The Drugs of Microbial Origin

Kenneth B. Raper, Robert G. Benedict

Tremendous progress has been made in the past decade in the development of new disease-preventing agents. Foremost among such agents are penicillin and streptomycin, both of microbial origin. Other antibiotic substances of similar origin, such as chloromycetin, aureomycin, and terramycin, are becoming especially valuable.

Still others, including bacitracin, subtilin, polymyxin, and circulin, offer promise for special applications. Although the new disease-preventing drugs are saving untold lives, there are yet many afflictions which take their toll and remain unchallenged.

The quest for useful drugs among the byproducts of micro-organisms was tremendously advanced by the findings of Professor Alexander Fleming, a bacteriologist working at St. Mary's Hospital, London, in 1928. Investigating staphylococci, which cause boils, carbuncles, and septicemia in man, he observed that a contaminating blue-green Penicillium inhibited the growth of these disease-producing germs. As a result of his discovery of the antibiotic penicillin, attention was drawn to the possibility of employing the product of one microbe to control or prevent the growth of another. It took the destruction of war, however, and its quickened search for new therapeutic agents, to bring penicillin into its rightful prominence as one of the greatest medical and scientific discoveries of all time.

Howard Florey, E. Chain, N. G. Heatley, and other men at Oxford University in 1939 took up the study of penicillin, and found it to be nontoxic and to have remarkable curative properties when tested on laboratory animals and man. Confirming Professor Fleming's results, they reported that penicillin inhibited the growth of many gram-positive disease-producing bacteria, such as staphylococci, streptococci, and pneumococci. They found also that it was relatively ineffective in combating gram-negative forms, such as the bacteria that cause typhoid fever and dysentery. They developed methods for producing penicillin in limited amounts and introduced a method for measuring its potency. They demonstrated unmistakably the remarkable potentialities of the new drug, but were unable to proceed with its production because of war conditions. They came to the United States in 1941 to enlist the aid of Government and industry. In the succeeding years, penicillin became the preeminent drug that has saved countless lives.

Encouraged by the phenomenal promise of penicillin, yet knowing its limitations, investigators soon began searching for other therapeutic agents of microbiological origin. They have discovered some outstanding drugs, each with its special field of application. Other agents have been reported and are being evaluated in animal experimentation and clinical trials. For some serious diseases of an infectious nature no satisfactory drugs are yet available, but the search for them among the antibiotic substances produced by micro-organisms continues with bright promise.

The production of penicillin in quantity required the solution of many difficult problems. A more productive
medium than that employed in England had to be devised. Improved methods of production had to be developed. More productive mold strains had to be discovered. Techniques for assaying penicillin had to be refined. Methods for recovering and purifying the drug had to be worked out.

Using corn steep liquor—a byproduct of the corn wet-milling industry—and lactose, or milk sugar, as the two principal ingredients, A. J. Moyer and R. D. Coghill, of the Northern Regional Research Laboratory, developed a production medium that increased yields of penicillin manyfold. Although this medium was developed specifically for penicillin production using surface cultures, the same basic solution was found suitable for submerged cultures when the concentration of the principal nutrients was reduced approximately one-half. Today, and after much experimentation in many different laboratories, this basic medium is still employed with only minor modifications.

A method for producing penicillin in submerged, or tank, culture was introduced, and mold strains especially suitable for this type of production were discovered and made available to industry. From an economic standpoint, this method was particularly attractive and soon supplanted all other methods of manufacture. It reduced labor costs and increased productive capacity enormously. At the same time it obviated the need for large and expensive incubators and the special machinery required to handle the tremendous number of bottles or other containers formerly used in producing penicillin by surface-culture techniques.

