Antibiotic Substances, Production by Microörganisms—Nature and Mode of Action*

SELMAN A. WAKSMAN, PH.D.

Microbiologist, New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick, N. J.

THE mounting interest in the production, utilization, and chemical nature of penicillin has recently focused considerable attention upon the whole subject of antibiotic substances and their formation by microörganisms. Although the nature and activities of some of these substances have been known for many years, the wide horizons that have now been opened for their practical utilization in combating human and animal diseases have attracted the attention of the bacteriologist, the chemist, the public health worker, and the clinician, not to mention the manufacturer who has envisioned a repetition, on a vastly wider scale, of the possibilities opened by the discovery of the sulfa drugs.

Formation and nature of antibiotic substances — Antibiotic substances are antimicrobial agents, produced by a variety of microörganisms found among the filamentous fungi or molds, yeasts, actinomycetes, and bacteria. They are chemically and biologically distinct from the common antiseptic and germicidal agents, although certain synthetic preparations may have properties similar to those of antibiotic agents. They are primarily bacteriostatic in nature, that is, they inhibit the growth of bacteria, whereas their bactericidal activities are of only secondary consideration and require a longer period of time than that needed for ordinary disinfectants.

One of the characteristic properties of antibiotic agents is their selective antibacterial action. Some affect largely Gram-positive bacteria and act only to a very limited extent upon Gram-negative organisms; others, however, have the capacity of inhibiting the growth of certain bacteria belonging to each of these groups, and act only to a limited extent against other organisms within the same group. The selective action of antibiotic substances is not only qualitative but also quantitative in nature, much larger concentrations being required to inhibit the growth of some bacteria than of others. The term "antibiotic spectrum" is, therefore, justifiably used to designate a range of activity of an antibiotic substance against a number of bacteria selected from various representative Gram-positive and Gram-negative groups.

Antibiotic substances vary greatly in their chemical nature, in their mode of action upon bacteria, in their toxicity to animals, and in their *in vivo* vs. *in vitro* activity. Some are destroyed by boiling, by exposure to light, and by

* Journal Series Paper, New Jersey Agricultural Experiment Station, Rutgers University, Department of Soil Microbiology. Presented before the Laboratory Section of the American Public Health Association at the Seventy-second Annual Meeting in New York, N. Y., October 12, 1943.

passage through various filters, whereas others are resistant to heat and to ultraviolet rays. Some are readily adsorbed by various adsorbing agents, such as active charcoal and infusorial earth; they usually can be removed from these by means of special solvents, such as ether, alcohol, chloroform, acetone, acids, or alkalies.

In order to obtain an antibiotic substance, an antagonistic organism is selected for its specific antibacterial activity and grown on an organic or a synthetic medium. The period of incubation usually varies from 3 to 20 days, and the temperature from 20° to 30° C. Since these organisms are aerobic in nature, proper aeration of the culture is essential; hence, shallow layers of liquid culture media for stationary cultures or forced aeration for submerged cultures are used. The formation of the antibiotic substance is greatly influenced by the strain of the organism, the composition of the medium, and the conditions of growth. Some antagonistic microörganisms produce more than one antibiotic substance. It has also been definitely established that some substances or closely related compounds may be produced by more than one organism.

Saprophytic and pathogenic microorganisms—There is no plant or animal now living that is not subject to infection by a number of bacteria, fungi, and protozoa, resulting in numerous diseases and epidemics. These disease producing microörganisms sooner or later find their way into the soil and into water basins, either in the excreta or in the dead and infected residues of the host. Fortunately, these natural substrates are not recognized at the present time as carriers of most of the common infectious diseases of man and animals. As a result of extensive investigations, the conclusion has been reached that most of the disease producing agents do not survive long in the soil, but die out there at a rather rapid rate, depending on the nature of the organism, the nature of the soil, climatic and other conditions.

The antagonistic effects of saprophytic soil microörganisms upon pathogenic bacteria and fungi were at first visualized as resulting from competition for food between these two groups. It was soon recognized, however, that this was not a sufficient explanation for all the observed phenomena, included by Ward [15] under the general term "antibiosis." Organisms living in associations were found to develop frequently certain characteristics that they did not possess when grown in pure culture. Schiller,[10] for example, observed that when beer yeasts were placed together with tubercle bacteria in a sugar-containing but nitrogen-free medium, the former developed antagonistic properties toward the latter and used the latter as a source of nitrogen. The antagonists secreted a bacteriolytic substance that was also active outside the living cells.

