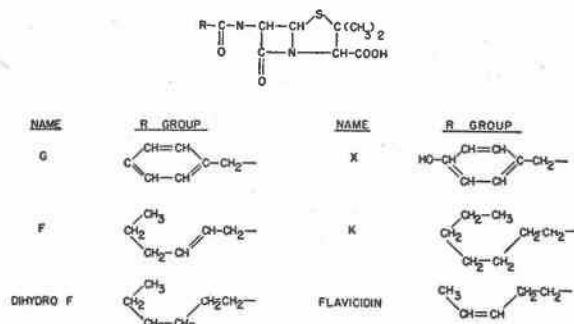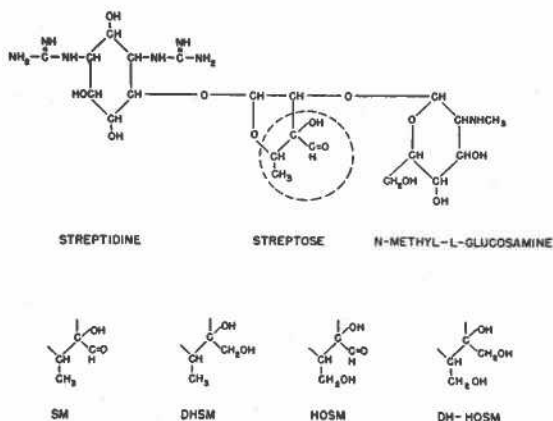

Figure 2. Active modifications of penicillin.

Figure 3. Some active modifications of the streptomycin molecule.

SM = streptomycin
DHSM = dihydrostreptomycin
HOSM = hydroxystreptomycin
DH-HOSM = dihydrohydroxystreptomycin

Taking the data as a whole for other antibiotics, a fifth general principle, that the particular antibiotic molecule is essentially the only one which is effective and that compounds of closely related structure are inactive, seems to hold. This principle would seem to be quite an important one, but its fundamental meaning is not yet understood.

The sixth generalization derivable from the experimental data is that there is no general mode of action of the antibiotics in the sense that there appears to be a general mode of action of sulfonamides. Such broad interpretations, as for example, that antibiotics as a class of substances affected enzymes containing sulfhydryl groups or indeed competed with an essential metabolite of the cell or similar inclusive interpretations, have not been fruitful. It is quite evident that the principle involved in the action of antibiotics varies considerably from one substance to another. Of course, this might well be expected from our present knowledge that there are several hundred such substances which are related only by the circumstances of having been discovered in a particular manner.

The seventh generalization is one which is certainly not a really fundamental principle but which I think needs some attention. It is that the availability of new and better antibiotics for the treatment of disease in the population as a whole is going to be a somewhat slower process than the development of the present antibiotics. This is hardly a principle. It is almost a prediction. The point is that there are factors already available which tend to limit the further development of new antibiotics, aside from those inherent in the nature of the present clinically available antibiotics themselves. This is my own opinion. Time will tell whether it is sound or not. I do not visualize an ever-expanding array of better and better antibiotics. Improvements will be made, of course, but the discovery and development of antibiotics for medical use is, to my mind, of the nature of a self-limiting reaction. Since relatively few people, particularly in research, are familiar with or consider these problems, it might be useful to discuss the matter, somewhat as a side issue to the main principles involved in antibiotics.

Since there are already five clinically effective agents, the search for new antibiotics of medical importance must, if it is to be successful, produce substances which are better, for one reason or another, than those we already have. Indeed, the new ones must be considerably better, because the trail to widespread effective clinical use is such a tortuous one and it is only worth while to follow it to the bitter end if there is much to be gained thereby. After all, if a doctor in his practice knows that drug A is effective in con-

dition *A*, why should he change to drug *B* unless it can offer him some marked advantage?

The process, the procedure, the approach to the discovery of a new antibiotic varies to a very minor extent in any of the organizations of which I have any knowledge. Some do it more effectively than others, some are luckier; but the ground has been somewhat worked over. The development of the substance begins by finding an antagonism. Studies are then made to determine how to grow the antagonist, how to assay the substance—in short, how to produce it on a small scale. Something must now be learned of the substance itself —under what conditions may it be preserved, how may it be separated? When this has been done, attempts are made to produce more of it, for two purposes: for a determination of its biological properties and for some study of its chemical nature. With reasonable luck, a gram may be made in perhaps six months to a year. The first gram is the hardest, because in the meantime—assuming all is going well—conditions of growth, selection of strains, alterations in media, etc., have been under study and some improvement has been made.

In a sense, one is getting the feel of the substance. The preliminary biological data are obtained. The substance, in crude form, has this degree of activity *in vitro*, it has or has not this degree of activity *in vivo*, it has this degree of toxicity, possibly due to impurities, etc.

Assuming all of this is favorable (and the substance is not one of the known antibiotics), the scale of the operations must now be increased. The pharmacologists, the chemists, the bacteriologists all cry for more material, and the project expands in all directions. The engineers start trying to make the substance on a larger scale: to 5 gallons, to 50 gallons, to 500 gallons, to 5,000 gallons. The chemists and chemical engineers try to find ways of isolating the material on a larger scale. Indeed, the whole complex machinery of industrial research (where such development is usually done) begins to hum.

When this process has made some progress, critical evaluation begins. Up to now, the substance has shown promise; will this promise bear a really critical test? While the development to this point ran on optimism, the substance now must prove itself against pessimism. The whole range of acute and chronic toxicities, the search for possible pathology, the blood levels, the tissue levels, the real effectiveness against experimental infection, the purity, the chemistry, the breakdown products, the stability, the formulations,

the development of resistance and the means of combating it, all of these must be critically examined. When all of these are favorable, then trial begins in reputable clinics with extreme care. If these too are favorable, the successes, the failures, the advantages, and the disadvantages are weighed and measured, are discussed with the Food and Drug Administration, and a larger trial is undertaken. From here on, the factors of the real value of the substance compared to other available agents and the economic forces begin their interplay. If the agent is only as good as present materials, it probably has little chance. If it is better in one or more aspects, it can progress. The economics are forgotten for a while, because the cost today can be reduced if life is at stake. If all this is still favorable, the substance eventually reaches the practicing physician. Here begins a process of education: a dissemination of where and how, and how much should be used when and for what.

At this point—and it seems to be unavoidable with any drug—if the substance is greeted with enthusiasm, it will undoubtedly be used for purposes for which it was not intended, and it will fail in this respect. Or, if hopes are set too high, it will not reach the expected result, even though it might be perfectly adequate in its own right. The pendulum will swing back from "this is a fine substance" to "this is no good at all." Eventually, as experience is gained with it, the pendulum of estimate will come to rest at the point that for these conditions and these circumstances it is the drug of choice. It may, of course, be unseated from even this position at any time by new developments which may or may not bear any relationship to the intrinsic value of the drug itself.

The point of all this is that the pathway from the antagonism observed on a plate to the pure substance on the shelf of the local druggist (or its name on the prescription pad of the doctor) is a long and critical one, as it should be. Many are called but few are chosen. Indeed, I consider it a most remarkable thing that in the past decade some twenty such materials should have passed these rigors of development and have been placed in the hands of the physician.

A final generalization, true, of course, in most fields of science, might be that the discovery and application of antibiotics has provided further problems. I would not have you believe that even a small part of this vast area is really known.

The contemporary problems of antibiotics may be divided into three aspects: those of fundamental scientific interest, those of a practical na-

ture, and those of medical importance. The establishment of present knowledge of antibiotics has profoundly influenced all three areas.

In the fundamental scientific area, one may well begin with the role played by such antagonisms in nature, the antagonisms between microorganism, plant, and animal, their relation to ecology, to agriculture, and to the transformations in nature. The production of antibiotics by microorganisms—why and how they produce them, what curious mechanisms may be involved in the synthesis of such complex molecules as penicillin or streptomycin—these matters are certainly of considerable interest. What is the mode of action of antibiotics? How do they inhibit or kill the susceptible organisms, or, indeed, what is the basis of their specificity? Why do they attack one organism and not another? These matters, too, are worthy of study. Further, attention is being devoted to the mechanisms by which resistance develops and to the even more curious phenomenon of dependence. Study of the chemical structures of antibiotics has as yet failed to reveal what it is in the arrangement of their atoms which permits their unique effects. Why does this particular arrangement of atoms permit these things to happen? Are there not principles of scientific importance underlying the occurrence and action of antibiotics the search for which we may well have been spared by these agents themselves? Indeed, what hath God wrought?

From the practical point of view there seem to be two vast areas which require research. First is in the use to which these substances may be put in order to operate upon the antagonisms of nature to our own lasting benefit. This requires more knowledge than we now possess, particularly so that we do not sacrifice immediate advantage for long-term loss. Their effects on plants and on plant diseases is so far only a microscopic part of antibiotic research, and it is possible that there are other areas of application as yet unopened.

Second, the process from discovery of antagonism to the preparation and evaluation of the substance needs some attention. It is too long in time and in money. We need to devise operational systems whereby we may "load the dice" in our own favor.

The medical problems are many and varied. I am not in a good position to speak of them, of course, but in the long run they reduce to the effective application of the substances to those conditions in which they will do the most good. Abuses we shall always find. Let us try to minimize them. One of the most insidious abuses, to my mind, is that apparent fact that we are a nation of faddists. We chase the latest, not the soundest. The experience of a lifetime is disregarded to follow the latest and sometimes "half-baked" opinion. This situation is not peculiar to antibiotics, but it does affect their use. One vast area of medical application is the virus diseases, and it does not yet have a suitable antibiotic. There is no assurance that one will be found. Problems of resistance, drug allergy, secondary infections, modified or new diseases, actual operation in the home or at the bedside, the relative relationship of prophylaxis or therapy, diagnosis, use and abuse of drugs, are not new problems. They are as old as the art of medicine. Yet, they are still problems, and they affect the use of antibiotics.

These comprise, in my opinion, the principal contemporary viewpoints of antibiotics. There are many hundreds of antibiotics. They all possess some degree of specificity, some to such an extent that they are exceedingly useful in the treatment of particular diseases. The chemistry of their molecules is highly varied, yet highly specific, and there is no general mode of action attributable to any large group of them. The reasons for this are yet to be discovered. Future development may progress somewhat more slowly, but an important group of problems, both for fundamental research and practical application, remains to be studied. With the antibiotics we have tools for treatment of disease, for fundamental research, for the promotion of growth, and for other yet uncertain purposes. Let us make use of them in the best possible way.

Unusual Aspects and Novel Uses of Antibiotics

JAMES C. LEWIS

The antibiotic compounds have a number of unusual aspects which it is my privilege to pursue with you this morning. When, in 1941, I left my classes and my friends at Oregon State College, René Dubos of the Rockefeller Institute had but recently announced the isolation of two crystalline substances from a *Bacillus* culture. Characterized by an extraordinarily high potency for inhibiting certain pathogenic bacteria, tyrocidine and particularly gramicidin keynoted an

intense interest among medical scientists, microbiologists, and biochemists. This interest culminated during the following decade in development of the remarkable drugs called penicillin, streptomycin, chloromycetin, aureomycin, terramycin, and others, about which we will hear much today. Along with the development of these notably successful antibiotics came the discovery and investigation of many hundreds of others which have more restricted commercial possibilities or only academic interest. Let us consider this array of compounds (the antibiotics in the strict sense) produced by certain micro-organisms and effective in inhibiting certain other micro-organisms in concentrations of 0.01 per cent or less.

The antibiotics are not a group closely knitted by chemical similarity. All contain carbon, hydrogen, and oxygen. No hydrocarbon has been recognized yet as being an antibiotic in the strict sense. Nitrogen and sulfur are present frequently, and non-ionic chlorine occasionally. The structures include simple acids, quinones, glycosides, polypeptides, and heterocyclic rings, both saturated and unsaturated. Chloromycetin is believed to be the first compound of biological origin to contain a nitrobenzene group. The structures frequently bear a weird resemblance to essential metabolites. Thus streptomycin contains 3 polyhydroxy residues in glycosidic linkage, an inositol twice substituted by guanidine groups, a new methyl dialdehyde pentose sugar, and N-methylglucosamine. The polypeptidic antibiotics are particularly interesting, because of the variety of new amino acids which have been isolated from nature for the first time as constituents of these strange compounds.

Gordon Alderton, who also went from this institution to the Western Regional Research Laboratory about 11 years ago, has isolated two sulfide amino acids, meso-lanthionine and beta-methyllanthionine, from the antibiotic subtilin which is produced by a strain of *Bacillus subtilis*. One or both of these acids have been recognized since as constituents of nisin, produced by a *Streptococcus lactis,* and of cinnamycin, produced by a *Streptomyces cinnamoneus.* These bacteria are not related closely in taxonomy. Other unusual amino acids include: beta, beta-dimethylcysteine from penicillin, alpha, gamma-diaminobutyric acid from the polymixins and polypeptin, ornithine from bacitracin, beta-lysine (the beta, epsilon diamino analog of lysine) from streptothricin, streptolin, and viomycin, and others that remain to be identified.

Like many other substances of biochemical interest, the antibiotics frequently occur in series of chemically closely related forms which have generally similar antibiotic and pharmacological characteristics. Thus, penicillin *G*, the usual penicillin of commerce, consists of benzyl alcohol linked through an amide group to the nucleus common to all of the penicillins. Other penicillins have the benzyl group replaced by the hydroxybenzyl group or by a wide variety of saturated and unsaturated aliphatic groups. It is possible to influence the course of microbial biosynthesis by supplying a precursor chemical, the nature of which is determined by the penicillin desired. Thus by addition of phenylacetic acid or derivatives the *Penicillium* mold is induced to make benzylpenicillin exclusively. Indeed, with certain strains, the yield is increased, as though the biosynthesis of the side chain had been the yield-limiting process. In the absence of added precursor n-heptylpenicillin is produced by an industrially important strain. By the use of appropriate precursors, it has been possible to biosynthesize substituted benzylpenicillins with fluorine, chlorine, bromine, iodine, or the nitro group in the side chain. The antibiotic activities of these analogs are similar to those of benzylpenicillin.

In addition to the customary form of streptomycin, another (hydroxystreptomycin) is known. In this form the methyl group of the dialdehyde sugar is replaced by a primary alcohol group. Streptomycin also occurs as an antibiotically active conjugate with a residue of d-mannose attached to the methylglucosamine residue. This conjugate resulted in a loss during purification of streptomycin in industrial operations until an enzyme was discovered in certain streptomycin-producing strains of *Streptomyces griseus.* By the proper control of this, it was possible to split off the mannose residue to give ordinary streptomycin.

The occurrence of closely related forms appears to be the rule with the polypeptidic antibiotics. Counter-current distribution or chromatography has shown that gramicidin, tyrocidine, polymixin, bacitracin, and others exist in several forms which differ by the substitution or addition of one or more amino acids.

