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CURRENT PROBLEMS IN RESEARCH

Antibiotics

The exploitation of microbial antagonisms is having a challenging impact on medicine and society.

Paul R. Burkholder

Antibiotics, in the broadest sense, are substances produced by living organisms, which, in small amounts, can inhibit the life processes of other organisms. The word *antibiotic, in sensu strictu,* is used for a chemical substance, of microbial origin, that has the capacity to inhibit the growth of bacteria and other microorganisms and even to destroy them. Antibiotic substances are of general interest in chemistry and biology, but their greatest impact on science and society has come about through practical applications in medicine and agriculture.

New Concepts of Antibiosis and Chemotherapy

It is well known that antibiotics were not the first effective, specific drugs used in the medical armamentarium for conquest of human disease. Very beneficial remedies of plant origin were introduced into Europe for use against malaria and amebic dysentery as far back as the 17th century. There was at that time no underlying rationale or basic conceptual scheme of chemotherapy. Cinchona bark and ipecacuanha rested on their own merits as simple remedies, produced in nature and discovered through early empiricism. Knowledge about the active drugs quinine and emetine seems not to have stimulated much scientific endeavor beyond continuing investigations of remedies found in the primitive pharmacopoeia.

The rationale of modern chemotherapy is based upon knowledge of the etiological agents of infectious diseases and knowledge of the properties of selected chemical compounds which differentially inhibit the growth of pathogens without doing undue harm to the host. It was Paul Ehrlich's concept that chemical constitution determines biological effect, which led to the development of Salvarsan against syphilis and, later, of the sulfonamides against streptococcal diseases and of pyrimethamine against malaria. The objective of Ehrlich's kind of chemotherapy is to discover drugs which act selectively upon essential constituents of pathogenic microbes —charmed bullets logistically designed for specific target sites inside the invading organisms.

When the conceptual scheme is clear, it is relatively unimportant whether 606 or 914 experiments are required to attain the desired goal. In rational chemotherapy, the elegant methods of organic chemistry can lead to successful synthesis of antimetabolite drugs and other types of nice remedies. Since the search is always more satisfying when desirable goals can be defined, we cannot afford to overlook the many indications provided by nature's antibiotic compounds, which may serve as guideposts toward a more rational chemotherapy. It appears that in our present state of relative ignorance it is no more and no less rational to test the inhibitory powers of synthetic analogs of growth factors against pathogenic bacteria than to assay the spectrum of microbial inhibition for a novel antibiotic "beer." One approach may confirm the educated guess about a possible mechanism of inhibition; the other may lead to discovery of some metabolic pathway that has been blocked; both approaches have provided desirable therapeutic agents—for example, *Salvarsan* and *penicillin.*

The natural phenomenon of antibiosis is not a new discovery of our present generation. A century ago, Louis Pasteur (1857) observed that onion juice inhibits growth of lactic acid bacteria without affecting certain other kinds of microorganisms. A little later, Pasteur (1877), Babes (1885), Garré (1887), and others noted how certain common bacteria could stop the growth of the anthrax bacillus and other kinds of microbes. It was even suggested by these early microbiologists that some day antagonistic properties of microorganisms might be used to combat infectious agents of disease. The scattered data on microbial antagonism in the late 19th century were not integrated into any formal scientific doctrine. Had it been otherwise, an earlier crystallization of the antibiotic concept might have directed research sooner toward desired goals—for example that of finding how better to prevent and alleviate human suffering by means of specific medication.

The word *antibiosis* was first used by Vuillemin in 1889, to describe the phenomenon where one organism is in opposition to the life of another. Actually, several antibiotic preparations, such as pyocyanin, pyocyanase, prodigiosin, and mycophenolic acid, were prepared before 1900, but unfortunately none of these could be developed for use in chemotherapy because of their ineffectiveness and toxicity. Antagonistic relations among microorganisms were not completely forgotten after 1900. According to a monograph on microbial association published by Papacostas and Gaté in 1921, an inhibition resulting from the association of two microbes in vitro is called *antibiosis* (*anti—against, bios—life*). Progress in this field seems to have

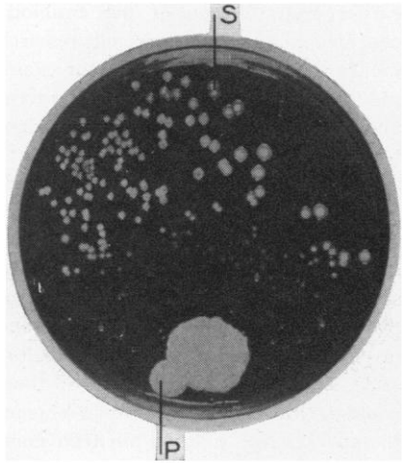
The author is director of research at the Brooklyn Botanic Garden, Brooklyn Institute of Arts and Sciences, Brooklyn, N.Y.

rested, in the first quarter of the 20th century, with recognition of the important but generally overlooked fact that microbial antagonism does indeed exist.

The Antibiotic Era

The next great and independent advance came in 1929, when Alexander Fleming observed a *Penicillium* mold destroying the bacterium *Staphylococcus aureus* in an old discarded petri dish. His imaginative genius moved Fleming to go beyond the usual explanation—that of stale products—to coin the term *penicillin,* the name for a new specific substance capable of killing bacteria. Fleming then proceeded with studies on the powerful action of penicillin against many common pathogenic bacteria and showed that it was not toxic to animal and human tissues. The development of penicillin was not deemed practicable, however, until 10 years later, when the Oxford group—Florey, Chain, Heatley, and others—began reinvestigations into the medical merits of Fleming's almost forgotten discovery. Penicillin soon became the first and greatest of antibiotic drugs, largely because of engineering accomplishments in the fermentation industry.

Another discovery in the field of antibiotics came at just the right moment. Although penicillin was being developed for chemotherapy, many persons came to believe that it was unique, and that probably there would be no more big antibiotics. Then, in 1939, René Dubos demonstrated two new crystalline antibiotics, gramicidin and tyrocidin, from a bacterium called *Bacillus brevis.* Publication of this research had great impact upon the thinking of bacteriologists and the strategy of investigators. Now it seemed clear that there might be many more antibiotic substances in nature.