Cultures that can produce substantially greater yields of penicillin were developed by Kenneth B. Raper and Dorothy I. Fennell. All early studies in England and the United States were made in surface cultures with the strain of Penicillium notatum originally isolated by Professor Fleming. Other strains were found to be more suitable for submerged production. Extensive search for more productive molds led to the discovery of a strain of the closely related species, P. chrysogenum, from which successively better substrains were developed through the selection of natural variants and the production of mutants by exposure of spores to X-ray and ultraviolet radiations. The latter steps were carried out at the Carnegie Institution of Washington in Cold Spring Harbor, N. Y., and at the Universities of Minnesota and Wisconsin. The cumulative result of all the studies was the development of a culture capable of yielding 900 to 1,000 units per milliliter of penicillin, in contrast to 75 to 100 units per milliliter obtainable from the unimproved parent. This culture is still universally employed for the manufacture of penicillin in this country and abroad, and additional selections and mutations have undoubtedly been developed in the research laboratories of the penicillin industry to increase productivity further.

Methods of assaying penicillin were much improved by W. H. Schmidt, R. G. Benedict, and others at the Northern Laboratory, by standardizing the composition and thickness of the nutritive agar layer and by controlling the density and uniformity of the test bacterial growth. Discs of filter paper saturated with penicillin-containing samples have been substituted for porcelain, glass, or metal cups, and various devices have been developed for rapid and more accurate measurement of the resulting inhibition zones. Despite the refinements, the method still generally employed rests squarely on the principles which underlie the technique developed by Heatley and his associates. Serial dilution and turbidimetric methods of assay have been perfected and find special applications in industrial practice. Some progress has been made toward developing an assay based upon chemical reactions and analyses.

Of the four types of penicillin known to be produced by Penicillium notatum and P. chrysogenum under natural con-
ditions, and commonly referred to as F, G, K, and X, only penicillin G is now manufactured. Penicillin F is comparatively unstable and therefore difficult to recover. Penicillin K, while showing high activity against staphylococci in the laboratory, is not a suitable drug because adequate blood levels cannot be maintained in the animal body. Penicillin X was at first thought to offer possibilities as a drug entity, because it was shown to be more effective than penicillin G against streptococci, pneumococci, and gonococci. Differences in effectiveness between penicillins X and G, however, were soon found to be quantitative rather than qualitative and did not warrant the manufacture of penicillin X with its much higher production costs.

Penicillin may be manufactured as a calcium, barium, or magnesium salt, but it usually comes from the factory as the potassium or sodium salt of penicillin G and is a highly purified, colorless, crystalline product. It is dispensed in or compounded into a variety of dosage forms to meet the physician’s needs. The foremost recent development has been the appearance of procaine penicillin formulations, either in aqueous solution or in oil. If given daily, these procaine preparations provide suitable blood levels, eliminating the necessity of painful and monotonous 3-hour injections. Various inhalator devices, which permit penicillin dust of high potency to be drawn into the respiratory tract, have also appeared and promise to provide effective means of combating bacterial infections commonly associated with colds, influenza, and other respiratory disorders. Few outstanding new uses for penicillin have emerged since 1945, but during the intervening period its use as a preventive agent has become increasingly commonplace. Very recently, penicillin, when incorporated in good rations, has been found to stimulate the growth of chickens and turkeys. The anticipated and widely dreaded development of penicillin-resistant strains of disease-producing microbes has not materialized.

The production of penicillin has increased steadily year by year since the industry was established—from less than 21 billion units in 1943 to more than 200 trillion units in 1950. Marked improvements have been made in the quality of the manufactured product. Prices have gone down steadily. The wholesale value of the penicillin produced in 1950 was estimated at more than 125 million dollars. Manufacturing facilities outside the United States constantly are being increased, but a large part of our domestic production is still sold abroad.

Many molds besides Penicillium notatum and P. chrysogenum have been shown to produce powerful antibiotic substances. Up to this time, however, penicillin is the only antibiotic derived from the higher fungi that has attained the status of an officially recognized and useful drug.

From the actinomycetes, a group of micro-organisms somewhat intermediate between the filamentous fungi and the bacteria, four important drugs of microbial origin are now obtained—streptomycin, chloromycetin, aureomycin, and terramycin.

Streptomycin, discovered in 1943 by Selman A. Waksman and his coworkers at Rutgers University, was the first antibiotic obtained from an actinomycete whose effective curative level in animals infected with disease-producing bacteria was considerably below that of its toxic level. It has been evaluated in far-reaching clinical trials and is now manufactured on a large scale here and abroad. It is particularly useful in the treatment of certain types of tuberculosis and other diseases not affected by penicillin.