In view of the disparity in chemical composition, it is not surprising that the antibiotics differ widely in other properties such as stability. A comparison of two antibiotics illustrates this. One, mycomycin, is a 13-carbon straight chain acid which contains 2 acetylenic bonds and 4 ethylenic bonds. It decomposes explosively at 75°C and is completely stable in solution only at

-40°C. The other, resistomycin, is stable to heating at 250°C and may be exposed to strong acid or alkali without loss of activity. Both are very active with test-tube cultures of the tuberculosis bacterium. At the recent meeting of the Chemical Society, James Dutcher suggested that perhaps in the case of mycomycin the bacteria were blown up by internal explosions, whereas with resistomycin the bacteria died of indigestion.

One of the most interesting characteristics of many antibiotics, as compared with other inhibitors of microbial activity, is their specificity. A considerable number are known which are active only against certain fungi and not against bacteria. Another large group are active for many gram-positive bacteria, but not for gram-negative bacteria or fungi. A large number have outstanding activity against certain *Mycobacteria*, as for example, *M. tuberculosis*, but have little activity against other types of micro-organisms. The clinically effective antibiotics obviously must combine high activity against the pathogen with low or negligible activity on animal tissues or organs. The most striking aspect of the specificity of antibiotics, however, is the natural occurrence of closely related strains of micro-organisms which differ widely in their sensitivity to a particular antibiotic, or the development of such strains. For example, in cultures of many species of bacteria which are highly sensitive to streptomycin, a minute fraction of the individual bacteria possess great resistance to the action of the antibiotic, as much as 1,000 times that of the majority. With other antibiotics, such as penicillin, great increases in resistance can be induced, but only slowly by exposure to gradual increases in penicillin concentration over many generations of the bacteria. The specificity of antibiotics is explained by a great diversity in their modes of action.

Frequently, an antibiotic which shows great activity in culture tubes is inactive in the animal. The example of actithiazic acid was mentioned recently by Nestor Bohonos. This antibiotic (discovered independently in different laboratories as so many other antibiotics have been) was found to be extremely active against tuberculosis bacteria *in vitro* but to have no effect in mice. The dose given to mice was very high. If recalculated for a man it would amount to about three-quarters of a pound per day.

Many factors affect the relative activities when effects in culture tubes and in animals are compared. One of the more important is the effect of constituents of the blood serum. In fact, a test for activity in the presence of serum is usually made early in any screening program for therapeutic antibiotics. In one case, that of the antibacterial agent lupulone which occurs naturally in hops, the antagonistic action of serum was traced to the phospholipid fraction of the serum. A similar inactivation was obtained with fresh egg yolk or with the phospholipids lecithin and cephalin from egg yolk. In view of some of the recent concern about the addition of antibiotics and other chemicals to foods, one might suggest that this observation may provide a rational basis for the old custom of putting an egg into one's beer.

Now let us consider what gives cohesion to this class of compounds that are so diverse in their chemical nature, physical-chemical properties, and biological behavior. Is it not a matter of the experimental technique of discovery as well as the common bond of interest in therapeutic use? In a common and efficient method of searching for new antibiotic agents, potential antibiotic-producing micro-organisms (usually derived from soil) are grown in bacteriological media solidified with agar. After the colonies are grown, the plate may be reinoculated with a test organism and reincubated. A zone lacking growth of the test organism will be seen around each colony which has excreted a growth inhibitor for the test organism. Many refinements are possible. Soil samples may be collected from all over the world. The bacteriological medium may be adjusted so that particular types of inhibitor-producing micro-organisms will be selected. The test organism may be a particular pathogen, and it may be one which is resistant to those antibiotics which are in general use. It is apparent that the organism selected may be literally one-in-a-million and that this selection is made without undue labor. This ease and efficiency of experimentation, used more or less effectively, is basic in any explanation of the antibiotic boom of the past decade.

The interest in antiviral agents is measured by tremendous sums being spent in organized searches among synthetic chemicals, plant extracts, and microbial filtrates; but here there is little semiautomatic screening of an efficiency such as is possible for antibiotics. The initial trial is made on an egg or a mouse. Obviously, any drastic simplification of technique would be most important, and probably essential if a range of agents is to be disclosed similar to that known now for the antibiotics.

The phenomenon of antibiosis is most general. Bacteriostatic agents are produced by all sorts of biological tissues. Hops, bananas, buttercups,

radishes, lichens, and scores of other plants produce agents that are fundamentally similar to those produced by micro-organisms. Indeed, the antibiotic citrinin is produced not only by fungi of the *Penicillium* and *Aspergillis* genera but also by the legume *Crotolaria crispata* to the extent of more than 1 per cent of the dry weight of the leaves. Blood serum contains an inhibitor of many bacteria. The hen's egg contains a complement of antibacterial agents: lysozyme, which hydrolyzes the mucopolysaccharide of the cell wall of many bacteria; avidin, which binds biotin tenaciously and inhibits the growth of biotin-requiring organisms; and conalbumin, which behaves similarly with respect to inorganic iron. Except that their modes of action may be somewhat better understood and that they resemble conventional proteins, which are rare or lacking among the well recognized antibiotics, they could be considered as antibiotics.

Also, the phenomenon of antibiosis is not restricted to actions on micro-organisms, as is testified by the examples of pyrethrum, curare, red squill, opium, poisonous mushrooms, and the many scores of other poisonous plants that the millennia of human experience have disclosed. The proteinaceous toxins of snakes, diphtheria, tetanus, and botulism are well known. Some antibiotics in the strict sense are remarkably toxic to animals. For example, actinomycin was suggested (perhaps facetiously) for use as a rat poison. It has been suggested that survival of a microscopic crustacean (*Daphnia pulex*), when suspended in a dilute solution of an antibiotic, may provide a better basis for an initial guess as to the acute toxicity of the antibiotic than the customary toxicity tests on rodents which require much more time and work.

According to a citation by Florey, a destructive species of leaf-cutting ant (*Acromyrmex striatus*) in South America could be controlled by fungal antagonism. As food, the ants use leaves on which particular strains of fungi (*Hypomyces ipomeae* and *Fusarium* spp.) are cultured. Other common fungi, such as *Penicillium*, *Verticillium*, and *Mucor*, are inhibited by the saliva of the ants and thus do not overrun the ant hotbeds or gardens. It was possible, however, to find an antagonistic fungus which inhibited the ant fungus both on agar and in the fungus gardens of the ants. When spores of the special fungus were strewn near the ant nest they were carried by the ants into the nest, whereupon within a few days not only the ant fungus but also the ants themselves were destroyed.

Antibiosis is but one aspect of the infinite number of interactions between cells of one sort and another, within an individual or between individual organisms, that are mediated through specific compounds. It is sufficient to mention the plant and animal hormones, and particularly the vitamins and growth factors. The latter stand in a special, inverse relationship to the antibiotics by virtue of the general hypothesis that antibiotics act by interfering with the utilization of compounds essential in metabolism. This provides for a number of interesting anomalies. Thus p-aminobenzoic acid gives a growth response under certain conditions for rats and chicks, and it is an essential vitamin for certain micro-organisms. The therapeutic action of some sulfa drugs is inhibited by the presence of small concentrations of p-aminobenzoic acid. This observation has given rise to one hypothesis for the mode of action of certain sulfa drugs. But p-aminobenzoic acid inhibits the growth of certain micro-organisms such as the *Rickettsia*; and, because it is produced by micro-organisms, it fits the strictest definition of an antibiotic. The bread mold *Neurospora*, with which George Beadle has originated much important biochemical and genetic work, requires p-aminobenzoic acid which most strains can biosynthesize for their own needs. Certain mutants of *Neurospora* have lost this biosynthetic ability and require an outside supply of p-aminobenzoic acid to be provided in the medium at low, non-toxic concentrations. Certain other mutants, on the other hand, produce such a toxic excess of p-aminobenzoic acid that they literally can not live with themselves. They grow, and can be detected, only in the presence of an antagonist for the excess p-aminobenzoic acid. Sulfanilamide serves this purpose; and, when first discovered, the *Neurospora* mutants appeared to require sulfanilamide.

A similar case is known with streptomycin and a mutant strain of virulent meningococcus. This mutant grows best in the presence of 100 to 400 ppm. of streptomycin and fails to grow with concentrations of streptomycin lower than 5 ppm. Mice infected with a normal strain of meningococcus quickly develop a fatal infection, which can be prevented by treatment with streptomycin. The streptomycin-requiring mutant, on the other hand, is not virulent in mice unless the mice are treated with streptomycin, in which case the infection follows its normal fatal course.

Now let us give some consideration to novel uses of antibiotics. I have already hinted at one, the use of hops in beer-making. This is a custom

which originated many centuries ago. Beer made with hops has a mild bacteriostatic action on certain gram-positive bacteria which would otherwise present a problem of spoilage in unpasteurized beer, such as is customarily used in many other countries than our own. Both the antiseptic action and the characteristic bitter flavor have their origin in one or the other of the two chemically similar antibiotics, humulone and lupulone, of hops, though it is recognized that extensive chemical changes in these constituents take place during the brewing process. It is a question whether the custom of using hops in brewing arose through a desire for the bitter flavor or for the improved stability. In either event hops are now used in this country primarily for their flavor. I am sure you will agree that while this is not a new use, it certainly is an unusual use for an antibiotic.

A few years ago the idea of feeding antibiotics to improve the growth rate and efficiency of feed utilization by poultry and swine would have been described as fantastic rather than as novel. Now it well deserves the special attention that Dr. James McGinnis will give it this afternoon.

Somewhat more conventional ideas are involved in certain proposed uses of antibiotics in the control of plant pathogens. A number of possibilities have been proposed or investigated. One type involves therapy of the infected plant. Some very encouraging results were reported last year with respect to the use of streptomycin in the control of a bacterial blight of beans. The antibiotic was applied to seedlings in a thin layer of lanolin on the internode just above the cotyledons. Three days later the primary leaves were inoculated by rubbing with the infective bacteria and fine carborundum powder. Only streptomycin and dehydrostreptomycin, of about a dozen major antibiotics tested, prevented development of the infection. It was apparent that the streptomycin was absorbed through the stem and translocated to the leaves, a finding most important to practical use of the observation. Tests were made with both halo blight (*Pseudomonas medicaginis* var. *phaseolicola*) and common blight (*Xanthomonas phaseoli*). Application of streptomycin to the soil was ineffective, although it has been shown that the roots of various plants can absorb various antibiotics and translocate them to the leaves. Also, streptomycin was not stored in the seeds of sprayed plants in sufficient amounts to be effective for the new seedlings.

Field plot trials with alternate rows of beans infected with halo blight and untreated and

treated with several streptomycin sprays throughout the growing season have given very encouraging results. The untreated plants were devastated by the blight whereas the treated plants matured normally. The cost is estimated to be within the economic possibilities. The very serious possibility that streptomycin-resistant variants of the blight bacteria may develop in field use of streptomycin remains to be tested.

Penicillin and streptomycin have been used successfully by local application in the treatment of crown galls on various plants. The causative bacterium, *Bacterium tumefaciens*, is killed and in some cases the tumors themselves have disintegrated and disappeared.

Other antibiotics have given promising results. Antimycin inhibits a scab-producing fungus of apples and pears (*Venturia inaequalis*) as well as other pathogens. It has retained its activity when applied with insecticide sprays, and it is not readily washed off the plants by rain.

Lettuce and tomatoes growing in water or sand cultures to which the antibiotic griseofulvin was added had considerably increased immunity to infection with the fungi *Botrytis cenerea* and *Alternaria solani.* Griseofulvin was shown to be translocated by assays of leaf tissue or, in the case of oat seedlings, by assay of the droplets of guttation-fluid induced by covering the oats overnight with a bell-jar. Griseofulvin continued to be detectable in the guttation drops for as long as three weeks after griseofulvin had been removed from contact with the roots.

Seed treatments also present an attractive method for economical application of prophylactic agents. Spring wheat dusted with finely powdered clay containing 0.5 per cent of actidione has given excellent protection against both natural and artificial contamination with covered smut (*Tilletia* sp.).

Streptomycin and other antibiotics have given effective sterilization of bean seeds contaminated with bean blight, although only streptomycin was effective as a spray, as has already been discussed.

The portion of the antibiotic applied to leaves or roots of plants that gets to the site of action must be small. It is of interest, however, that absorption of certain water-soluble fungicides by roots, in the presence of soil, is much more effective than absorption from injections into the trunks of trees infected with Dutch elm disease. The practical modes of administration necessarily involve much more wastage than is usually the case in the treatment of animal or human diseases. An exception is the use of streptomycin in the

treatment of tuberculosis and resembles the plant diseases in terms of the difficulty of obtaining effective concentrations of the antibiotic at the site of the infection.

It is clear that costs become particularly important in any consideration of control of plant diseases. It must be understood, however, that the cost of antibiotics for human use includes the costs of special purification and control which could probably be reduced greatly if crude antibiotics were to be prepared especially for the control of plant pathogens.

One may note that knowledge of effective agents of microbial origin also could be very important as a source of clues for the synthesis of effective, more readily producible agents.

Another basic mode of approach attempts to minimize cost by establishing a soil flora that is unfavorable to the pathogenic micro-organism. It is to be noted that the soil is one of the important means for the transmittal of plant pathogens. Although this approach is subject to grave difficulties and complications, certain observations from nature have encouraged work of this sort. For example, the Panama disease of bananas is caused by a fungus *Fusarium oxysporum cubense*. Certain areas have a natural resistance to the disease, whereas other nearby areas lack such a resistance. One example involves a locality on a river in Honduras which is flooded regularly by waters carrying debris from infected areas up river. This locality has remained free of the disease for about fifty years. Actinomycetes antagonistic to the *Fusarium* have been isolated from various soils of Jamaica, but a correlation with the incidence of the disease has not been reported yet.

Manuring practices can sometimes be used to control plant pathogens. The manures stimulate and alter the microbial activity of the soil, and it seems likely that the action on the pathogens is mediated through diffusible antibiotics. For example, certain green manures, such as soybeans, aid in controlling potato scab and strawberry root-rot. The green manures are known to stimulate growth of *Bacillus*, a group noted for production of a diversity of antibiotics. "Take all" disease of cereals (*Ophiobolus graminis*) is controlled by low nitrogen manures but not by high nitrogen manures. Deep covering of large quantities of hay permits successful control of cotton root-rot disease (*Phymatotrichum omnivorum*). Traces of antibiotic activity have been demonstrated in extracts of natural soils which have been treated with carbohydrate, but no identification of the antibiotics has been made.

The role of an antibiotic in the ecological relationships that influence plant growth is well illustrated by the classic case of certain heath soils at Wareham Forest, Dorset, England. Poor growth of pine trees in these areas correlated well with absence or poor development of the normal mycorhiza or root fungi, of which *Boletus bovinus* is dominant. Field observations indicated the poor growth was most evident under waterlogged conditions and that it tended to be corrected by drying, or particularly by the addition of compost to the soils. However, erratic distribution of the infertile condition and rapid, spontaneous recovery in scattered localities strongly suggested the intervention of some sort of soil infection.