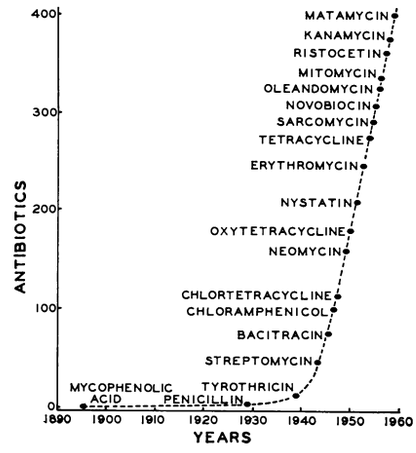


Petri plate of bacteria contaminated with a large colony of green mold. As he looked at this plate in 1929, Sir Alexander Fleming envisioned a special antibacterial substance, penicillin, produced by the mold *Penicillium notatum* (P) and inhibiting growth of nearby colonies of *Staphylococcus aureus* (S). [From Florey *et al., Antibiotics* (Oxford University Press, 1949).]

Search for the new substances went forward in many laboratories. Among several antibiotics discovered in Waksman's laboratory, streptomycin, produced by an actinomycete, *Streptomyces griseus,* soon came to be recognized as a powerful therapeutic drug against the microbe that causes tuberculosis. Two important results of the discovery of streptomycin may be noted. First, the battle against tuberculosis began to be waged successfully, and second, some of the accumulated profits from gigantic drug sales made possible the organization of an unusual Institute of Microbiology at Rutgers University, where students are trained and research goes forward in microbiology.

Knowledge in various fields of human endeavor, in that of antibiotics or any other, seems to grow like living populations of organisms. The number of publications on antibiotics has already grown into tens of thousands. Beginning with mycophenolic acid, the first mold antibiotic prepared in crystalline form in 1896, the number of "discovered" antibiotic substances has grown to many hundreds. Along the antibiotic growth curve a few noteworthy compounds have appeared. Some of these are very important in medicine, because of their desirable properties in relation to great human needs. To be very useful in medicine an antibiotic must be more injurious to pathogenic organisms than to the human body. The drug should be comparatively stable, easily absorbed, decisively active against living agents of disease, and without harmful side effects, and when its work is done it should disappear from the patient's body. Only a few antibiotics have measured up to these rigorous requirements.

Following development of the antibiotic concept and successful production of several very important narrow-spectrum antibiotic drugs, such as penicillin and streptomycin, the next great advance came with the advent of broad-spectrum antibiotics. The broad-spectrum antibiotics are capable of inhibiting growth of a broad range of microbes, including Gram-positive and Gram-negative bacteria, rickettsiae, large viruses, and some



Observation of curious microbial antagonisms in the 19th century led to a virtual era of antibiotics in the 20th century. The "growth curve" shown here represents increase in the number of antibiotic compounds reported in the literature plotted against time, in years. A few named substances are listed as examples of the hundreds of known antibiotics.

other organisms. Examples are chloramphenicol and the group of tetracyclines, including oxytetracycline and chlortetracycline. Having a relatively simple structure, chloramphenicol was the first antibiotic to be synthesized on a commercial scale. Because of the effectiveness of chloramphenicol against enteric infections, typhus, and other diseases, its relative freedom from undesirable side reactions, and the comparatively low rate of associated microbial adaptations to resistance, this drug has found an important place in chemotherapy. It is sold under the trade name Chloromycetin. The chemical structures of chlortetracycline (trade name, Aureomycin) and oxytetracycline (trade name, Terramycin) and their relation to the basic tetracycline are completely known. The tetracycline antibiotics are widely used against many bacterial infections and diseases caused by large viruses. Some other antibiotics have also been proved useful in the treatment of animal and human diseases, as is mentioned later in this article.

Numerous attempts are now being made to expand the inhibitory spectrum of antibiotics to include destruction of neoplastic cells. Various methods have been developed recently to make possible the screening of antibiotics against cancer grown in rodents, in embryonated eggs, and in tissue culture. With these techniques, encouraging results are being attained. Tumor-retarding agents have been demonstrated in the mold *Aspergillus fumigatus.* Azaserine is a unique compound showing activity against sar-

coma 180 in mice and against some kinds of microorganisms. Sarkomycin inhibits growth of ascites tumor cells in rabbits. Alazopeptin and puromycin are new antitumor substances. Actinomycin inhibits growth of tumors in rodents and arrests certain types of spontaneous tumors in human beings. Sulfocidin and 6-diazo-5-oxo-L-norleucine (DON) depress the growth of experimental tumors in mice. Some species of mushroom-like fungi are known to produce antitumor substances.

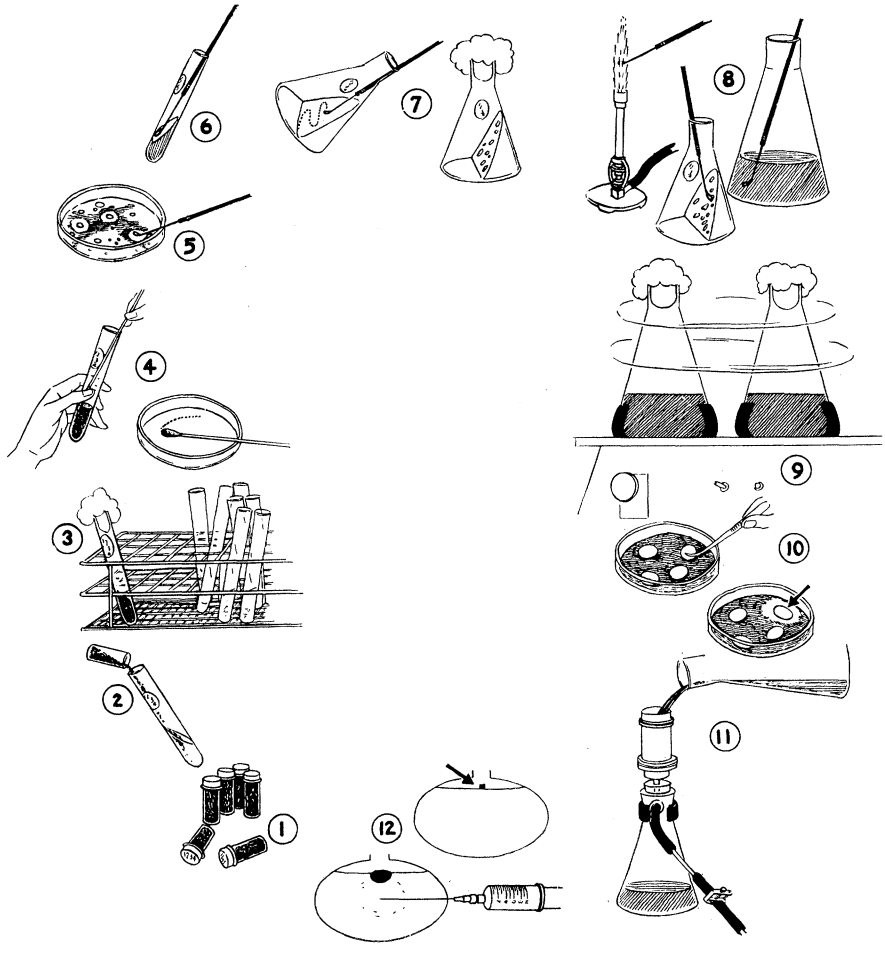
From recognition, in the 19th century, of curious microbial antagonisms has evolved the "antibiotic era" of the mid-20th century. The broadening spectrum of antibiotic action has now been extended to include inhibition of various bacteria, molds, yeasts, algae, protozoa, rickettsiae, large viruses, phages, and finally neoplasia. Perhaps new antiviral antibiotics may provide an approach to the unknown realm of virus-tumor chemotherapy. It is hoped that we may be standing on the threshold of a new era of cancer chemotherapy, and of ultimate prevention or cure of all infectious diseases.