Streptomycin is highly active against various gram-positive, gram-negative, and acid-fast bacteria, including the tubercle bacillus.

Streptomycin is effective in the treatment of tularemia, Hemophilus influenzae infections, urinary tract in-
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Infections, bacteremia, and meningitis caused by gram-negative bacteria. The results in typhoid fever, undulant fever, and Salmonella infections have been somewhat disappointing and inconclusive. However, much better results are now obtained with aureomycin, chloromycetin, or terramycin, with less danger of the development of resistant organisms. A new and important use for streptomycin is emerging through its use in combination with one or more other antibiotic agents.

For commercial production, a culture of Streptomyces griseus producing high yields of streptomycin is required. It is grown in a medium of meat or vegetable protein, minerals, and a carbohydrate such as dextrose (corn sugar). The operation is carried out aseptically in large fermentors under controlled conditions of temperature, aeration, and agitation. The organisms break down the nutritive components of the medium and usually produce maximum amounts of streptomycin by the third day. The culture liquor is clarified and passed through a large column containing a special ion-exchange resin. The resin takes up the streptomycin which is later removed from the column with a dilute mineral acid, and a crude preparation obtained from the neutralized eluate (washings) by evaporation and precipitation with acetone.

Streptomycin is used in the treatment of some forms of tuberculosis in humans. The former method of overwhelming the infecting tubercle bacilli with large doses of the antibiotic (3 to 6 grams daily) for periods up to 4 months resulted in toxic reactions in the patients, including loss of a sense of balance, neurological disturbances, fever, and skin eruptions. The dosage now recommended is 0.5 gram each 12 hours for 42 days. Miliary or meningeal tuberculosis cases receive 0.75 gram twice a day for 90 to 120 days. p-Aminosalicylic acid, also effective against tubercle bacilli, is given by mouth in conjunction with streptomycin.

Failure of streptomycin to eliminate infections is often due to the development in the patient of certain resistant forms of the disease-producing bacteria. Such resistant organisms may tolerate as high as 10,000 times the amount of drug ordinarily required to inhibit the pathogen when treatment was begun.

Credit for elaborating the chemistry of streptomycin goes to research investigators at Merck & Co., E. R. Squibb & Sons, Abbott Laboratories, the University of Illinois, and Ohio State University.

A valuable byproduct of the streptomycin fermentation is vitamin B_{12}, a member of a new group of interesting and essential growth factors. Vitamin B_{12} is effective in treating pernicious anemia and other forms of anemia in humans. It also is a powerful growth factor for certain farm animals. Members of this new group of vitamins are produced also as byproducts of other antibiotic fermentations (for example, aureomycin, chloromycetin, and terramycin) conducted with different species of Streptomyces. Vitamin B_{12} supplements added to normal feeds markedly stimulate the growth of chickens, turkeys, and swine. Certain antibiotics (streptomycin, penicillin, bacitracin, polymyxin, aureomycin, and terramycin) when incorporated individually in the feeds produce further gains in weight beyond those encountered with adequate B_{12} supplements.

The commercial production of streptomycin has increased steadily. By January 1950, the monthly production rate was more than 8 million grams (about 8.5 tons) a month, and the wholesale price had dropped to 60 cents a gram.

In 1950, a new form of streptomycin was discovered by Robert G. Benedict, Frank H. Stodola, and co-workers at the Northern Laboratory. The same antibiotic was subsequently reported by W. E. Grundy and co-workers at Abbott Laboratories. The Northern Laboratory workers assigned
the name hydroxystreptomycin to the antibiotic since it differs from regular streptomycin in having one more oxygen atom in the molecule. Hydroxystreptomycin is produced by a new actinomycete, *Streptomyces griseocarneus*. At present, the antibiotic is undergoing toxicity and pharmacological tests to determine whether it possesses advantages over streptomycin.