The poor growth was found to be related to the presence in the soil of a diffusible toxic substance, which was particularly active against *Boletus bovinus*, the normal root fungus. The toxin was unstable, being destroyed in soil samples by steaming or by drying. Soil that was rendered nontoxic by a short period of steaming slowly regained its toxicity, whereas soil which was steamed for an hour, or autoclaved, or soaked in alcohol for several hours, failed to regain its toxicity. Such a sterilized soil, however, regained its toxicity rapidly under favorable conditions if it was inoculated with a small portion of toxic soil. Thus, it appeared that a biological agent is responsible for the production of a toxin against the mycorhizal fungi and that the low activity of these fungi led to long-lasting effects on the growth of pines—that is, to an infertile soil.

It has been shown subsequently that the toxicity is probably due at least in part to gliotoxin, a somewhat unstabile antibiotic with high activity against the mycorhizal fungi, which was found to be produced by certain strains of *Penicillium* (*P. jenseni* and others) which are among the characteristic mold flora of the Wareham soil.

Inoculation of soils with antibiotic-producing micro-organisms represents another possibility that has attracted attention and which, if successful, might prove quite economical. It represents a very difficult problem, because not only must the antibiotic have favorable characteristics with respect to activity on the pathogen, mobility in the soil and in the plant, and stability, but also the productive micro-organism must be adapted both to production of the antibiotic in nonsterile soil and to the difficult feat of self-survival. This consideration would seem to make particularly worth while a search for producers of suitable

antibiotics among the normal flora of the rhyzo-sphere and the regions of the soil immediately adjacent to the roots which are characterized by the most intense microbial activity.

In this connection the supposed "immunity" of corn seedlings to *Phymatotrichum omnivorum* was traced to the protective action of other micro-organisms associated with the roots. A similar situation was induced for the sensitive cotton plant by treatments such as partial defoliation, which raised the carbohydrate content of the root bark. Increases in the blue-green fluorescent bacteria associated with the roots were considered possibly to play a role in the resistance thus induced.

The general result so far of experiments with inoculation has been that, whereas experiments in sterilized soils frequently have been effective, the results in nonsterile soils have been much less promising. Soils sometimes are partly sterilized, as in nurseries. It is possible that inoculation and manuring might be feasible here. Such a situation would also be most favorable for higher-cost practices such as the direct application of crude antibiotic preparations.

An interesting search has been made for a soil organism that would produce an antibiotic effective in the control of damping-off fungi (*Pythium* spp.) in legume seedlings but which would not interfere with the nitrogen-fixing *Rhizobium* spp. The antibiotics actidione and fradicin have these properties and unlike many other antibiotics they remain active in the soil for the requisite two-week period. These antibiotics exhibited the curious property of interfering with each other so that the *Pythium debaryanum* being tested grew better in the mixture of the two antibiotics than with each one by itself. Further investigations of these agents appeared to be indicated.

There is yet another phase of the use of antibiotics in the control of plant pathogens, if you will permit me to apply the term to chemicals of plant origin. This involves selecting or breeding plants for a suitable content of a natural constituent which has the desired effect. Thus Greathouse surveyed 60 different alkaloids from approximately that many species of plants and found that the relative resistance of the plants to the root-rot fungus *Phymatotrichum omnivorum* correlated well with the occurrence of certain alkaloids in the roots and with the specific activity of the various alkaloids. Thus the most active alkaloid tested, sanguinarine, was isolated in a yield of 0.2 per cent from powdered rhizome

and root tissue of *Sanguinaria canadensis,* a plant which is highly resistant to the *Phymatotrichum* root rot. Other alkaloids from the same tissue were much less effective. As another example, the roots of *Mahonia trifoliolata* and *M. swaseyi* contained 1.3 to 2.5 per cent of berberine, dry basis. The lower value is at least 60 times as great as the minimal inhibitive level for the root rot fungus in a laboratory culture. The berberine content in the above-ground portions of the plants was much lower, and the alkaloid could not be detected in young leaves. Histological studies of the roots showed that most of the berberine was located in the bark of the roots. The two species, which grow in a region of central Texas where cotton is infested with root-rot fungus, are considered to be highly resistant or immune to the root rot.

Another example is the presence of protocatechuic acid and catechol in the dry outer scales of yellow and red skinned onions. Diffusion of these water soluble compounds into the soil is considered to prevent growth of various decay fungi. Thus, certain onion hybrids which have split or otherwise defective outer scales are vulnerable to fungal attack.

Such observations point up the possibilities of extensive basic studies of the occurrence within the economically important plants or their genetically miscible relatives of inhibitors of important pathogens and pests.

The germination of seeds is inhibited by a number of antibiotics, among them penicillin. I do not pretend to see a use for this observation, but in case one were to be foreseen it may be noted that it is possible to search efficiently for such agents by much the same technique that is used for conventional antibiotics. Instead of a test micro-organism, surface sterilized fine seeds may be placed on an agar plate with mixed colonies of micro-organisms such as are obtained from a soil suspension. Germination of seeds in the neighborhood of certain colonies has been observed to be inhibited.

Antibiotics recently have been reported to stimulate plant growth without the intervention of micro-organisms. A variety of antibiotics stimulated growth of a tumor tissue from the root of the sorrel plant (*Rumex acetosa*) when this tissue was grown under aseptic conditions. The growth of germinating seeds of *Agave toumeyana* was stimulated by a few ppm. of thiolutin under standard germination test conditions whether the seeds were sterilized or not. In soil flats the germination and growth of sorrel seeds and of seed

corn was promoted substantially by watering with tap water containing 5 ppm. of terramycin.

The possibility of using antibiotics in the preservation of food has attracted much attention. The use of hops in brewing has been mentioned. I have not been able to find any other use as clearly established as this one is, but it appears that antibiotics which are formed naturally during the process of making cheese may aid in the preservation of this food. Hirsch has pointed out recently that the factors which generally govern the development of botulism in foods are present for cheese but that on the other hand botulism is rarely associated with cheese. The factors are (1) the presence of considerable numbers of spores of *Clostridium botulinum,* (2) anaerobic conditions, (3) lack of thorough heating or cooking, and (4) use without a final cooking. The restraining factors in cheese may be complex. It has been suggested that fatty acids as well as hydrogen peroxide produced by certain lactobacilli may be involved. Certain strains of *Streptococcus lactis* when grown in milk produce an antibiotic (nisin) which is active against many strains of *Clostridium.* A natural type of infection caused by *Cl. butylicum* and *Cl. sporogenes* was prevented even in cheese inoculated with these bacteria if the cheese was made with milk especially cultured to have a high nisin content.

Soon after Fleming discovered lysozyme in egg white, it was reported that the Russians were using this bacteriolytic protein for the preservation of caviar. More recently the addition of known antibiotics has been investigated in connection with the preservation of fish, fresh and processed meat, fresh fruit and vegetables, canned vegetables, custard, milk, tomato juice, and the Japanese rice wine "saké". While there have been a great many disappointments, certain observations encourage much more investigation of this field.

One type of application looks toward an enhanced shelf life of food products. The Bureau of Plant Industry has been conducting an empirical search for chemicals that will retard the development of rot in citrus fruit and fresh vegetables. Spinach in cellophane bags is prone to develop bacterial slime. It has been reported that if the spinach is dipped in dilute streptomycin solution and then rinsed thoroughly before being placed in the bags the shelf life is prolonged by several days.

It has been reported from the Great Atlantic and Pacific Tea Company that substantial concentrations of subtilin, preferably in combination with low concentrations of terramycin, have sub-stantially lengthened the shelf life of custards with the natural bacterial flora and also with artificial inocula of the food-poisoning bacterium *Staphylococcus aureus.* These foods present a serious problem, because enough of the toxin may be produced in the foods that apparently are still edible to cause digestive disturbances that are severe although rarely lethal (as with botulism). A method of repressing the food-poisoning strains of *Staphylococcus,* at least until the other bacteria give rise to obvious signs of putrefaction, would be very desirable.

Studies on the possibility of using penicillin and streptomycin in the preservation of milk were reported from the Bureau of Dairy Industry as early as 1946. Penicillin was added to inoculated milk after a mild heat treatment which had the purpose of inactivating penicillinase, killing vegetative cells, and activating the spores so as to promote their subsequent germination. Although preservative effects were obtained with some of the strains of bacteria used for inoculation, the results were not sufficiently general or reliable to encourage further work with these agents.

In 1949 Andersen and Michener of the Western Regional Research Laboratory reported the novel observation that when the antibiotic subtilin was present during a mild heat treatment at 85° or 100° C, a variety of canned vegetables and milk were preserved with excellent effects on quality of the products. Unfortunately, the only inoculated controls were inoculated with spores of a strain of *Bacillus stearothermophilus* which was particularly sensitive to the simultaneous action of subtilin and heat, and this led to some overly optimistic conclusions. In the flush of enthusiasm that followed various people acted on the assumption that the phenomenon was general. Thus there was considerable disappointment when this was found not to be true. In particular, large inocula of spores of *Clostridium botulinum* and other heat-resistant spoilage bacteria were not controlled with mild heat treatments of moderate duration even though high concentrations of subtilin were used.

It may be well to pause for a few minutes to consider the nature of heat sterilization. Modern analysis of death of spores treats this type of reaction as a statistical phenomenon. This implies that there is no one particular level of heat treatment for a particular lot of spores dispersed in a food or nutritive material which gives complete killing regardless of spore density and size of container. Instead, it is assumed that the thermal inactivation of spores follows a first order

reaction, such as is found for radioactive decay, so that during successive equal intervals of time the number of spores is reduced to a constant fraction of the number present at the start of the interval. Thus, if only 10 per cent survive after a 1 minute treatment it would take 3 minutes to reduce 1,000 spores to a single spore whereas it would take 6 minutes to reduce 1,000,000 spores to a single spore. I am belaboring this point a little bit, because one occasionally hears statements such as "Where foods are concerned not one *botulinum* spore must escape destruction." In reality a small but finite number will escape destruction, and whether this is to be one in a billion cans or one in a quadrillion cans represents a practical compromise between considerations for public health and for consumer acceptance. For convenience, the heat sensitivity of spores is expressed by two constants, one expressed as the number of minutes at 250°F required to achieve a reduction of the spore count to 10 per cent and the other related to the killing intensities at different temperatures. C. R. Stumbo of Washington State College is engaged in an extensive program for the determination of the effect of subtilin and other antibiotics on the thermal death constants for a variety of strains of *Cl. botulinum* and other spore-forming food spoilage bacteria in various vegetable media. He has put the findings of Andersen and Michener on a quantitative basis and extended them considerably. The effect of subtilin on several strains of *Cl. botulinum* and related bacteria is equivalent to a reduction of approximately 40 to 50 per cent in the number of minutes at 250°F for reduction to 10 per cent of the initial count, whereas there was no significant change in the constant relating the relative effectiveness at various temperatures. He considers that the reduction possible in sterilization schedules would have a marked effect on the quality of certain canned vegetables, such as corn and particularly cauliflower and other winter vegetables which deteriorate so badly under the presently prescribed processing schedules that these vegetables ordinarily are not packed in this country.

I hasten to add that considerable more investigational work must be done before any practical recommendations are made. Furthermore, subtilin is not available commercially, so that accurate cost estimates cannot be made as yet. It seems clear that the cost would be appreciable though not necessarily prohibitive.

Less processing is required for acidic foods such as fruits and tomato juice, because botulism does not develop in such foods and because heat sterilization tends to be more effective at low pH levels. In order to obtain the best quality, tomato juice is not processed ordinarily to the point of complete destruction of the heat-resistant, flat, sour spoilage bacteria. This is feasible since tomato juice ordinarily is too acidic for germination of spores of such bacteria. Occasionally, severe losses are suffered, however. Subtilin has been found to be very effective against this type of bacteria. Heavily inoculated packs containing low concentrations of subtilin became sterile when they were processed with mild heat. It is possible that such a use of subtilin might be justified as insurance against occasional spoilage.

The same considerations that apply in prolongation of the shelf life of perishable products might be applied to canned goods if public health considerations could be met. In other words, it might not be necessary to produce a sterile pack if spoilage could be put off indefinitely. One might destroy enzymes and vegetative cells, together with a proportion of the spores in canned vegetables, and hope to preserve the vegetables by dependence on an antibiotic which would prevent any microbial reproduction although it might be without any effect on the ungerminated spores. Indeed, the initial success of Andersen and Michener's subtilin-mild heat treatment supported hopes of this sort. The action of subtilin in killing germinating spores of many species and strains is far more intense than the effect on thermal death of spores that I have discussed. This approach, however, is fraught with difficulties which I shall enumerate. First, the antibiotic or mixture of antibiotics would have to be active against all spore-bearing spoilage bacteria. Complicated and expensive mixtures would probably be required to achieve this. Secondly, unless all spores were to germinate and be eliminated promptly, the antibiotics would have to be sufficiently stable to maintain their effectiveness during the normal shelf life of the product. Subtilin is fairly stable as antibiotics go but the subtilin content of canned vegetables drops markedly in a matter of weeks. As an alternate to this second requirement, I have mentioned prompt germination and death of all spores. Although many of the factors that influence spore germination are now being discovered, the outlook for this approach is not encouraging. Indeed, it appears that spores may have mechanisms that tend to favor survival by avoiding prompt or simultaneous germination.

In addition, this approach would require a

complete revolution in public health considerations with respect to canned goods. At present the standards require that extremely heavy inoculations of *C. botulinum* be destroyed with extremely low probability of failure. Such standards, however, are not applied in the pasteurization of milk. If milk is inoculated heavily with *C. botulinum* and pasteurized, it becomes toxic. Obviously, however, the use of pasteurized milk does not present a serious public health hazard. Aside from the benefit of strict control of sanitation during handling, milk does not become toxic, because other types of bacteria develop preferentially and prevent the development of *Clostridium botulinum*. Possibly, such a built-in protection could be given to vegetables canned with mild heat, but it would probably not be essentially automatic as is the case with milk.

There are a number of other minor but interesting uses for antibiotics. Penicillin, streptomycin, and other antibacterial agents have been used in the suspending fluids for bovine spermatozoa with good results that have been interpreted to indicate a reduced embryonic mortality associated with control of infectious agents in the semen.

Occasionally, the use of antibiotics may give rise to new problems. Penicillin and aureomycin, which are used for the control of mastitis in dairy cows, are excreted into the milk for considerable periods after therapy. For several years they have been observed to interfere with the cheese-making process, unless such milk is diluted sufficiently with normal antibiotic-free milk. The antibiotics act by inhibiting the normal lactic-acid producing streptococci. As little as 0.5 ppm. of aureomycin or less than that of penicillin interferes seriously.

A somewhat similar observation has been made that eggs from hens fed aureomycin may contain sufficient of the drug to affect their use in the culturing of *Rickettsia* in chick embryos.