Sources of Antibiotics

Among the numerous kinds of antibiotics which have been discovered, chiefly over a span of the past two decades, many diverse patterns of chemical structure occur. Some species of microbes produce several different kinds of antibiotic compounds, and it is also true that a given compound may be synthesized by more than one species of mold or actinomycete. The rapid growth of the list of new compounds comes about as a result of intensive efforts to find drugs that may be useful against special pathogens and against disease agents that have become resistant to the older antibiotics, making them no longer effective. The majority of the new compounds seem to come from fresh isolates of *Streptomyces,* studied principally in industrial laboratories, in universities, and in governmental institutions in Japan, England, Germany, the Soviet Union, and the United States. Common sources of antibiotics are found among the actinomycetes, molds, and bacteria that live abundantly in soils, composts, and other places. Larger organisms, such as mushrooms and lichens, also produce antibiotic substances which are active against diverse living systems, ranging from bacteria to human cancer. In many flowering plants and in some coniferous trees antimicrobial compounds, of unusual nature or with chemical structures not unlike those of some of the mold antibiotics, are known to occur. A few kinds of algae elaborate antibacterial substances. Some kinds of corals produce compounds which strongly inhibit the acid-fast group of bacteria, to which the tuberculosis germ belongs.

Perhaps it would be stretching the definition of antibiotic substances too far to include the defensive secretions of cockroaches, carabid beetles, and millipedes, although some of these substances bear close structural similarity to certain antibiotic quinones synthesized by some kinds of molds. In the widest usage of the term *antibiotic,* some persons might want to include also Indian arrow poisons, snake venoms, the paralyzing substances of sea cucumbers, the stinging material of the Portuguese man-of-war, or even dinoflagellate toxins which can cause widespread poisoning of fish life in the ocean. The scope of antibiotics in this discussion is restricted chiefly to the various substances produced by certain microbes and active against other microbes.

One of the current problems in antibiotic research is that of developing new ways to find novel antibiotic compounds. The study of specialized source materials from unusual habitats is one approach. Another involves highly specialized enrichments of soil or other material, made for the purpose of encouraging the growth of a minor constituent of the varied microbial population. A



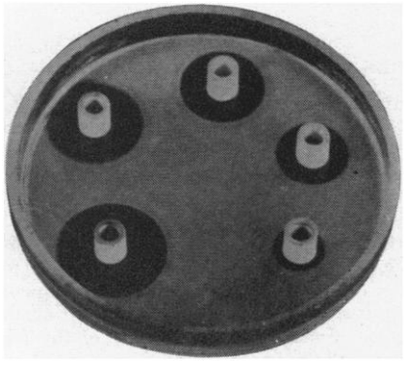
Schematic diagram that shows the 12 steps in the isolation and testing of potential antibiotic cultures obtained from soil. (1) Soil samples are selected. (2) Soil is added to water in test tube. (3) Soil and microbes are now suspended by shaking. (4) A small portion of the suspension is streaked on the surface of nutrient agar in a petri dish. (5) Colonies of microbes grown after 2 days are selected with a bacteriologist's needle. (6) These are transferred to an agar slant. (7) The culture is transplanted into a flask in order to allow further growth. (8) The inoculum is seeded into a flask of nutrient broth. (9) The broth culture undergoes fermentation on a shaker. (10) Potency of the fermented "beer" can be tested with paper pads wet with "beer" and placed on agar plates seeded with living indicator bacteria. (11) An interesting "beer" is filtered to remove microbes. (12) Sterile "beer" is then injected into embryonated chicken eggs bearing implanted human tumors. Inhibition of bacterial growth in step 10, or arrested growth of tumor tissue in step 12, may suggest antibiotic activity of sufficient significance to warrant further studies on selected cultures.

new approach would be the production of mutants which might be blocked in metabolic pathways to yield unusual products.

Quest for Antibiotics

In the search for organisms which produce antibiotic substances, various techniques have been developed, in many laboratories, which are applicable to the survey of large numbers of cultures derived from nature. Small samples of soil, compost, mud, or organic debris are collected from likely places. A portion of the sample is suspended in water and shaken for the purpose of dispersing cells or spores of the numerous microorganisms. Then a small amount of the suspension is spread in a suitable nutrient agar in glass petri dishes. After several days, numerous colonies of diverse microbes make their appearance. The different kinds of molds or bacteria are carefully transplanted into test tubes of sterile media, one at a time, in order to obtain pure cultures. The possible antibiotic activity of each culture may be tested by growing the culture in a suitable broth on a shaking machine and then assaying the resultant "beer" against indicator microbes.

One very convenient method of performing a test consists of seeding indicator bacteria onto an agar plate and then placing a paper impregnated with beer on the agar. After incubation overnight, zones of growth inhibition may occur around some of the pads wherever the beers contain antibacterial substances. The larger the zones of inhibition, the greater the potency of the beer. There are other methods too; each laboratory has its own special techniques for isolating and surveying organisms. New methods would doubtless lead to the discovery of new microorganisms and then of new antibiotics. For testing larger organisms, such as lichens, mushrooms, flowering plants, and corals, extracts of the materials may be prepared and assayed in the same way as when fermented beers of molds or bacteria are used.



The diameters of zones of inhibition around paper pads or cups that contain antibiotics and that are placed on a plate seeded with bacteria are proportional to the concentrations of antibiotic used.