One of the newer antibiotics that offers promise as a therapeutic agent is obtained from culture solutions in which the actinomycete *Streptomyces venezuelae* has been grown. This drug, discovered in 1947, has been named chloromycetin (chloramphenicol), because chemical analysis shows that it contains nonionic chlorine, besides carbon, hydrogen, nitrogen, and oxygen. The empirical formula is $C_{11}H_{12}N_2ClO_5$, and chemists at the laboratories of Parke, Davis & Co. have successfully synthesized the antibiotic. Chloromycetin is effective against a number of gram-negative bacteria and has been successfully used in treating rickettsial diseases in man. The rickettsia are minute, bacterialike forms, which had successfully resisted all previous antibiotic therapy. Included among the rickettsial diseases that have shown favorable responses to chloromycetin are Rocky Mountain spotted fever, epidemic typhus, and scrub typhus. Success has been attained in the treatment of typhoid fever with this new drug. Tests on embryonated eggs and mice reveal that one virus is susceptible to it.

Chloromycetin is now produced on a large scale synthetically and by fermentation.

The third antibiotic of increasing clinical significance is obtained from the actinomycete *Streptomyces aureofaciens*. It was discovered by B. M. Duggar and associates in 1948 at the Lederle Laboratories, Pearl River, N. Y., and was named aureomycin because of the yellow color of the culture and the golden color of the crystalline product. It resembles chloromycetin in that it contains nonionic chlorine. The free base has the probable empirical formula $C_{21}H_{22}O_NCl$.

It resembles chloromycetin in its activity against the rickettsia, undulant fever, and typhoid fever. It is strikingly effective against the virus that causes lymphogranuloma venereum in humans. It has been used successfully for the treatment of complicated ocular infections. It is successfully used for the treatment of atypical pneumonia, probably of viral origin. In addition to its antibacterial, antirickettsial, and antiviral activity, aureomycin is effective against the parasites of amoebic dysentery in test monkeys and may be valuable in treating human cases of this disease.

Aureomycin is available for general clinical use, and it is understood that the antibiotic can be produced in good yield. It is now being produced in quantity by one large pharmaceutical manufacturer.

Neomycin, discovered by Professor Waksman and H. A. Lechevalier in 1948, appears to offer promise for the treatment of urinary infections. It is produced by an actinomycete similar to *Streptomyces fradiae*, and generally resembles streptomycin in its activity—but with certain important exceptions. Strains resistant to streptomycin are highly susceptible to the new drug, and organisms sensitive to neomycin show little tendency to become resistant. Neomycin is a complex of possibly three closely related factors. One of these, neomycin A, has been crystallized, and work is progressing toward the separation of the others. The neomycin mixture is somewhat more toxic than streptomycin, but there remains the possibility of combining the two drugs for treatment of various diseases with beneficial results.

Terramycin is the most recently discovered antibiotic of medical significance derived from a species of *Streptomyces*. It is produced by *Strep-
tomyces rimosus and was discovered by A. C. Finlay and coworkers at Chas. Pfizer & Co., Brooklyn, N. Y. The pure hydrochloride of terramycin is yellow in color and the crystalline dihydrate has the probable empirical formula \( \text{C}_{81}\text{H}_{119}\text{N}_{33}\text{O}_{24} \). Preliminary reports indicate that it is similar to aureomycin and chloromycetin in its antirickettsial and antibacterial activity. In high concentration, it has shown some antiviral action against influenza A virus in chick embryos.

In May 1947, Robert G. Benedict and A. F. Langlykke, of the Northern Laboratory, reported the production by certain strains of Bacillus polymyxa of a water-soluble factor that strongly inhibited the gram-negative bacteria. They did not assign a name to the antibiotic, because it had not been purified sufficiently to compare its chemical and physical properties with those of known antibiotics from other members of the genus Bacillus. Independently, P. G. Stansly, R. G. Shepherd, and H. J. White, investigators at the American Cyanamid Co., discovered the same antibiotic and designated it polymyxin. In August 1947, G. C. Ainsworth, A. M. Brown, and G. Brownlee, working at the Wellcome Laboratories in England, independently reported the production of an antibiotic, called aerosporin, from Bacillus aerosporus, which is synonymous with Bacillus polymyxa.