It is hardly necessary to point out that the antibiotics have many uses of a purely research nature. Some of these perhaps will become apparent with the talks to be given later today. One that I shall mention deals with their use in selective media. These media have great importance in bacteriology for distinguishing various groups of micro-organisms. Crude broth filtrates containing penicillin were used for this purpose early in the 1930's. Such selective media have had great importance in studies of bacterial genetics since antibiotic-resistant mutants which occur at low frequencies could be isolated or enumerated

readily. One of the most interesting techniques was proposed only a few years ago by Bernard Davis. This technique is intended to isolate bacterial mutants which have additional nutritional requirements, a task which would be extremely tedious by any conventional pick-and-test method. The principle depends on the fact that penicillin kills only growing bacteria and is harmless to non-growing cells. Suppose it is desired to obtain a strain of *Escherichia coli* which requires the vitamin niacin from a usual niacin-non-requiring strain. The latter is placed in a medium suitable for growth but lacking niacin. After growth has started, penicillin is added. The dividing cells are killed and those that cannot grow for lack of niacin or other reasons are not killed. The penicillin is then destroyed with penicillinase, and the bacteria are plated onto the same medium to which niacin has been added. The bacteria that grow now have a greatly increased frequency of niacin-requirement, and individual colonies may now be picked and tested with assurance that niacin-requiring strains will be found.

You have listened with forbearance to a smattering of facts about antibiotics. Particularly well-developed aspects will be discussed much more intensively by others. Perhaps some day the actual use of antibiotics in horticulture, in food technology, or in fields yet untouched will warrant similar attention.

SELECTED LITERATURE

BRADFIELD, A. et al., The presence of aureomycin in milk and its effect on cheese making and starter activity. *J. Dairy Sci.* 35: 51 (1952).

DAVIS, B. D., The isolation of biochemically deficient mutants of bacteria by means of penicillin. *Proc. Nat. Acad. Sci.* 35: 1 (1949).

FLOREY, H. W., et al., Antibiotics. Oxford Univ. Press (1949).

FOOTE, R. H., and R. W. BRATTON, The influence of antibiotics on delayed returns in artificial breeding. *J. Dairy Sci.* 35: 261 (1952).

GREATHOUSE, G. A., et al., The chemistry of resistance of plants to Phymatotrichum root rot. *Phytopathology* 30: 475 (1940).

GREGORY, K. F., et al., Antibiotics as agents for the control of certain damping-off fungi. *Am. J. Bot.* 39: 405 (1952).

GROSSBARD, E., Antibiotics and microbial antagonism in plant pathology. *Endeavor* 10: 145 (1951).

HART, H., Nature and variability of disease resistance in plants. *Ann. Rev. Microb.* 3: 289 (1949).

HIRSCH, A., Antibiotics in food preservation, lecture presented before the Summer Conference (Cambridge) of the Society for Applied Biology (1952).

HORSFALL, J. G., and A. E. DIMOND, Plant Chemotherapy. *Ann. Rev. Microb.* 5: 209 (1951).

KELNER, A., A method for investigating large microbial populations for antibiotic activity. *J. Bact.* 56: 157 (1948).

LE BLANC, F. R., et al., Antibiotics in food preservation I. The influence of subtilin on the thermal death of spores of *Clostridium botulinum* and the putrefactive anaerobe 3679. *Food Technology* 7: 181 (1953).

MEREDITH, C. H., The antagonism of soil organisms to *Fusarium oxysporum cubense. Phytopathology* 34: 426 (1944).

MILLER, C. P., and M. BOHNHOFF, Two streptomycin-resistant variants of meningococcus. *J. Bact.* 54: 467 (1947).

MITCHELL, J. W., et al., Translocation of streptomycin in bean plants and its effect on bacterial blights. *Science* 115: 114 (1952).

NEILSON-JONES, W., Biological aspects of soil fertility. *J. Agr. Sci.,* 31: 379 (1941).

NICKELL, L. G., Stimulation of plant growth by antibiotics. *Proc. Soc. Exptl. Biol. Med.* 80: 615 (1952).

TURNER, J. C., and T. J. LANNON, Effects of antibiotics on Daphnia. *Proc. Soc. Exptl. Biol. Med.* 80: 684 (1952).

WEINDLING, R., et al., Antibiosis in relation to plant disease. *Ann. Rev. Microb.* 4: 247 (1950).

Social and Economic Impact of Antibiotics*

ROBERTSON PRATT

Man has made empirical use of natural antagonisms among micro-organisms for the control of infectious microbes for at least thirty centuries. But it was only after the birth of bacteriology as a science in the last half of the nineteenth century that rational application of microbial antagonisms according to definite principles became possible. No development in the field of biologic research since Pasteur's enunciation of the germ theory of disease (about 1860) has been of greater importance in the treatment of disease than discovery and development of practical means of using antibiotics clinically for systemic chemotherapy.

Only a few years ago a diagnosis of pneumonia, of septicemia, of typhoid fever, of tuberculosis, or of other infectious diseases struck a note of terror in patient and relatives alike and, at best, foretold a long, painful, expensive course of treatment and convalescence. Today, with proper use of antibiotics, this is changed. Some of the most dreaded diseases can be cured in a matter of days. This change is the outcome of the application in medicine of the results of fundamental research in microbiology and chemistry.

While solving one problem, research frequently gives rise to new problems. These are not always scientific or technologic problems; often they are social or economic. Research in the health sciences, despite the fact that it provides for improvement of health and prolongation of life, is not exempt. Indeed, the very success that has been achieved is increasing the complexity of socio-economic problems by favoring a rapid change in the population structure. Antibiotics are contributing to this change.

All highly industrialized and technologically advanced nations where modern technics of health care and sanitation are practiced are rapidly becoming nations of older people. In United States three major factors have contributed to the increase in the proportion of elderly people in the present population. These are immigration, declining birth rate, and declining death rate.

Immigrants who entered the United States in great numbers between 1890 and 1910 constitute a significant part of the present day older population (about 20 per cent as compared with 10 per cent foreign born in the population as a whole), but immigration will not be so important in determining the future population structure because entrance into the country is now restricted by quotas. The two factors that continue to contribute markedly to our aging population are decreased birth rate and declining mortality rate. Life expectancy at birth has increased remarkably in the last fifty years, as shown by the following statistics and by Figure 4.

	Years
1900	47.3
1905	48.7
1910	50.0
1915	54.5
1920	54.1
1925	59.0
1930	59.7
1935	61.7
1940	62.9
1945	65.9
1950	68.3
1952	68.5

Note: These composite figures are based on data for both sexes and all races in the United States from 1900 to 1952.
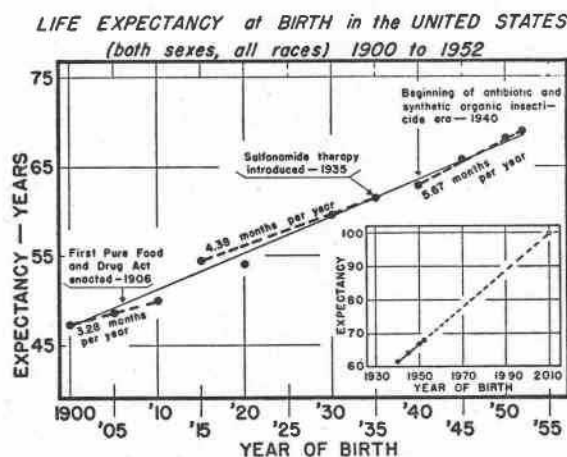
Figure 4. Life expectancy at birth in the United States (both sexes, all races) 1900-1952. Inset: Extrapolation of curve for last decade shows that approximately three generations hence life expectancy at birth will reach 100 years if the rate of the past decade continues unabated.

Figure 4 is a graphic representation of the tabulated data. A single straight line (solid in graph) fits the points reasonably well, but careful examination of the data suggests that actually the rate of increase has been accelerating since 1900. The dashed curves show that the over-all picture can be considered as composed of three periods, namely 1900 to 1910, 1915 to 1935, and 1935 to the present time. Within any one of these periods the rate of increase was essentially constant, but in each successive period the rate was appreciably greater than during the preceding one. The rate of increase characterizing each period is shown adjacent to the dashed curve. Im-

portant developments that may have influenced the rate of increase in subsequent years also are indicated. Many biologists and anthropologists hold that the natural life span (not to be confused with longevity, life expectancy, or average length of life) of man probably is about one hundred years. The inset in Figure 4 shows that if the present rate of increase in life expectancy continues unchanged, life expectancy at birth in the United States may reach the century mark approximately three generations hence.

In the short term view, declining mortality reduces the average age of the population, because the most remarkable gains have been made in curtailing infant mortality. But, in the long term view, the gains in the early years combined with the declining birth rate can only increase the ratio of older individuals in the dependent age groups to the younger individuals in the productive age groups and result in an increase in the average age (Figure 5). Although some individuals under 20 and some over 60 are employed or are otherwise self-supporting, the number is small compared with the total population of the two age groups. Therefore, it is conventional to class these two groups as dependent and to consider the industrially or economically productive population as comprising those individuals between 20 and 60 years of age.

The declining mortality rate and increasing life expectancy can be attributed largely to the many technologic advances that have been made, including improved techniques of health care and sanitation. Biologic research has had a prominent part in these developments. Some of the reduction in disease mortality rates since 1930 unquestionably is due to introduction of sulfonamide therapy in 1935 followed in the 1940's by antibiotic therapy. But the real impact of chemotherapy and especially of antibiotic therapy on the population structure will not be felt until the latter part of this century when the sharp decrease in infant mortality will be reflected in a larger proportion of people in the later decades of life (Figure 6).

Figure 7 shows graphically the per cent change in the ratio of dependent age groups to the productive age group in the United States during the century 1850 to 1950. It is apparent that the net increase has been about one hundred per cent. This means that today, in addition to a tax burden which has been ever increasing, the average worker carries about twice the economic load for support of dependents that the average worker carried one hundred years ago. The inset in Figure 7 shows, by extrapolation, what may be
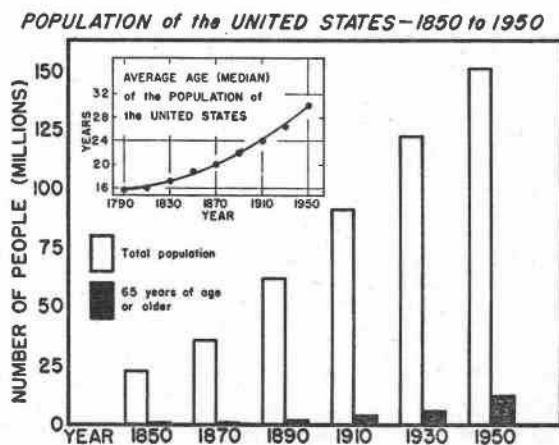

Figure 5. Population of the United States, 1850-1950. Inset: Average age (median) of the population of the United States, 1790-1950.
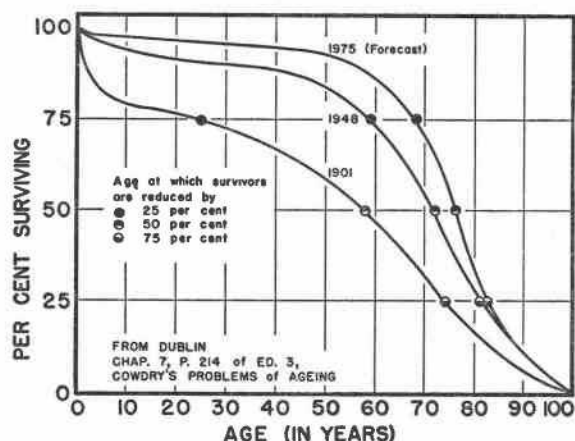
Figure 6. Survivor curves based on life tables for the United States, 1901, 1948, and 1975 (forecast). One-fourth of children born in 1901 failed to attain age 25. Corresponding depletion in children born in 1948 will not occur until the surviving individuals are almost 60 years. The corresponding forecast for the 1975 life table is more than 65 years.

expected less than one generation hence if the present rate of increase continues unchanged. The total population of the United States has approximately doubled since 1900. In the same period of time the number of people over 65 years of age has quadrupled. At least some of this disproportion can be attributed to use of chemotherapy and especially of antibiotics. To cite only three examples, in 1900, pneumonia claimed the life of 159 individuals out of every 100,000 of the population while today it claims the life of less than 12 per 100,000; in 1900, pulmonary tuberculosis killed 181 of every 100,000 people in the
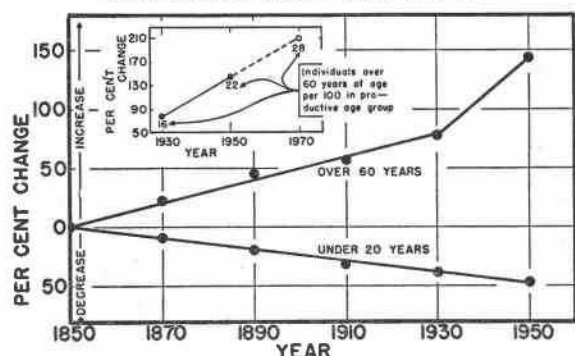

Figure 7. Per cent change in the ratio of dependent age groups to productive age group in the United States, 1850-1950. Inset: Extrapolation of upper curve to 1970.

United States, while today it kills about 20 of each 100,000; and ten years ago the case fatality rate for Rocky Mountain spotted fever was 40 to 50 per cent, while today, with proper use of broad spectrum antibiotics, the disease is usually cured in a few days and is seldom fatal.

What do these changes in mortality rates and in the population structure mean? They mean that a decreasing proportion of the population is faced with the responsibility of providing for an increasing proportion of older people who, largely because of compulsory retirement ages and other factors beyond their control, are denied the privilege of useful occupation and who, industrially speaking, are nonproductive. A partial solution to this problem will be available when it is recognized generally that time is relative. Age can be measured in years or it can be measured in physiologic terms. Physiologically, many people today are younger than were their parents and grandparents at the same chronologic age. Partly because of chemotherapy, the concept developed by Lecomte du Noüy* that biologic time differs from physical time gains greater significance. Over and above the economic problems arising from a rapid change in the population structure with regard to age distribution are serious medical and social problems which may be even more important. Prominent among these are problems of physical and mental health. Coincident with the rapid increase in the proportion of older people in the population, there has been an increase in the percentage of chronic illness, disabilities, and degenerative diseases. The diseases of childhood, adolescence, and early adult life are largely infectious; many can be avoided by proper prophylaxis. In the older age groups a different situation exists; the most common incapacitating ailments are not infectious. Although methods of diagnosis and treatment are being improved constantly, there are not now known any universally effective prophylactic measures against cancer, many heart disorders, and organic and mental disturbances that arise from degenerative changes in the tissues. The major source of disability in the elderly is internal and not external. Treatment is largely therapeutic and not prophylactic. Thus by reducing deaths due to infectious diseases in the earlier years, and thereby markedly prolonging the average length of life, widespread use of antibiotics may tend to focus medical attention on the degenerative diseases that are most prevalent after middle life.

* *Biologic Time*, Macmillan, New York, 1937.

Market forecasts in the pharmaceutical industry predict that the next great field of expansion, following close on the heels of antibiotics, will be in the area of supplying medicaments that will aim at preventing, or at least arresting, the degenerative diseases that threaten the elderly. It is not enough merely to add years to one's life—the added years must be made healthful and worth while for the individual and for the community. This calls for a change in a common point of view. The pattern—youth, middle age, "retirement"—is incompatible with the trend of our society. A new cycle of life is in order.