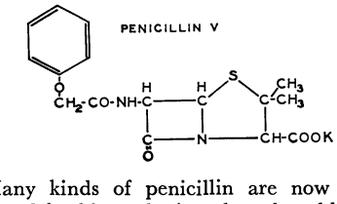
The investigator who has accumulated large numbers of antagonistic microbes must determine which of his cultures produce new and valuable antibiotics. Several means may be useful in discovering whether the activity is caused by one of the many known substances or by some new compound. The colonial morphology, microscopic structure, and physiological characteristics of the organism are studied with a view to determining the nature of the antibiotic-producing organism. Varying sensitivities of different kinds of test organisms permit the construction of an antibiotic spectrum. The pattern of this spectrum, consisting of a list of values for microbial sensitivity, sometimes throws light upon the identity of a given preparation. Drug-resistant strains also serve as precise indicators for the identification of unknowns. Thus, if a penicillin-resistant bacterium is found to be susceptible to the beer of a mold obtained from soil, it may be concluded that among the various products of this mold is a substance different from penicillin. Concentration and purification of a compound may be achieved through differential solubility by the use of countercurrent apparatus, by precipitation techniques, through the use of absorption columns, or by means of chromatography. The mobility of active compounds in paper chromatograms is an extremely useful indicator for purposes of identification, the relative positions of invisible active spots being readily detected by auxanographs in agar plates seeded with susceptible organisms. In this method, paper chromatograms are placed upon the seeded agar plates to allow transfer of antibiotic spots from paper to agar, with the resultant appearance, eventually, of growth-inhibition zones corresponding to the original location of the antibiotic substances on the paper.

Once it has been found that an organism produces an interesting and novel compound, the physical, chemical, and pharmacological properties of the compound are determined, and its probable value as a chemotherapeutic agent is studied. The big hurdle for every antibiotic candidate is the test for toxicity to experimental animals. Many promising drugs turn out to be rat poison and never reach the stage of clinical trial. The therapeutic index must be satisfactory before large-scale production is warranted. Out of the many initial candidates, only a few drugs ever reach the practicing physician and the impatient and willing consumer of antibiotics.

Significance of Antibiotics in Nature

The question has often been asked, "Are antibiotics formed in nature or are they artifacts of the laboratory?" It is well known that many fungi and actinomycetes produce antibiotics in sterilized soil supplemented with organic matter. A few organisms have been shown to produce antibiotics when they are grown in sterile soils without added organic matter. Formation of such substances as gliotoxin, chloramphenicol, and actinomycin occurs only in trace amounts under conditions of low nutrient level. In general, it has proved difficult to show that soil microbes produce active compounds in soils containing the normal mixed flora and fauna, unless there has been excessive addition of organic matter. The evidence suggests that antibiotics are detectable in microhabitats of the soil where nutritive conditions are suitable for production of these substances in excess of amounts destroyed by absorption on clay colloids and through breakdown by chemical and biological action.

Several kinds of molds are known to form antibiotic substances such as patulin, alternaric acid, and fusaric acid, found in rotting apples and infected tomato plants. About half of the many lichens which have been examined produce antimicrobial substances in nature. It seems probable, also, that algal antibiotics are not unusual products in the



Many kinds of penicillin are now produced by biosynthesis, selected molds being grown in fermentation broths containing special substances. The structure of penicillin V, shown here, includes a methoxy benzyl moiety joined to the fundamental penicillin portion, consisting of B-lactam and thiazolidine rings, resembling fused structures of the amino acids and cysteine.

natural planktonic environment of lakes and seas. Certain kinds of bracket fungi and toadstools regularly contain extractable antibiotics. Gorgonian corals represent a recently uncovered source of antibacterial substances, but it is not certain whether the animal polyps or their associated algae are the producers. Antimicrobial substances are fairly common among higher plants—for example, in mountain ash, in onions, and in some conifers. The natural functions of antibiotics as products of organic evolution need to be studied much more thoroughly. Formation of antibiotics in nature seems to be compatible with the view that these substances have survival value for the organisms which possess the ability to produce them, as well as for the human beings who have the skill to employ nature's products advantageously in the nutrition of domesticated animals, for crop protection, in food preservation, and for chemotherapy of infectious diseases.

Chemical Patterns of Antibiotics

Perhaps the most interesting aspect of antibiotics from the standpoint of science lies in the comparative biochemistry of their molecular forms and functions. Only a few examples can be mentioned here to illustrate diverse types of organic structures and some of their relationships. It is hoped that investigators may be encouraged to study the metabolic pathways which lead to biosynthesis of antibiotics. Some of the simplest antibiotics bear a close resemblance to the structure of glucose, from which they may be derived in the peculiar metabolism of the antibiotic-producing organisms. Examples are kojic acid, from a species of *Aspergillus* mold; claviformin, from *Penicillium claviforme*; and parasorbic acid, which can be extracted from fruits of the mountain ash, *Sorbus aucuparia*. Antimicrobial substances related structurally to the sweet-clover compound coumarin, are dicoumarol and datiscetin. Related to vitamin C are the antibiotics penicillic acid and lichesterinic acid. Odd 7-carbon-ring tropolones are represented by the fungus compounds puberulonic acid and puberulic acid and by thujaplicin, contained in the cedar tree *Thuja plicata*. If one asks how it can be that molds and trees produce similar antibiotics, any simple answer would have to be based upon their inheritance of genes and enzymes through a common ancestry.

Numerous examples of antibiotics are found among the quinones—antibiotics such as fumigatin, spinulosin, and phoenicin, all of which are related to parabenzoquinone. Among the somewhat more complex structures, showing similarity to α-naphthaquinone, may be mentioned juglone, plumbagin, phthiocol, and javanicin. A whole new group of quinocycline antibiotics resemble substituted anthroquinones. The general structure of aspergillic acid is repeated among the products of other microorganisms—for example, pyocyanin, hemipyocyanin, iodinin, and chlororaphin.

Among the numerous antimicrobial substances which occur in lichens and fungi, usnic acid is a basic type, with related substances such as didymic acid, strepsilin, and griseofulvin. Somewhat more complicated are the derived depsides, perlatoric and olivetoric acids, and the depsidones, sekikaic acid and diploicin. Some of these lichen compounds are now being used for medical purposes in Finland.

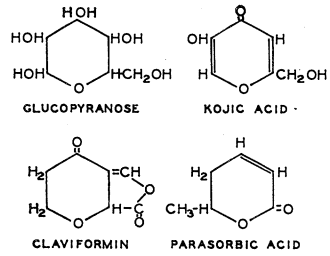
The important tetracycline family of antibiotics is illustrated by oxytetracycline and chlortetracycline. The new antituberculosis drug kanamycin contains two amino glucopyranose moieties coupled to diamino cyclohexane. Steroid antibiotics are known among the metabolic products of bacteria and fungi, such as, for example, polyporenic acids, helvolic acid, and cephalosporin P. It appears likely that these steroids are synthesized from acetate through squalene. The precise structural features which confer on these steroids inhibitory properties against *Staphylococcus* and some other bacteria are not well understood.