Stansly and his coworkers showed that high levels of polymyxin could be tolerated by mice and that the antibiotic was effective in counteracting infections in mice, rabbits, and fowls caused by various gram-negative pathogenic bacteria. It was difficult for the bacteria to build up resistance toward polymyxin. From a clinical viewpoint, it thus seemed that the new antibiotic might have an advantage over streptomycin.

A medium consisting of corn steep liquor, dextrose, and calcium carbonate, developed and recommended by Benedict and Langlykke, was cheaper than Stansly’s yeast extract-mineral salts-glucose medium. However, the Stansly medium gave higher yields of polymyxin, and the substitution of soy or peanut meals as protein sources in place of yeast extract lowered its cost.

Employing a technique previously used with remarkable success in the search for better penicillin-producing molds, investigators at the Northern Laboratory obtained ultraviolet-induced mutants, which produced polymyxin in greatly increased yields.

By the addition of sterile dextrose solution to the fermentations between the forty-eighth and seventy-second hours, yields were increased still further, and potencies up to 1,000 units per milliliter were often attained. In general, polymyxin yields were highest when the fermentations were conducted at 25° to 28° C., with controlled rates of aeration and agitation.

Just as in the case of the penicillins, where the name is applied to several chemically related compounds, the name “polymyxin” can be properly applied to a family of chemically related antibiotics produced by various strains of Bacillus polymyxa. Brownlee and his coworkers showed that there are four polymyxins, A, B, C, and E, chemically different from type D studied by Stansly and by Benedict. Investigators at the American Cyanamid Co. and Lederle Laboratories have shown that polymyxin D has the probable empirical formula \( \text{C}_{81}\text{H}_{119}\text{N}_{33}\text{O}_{24}\text{HCl}\cdot\text{H}_{2}\text{O} \), with an approximate molecular weight of 1210.

In 1948, F. J. Murray and P. A. Tetraault, of Purdue University, reported the production of an antibiotic, termed circulin, from a strain of Bacillus circulans. Upon careful microbiological and chemical examination, D. H. Peterson and L. M. Reinecke, at the Upjohn Company Research Laboratories, proved it to be qualitatively the same as polymyxin A and E. It differs from polymyxin A in that it is unaffected by the enzyme lipase; insofar as is known, it has not been differentiated from polymyxin E.

All the known polymyxins are poly-
peptide antibiotics, very soluble in water and methyl alcohol, only slightly soluble in ethyl or butyl alcohol, and insoluble in other organic solvents.

The polypeptide antibiotics, including the polymyxins and bacitracin, tend to damage the kidneys in man and experimental animals. Brownlee and his coworkers maintained that polymyxin B is relatively free from nephrotoxic action, but further trials with this antibiotic have failed to substantiate the validity of their original claims.

P. H. Long and associates at The Johns Hopkins Hospital used polymyxin D to treat 21 human cases, which had not responded to previous treatments with streptomycin. The clinical course and laboratory examinations of the patients indicated that polymyxin D was beneficial despite evidence of kidney toxicity, which limited the use of the antibiotic. Promising as were the results obtained by the investigators, unless some means is found to reduce the renal toxicity of antibiotics belonging to the polymyxin family, their use in human medicine will be seriously curtailed. It now appears possible, however, that they may prove applicable for the treatment of infections in lower animals, particularly fowls. Like some of the antibiotics from species of Streptomyces, crude polymyxin D, adsorbed on carbon, and fed to normal chickens, was also found to stimulate markedly their growth.

Bacillus subtilis is a common spore-bearing bacterium present in most of our soils. Many strains of the organism produce antibiotics; thus far 11 different substances of this kind have been reported. Of these factors, the best known and most interesting from the viewpoint of possible clinical use is bacitracin.

Bacitracin was discovered by H. Anker, B. A. Johnson, and F. L. Meloney at Columbia University in 1945. The antibiotic is highly active against gram-positive bacteria and has shown promise in the treatment of surgically infected wounds and in eradicating early syphilitic infections in rabbits. In conjunction with penicillin G, it may find use in the treatment of the approximately 10 percent of syphilitic patients who do not respond to penicillin alone. Because its toxicity limits its internal use, it has been approved by the Federal Food and Drug Administration for sale to the public for topical application only. Bacitracin is now produced in fairly large amounts.