About fifty years ago, Metchnikoff* expressed the thought that aging, in the physiologic sense of organic deterioration, is not the inevitable consequence of a long life, and he was ridiculed. About the same time, many people were ridiculing the Wright brothers for thinking they could build a machine heavier than air to carry them into a third dimension. When Wilbur and Orville Wright made their first flight, people were incredulous, and some newspapers refused to print the story. Today rocket flights to the moon are not regarded as being beyond the realm of possibility.

Society must change its views regarding the restriction that time imposes on human life and activities, and about the aging process generally, as it has about flying. Metchnikoff expressed the hope and the belief that means could be found to retard the aging process. Progress toward that goal and toward extending the physiologically and mentally active life span of a larger proportion of those in the seventh and later decades of life may be anticipated from developments in the study and application of endocrinology paralleling the expanding use of antibiotics and from the study of mental health.

If antibiotics are contributing to the complexity of present and future social and economic problems, they are also contributing in several ways to at least partial solution of them. It has been estimated that twenty years ago lobar pneumonia cost a patient about $1,000 in hospital and medical bills and loss of wages for a month or more†. Today, despite inflationary trends and decreased purchasing power of the dollar, proper use of antibiotics cures the patient in about a week at less than one-fifth the former cost. This is but one of many common examples.

Antibiotics contribute in other ways also. They play an important role in the economy of the pharmaceutical industry and of the nation at large. The annual production of approximately 325 tons of penicillin, 125 tons of streptomycin and dihydrostreptomycin, and more than 150 tons of broad spectrum antibiotics (Chloromycetin, Aureomycin, and Terramycin), to mention only the major antibiotics, and the continuing research on which their production depends have created a new industry and a new source of national income. The antibiotics comprise a new and very large segment of chemical industry. They account for more than 40 per cent of all pharmaceutical exports and on the domestic scene far exceed, in dollar value, any other single portion of the pharmaceutical chemical industry. Dollarwise, at the retail level, antibiotics annually account for more than one-half of the total value of prescriptions filled in the United States.

Antibiotics—the chemotherapeutic use of which has been incriminated as contributing to the increasing population that may tax the productive capacity of the earth—may simultaneously provide a partial solution for that problem too. Small amounts of antibiotics added to animal feed accelerate and increase the production of highly nutritional animal proteins and, therefore, may help make it possible to maintain a greater population, without reduction in living standards, even with a lower proportion of productive workers. Moreover, antibiotics contribute therapeutically also. Extensive and expanding use of antibiotics in veterinary medicine annually saves millions of dollars worth of animals that in the preantibiotic years would not have matured to reach food markets. Further gains may be made in the future by applying antibiotics in the field of phytopathology.

It is apparent that as knowledge develops, microbes and microbial antagonisms can be more efficiently utilized to solve not only therapeutic problems but economic problems as well. Commenting in their observations concerning microbial antagonisms, Pasteur and Joubert wrote more than 75 years ago "these facts perhaps justify the highest hopes for therapeutics." Today we can expand their statement to say that perhaps our increasing knowledge of microbes and their behavior justifies the highest hopes for the future.

---

* Metchnikoff, E., *Ann. Inst. Pasteur*, 15: 865. (1901)
† Klumpp, T. G. *J. Am. Pharm. Assoc.*, Pract. Pharm. Ed. 13: 789-793 (1952).

Current Status of Antibiotics in Human Medicine

Norman A. David

Laboratory and Research Efforts

Many of the more important aspects of antibiotic action from the laboratory and experimental standpoints have been considered by other speakers of this colloquium. No one appreciates more than the pharmacologist the importance of and need for continued intensive studies to be carried on in research laboratories to further our knowledge, scientifically and clinically, of the antibiotics. This information may be directly transferred to clinical practice to allow the physician to use the antibiotics more intelligently in the treatment of infectious disease. Indirectly, it serves to provide a better understanding of infectious disease processes in both man and animals, it allows an insight into the various factors involved in the development of immunity and resistance, and it reinforces our respect and appreciation of Vis Medicatrix Naturae, the great healing power of the forces of nature. It is not too optimistic to hope that through further knowledge in this field of chemotherapy eventual control, if not complete disappearance, of pathogenic bacterial disease in mankind may be achieved. Already, thanks to the widespread use of penicillin almost continuously in millions of patients for the treatment of a variety of conditions, the incidence of primary or secondary syphilis has dropped to practically zero.

Due credit should be given to the many indefatigable workers who have combined their mutual efforts in these investigations. One thinks of the pharmaceutic chemist who, working with the biochemist, the engineer, the physicist and the enzyme chemist, and the bacteriologist, has made possible the provision of adequate supplies of the antibiotic agents for clinical use. The organic chemist has provided information of the chemical composition and structure of many of the antibiotics, thus paving the way for their synthesis. The bacteriologist deserves credit for evaluating the specificity and spectrum of antibacterial activity of the antibiotics. Working with the pathologist, immunologist, and pharmacologist, the bacteriologist through in vitro studies in the test tube and in vivo observations in infected animals has been able to select the most promising substances from the thousands screened for clinical trial. Much of our knowledge of the mode of action of the antibiotics and the effect of enhancing or hindering substances influencing their activity within the body is due to the efforts of the enzyme chemist.

Since the antibiotics are relatively nontoxic within the animal body as far as pharmacologic action, the pharmacologist has turned his efforts toward investigation of the factors concerned in the development of drug resistance, of the nature of drug sensitivity in humans, and of a better understanding of the rare, but occasionally fatal, toxic manifestations of these drugs. Actually, in many instances the pharmacologist has sought to make his contribution in this field by aligning himself with the clinician in an effort to solve the many problems encountered when the antibiotics are used therapeutically.

Useful as is all this information gained in the laboratory, it frequently is of little help to the clinician in explaining, ameliorating, or preventing certain undesirable phenomena appearing in man when antibiotics are used. For example, many antibiotics which give great promise in the laboratory by showing high specificity against certain organisms, both in vitro and in vivo in infected animals, prove useless when used in man. Another annoying and unsolved problem is that often the results obtained from studying the effects of certain antibiotics on the intestinal flora of animals can not be transferred to man because of dietary and species differences. The clinician would like to see laboratory assistance forthcoming in helping him solve the still not well understood and vexing problem of combating the overgrowth of yeast and fungi in body cavities when antibiotics are required to suppress infection. Similarly, we can not reproduce too well for study in the rabbit, guinea pig, rat, or dog the hypersensitivity phenomena encountered clinically nor the occasionally seen toxic effects on blood cells. In this respect, some help might be forthcoming from the pharmacologist who through his knowledge of the relationship of chemical structure to pharmacologic (and toxic) action could point out certain possible dangers inherent in antibiotic compounds when their structure is known. The clinical pathologist and laboratory technician can aid the physician, through routine studies, such as blood counts, urine examinations, spinal fluid examinations, roentgenologic studies, sputum examinations,

blood cultures, etc., to inform him of the severity of the infectious process in the patient and whether or not the antibiotic selected is effective. In the final analysis, however, it remains for the physician or the veterinarian to make the decision as to the usefulness of antibiotics in treating disease.

SOME CLINICAL ASPECTS OF ANTIBIOTIC USAGE

Turning now from the role of pharmacologist to that of a physician who has had some opportunity to use antibiotics, I should like to consider the present status of the antibiotics in human medicine and some of the problems encountered clinically. During the War years I had to mix penicillin powder (it was probably mostly penicillin "K"!) in proper amount and in a suitable medium for intravenous administration, to do the intravenous injection, and then to observe its almost miraculous effects in saving the life of a patient dying with lobar pneumonia. Since then, I have constantly marvelled at its almost unbelievable curative powers. I recall the hesitancy and apprehension with which I administered intramuscularly what was probably the first preparation of penicillin in beeswax used in this area. This was made by a local pharmacist soon after news first reached us of such a preparation. It proved effective on single injection in curing a severe infection of trench mouth, and the patient suffered no untoward effects other than having a lump in the injection area which was still there when I happened to see her four years later.

Few people know that credit for the discovery of procaine penicillin goes to Dr. Nicholas P. Sullivan (1), who received an M.S. in bacteriology and the M.D. degree from the University of Oregon Medical School and was the former director of the Portland Public Health Clinic. In his work at this Clinic he realized the need for a better preparation than penicillin in oil and beeswax and, while at Eli Lilly in Indianapolis, was able to carry on researches leading to this discovery. In the laboratory, my associates and I have been interested in studies aimed at elucidating the mechanisms concerned in causing vestibular or cochlear damage when streptomycin or neomycin is used. Clinically, some modest studies on the clinical trial of dihydrostreptomycin, Penicillin "O", and erythromycin and terramycin (which will be considered later) have been accomplished.

Since the introduction of penicillin in 1940 (2) by Alexander Fleming and Chain and Florey, a chemotherapeutic substance extracted from the mold *Penicillium Notatum,* and of streptomycin

by Waksman of Rutgers from the soil bacterium *Streptomyces griseus* in 1944 (3), of aureomycin in 1948 (4) by Wright, et al., and of chloramphenicol in 1947, by Ehrlich (5), literally no stone has been left unturned in the world-wide search through the dung of barnyards, the muck of tropical swamps, and the loam at the foot of giant redwoods for new antibiotic substances from plant, organic, or soil sources. Millions of dollars have been spent and hundreds of thousands of samples screened for the isolation of new antibiotics, but little of value has been forthcoming. Of the several new antibiotic substances isolated, only a handful give promise of clinical usefulness. A more profitable field of endeavor might possibly be the chemical identification of the structure of those antibiotics now available with the synthesis of new congeners possessing higher activity and specificity along with less toxicity.

SELECTION OF ANTIBIOTICS FOR CLINICAL USE

Penicillin. This antibiotic still remains the most effective in the hands of the practitioner for the treatment of the most commonly seen infections. It is still the antibiotic of choice in infections due to pneumococci, meningococci, group A beta hemolytic streptococci, gonococci, and treponema pallidum. On the other hand, while penicillin was effective in about 80 per cent of hemolytic Staphylococcus aureus and Staph. albus infections when first available, today because of the development of drug resistance less than 40 per cent of infections due to these strains respond to penicillin.

An advance in penicillin therapy is the realization that oral administration, now made possible by inexpensive supplies of this drug, provides effective blood concentration levels for the control of moderately severe infections. Divided doses given every 4 to 6 hours to a total of one to one and one-half million units per day for several days have been found curative in septic infections and lobar pneumonia. In the face of serious fulminating infections, however, the physician places more reliance on intramuscular injections of depot preparations such as Procaine penicillin given in 300,000 unit amounts once or twice daily. Intravenous penicillin is still resorted to in the treatment of critical cases which are best managed in the hospital.

At the Mayo Clinic (6) patients with bacteremia or subacute bacterial endocarditis are given penicillin by intravenous drip administered through a polyethylene (polythene[B]) tube inserted into a vein on the dorsum of the hand. The

use of such a tube prevents thrombosis and clotting and penicillin has been given constantly through the same tube without reinsertion or removal for as long as two weeks. With the certain repository type of penicillin preparations now available and claimed to maintain effective blood levels for several days, or even longer (Bicillin⁽ᴿ⁾), it is possible to treat syphilis satisfactorily by administering 600,000 units intramuscularly thrice weekly for a total of 10 or more injections. Recent reports show that as little as 1,000,000 units or even less is curative in early syphilis. Weekly or biweekly injections of depot penicillin administered to children has proved effective in preventing relapses of rheumatic fever or in preventing the initial attack of rheumatic fever when given daily in the treatment of streptococci infections. The American Heart Association now recommends the oral administration of 100,000 to 200,000 units of penicillin three times daily for persons who have had one or more previous attacks of rheumatic fever or for prophylaxis in susceptible children (7).

Oral penicillin has been shown to be less likely to lead to hypersensitization of the individual. While there are many preparations of penicillin on the market intended for topical or local application, the best authorities ban their use since there is abundant evidence that such application to a skin or mucous membrane surface leads to sensitization to the antibiotic. Moreover, topical application is often ineffective or, when used in troche form in the face of serious infections such as diphtheria, may temporarily lead to a false sense of security by eliminating secondary invasive organisms.

Among the newer preparations of penicillin recently introduced is Neo-penil⁽ᴿ⁾ (penicillin G. diethylaminoethyl ester hydriodide). By virtue of its iodide fraction, this preparation is claimed to produce high concentrations of penicillin in lung tissue and in sputum. We have used it in a few hospitalized patients, and in two cases we believe that Neo-penil⁽ᴿ⁾ was instrumental in recovery, because the other previously used antibiotics had failed to control the respiratory infection. Bicillin⁽ᴿ⁾, a special repository form of penicillin, is claimed to maintain effective blood or tissue concentrations of penicillin for long periods after its injection. It is now being studied in the prophylaxis of rheumatic fever.

Our studies using Penicillin "O" (allylmercaptomethyl-penicillin) in the treatment of venereal disease in over 200 patients during 1951 and 1952, showed that it is as effective as penicillin and may be used without exciting a sensitivity reaction in a majority of patients who have previously shown reactions to ordinary penicillin "G" preparations. We did find, however, that penicillin "O" given to several patients who had not previously received penicillin is in itself capable of inducing sensitivity reactions.

Streptomycin. Early laboratory studies revealed the effectiveness of streptomycin against not only the organisms susceptible to penicillin but a wider spectrum including such organisms as the tubercle bacillus, hemophilus influenzae, tularemia, undulant fever, Klebsiella and some other infections not affected by penicillin. When streptomycin was first introduced, it was thought necessary to use dosages of the order of 2 to 4 grams injected daily over a considerable period of time. The use of these large doses, particularly in tuberculosis, soon showed that drug tolerance or resistance on the part of the tubercle bacilli developed rapidly. Some toxicity also was apparent. Many patients developed ringing in the ears, dizziness, or deafness. Further experience with this antibiotic demonstrated that the use of lesser amounts of streptomycin in the order of 1 gram per day, or on alternate days, achieved the same curative effects with much slower development of drug resistance and less neurotoxicity. Because the injectable solution of streptomycin, although somewhat painful, acts similarly to depot forms of penicillin in so far as fairly prolonged absorption is concerned, and because of the danger of toxicity repository types of this antibiotic are not used. When given orally, streptomycin is poorly absorbed but may be so used in the treatment of certain intestinal infections. Present day therapy with streptomycin calls for its combined use with either penicillin in specially prepared forms such as Dicrysticin⁽ᴿ⁾ or in conjunction with para-amino-salicylic acid and isonicotinic acid hydrazide as in the treatment of tuberculosis. Such combinations provide a synergistic action better than single doses of any of these two or three agents. They allow much less dosage, thus reducing the danger of toxicity from any of these compounds. Such therapy also forestalls the development of drug resistance. Depot forms of penicillin-streptomycin combinations, given in 2 cc. amounts twice daily over several weeks' time, have proved effective in the treatment of bacterial endocarditis.