A new group of important antibiotics are the macrolides, which have in common a large lactone ring. Included among the macrolides are erythromycin, novobiocin, methymycin, carbomycin, and probably also oleandomycin, pikromycin, and narbomycin. The type compound erythromycin is composed of two sugars, desosamine and cladinose, connected by glycosidic linkages to the 14-carbon lactone erythronolide. This drug is valuable in treating refractory infections caused by various kinds of bacteria. Finally, an important series of antibiotic compounds related to amino acids and peptide groups of these acids should be mentioned. The penicillin group, including penicillin G, V, and so on, as well as the peptide compounds cephalosporin N, micrococcin, and bacitracin, have in common special sulfur- and nitrogen-containing rings. Numerous kinds of penicillin can be biosynthesized by molds in the presence of precursors added to the growth medium. Apparently many kinds of microbes contain enzymes which are able to effect synthesis of compounds containing the thiazoline ring. Not all such moieties possess antimicrobial properties; the thiazole of vitamin B_1 is an important part of the growth-promoting factor required by numerous fungi and bacteria.

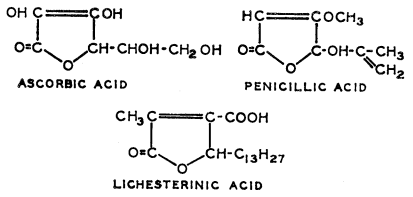
Some of the streptothricin-like antibiotics contain β-lysine. Chloramphenicol may be portrayed as a modified β-phenylalanine. Cycloserine and azaserine are structurally related to serine. 6-Diazo-5-oxo-L-norleucine is a derivative of norleucine. Puromycin contains phenylalanine.

A very large group of polypeptide antibiotics deserves special mention. Numerous kinds of actinomycins have been demonstrated in the fermentation broth of certain kinds of soil actinomycetes. Seven of these compounds have been crystallized and found to contain six amino acids, among which D-valine and D-isoleucine appear to be important in relation to inhibitory properties of the polypeptides. Among other peptide antibiotics containing D-amino acids are tyrocidin, gramicidin, the polymyxins, cephalosporin, and bacitracin. Though many substances of this kind come from actinomycetes and bacteria, some are known to occur in larger fungi—for example, lycomarasmine in *Fusarium* and phalloidine and amanitin in the poisonous toadstool *Amanita phalloides*.

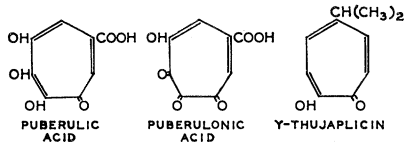
Some of the newer antibiotics possess special properties which make them valuable for therapeutic treatment of specific infections. Thus, nystatin is used in the control of bronchial and intestinal infections caused by *Candida* (yeast), which tends to cause secondary infections in patients following a course of broad-spectrum antibiotics. Fumagillin is particularly valuable for its effective amebicidal action. Streptothricin-like antibiotics include streptocin, roseomycin, neomycin, and xanthomycin. These are not peptide antibiotics, though β-lysine has been identified in some of them. The neomycin complex of substances can be separated by chromatography into a series of different fractions, each with special properties. A group of yellow sulfur-containing compounds include thiolutin, thioaurin, and aureothricin, all of similar structure. Numerous polyenes, isolated from soil actinomycetes, show antifungal activity. The classical polyacetylene structure is illus-



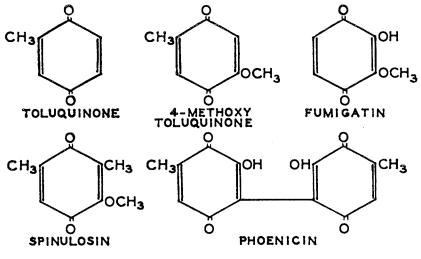
Three antibiotic compounds, kojic acid, claviformin, and parasorbic acid, related in structure to glucopyranose. Kanamycin also contains two amino-glucopyranose moieties.



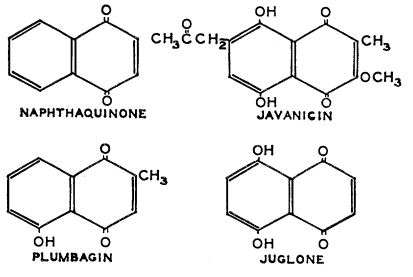
Lichesterinic and penicillic acid resemble ascorbic acid (vitamin C).



Seven-carbon ring tropalones are represented by mold antibiotics, puberulic and puberulonic acids, and thujaplicin, produced by the cedar tree *Thuja plicata*.



Numerous toluquinones are produced by fungi.



Compounds related to α-naphthoquinone are javanicin, of microbial origin; plumbagin, from Indian shrubs of the genus *Plumbago*; and juglone, found in black walnut fruits.

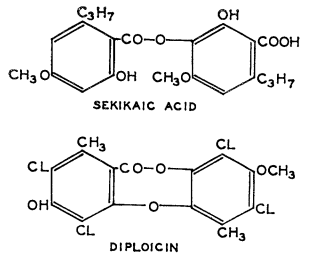
trated by the antibiotic mycomycin. Many other new antibiotics, such as ristocetin, hygromycin, actathiazic acid, and cycloserine, inhibit growth of a variety of different organisms. It is beyond the scope of this discussion even to list all of them by name.

Modes of Action

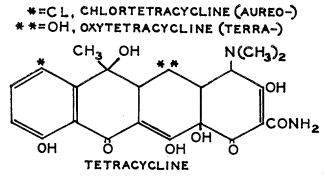
Different kinds of antibiotics work against susceptible microbes in different ways, depending upon special biochemical properties of the drugs and physiological systems of the "bugs." Much research has been done on the ways in which penicillin inhibits growth of Gram-positive bacteria and causes morphological changes of spherical cocci to rod-shaped organisms, or of rods to bizarre filamentous forms. Recent evidence points to blocking of uridine nucleotide incorporation into the cell-wall structure of penicillin-inhibited bacteria. By the chelation process, tetracyclines probably prevent the use of such essential ions as Mg, Fe, and Mn in enzyme systems essential for protein synthesis in growing bacteria. Evidence obtained in experiments with a C^{14}-labeling technique indicates that the inhibition site for tetracyclines is the surface of a catalytic enzyme which is probably involved in the metabolism of glutamic acid. The reversal of chlortetracycline inhibition of respiration in *Azotobacter* by magnesium salts offers evidence for chelation of certain enzyme-activating metal ions by this group of antibiotics.

The structural resemblance of some antibiotic compounds to essential metabolites suggests a possible mode of action through interference with specific steps of normal metabolism. In special cases, chloramphenicol appears to be antagonized by phenylalanine. Microbial inhibition by chloramphenicol may be explained as the result of blocked steps in protein formation; such blocking would allow the accumulation of D-glutamic peptides but not of peptides with the L-configuration.