It is finding application in the treatment of superficial lesions, for the relief of respiratory congestion, and, in combination with streptomycin and polymyxin B, for sterilizing the gut prior to intestinal surgery. It is valuable also as an antibiotic feed supplement comparable to penicillin, polymyxin, and the three antibiotics from species of Streptomyces previously cited.

Subtilin, an antibiotic produced by a different strain of Bacillus subtilis, was discovered in 1944 by E. F. Jansen and Doris J. Hirschmann at the Western Regional Research Laboratory. Subsequent studies by J. C. Lewis, K. P. Dimick, and others of the same laboratory showed it to be very active against gram-positive bacteria and to inhibit strongly various acid-fast species. A. J. Salle and his coworkers at the University of California found that subtilin protected experimental animals against heavy doses of pneumococci and anthrax organisms. A definite suppressive effect on the course of experimental tuberculosis in guinea pigs was also noted. Its clinical use with humans has been limited because of its low solubility in water and body fluids. More recently this factor has been substantially improved by chemical treatment to increase solubility without materially lowering its antagonistic action.

Investigators at the Western Laboratory found that subtilin might be helpful in processing canned vegetables and meats. The addition of a few parts per million of subtilin before canning may offer promise of reducing the time and temperature of processing.
Much has been accomplished. Curative agents have been developed to control some of man's most serious diseases. Other diseases remain unchallenged, however. A potent and less toxic substance than streptomycin and neomycin is needed for the treatment of tuberculosis. A few factors have been found that will act on some of the larger viruses, but none that will arrest or cure infections by many of the smaller viruses, including those of poliomyelitis, influenza, and equine encephalomyelitis. Also needed are agents that will effect more rapid cures of the deep and superficial fungal infections of man.

More than 150 antibiotic substances produced by micro-organisms, higher fungi, and green plants have been reported. Less than one-tenth of them, however, offer promise for the control of human and animal diseases. Some are inactive in the animal body; many are highly toxic. But the search continues, and periodically, from among the many new antibiotics discovered, one is found to combat or forestall some infection against which previously there has been no satisfactory weapon. The list of curative agents is thus constantly enlarged, and in this expanding array of new drugs, those of microbial origin occupy an increasingly prominent position. The vision of a cure for every type of infection gradually assumes reality.

Kenneth B. Raper is principal microbiologist in charge of the culture collection section in the Northern Regional Research Laboratory. He has been associated with the Department of Agriculture most of the past 20 years. Dr. Raper holds degrees from the University of North Carolina, George Washington University, and Harvard University. At the Northern Laboratory, Dr. Raper and his co-workers have built a large collection of cultures of fungi, yeasts, and bacteria, many of which are essential in industrial fermentation processes. During the period in which the penicillin fermentation was being studied, he was instrumental in developing the mold culture from which were derived the high-yielding substrains now used in industry.

Robert G. Benedict is a bacteriologist in the survey and development section, Northern Regional Research Laboratory. He joined the Department of Agriculture in 1942. Dr. Benedict holds degrees from Michigan State College, Virginia Polytechnic Institute, and the University of Wisconsin. He began research work in antibiotics in 1944, and has studied penicillin, polymyxin, and other antibiotics.

Just as some disease-producing germs lose their pathogenicity upon continued cultivation in the laboratory, so some industrially important micro-organisms lose their capacity to produce specific chemicals. The best example of this phenomenon of degeneration is the failure, reported by C. Wehmer, of Aspergillus fumaricus, which originally produced fumaric acid in 70 percent yield, to yield any after 10 years in stock culture, although it did yield citric and gluconic acids. So valuable to industry are the strains that produce high yields of penicillin, streptomycin, butyl alcohol, gluconic acid, and so on, that every effort is made to preserve their biosynthetic characteristics. That is best accomplished by storing the dried spores in a high vacuum, so that growth is stopped and change is reduced to a minimum.—Frank H. Stodola, Northern Regional Research Laboratory.