Dihydrostreptomycin. Chemical studies of streptomycin allowed the derivation of its structural formula and, following this, attempts to

rearrange molecules within its structure. Dihydrostreptomycin is formed by hydrogenation with the formyl group (-CHO) taking on 2 atoms of hydrogen to become a carbinol group (-CH$_2$OH). It was at first thought that this new preparation would be less likely to develop drug resistance in the tubercle bacillus and to cause sensitivity reactions or to result in eighth nerve damage. Dihydrostreptomycin was immediately given extensive trial in many tuberculosis hospitals and veterans' hospitals throughout the country. It quickly became apparent, however, that this new antibiotic substance was prone to damage the cochlear branch of the eighth cranial nerve leading to permanent deafness. Because of this, as well as its increased cost, its use has been banned from veterans' hospitals for the past year.

Recently, a preparation consisting of equal parts of streptomycin and dihydrostreptomycin called Distrycin[R] has been made available. It is recommended for use in place of either of the parent substances. Administered in the same dosage as either forerunner, the patient gets only half as much of each with equal if not more effectiveness and he runs much less risk of neurotoxicity.

Soon after dihydrostreptomycin became available in 1948, we had the opportunity to use it clinically. When given intramuscularly in single doses of only 250 mgms. it proved curative in 24 patients with gonorrhea, in 2 with chancroid infection, and in several other cases of nonspecific urethritis. Only 1 case of Neisserian infection failed to respond to this low dosage, thereby confirming Chinn et al. (8) who found that 200 mgms. of dihydrostreptomycin cured 20 of 22 patients with gonorrhea.

Chloramphenicol. This antibiotic was introduced as the proprietary Chloromycetin[R] in 1947. It was derived from bacteria isolated from a soil sample collected in a mulched field near Caracas, Venezuela, by Burkholder (5). Chloramphenicol was found effective when given orally in the treatment of a wide variety of gram-positive and gram-negative organisms. In contrast to its immediate predecessor streptomycin, it was found to be rapidly absorbed from the gastrointestinal tract and to be apparently non-toxic even when given intravenously in massive doses to animals. Some of the infections responding to this new antibiotic were those due to Staph. aureus, Brucella abortus, Escherichia coli, Salmonella schottmuelleri, Shigella paradysenteriae, and Klebsiella pneumoniae. It early showed high effectiveness against rickettsia in the treatment of

scrub typhus and Rocky Mountain spotted fever. Later trial showed it superior to other antibiotics in the treatment of typhoid fever. In early syphilis it has been shown to be curative when given orally in doses of 250 mgm. four times daily for two weeks. Chloramphenicol is still the preferred antibiotic for the treatment of whooping cough where it may be given orally, intravenously, or in suppository form (9). As yet, the intramuscular injection of chloromycetin is not convenient since the drug is only slightly soluble and solvents such as propylene glycol, diethylacetamide, or beta-phenoxyethanol must be used.

In 1949 Mildred Rebstock (10) reported the analysis and synthesis of chloramphenicol showing that it contained a nitro-benzene ring structure. It is this nitro-benzene fraction which is believed responsible for causing aplastic anemia in man as first suggested in 1949 by Smadel (11). After extensive trial had been given chloramphenicol for two years, several fatal cases of aplastic anemia were reported in the medical literature. Following the widespread publicity given to this danger of chloromycetin therapy in *The Journal of the American Medical Association* in the July 5, 1952, issue (with three articles on this subject), the use of chloromycetin for anything other than typhoid fever or fulminant septic conditions quickly ceased. The question of whether or not chloromycetin, because of the nitro-benzene fraction, is the responsible agent or whether this simply acts as a trigger mechanism in causing aplastic anemia is still debated. We have heard that a new analog of chloramphenicol, with the NO$_2$ radical of the benzene ring replaced by a CH$_3$ group is soon to be made available. No longer does the physician indiscriminately prescribe chloromycetin for minor infectious conditions and this, in itself, is a good thing.

Experimental studies have shown that chloromycetin is antagonistic *in vitro* to penicillin when tested with Streptococcus and Klebsiella organisms and *in vivo* in streptococci infection in mice. If penicillin is given first, however, no antagonism occurs. Despite these findings, the combination of penicillin and chloromycetin has been found effective in severe staphylococci septicemia (12). Drug resistance develops to chloromycetin, but much more slowly than is the case for penicillin. The incidence of hypersensitivity phenomena is least for all antibiotics with this drug.

Aureomycin. This antibiotic agent has a similar spectrum of activity to chloramphenicol and, in addition, is active against certain virus infections. Early pharmacologic studies indicated

that aureomycin was active when administered orally and possessed a low degree of toxicity. Perhaps even more so than chloromycetin, which was less actively exploited or advertised, aureomycin has been widely used in the treatment of minor infectious conditions, such as septic sore throat, to suppress secondary invaders during the common cold, and as an effective agent against hemophilus influenzae and various streptococci or staphylococci infections. When aureomycin was first introduced, it was thought necessary to use large doses of the order of 3 or 4 grams daily, administered orally in divided doses of 3 or 4 of the 250 mgm. tablets every 4 or 6 hours. Recent studies, however, show that as little as 1 or 2 tablets (0.5 gm.) four times daily is effective in controlling serious septic infections such as bacteremia and lobar pneumonia. Although attempts have been made to prepare aureomycin in an injectable intramuscular form, so far such a preparation has not been forthcoming. In serious infections it may be given intravenously (6).

Probably the most distressing symptoms accompanying the oral ingestion of aureomycin are gastrointestinal upsets such as nausea, emesis, diarrhea, stomatitis, proctitis, and anitis. Often the diarrhea and anal pruritus following aureomycin therapy are long lasting and difficult to ameliorate. Most workers now agree that these persistent symptoms are due to the overgrowth either in the lower bowel or, in the case of women, in the vaginal tract of ordinary nonpathogenic yeast organisms such as Monilia or of fungi. To correct this the manufacturers of aureomycin have added 90 mgm. of methylparaben and 22.5 mgms. of propylparaben to each 250 mgm. tablet of aureomycin to act as an inhibitor against yeast infestations. My experience personally with the use of this new preparation and in medical students complaining of minor infections is that it is much less likely than plain aureomycin to cause annoying pruritus ani or vaginal itching or the prolonged diarrhea which, in the past, I am sure some of us have experienced after plain aureomycin. While aureomycin has, perhaps, been used more extensively than chloromycetin, in a way this may be fortunate, because aureomycin apparently does not cause blood dyscrasias. *In vitro* antagonism between penicillin and aureomycin has also been demonstrated although clinically this phenomenon does not seem a hindrance to therapy if aureomycin is given after penicillin. Combined therapy with aureomycin orally and intramuscular injection of streptomycin has proved effective in bacteremia due to coliform organisms. Intravenous injection of aureomycin may cause liver injury if the daily dose exceeds 2 grams. Drug resistance to aureomycin when therapy is continued over a period of time may occur although, so far, this has not been troublesome clinically.

According to an editorial comment in the January 3, 1953, issue of *The Journal of the American Medical Association* (page 46) the chemically descriptive generic name of chlorotetracycline (New and Non-Official Remedies) has been proposed for aureomycin.

Terramycin(R). Since this antibiotic has about the same spectrum of activity as aureomycin, causes the same bothersome gastrointestinal difficulties, develops drug resistance occasionally when given over long periods, and is nearly as widely used in clinical medicine as aureomycin, little additional comment seems necessary here. In fact, as the editorial referred to above points out, it was at one time considered that terramycin was identical with aureomycin since preliminary studies indicated many similarities in chemical and physical properties. Chemically, aureomycin has been shown to have a chlorine atom at ring one while terramycin has a hydroxyl group at ring three; accordingly, the chemically descriptive name of Oxytetracycline N.N.R. has been adopted for this preparation by the Council on Pharmacy and Chemistry of the American Medical Association.

THE NEWER ANTIBIOTICS

Bacitracin(R). This is obtained from B. Subtilus and is now available in sufficient supply for clinical use. Introduced in 1947, it was found to have a wide spectrum of activity including E. histolytica and to be useful where other antibiotics failed. It has been used in the past for topical application, and no objection to this mode of administration can be found since bacitracin is non-allergenic. The antibiotic also has the advantage that staphylococci drug resistance does not seem to develop and, when administered orally, it does not damage the kidney nor have other toxic effects. While not too well absorbed when given orally, bacitracin has been found to potentiate the action of penicillin. Orally, it is useful in treating infections of the lower bowel such as those due to C. Welchii infection.

Polymyxin (Aerosporin(R)*)*. This antibiotic is only active against gram negative organisms. While not absorbed orally, when so given it has been found effective against certain gram negative infections of the bowel. It is now routinely used

for gut sterilization previous to surgery. Occasionally, oral therapy over a period of several days leads to parasthesias, flush reactions, transient kidney damage, and other annoying but non-serious conditions. Polymyxin has been highly effective against gram negative types of pyelonephritis, against B. pyocyaneus sepsis and pyelonephritis and other stubborn urologic infections resistant to other antibiotic therapy.

Neomycin[R]. This is obtained from the soil bacterium S. fradiae and is related to streptomycin, aureomycin, and terramycin. Neomycin possesses a wide spectrum of activity against both gram positive and gram negative organisms. At first, it was thought that neomycin might prove useful in the treatment of tuberculosis, because it does not lead to a rapid development of drug tolerance; but it is relatively ineffective in this condition. While neomycin may be given orally, it is more economical to use it in doses of from 200 to 500 mgm., given intramuscularly three times daily. Neomycin has been found toxic to the kidneys and to affect the cochlear portion of the eighth nerve, similar to dihydrostreptomycin, so that some hesitancy in its use has been shown by the clinician. Used topically in a dose of 5 mgm. per gram of ointment base, it has been found effective against local infections. So far, sensitivity has not been reported for this mode of administration.

Viomycin[R]. This is obtained from Streptomyces floridae found in a Florida soil specimen and has been found active against M. tuberculosis organisms. Given intramuscularly, it seems well tolerated and effective although it is too new to compare its efficacy with streptomycin in the treatment of tuberculosis.

Erythromycin. This new antibiotic, released under the trade names of Ilotycin[R] or Erythrocin[R], was discovered from a strain of streptomyces erythreus originating from the Philippine Islands. It was released for general prescription use some months ago. It is now widely advertised as a substitute or as a replacement orally administered antibiotic for penicillin in the treatment of conditions where, ordinarily, penicillin would be chosen. Having about the same spectrum of activity as penicillin but, so far, showing no evidence of sensitivity or the development of drug resistance, erythromycin should prove a useful addition to our antibiotic armamentarium. Recently, the practitioner has shown, so to speak, a tendency to go over to the use of erythromycin for routine treatment of minor infectious conditions such as colds or streptococci sore throat in preference to either oral penicillin or aureomycin.

We have just completed a preliminary clinical study using Ilotycin[R] and terramycin in the treatment of 60 cases of acute gonorrhea. Doses of from 3 to 4 grams of Ilotycin given in amounts of three of the 100 mgm. tablets four or five times daily for two and one-half days proved effective in about 90 per cent of the treated cases. However, these rather large doses provoked severe diarrhea in the majority of patients which, in some cases, led to stopping further therapy. Terramycin, given in similar amounts, was found more curative and less likely to cause gastrointestinal complaints although some patients experienced severe diarrhea. Since it has been shown experimentally that erythromycin given intravenously to dogs causes increased intestinal activity and peristalsis, we (13) believe that these undesirable symptoms represent a definite pharmacologic action of the drug rather than a change in intestinal flora, which also might cause this difficulty. When given in lower doses of about 1 or 2 of the 100 mgm. tablets four or five times daily for 3 or 4 days, erythromycin does not usually cause any intolerance.

THE FUTURE OF ANTIBIOTIC THERAPY

This rather lengthy review of the uses and problems encountered when antibiotics are used in the treatment of human infectious disease is best summarized by speculating on the future place antibiotics may occupy in therapy. First, it is apparent that those antibiotics now available will remain useful for a long time and, unlike the sulfonamides, are not likely to be soon replaced by other chemotherapeutic agents. Certain vexing problems, such as the rapid development of drug resistance to penicillin which poses a serious threat to its continued usefulness, can be solved in large part by the medical profession by less promiscuous administration, by restricting medication to the oral or parenteral route, and by the use of either combined therapy or counteracting agents. At present, I understand that an organized research program is now under way to study ways and means to decrease or prevent the development of drug sensitivity in the human to various drugs including the antibiotics.

The answer to the problems of bacterial drug resistance and of human sensitization does not seem to lie in the introduction of further new antibiotics. In fact, it is doubtful whether many more new antibiotics are likely to be forthcoming

since exhaustive researches along this line for the past few years have been practically fruitless. One logical solution to the development of more effective and less toxic antibiotics would seem to be through further chemical researches. With the identification of the chemical structure and make-up of those antibiotics now available and the synthesis of new related analogs differing in certain respects chemically, but not essentially, from the parent antibiotic, it is hoped that better antibiotics may be made available to the physician. In the meantime, however, the physician or the veterinarian should use these miracle drugs with due discrimination and respect so that these fruits of the research worker and these lifesaving drugs may not be too soon "wasted upon the land."

REFERENCES

1. SULLIVAN, NICHOLAS P., A. T. SYMMES, H. C. MILLER, and H. W. RHODEHAMEL, JR. A New Penicillin for Prolonged Blood Levels. *Science* 107: 169 (1948).

2. CHAIN, E., H. W. FLOREY, A. D. GARDNER, N. G. HEATLEY, M. A. JENNINGS, J. ORR-EWING, and A. G. SANDERS. Penicillin as a Chemotherapeutic Agent. *Lancet* 2: 226 (1940).

3. SCHATZ, A., E. BUGIE, and S. A. WAKSMAN. Streptomycin, Substance Exhibiting Antibiotic Activity Against Gram-positive and Gram-negative Bacteria. *Proc. Soc. Exper. Biol. and Med.* 55: 66-69 (1944).

4. WRIGHT, L. T., M. SANDERS, M. A. LOGAN, A. PRIGOT, and L. M. HILL. Aureomycin: A New Antibiotic With Virucidal Properties, *J.A.M.A.* 138: 408 (1948).

5. EHRLICH, J., Q. R. BARTZ, R. M. SMITH, and D. A. JOSLYN, and P. R. BURKHOLDER. *Science* 106: 417 (1947).

6. HERRELL, W. E. Antibiotic and Chemotherapeutic Agents in Infections of the Blood Stream and Heart. *J.A.M.A.* 150: 1450 (1952).

7. KOHN, K. H., and A. MILZER. Prophylaxis of Recurrences of Rheumatic Fever with Penicillin Given Orally: Final Report of a Five Year Study. *J.A.M.A.* 151: 347 (1953).

8. CHINN, B. D., L. E. PUTNAM, S. R. TAGGART, and R. P. HERWICK. The Treatment of Gonorrhea with Streptomycin. *Amer. J. Syph., Gonorrhea and V. D.* 31: 268 (1947).

9. PAYNE, E. H., M. LEVY, G. M. ZAMORA, M. S. VILARROEL, and E. Z. CANELAS. Pertussis Treated With Chloramphenicol. *J.A.M.A.* 141: 1298 (1949).

10. CONTROULIS, J., M. C. REBSTOCK, and H. J. CROOKS. Chloramphenicol (Chloromycetin). *V.* Synthesis. *J. A. Chem. Soc.* 71: 2463 (1949).