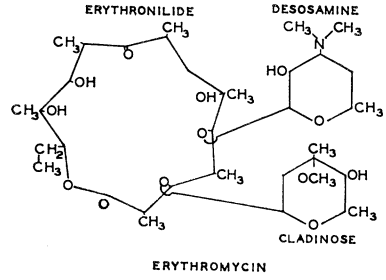
Studies on azaserine and 6-diazo-5-oxo-L-norleucine suggest interference with the incorporation of glycine and formate into nucleic acids. It has been proposed that streptomycin interferes with condensation of pyruvate and oxalacetate in the pathway of intermediary carbohydrate metabolism. The presence of an amino sugar in streptomycin, erythromycin, carbomycin, picromycin, and puromycin suggests the possible sig-



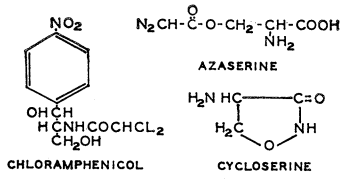
Depsidones occur commonly in certain species of lichens.



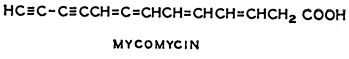
Tetracycline, chlortetracycline, and oxytetracycline are important broad-spectrum antibiotics.



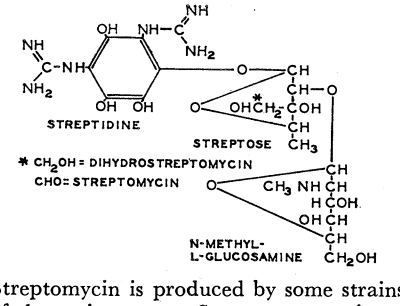
Erythromycin illustrates a macrolide ring structure with two satellite sugars, desosamine and cladinose.



Chloramphenicol bears a resemblance to B-phenylalanine, and azaserine and cycloserine are related to the amino acid serine.



Mycomycin is a polyacetylene.



Streptomycin is produced by some strains of the actinomycete *Streptomyces griseus*.

nificance of this structure in their mode of action. Recent evidence that sterols interfere with the inhibition of fungus growth by polyene antibiotics, such as filipin and amphotericin, provides an interesting hypothesis concerning their mode of action. The general body of evidence is compatible with the concept that biological action of antibiotics occurs by interference with enzymatic processes in susceptible organisms. Researches in this field have only scratched the surface of a gold mine of problems.

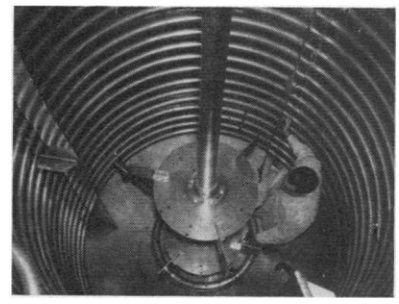
Current Trends

Numerous new compounds of remarkable organic structure and biological function continue to emerge from the large-scale research programs of government and industrial laboratories. One of the main activities for several years has been the screening of thousands of cultures isolated from soils of the world. The principal effort in antibiotic research is still directed toward the development of new therapeutic drugs. One reason for this is the need to fill gaps in the list of therapeutics; the other, the need to supplant old drugs with new ones in order to combat drug resistance.

The availability of new growth inhibitors for physiological studies helps in the making of numerous significant contributions to our knowledge about the life activities of living cells. The continuing research that is being done on modes of action of antibiotics is an important source of information for geneticists and biochemists. The molecular structure of an inhibitory compound may suggest a possible mode of action through resemblance to the structure of a known metabolite; sometimes the mode of action of a drug reveals much concerning incompletely understood pathways of metabolism. Knowledge about the structure and biochemical action of therapeutic agents, such as antibiotics, should eventually lead to the synthesis of effective "tailor-made" molecules for a more rational chemotherapy.

With the advent of so many kinds of drugs, clinicians are experimenting with combination therapy in the hope that mixtures of antibiotics may accomplish more good results than can be achieved with single drugs. Emergence of drug-resistant cells from a general population of drug-susceptible pathogenic organisms can create serious problems. The mechanism seems to be based upon spontaneous mutation of a few cells to resistance against, or dependence upon, a certain drug. The mutants subsequently respond by growing rapidly, and a new strain of pathogens emerges to challenge the patient's life and the physician's wisdom. Examples of drug resistance are found, all too frequently, among "hospital strains" of penicillin-resistant *Staphylococcus*. Other notorious offenders are the streptomycin-resistant tubercle bacteria, which may acquire resistance while the host is receiving low-level drug therapy. Among the various objectives behind the new trend toward polyantibiotics in medicine are the following: (i) to suppress different kinds of pathogenic microbes without specific diagnosis of the causal agents of disease; (ii) to prevent the emergence of drug-resistant strains; (iii) to achieve synergism; (iv) to avoid superinfection by opportunist fungi and other organisms; and (v) to arrest infections caused by mixed populations of unlike organisms.

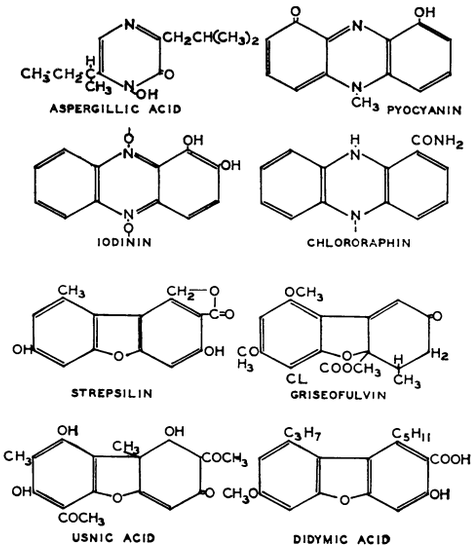
In the successful treatment of tuberculosis with a combination of streptomycin plus isoniazid or para-aminosalicylate, the emergence of resistant strains of tubercle bacteria is largely prevented. Another combination, designed to combat the overgrowth of pathogenic yeasts as secondary invaders, is found in Comycin, an antibiotic mixture which contains tetracycline phosphate plus the antifungal drug nystatin. A recent survey of the interactions of drugs against many kinds of pathogenic bacteria indicates that four predicted classes of interactions are actually found: (i) synergistic, (ii) additive, (iii) interfering, and (iv) negative. When two popular drugs were used alone and in dual combination with any one of 15 other antibiotics, the frequencies of occurrence of these types of interactions in results of antimicrobial tests were as follows: synergism, 24 percent; summation, 51 percent; antagonism, 3 percent; and no interaction, 22 percent.



Interior of a large fermentation tank used in producing antibiotic substances. [Courtesy of Parke, Davis and Co.]

A conspicuous recent trend is that of increasingly extensive manufacture of antibiotics for uses other than in human medicine. Large quantities of antibiotic drugs are used in veterinary medicine. Sometimes there may be some carryover of antibiotics from treated cows into the milk supply. Occasionally the antibiotic level in milk may be sufficient to interfere seriously in the microbial conversion of milk to cheese, or to cause allergic reactions in extremely sensitive persons.

Important applications of antibiotics have also been found in agriculture, as supplements in animal feeds. Practically all commercial poultry feeds contain low-level supplements of antibiotics such as penicillin, the tetracyclines, erythromycin, or bacitracin. Antibiotic-fed pigs, poultry, horses, and mink grow faster, require less feed, and show lower mortal-



(Top) Phenazine antibiotics of bacterial origin bear some resemblance to the mold product aspergillic acid. (Bottom) Numerous antimicrobial substances resemble usnic acid, commonly found in lichens.

ity rates than those not fed antibiotics. Of considerable interest is the use of antibiotics with sex hormones to produce better capons and superior beef. Growth in human beings seems also to be increased through low-level administration of certain antibiotics. Premature infants as well as young naval recruits have shown very significant gains in weight when kept on diets which included antibiotics as compared with controls maintained on the same diets without antibiotics. It is thought that these favorable effects may result from suppression of undesirable microbes, with consequent lessening of intestinal toxins and increase in the availability of essential nutrients and growth factors.

Antibiotics are being developed for a variety of other uses; these include everything from sprays for the arrest of bacterial and fungus diseases of plants to agents for increasing fecundity in cattle, agents for short-term preservation of foods, and so on. Preparation for slaughtering steers in some countries includes injection of antibiotic preservatives; these are carried into the tissues of the animal, so that later on the meat can be hung in warm rooms to bring about tenderization without spoilage. Extensive studies have shown the value of antibiotics in the preservation of fish, chickens, hamburger, vegetables, and other perishable foods. The propriety of such uses is under consideration by the Food and Drug Administration. In the control of certain plant diseases, antibiotics such as penicillin and streptomycin may act systemically throughout the plant and thereby have some advantage over conventional pesticides. Duramycin and actidione are examples of antifungal antibiotics employed for control of plant diseases. Antibiotic crop sprays are used to prevent blight in fruit trees and on bean plants and to control bacterial diseases of tobacco, tomatoes, cherries, lettuce, and other vegetables.

Over the past 15 years, antibiotics have become big business. The total production for all uses was over 2.7 million pounds in 1956 in the United States. The value of all antibiotics sold during 1956 in the United States came to a total of over 299 million dollars. The reports of the U.S. Tariff Commission show great increases, over the past 5 years, for antibiotics used as feed supplements and in veterinary medicine. It seems probable that production of antibiotics for agriculture, food preservation, and animal feeding, may eventually become greater than the production for medicinal purposes.

Areas of future research in the field of antibiotics will probably include methods for producing new genetic types of antibiotic-producing organisms, as well as ways for enormously increasing the yields of antibiotic substances through novel techniques. Methods may be found for developing new kinds of antimicrobial compounds with short half-life, suitable for the preservation of foods. The problems of finding more satisfactory controls of fungus diseases, tropical protozoa, severe virus infections, and the common cold offer challenges to the courageous investigator. New drugs will be needed to combat rare and dangerous diseases which may arise from microbial mutations in the future. Eradication of venereal diseases and other infections through a big public-health movement would be appropriate for an enlightened world civilization. Biological control through management of antibiotic soil microbes in nature might aid in bringing about extensive reduction of certain phytopathogenic fungi and undesirable nematodes, insects, and other agricultural pests. Discovery of microbial substances suitable for regulating fecundity in animals and man lies within the realm of possibility. Studies on the mode of action should lead to deeper understanding of the biochemistry of antibiotics and possibly to the rational synthesis of therapeutic agents far better than those that have become available through present trial-and-error methods.

Use and Abuse of Antibiotics

In this antibiotic age of chemotherapy problems arise out of the unwarranted widespread use of these powerful and sometimes dangerous drugs. The general population soaks up drugs like a huge sponge. On the basis of popular articles which they may have read, patients often demand access to the newest wonder drugs, through their physicians. The physicians, in turn, are subjected to high-pressure advertising which makes strong claims for (i) the potency of antibiotics; (ii) the synergistic power of antibiotics in combination therapy; (iii) the almost complete absence of side reactions; (iv) the ready absorption of these drugs by the body; (v) the prompt attainment of high blood levels; (vi) the slight extent of patient sensitivity; and, of course (vii) the applicability of antibiotics in most situations. It is not surprising to learn from a recent survey that in a typical small community in North Dakota, only 7.9 percent of the total population failed to receive antibiotics in a 5-year period. It is believed that the prescribed medication in this town was justified in less than half of the illnesses which were studied as case histories.

Indiscriminate use of antibiotics, and improper dosages, can cause harm through allergic reactions, blood dyscrasias, alteration of the normal flora, superinfections, emergence of resistant mutants, and fostering of persistent pathogens. As a result of nationwide surveys of severe reactions to antibiotic therapy, it was concluded that the highly potent antibiotics must be regarded as potentially dangerous drugs in the hands of uninformed persons. When these drugs are improperly used they can be dangerous; when properly used they are lifesaving. They should only be taken on the advice of a competent physician.

Antibiotics and the Public Health

Public health in the Western World has improved immensely over the 20 centuries which have passed since the time of the Roman Empire. In Caesar's Rome, the average life expectancy was about 22 years. Europeans of the Middle Ages could expect to reach, on the average, 33 years of age. In the United States today the newborn's statistical life expectancy is almost 70 years. Increased public health measures, improved diets, skillful surgery, better medication, and the general rise in standards of living all contribute to our living better and longer lives.

It is difficult to say how great have been the contributions of sulfa drugs and antibiotics to the public health. It seems quite clear, however, that marked reduction in mortality from many infectious diseases has occurred during the years since the introduction of "miracle drugs." This is true for meningococcal and streptococcal infections, influenza, pneumonia, tuberculosis, syphilis, and appendicitis, all of which yield to treatment with sulfonamides or antibiotics.