11. SMADEL, J. E. Chloramphenicol (Chloromycetin) in Treatment of Infectious Diseases, *Am. J. Med.* 7: 671 (1949).

12. ROMANSKY, M. J., and G. A. KELSER. Antibiotic and Chemotherapeutic Agents in Infections of the Respiratory Tract. *J.A.M.A.* 150: 1447 (1952).

13. DAVID, N. A., W. S. WELBORN, and D. T. SMITH. Erythromycin Therapy in Acute Gonorrhea: Untoward Intestinal Effects. *Fed. Proc.* 12: 314 (1953).

Growth Stimulation by Antibiotics

JAMES McGINNIS

QUESTION: I know there have been some studies on the growth of animal parasites, in hogs for instance. Are there any available studies on the subject—such as the effect on worms in regard to antibiotics?

DR. McGINNIS: There are a couple of papers on worms. It seems that worms grow bigger and better in the G-I tract along with bigger and better chickens. I think there is at least one paper on worms in poultry. One other comment I meant to bring up in terms of absorption is that some rather peculiar results have been obtained in mink when vitamin E and aureomycin were used as a supplement, alone and in combination. The peculiar thing is that the condition was one that would cause yellow fat disease: a high level of salmon waste was being used. Both the antibiotic and the vitamin E prevented yellow fat disease.

The antibiotic prevented mortality. The only way I can interpret that is there was more efficient absorption of what little vitamin E was present in the diet to start with.

QUESTION: What can you tell us of the use of inactivated penicillin or antibiotics in experimental work?

DR. McGINNIS: Dr. Couch at Texas and his group have done considerable work on that problem. When they inject inactivated penicillin, they can still get a growth response in chicks. A rather interesting paper was reported at Chicago last week. Rats and two or three inactivated antibiotics were used, and the investigators got growth responses. Now, keep in mind, *inactivated.* They were inactivated in terms of a specific group of organisms. The work at Chicago last week, I believe, was even more precise than the work that Dr. Couch has been talking about because at Chicago they used ten test organisms, micro-organisms, to determine whether inactiva-

tion had taken place. The antibiotics were inactivated for ten test organisms, whereas in the animal they might be acting against hundreds and thousands of organisms. Maybe the ones they are acting against have no relation at all to the ten test organisms chosen. One other thing, too, antibiotics have been demonstrated to be fully active when given by injection, and I think we often make the mistake of considering that something injected has no effect on what is happening in the gut. I don't think that is the case at all, because the evidence is continuing to pile up that a lot of what you put into the body by injection might end up in the intestinal tract.

QUESTION: Does it seem likely that the antibiotics at the extremely low level where they are promoting growth are also acting against micro-organisms?

DR. McGINNIS: I think it is very possible, because if you figure out the levels in terms of parts per million in the intestinal tract, such levels are effective against many organisms in vitro. As a matter of fact, probably even lower levels are effective in vitro than the levels that you have with this feeding of antibiotics. There is one thing that we haven't paid much attention to, unfortunately. There has been a tremendous amount of counting of micro-organisms, and we have no way of knowing how significant these counts are. The unfortunate part is that a lot of people have done the work—and I have been guilty myself—just taking an established method of doing a particular thing and saying, "Well, this is the method we will use." We might not be growing a tenth of the micro-organisms that are there. Many of the ones that are really responsible we don't even know how to grow yet. Antibiotics in almost unbelievably low concentrations might be effective against these organisms.

QUESTION: Is there a chance that antibiotics getting into the milk as a result of feeding dairy cows is going to have an effect on the infant population?

DR. McGINNIS: I should like to add: Are you asking *favorable* or *harmful* effect? My feeling is that if we could get some into the infants we might even have a favorable effect.

QUESTION: In other words, you mean to feed them antibiotics just as you would a cow?

DR. McGINNIS: I'd like to see it done. Perhaps some of the M.D.'s here would comment on that. There is no hesitation—at least I doubt that there is—of treating a sick infant by injection.

FROM THE FLOOR: But I am speaking of a well infant. I am not speaking of a sick infant.

DR. McGINNIS: By our classical definition the experimental animals are well animals. People used to think that animals to be good, hardy, thrifty animals ought to have a pretty tough time of it in growing up and that they will live to be tougher. Maybe that is true, but one fellow at Illinois said two weeks ago that he hadn't seen any evidence yet that the animals that were grown to maturity and kept on as breeders were any worse off by having grown to their mature size at a more rapid rate. He actually had a good bit of work that is of further interest. He has checked the susceptibilty of chicks to diseases, salmonella pullorum infections, paratyphoid infections, and so on, and found that the healthier, faster-growing chicks have better resistance to diseases. What you might call their general resistance is better. I think the answer you are looking for is being obtained in some of the Latin American countries. Infants are being fed antibiotic supplements in some of the experiments being carried on in these countries, particularly in places where there is a terrific infant mortality rate. That is where we stand to benefit—I say *benefit* with a question mark in view of some of the discussions we have had, because if we succeed there, the population is really going to skyrocket.

QUESTION: Does the use of antibiotics have an effect on subsequent growth?

DR. McGINNIS: Certainly with chicks we get no bigger hatching weights if the hens are receiving antibiotics, and we get no better livability. We've tested animals from different breeding stock and their subsequent responses. Their subsequent growth is not modified by the fact that the hens had antibiotics or didn't have antibiotics.

QUESTION: When, or how old may the animal be, and still get a growth response?

DR. McGINNIS: I don't think we know. There are conditions where we can get a gain in weight and improve egg production with hens by feeding antibiotics, but there I think we are dealing with disease. I am not so sure we are not dealing with disease all along the line. Do you want to classify disease as something that causes a depressed action or physiological state or something of that type? Not contagious, of course.

QUESTION: Could you tell us more of the mink experiment you mentioned?

DR. McGINNIS: It was a very large experiment—in Connecticut, I believe it was—at one of

the large mink farms. Terramycin was used. Better fur coats, better pelts, resulted, but again, I think the animals were healthier. Far, far fewer died—mortality was greatly reduced.

QUESTION: Is there any effect on milk production and the constituents?

DR. McGINNIS: I really don't know. According to what little information I have on the use of antibiotics as supplements for dairy cows, I believe Cornell reported no effect on milk production. That is the only experiment I know of.

DR. MACY: As chairman, I expect that it is my duty to bring this to a close. I am sure that if you have further questions it may be possible to consult Dr. McGinnis directly.

Mode of Action of Antibiotics

W. W. UMBREIT

Studies on the mode of action of antibiotics have been numerous and extensive, but we do not have today the consolidated knowledge which one might expect to result from the effort that has been expended in many laboratories. In fact, in a sense we suffer from too much information rather than too little. It is possible to make some sense out of the conflicting viewpoints, however, if one keeps in mind certain laws of logic and remembers that we are dealing with living organisms. At the outset, it is quite desirable to outline what it is we are trying to find out, for the term "mode of action" means different things to different people. For the present, we shall take mode of action of antibiotics to mean the study of how the antibiotic interferes with the development of the sensitive organism. In a very general way, this interference could be brought about by alteration in the physical status of a cell or by alteration in its chemical status. There are some antibiotics—tyrocidine, for example—which are surface active agents (1)* and may be regarded as altering the physical status of the cell. Because there are so many antibiotics, the possible modes of action are many and much time and effort might be spent upon the various known kinds of activity. The characteristic of antibiotics, however, is the presence of a degree of specificity, and it is of most interest to examine those in which the degree of specificity is the highest—particularly those of widespread, effective clinical use. There are five of these about which knowledge is sufficient to warrant discussion. These are penicillin, streptomycin, chloramphenicol, aureomycin, and terramycin. It seems only sensible to confine our attention to these five.

There are certain aspects of their action which all of these substances have in common. First, they are all adsorbed to or absorbed by the susceptible cells. In the cases adequately studied, there is a "specific" irreversible absorption, although this may not be true for all five. The action of all is primarily biochemical, not physical; and they all seem to interfere, in a most curious manner, with a reaction or a reaction type within the cell. A great many enzymes are not in the least affected by them, and a wide array of cell processes continue in their presence.

The five substances are all relatively complex molecules containing a fair number of substituent groups which may bear a resemblance to other structures in the cells. It seems reasonable that molecules of this complexity may interfere with reactions in which similar groups are involved, if only the case of "the butter not suiting the works." Some of these interferences may be quite specific and directly related to how the antibiotic kills the organism, but some may be due to the fact that similarity in structure causes interference in reactions in which such structure is of importance, even though this has nothing to do with its mode of action. To distinguish between the antibiotic effects and what we may call the structural effects of the antibiotic, there are two criteria in use at present.

1. The effects observed must be evident for the antibiotically active forms of the antibiotic but show no reaction with structurally similar derivatives which possess no antibiotic activity.
2. The concentrations of the antibiotic required to act on a given reaction must be comparable to those required to inhibit growth.

More stringent criteria of antibiotic action may be required later; for the moment these suffice.

In considering the mode of action of antibiotics, it is now necessary to consider each of the chosen five individually, because they each act in a different manner. The first to be considered is streptomycin, because with it various

* Reference to bibliography on page 39.

effects may be demonstrated in resting cell suspensions. In fact, far too many effects have been observed and not all of these can be the "grandest tiger in the jungle." I have elsewhere (2) surveyed these effects in the light of the two criteria of antibiotic effect. One reaction survives such a scrutiny. Unfortunately, it is an unknown reaction and occurs simultaneously with an array of reactions comprising the entrance to the citric acid cycle. If it were not for streptomycin, I doubt whether this reaction would have been recognized for many years. Indeed, it is ignored except by those who study streptomycin.

What streptomycin does when properly applied to either bacterial or animal tissue is to prevent the entrance of pyruvate into the respiratory cycle. For example, when bacterial cells are so grown that they do not oxidize added acetate, pyruvate is oxidized to acetate and then stops. But pyruvate plus oxalacetate are oxidized to completion. When streptomycin is applied, pyruvate and oxalacetate are oxidized, not to completion but to the oxidation state of acetate and indeed acetate accumulates. This certainly looks as though streptomycin is inhibiting one or more of the steps involved in entering the citric acid cycle.

The type of data obtained is shown in Table 1. In the absence of streptomycin, succinate, fumarate, malate, and oxalacetate are oxidized toward completion. In the presence of streptomycin, oxidation of all proceeds to the stage of acetate, and there stops.

TABLE 1. AMOUNTS OF OXYGEN UTILIZED ON VARIOUS SUBSTRATES

Substrate	Moles O_2/Mole substrate		
	Normal	+ Streptomycin	Theory to acetate
Pyruvate	0.44	0.42	0.5
Oxalacetate	>0.87	0.5	0.5
Malate	>2.3	0.77	1.0
Fumarate	1.42	1.0	1.0
Pyruvate + Oxalacetate	>0.83	0.56	0.5

The known pathways involved in this oxidative process are shown in Figure 8. When cells are grown in air, comparable to the conditions used by Ajl (3), an acetate oxidation system is present. We do not know the mechanism of the acetate oxidation system and have merely indicated its further oxidation. Since it is not inhibited by
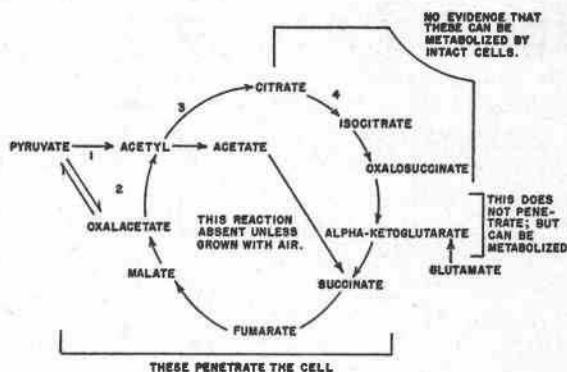
Figure 8. Known oxidative pathway

streptomycin, the action of the inhibitor is not at this point and we may ignore it from here on. In cells grown without air in which the acetate oxidizing system is absent, streptomycin inhibits the oxidation of pyruvate and oxalacetate. One might well assume that inhibition occurs of the reactions pyruvate to acetyl plus oxalacetate to citrate and thus around the cycle, and the problem merely becomes one of which step is inhibited by streptomycin. It is true that such cells do not oxidize added citrate, cis-aconitate, alpha-ketoglutarate, but this may mean merely that these compounds cannot penetrate through the intact cell surface. Actually, in the case of alpha-ketoglutarate, this seems to be the case. Glutamate is readily oxidized to completion, and it can be easily shown that alpha-ketoglutarate accumulates in the early stages of the oxidation inside the cell and later disappears. Hence, citrate, isocitrate, etc., may be inactive, because they do not penetrate. The enzyme condensing acetyl and oxalacetate is present as well as aconitase. It would seem that the only problem is at which point in reactions 1, 2, 3, or 4 does streptomycin act? What happens experimentally, however, is that streptomycin cannot be shown to inhibit any of them. Streptomycin does not inhibit the conversion of pyruvate to active acetyl nor the condensation of this with oxalacetate to form citrate nor the further metabolism of citrate nor, indeed, oxalacetate decarboxylase which would not be a necessary reaction when both pyruvate and oxalacetate were added.

The experimental data supporting these conclusions are contained in a series of papers published during the course of the past five years (4-9). Over this period, many hundreds of papers have been published on streptomycin, some of which provided the same type of information but

many of which were unable to obtain a similar type of result. Usually this was due to the presence of the acetate oxidizing system in great preponderance either because of the growth conditions employed or because of the genetic constitution of the bacterial strains used. Since this system is not inhibited by streptomycin, when one measures oxidation one may obtain any of a number of effects depending upon such circumstances. A small residuum of papers report effects which are clearly due to adaptive enzyme formation in resting cells, a process inhibited by all of the antibiotics here considered except penicillin. In some further cases we seem to have been unable, either by data or by logic, to make ourselves understood, and straw men have been victoriously vanquished. There was, indeed, one difficulty. If there were another reaction, an oxalacetate-pyruvate reaction (as distinct from an oxalacetate-acetyl reaction), one would like to have some positive evidence of its nature. However, the experimental problem as to the nature of this reaction was not easily approachable. Two very large experimental difficulties prevented further progress. First was the inability to make any kind of preparation which would contain the streptomycin sensitive reaction. There was just enough indication that this would eventually be possible shown in a very few active preparations, but as an experimental tool this method of approach was, and still is, impossible. Second, there was the lack of any way to measure this reaction except its streptomycin sensitivity, under which circumstances, of course, the reaction did not go so that the search for end products was fruitless. It was obvious that we were dealing with an active intermediate, present in small amounts, whose nature we did not know and for which we had no method of measurement except to prevent its

$$\begin{array}{c} COOH \\ | \\ HC - O - PO_3H_2 \\ | \\ CH_2 \\ | \\ HO - C - COOH \\ | \\ CH_2 \\ | \\ COOH \end{array}$$

Figure 9. Structure of the seven-carbon phosphorylated intermediate.

formation by streptomycin. Always, however, when streptomycin was active there were both 3-carbon and 4-carbon compounds present. As mentioned, this would suggest a 7-carbon intermediate, but studies of these had shown those which could be synthesized to be essentially inactive.