Before the antibiotic era, public health programs placed major emphasis on prophylaxis and the difficulty of therapy in venereal diseases. With the advent of penicillin, a remarkable reduction in the incidence of early syphilis has occurred. In New York City the decline in syphilis over 9 years following the 1946 postwar peak was from 78.5 to 7.7 per 100,000 of the population. Complete eradication of a disease is seldom achieved, and it is now important that attention be given

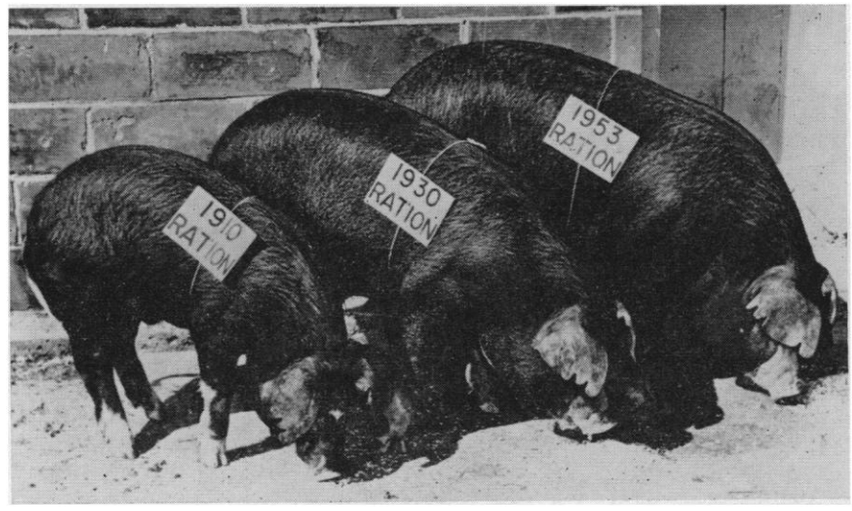
to gaining further ground in the conquest of syphilis. Gonorrhea also has yielded to penicillin therapy, but the emergence of drug-resistant strains of the pathogenic bacteria gives some grounds for concern. Here again, too much confidence in the merits of an established mode of therapy could lead to disaster.

As in the venereal diseases, so also in other common diseases a satisfying degree of control has been obtained by the use of antibiotics. Great changes have recently occurred in the management of tuberculosis. Whereas long periods of hospitalization were once regarded as necessary, now the tubercular patient can be trained to follow a prescribed regimen of combined therapy (with streptomycin and adjuvants) and sanitation during most of the convalescent period, spent at home. The serious effects of subacute bacterial endocarditis and rheumatic fever, and relapses of these diseases, have been greatly lessened by penicillin therapy and prophylaxis.

Recent estimates indicate that during the period from 1937 to 1952, approximately 1.5 million lives were saved through chemotherapy of the common infectious diseases mentioned above, exclusive of tuberculosis. When the data are brought up to date and other diseases are included, such as the enteric disorders, tuberculosis, and rickettsial diseases, the figures on conservation of human lives are much greater. Perhaps lifesaving with drugs has more than offset the losses sustained in the United States during the same period through wars and automobile accidents.

The impact of antibiotics and other wonder drugs on society is not wholly revealed in terms of improved medical care and prolongation of human lives. Modern miracle drugs are creating changes which lead to ponderable social problems. Some of these deserve to be mentioned here briefly. Since improved medication has tended thus far to conquer infections and steadily to increase the proportion of older people, the main medical challenges are being shifted to other areas. We will have to learn, more than ever, how to reduce disability, and how to make more effective use of the talents of healthy older people. This will come about more easily when science shows the way to extend the normal vitality of youth into what is now regarded as inevitable old age.

There are at present some distressing trends in our society which are aggravated by large-scale use of miracle drugs. Antibiotics today are keeping alive idiots who used to die under 12 years of age, as well as many thousands of persons who constitute a social liability. Half of the hospital beds in the United States are now occupied by mental patients, and there are tremendous numbers of mental defectives at large, making their contribution to the common gene pool. Insulin now prolongs the lives of numerous diabetic children, who marry and produce more victims of diabetes. Vitamin B_{12} gives patients with pernicious anemia ample time to propagate anemic offspring.



Advances made in livestock production are illustrated by the growth of pigs fed on rations typical of the years 1910, 1930, and 1953, respectively. The pig at left was fed yellow corn and minerals; the pig in the center received yellow corn, tankage, and minerals, and the pig at right was given a well-balanced ration fortified with vitamins and an antibiotic. "Miracle" drugs are bringing about great changes in agriculture. [Courtesy of Chas. Pfizer and Co., Inc.]

It is time to consider the possible untoward implications of an artificial system which fosters the survival of unfit germ plasm. Science has brought changes to society, and, as old problems become obsolete, new difficulties present their challenge.

One of the greatest problems of all time is destined to arise when miracle drugs and better nutrition are introduced into underdeveloped areas of the world. Almost three-fourths of the earth's human population live in countries where life expectancy is about 30 years, rather than 70. In many of these areas the birth and death rates are at least twice those of the United States. When infectious diseases in backward areas are overcome by the introduction of measures which have been found effective in the Western World, enormous numbers of Asians and Africans, as well as Americans, will live longer and have extended periods of fecundity. Perhaps these peoples can learn much, in advance, from the difficulties and experiences in Europe and North America. It is hoped so, because without knowledge and the right leadership, population pressures automatically produce poverty, famine, epidemics, and war. If birth control is felt to be desirable and if it is desired by people in overpopulated areas, what inexpensive, practical, and safe methods can be recommended to vast illiterate hordes of human beings?

Science has provided society with lifesaving drugs, and many times over it will need to redefine its objectives and seek solutions to difficulties as they arise. In the immediate future some problems of greatest concern to mankind will be cancer, degenerative ailments, mental diseases, population pressures, and management of our destiny in an all too complex society. Perhaps the methods of science, which generated knowledge about atoms and antibiotics, can continue to serve as Aladdin's lamp.

Bibliography

Antibiotics and Chemotherapy (MD Publications, New York), 9 vols. to 1959.

Antibiotics Annual (Medical Encyclopedia, New York), 5 vols. to 1958.

E. B. Chain, "Chemistry and biochemistry of antibiotics," *Ann. Rev. Biochem.* 27, 167–222 (1958); see also earlier volumes.

H. W. Florey *et al.*, *Antibiotics* (Oxford Univ. Press, London, 1949), 2 vols.

H. S. Goldberg *et al.*, *The Chemistry and Non-Medical Uses of Antibiotics* (Van Nostrand, New York, 1959).

G. W. Irving and H. T. Herrick, *Antibiotics* (Chemical Publishing Co., Brooklyn, N.Y., 1949).

J. Antibiotics (Japan). (Japan Antibiotics Research Assoc., Tokyo), 12 vols. to 1959.

L. Karel and E. S. Roach, *A Dictionary of Antibiotics* (Columbia Univ. Press, New York, 1951).

S. A. Waksman, *Microbial Antagonisms and Antibiotic Substances* (Commonwealth Fund, New York, 1945).