Naturally, there might exist others of which we had as yet no indication. By a curious set of circumstances, this actually appears to be the case. In 1951, Rapoport and Wagner reported (10) that they had succeeded in isolating from dog liver a 7-carbon *phosphorylated* compound, 2-phospho - 4 - hydroxy - 4 - carboxy - adipic acid, whose structure is illustrated in Figure 9. This offered the possibility of tracing the metabolism of this compound by way of radioactive phosphorus. By such methods it could be shown to be an active metabolic intermediate, at least in the liver of the rat (11, 12). In *E. coli* it is also an active metabolic intermediate and, as shown in Table 2,

TABLE 2. FORMATION OF THE NEW COMPOUND BY *E. COLI*, AIR-GROWN CELLS

Substrate added	Streptomycin	Specific activity of new compound
None	0	3.9
Pyruvate (1 mM)	0	3.8
" "	50 μg./ml.	3.3
Pyruvate (1 mM) + Oxalacetate (2 mM)	0	17.3
" "	50 μg./ml.	5.0
" "	25 μg./ml.	1.7
" "	50 μg./ml. (2 hours)	3.0

it is formed only when pyruvate plus oxalacetate (or other 4-carbon acid convertible to oxalacetate) is present, and its formation is completely inhibited by streptomycin. This thus appears to be the site of action of streptomycin. The function of this compound *per se* can at present only be surmised, but the indications certainly point to a direct relation to vital processes.

It is evident, of course, that this type of reaction—i.e., entrance into the terminal respiration system—is an important part of animal metabolism. Why, then, can streptomycin be put into the animal without harm, or at least comparable harm, to the host? Without going into detail, the evidence shows (7) that a permeability barrier to streptomycin exists at the cell wall and at the surface of the mitochondria. The reaction we are dealing with is located within the

mitochondria. Streptomycin is an antibiotic because it does not penetrate to sites of the sensitive reaction in the animal cell. If it is put there artificially, however, it is a very powerful inhibitor of the animal enzyme.

What happens metabolically when resistance develops? Evidence shows that the oxalacetate-pyruvate reaction is lost (6). In the case of the resistant strains described in published data (6), the oxidation of pyruvate and oxalacetate even to the state of acetate is extremely low and the oxidations of succinate, fumarate, malate, etc., were also similarly affected. In a variety of other strains studied, it was found that the loss of the pyruvate to acetate reaction, or the loss of ability to oxidize the dicarboxy acids, was largely a matter of chance, because some strains retained these abilities but consistently, whenever a resistant strain was obtained, the oxalacetate-pyruvate reaction was missing. It is curious that in resistant strains, even though oxidation of oxalacetate and pyruvate is lost, the ability to form citrate from these compounds is present and is enhanced (9). Similarly, in the strains dependent upon streptomycin for growth, the oxalacetate-pyruvate reaction is missing (6). The data are sufficient to conclude that whenever one obtains growth in the presence of streptomycin, the oxalacetate-pyruvate reaction is not present in the cells.

Considering the mode of action of penicillin, before a drug can react it must be adsorbed by the organism and be in physical contact with sensitive loci. After some confusion, such an adsorption is now well established. Maass and Johnson (13, 14), for example, found a specific adsorption of penicillin independent of the extracellular penicillin concentration comprising close to 750 molecules of penicillin per cell as well as a diffusion of penicillin into the cells over and above this point. This specifically absorbed penicillin was not exchangeable and remained bound during subsequent growth of the cells in a penicillin free

medium. The interpretation (Figure 10) placed upon the data is that during growth the cell synthesizes the penicillin "binding component," BC. The synthesis of this component is not inhibited by penicillin, but the penicillin unites with it once it has been formed and thus prevents either its activity (if one supposes it to be an enzyme or coenzyme) or its further metabolism or utilization. The lowest bacteriostatic penicillin concentration is that at which the union with the specific penicillin binding component is slightly more rapid than its resynthesis by the cell. Since the rate of synthesis of "binding component," BC, may vary with the species of organism, one would not expect too close a correlation between the sensitivity to penicillin and the amount of specifically bound penicillin, but in general a fair correlation is found.

The next problem is the nature of "binding component." This is not known, but several observations give one a hint as to the type of reaction with which "binding component" is concerned. The location of this material is most probably at or near the cell surface; but, wherever it may be, one may determine a variety of effects due to its lack of function in the presence of penicillin. Since all measurements of the lack of activity of binding component must be, at the moment, quite indirect, it shall suffice to mention the general conclusion rather than to particularize on any phase with specific data—particularly since this has been reviewed elsewhere (15, 16). It seems apparent that binding component, the material inactivated by penicillin, is concerned with the synthesis of pentose nucleic acids, probably of a particular type. The exact details of the reaction are not yet known.

The question, "Why is penicillin able to enter the animal, kill off the susceptible bacteria therein without harm to the host?" is not answerable experimentally by any data known to me. Perhaps the "binding component" does not exist in the animal cell; perhaps the reactions catalyzed by the "binding component" are not important in animal cell metabolism. Undoubtedly, there are other possibilities. Because we know of no studies devoted to this problem, one can only point out that there appears to be a difference in the metabolism of gram positive and gram negative or animal cells which makes this specificity of penicillin possible.

A third question concerns the alteration of metabolism when resistance develops. With respect to resistance to penicillin, several phenomena have been noted. First, there are certain or-
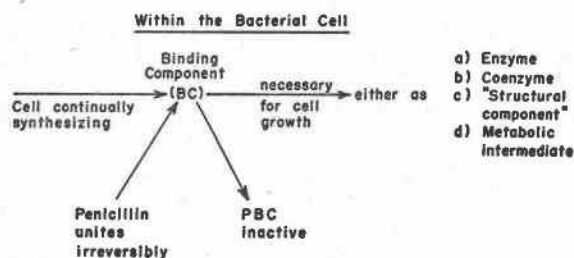

Figure 10. Diagram representing the action of penicillin.

ganisms, such as most of the yeasts, which are resistant to penicillin because of the inability of penicillin to penetrate into the cell. The vast majority of organisms, however, are resistant to penicillin because they possess or develop an enzyme, penicillinase, which destroys the drug. But finally, some organisms exist (and this is the group we are concerned with) into which penicillin penetrates and which possess no penicillinase, yet they are still resistant. With these there is sufficient data so that it is possible to make a generalization that the most probable mechanism of resistance is the development of an alternative pathway to the products of the inhibited reaction.

It should be emphasized that the changes in metabolism associated with the development of resistance to drugs may be neither singular nor necessarily specific. If we consider that an organism must dispense with the sensitive reaction series, it must then develop another series to accomplish the same end. This so-called "by-pass" may put additional strain on the organism, and it may be necessary to make several other metabolic alterations to accommodate the new pathway. Because of these possibilities one may, indeed one does, find a variety of alterations in resistant cells, some closely related to the by-pass mechanism itself and some secondary changes arising from the considerations just described. An experimental problem of some importance is to distinguish between the primary and secondary changes. Admittedly, because of certain difficulties in working with penicillin, this has not yet been done with certainty. What such possibilities mean in terms of an experimental approach to problems of mode of action is that one cannot compare the metabolic properties of sensitive and resistant strains and hope thereby to deduce the mode of action of the antibiotic.

With respect to chloramphenicol, it does not inhibit a wide array of reactions, including proteolytic enzymes; but it does have a curious effect upon bacterial esterases (17) and on the crystalline liver esterase. Inhibition is observed at concentrations about tenfold higher than those required to inhibit growth but within the physiologically effective range. In the animal mitochondria, the esterase is not inhibited, which suggests a barrier, as with streptomycin, preventing the antibiotic from reaching the site of the sensitive reaction. This is not quite a reasonable explanation, because chloramphenicol does apparently penetrate the red blood cell and further acts upon certain rickettsial infections where the parasite is intracellular. At the moment, it is difficult to relate this action of chloramphenicol on esterases to its mode of action in killing the organism, partly because of our lack of knowledge of the critical metabolic importance of esterases but more because of the curious response of esterases to chloramphenicol involving stimulation as well as inhibition.

A variety of enzyme systems acting on chloramphenicol itself has been described but these reactions do not seem to be pertinent to its mode of action inasmuch as alterations in the chloramphenicol molecule reduce or eliminate its antibiotic effect. The inhibition by chloramphenicol for *E. coli* and *L. casei* is decreased by phenylalanine and, to an extent, by tyrosine and tryptophan (18). This antagonism was noncompetitive, but is demonstrable over only a narrow range of concentration of the drug and only with minimally effective doses of chloramphenicol. This was taken to mean that, since chloramphenicol is a naturally occurring analogue of phenylalanine, it might owe its antibacterial properties to interference with the action of phenylalanine and that over a narrow range more phenylalanine could compensate for the loss of certain reactions inhibited by chloramphenicol. Other workers (19) feel that the antibiotic interferes with the early stages of tryptophan synthesis, especially the formation of indole from anthranilic acid. Further, a growth factor for *L. citrovorum*, not folinic acid but produced by incubation of folic acid with hemopoietic tissue, is claimed to reverse chloramphenicol inhibition. Finally, chloramphenicol has been shown (20) to inhibit protein synthesis in *Staph. aureus* without interference with glucose fermentation, extracellular peptide formation, or nucleic acid synthesis. All of these experiments have not yet been integrated into any common explanation or any picture of a possible mode of action of chloramphenicol. They seem to point in somewhat opposite directions and it is apparent that the definitive experiments have yet to be conceived and executed.

If the status of knowledge of the mode of action of chloramphenicol is in a somewhat immature state, that of the action of aureomycin and terramycin is dominated by a singularly illogical conception. The majority of the studies have been done not with susceptible bacteria but with animal tissue, where one might suppose the real problem is why do these antibiotics *not* act on the animal? Aureomycin, applied to animal homogenates at relatively high concentrations, inhibits aerobic phosphorylation, possibly by blocking some part of the Krebs cycle. Terramycin

apparently acts in the same manner, as indeed do dinitrophenol, atabrine, gramicidin, usnic acid, and barbiturates. There appears to be some difference of opinion with regard to the similarity of the mode of action of aureomycin and terramycin; but, in view of their similarity in structure, we shall consider them simultaneously, although their mode of action may differ considerably. At these relatively high concentrations such phosphorylation "uncoupling" is evident with aureomycin in *Staph. aureus* (20), but it seems quite unlikely that this uncoupling reaction can explain the antibiotic activity even in *Staph. aureus,* because growth and protein synthesis are sensitive to much smaller concentrations of the drug. Further, (20) there is a difference in the action of aureomycin (which inhibits glutamate accumulation but not glucose fermentation) and terramycin (which inhibits both) at higher levels of concentration. Both agents inhibit protein synthesis at concentrations comparable to those required to inhibit growth.

For chloramphenicol, aureomycin, and terramycin there is no indication so far as to what may underlie their ability to be used in the animal, nor of the metabolic changes which accompany resistance. Some most interesting and important problems remain for the future.

These descriptions of the mode of action of the antibiotics reveal, I think, that they are acting in a manner not exactly predictable from our previous knowledge of metabolic processes. In their action we appear to be dealing with a somewhat higher organization of processes. This is a very good thing. As Francis Bacon said some centuries ago, "Some of the inventions already known are such as before they were discovered it would hardly have entered any man's head to think of; they would have been simply set aside as impossible. For in conjecturing what may be, men set before them the example of what has been, and divine of the new with an imagination preoccupied and colored by the old; which way of forming opinions is very fallacious; for streams that are drawn from the springheads of Nature do not always run in the old channels."

BIBLIOGRAPHY

1. HOTCHKISS, R. D., Gramicidin, tyrocidine, and tyrothricin. *Advances in Enzymology* 4: 153 (1944).
2. UMBREIT, W. W., Streptomycin. Trans. *N. Y. Acad. Sci.,* 15:8 (1952).
3. AJL, S. J. Acetic acid oxidation by *Escherichia coli* and *Aerobacter aerogenes. J. Bact.,* 59: 499 (1950).
4. UMBREIT, W. W., A site of action of streptomycin. *J. Biol. Chem.* 177: 703 (1949).
5. OGINSKY, E. L., P. H. SMITH, and W. W. UMBREIT. The action of streptomycin. I. The nature of the reaction inhibited. *J. Bact.* 58: 747 (1949).
6. SMITH, P. H., E. L. OGINSKY, and W. W. UMBREIT. The action of streptomycin. II. The metabolic properties of resistant and dependent strains. *J. Bact.* 58: 761 (1949).
7. UMBREIT, W. W. and N. E. TONHAZY. The action of streptomycin. III. The action of streptomycin in tissue homogenates. *J. Bact.* 58: 769 (1949).
8. OGINSKY, E. L., P. H. SMITH, and M. SOLOTOROVSKY. The action of streptomycin. IV. Fatty acid oxidation by *Mycobacterium tuberculosis,* Avian type. *J. Bact.* 59: 29 (1950).
9. UMBREIT, W. W., P. H. SMITH, and E. L. OGINSKY. The action of streptomycin. V. The formation of citrate. *J. Bact.* 61: 595 (1951).
10. RAPOPORT, S., and R. H. WAGNER. A phosphate ester of a tricarboxylic acid in liver. *Nature,* 168: 295 (1951).
11. UMBREIT, W. W. Respiratory cycles. *J. Cell. Comp. Physiol.* (In press 1953).
12. UMBREIT, W. W. The action of streptomycin. VI. A new metabolic intermediate. *J. Bact.* (In press 1953).
13. MAASS, E. A., and M. J. JOHNSON. Penicillin uptake by bacterial cells. *J. Bact.* 57: 415 (1949).
14. MAASS, E. A., and M. J. JOHNSON. The relations between bound penicillin and growth in *Staphylococcus aureus. J. Bact.* 58: 361 (1949).
15. UMBREIT, W. W., and E. L. OGINSKY. The mode of action of antibiotics: Penicillin and streptomycin. *J. Mt. Sinai Hospital* 19: 175 (1952).
16. UMBREIT, W. W. The mode of action of streptomycin. Symposium on mode of action of antibiotics. *2nd Int. Cong. Biochem.,* Paris, 63 (1952).
17. SMITH, G. N., C. S. WORREL, and A. L. SWANSON. Inhibition of bacterial esterases by chloramphenicol (Chloromycetin). *J. Bact.* 58: 803 (1949).
18. WOOLLEY, D. W. A study of non-competitive antagonisms with Chloromycetin and related analogues of phenylalanine. *J. Biol. Chem.* 185: 293 (1950).
19. BERGMANN, E. O., and S. SICHER. Mode of action of chloramphenicol. *Nature* 170: 931 (1952).
20. GALE, E. F. Points of interference by antibiotics in the assimilation of amino acids by bacteria. Symposium on mode of action of antibiotics. *2nd Int. Cong. Biochem.,* Paris, 5 (1952).

BIOLOGY COLLOQUIUM PROCEEDINGS

No proceedings were published for the Colloquia held before 1943.
No Biology Colloquium was held in 1945.

Address order to: BIOLOGY COLLOQUIUM, 107 Commerce Hall
Oregon State College
Corvallis, Oregon