Dubos [4] used the method of enriching a soil with cultures of living pathogenic organisms in order to stimulate the development of antagonists that brought about the destruction of the pathogens. In order to isolate an antagonist from an ordinary soil or from one thus enriched, it is sufficient to inoculate a suspension of living bacteria with the soil, or plate out the soil on an agar medium with these living bacteria as the major nutrient, as suggested by Waksman and Woodruff.[13, 14] A number of different antagonistic organisms have thus been isolated.[12]

The most important organisms known at the present time capable of producing antibiotic substances can be classified into four broad groups: 1. non-spore-forming bacteria, comprising *Pseudomonas aeruginosa* and various others; 2. spore-forming bacteria including *Bacillus brevis, Bacillus mesentericus*

and *Bacillus mycoides;* 3. filamentous fungi, especially members of the genera *Penicillium* and *Aspergillus;* and 4. actinomycetes, especially the aerial mycelium producing types of the genus *Streptomyces.*

The antagonistic action of *Pseudomonas aeruginosa* was first established in 1888 by Freudenreich,[8] who believed that the lack of growth of various bacteria in the sterile filtrate of the antagonist was due to the exhaustion of nutrients in the medium. The filtrate possessed bacteriolytic properties, believed to be due to an enzyme designated as "pyocyanase" and to the pigment pyocyanin. Since pyocyanase is thermostable, not sensitive to pH change and not precipitated by ammonium sulfate and alcohol, but is soluble in alcohol, a lipoid-like nature has later been suggested. This organism is now recognized as producing three antibiotic substances, including also hemipyocyanin. These substances differ in chemical nature and in their mode of action upon bacteria.

The production of antibiotic substances by spore-forming bacteria has long been established. This culminated in the isolation by Dubos[5] of the antibiotic agent tyrothricin, which consists of two distinct substances, gramicidin and tyrocidine; these differ in their chemical nature, mode of action upon bacteria, and toxicity to animals.

Among the fungi, various green molds belonging to the genera *Penicillium, Aspergillus,* and *Trichoderma* have long attracted attention as organisms possessing antibacterial properties. Vaudremer,[11] for example, demonstrated in 1913 that *Mycobacterium tuberculosis* loses its virulence in the presence of *A. fumigatus* or its products.

The ability of a member of the *Penicillium* group to produce an antibacterial substance was first established by Fleming[6] in 1929. This substance was designated as "penicillin." Various strains of *P. notatum* and *P. chrysogenum* are now known to produce this antibiotic agent. Clutterbuck, Lovell, and Raistrick[3] reported, in 1932, that these fungi produce three compounds, penicillin, a protein, and a pigment, only the first of which showed antibacterial properties. It was active largely against Gram-positive and certain Gram-negative bacteria, but not against most other Gram-negative bacteria. It was non-toxic to animals and was active *in vivo.*

This work attracted very little attention for nearly a decade until a group of Oxford chemists and pathologists[1,2,7] decided in 1940 to reëxamine the potentialities of penicillin for the treatment of various diseases. These investigations resulted in a series of contributions concerning the isolation, antibacterial properties, chemical nature, and mode of action of this important antibiotic substance.

It has been recently established that *P. notatum* produces, in addition to penicillin, another antibacterial factor, active not only against Gram-positive but also against Gram-negative bacteria. This substance was designated variously as the *Escherichia coli* factor, penatin, notatin, and penicillin B. It is a protein and functions as a glucose-oxidase. It has also been demonstrated that other fungi, notably members of the *A. flavus* group, produce a substance designated as flavicin that is similar to if not identical with penicillin.

Actinomycetes yielded several antibiotic substances; the most promising of these is streptothricin[14] which is active against many Gram-negative as well as Gram-positive bacteria, has a low toxicity to animals, and is active *in vivo.*

A fairly large number of other antibiotic substances have now been isolated, but many of these are toxic to animals, possess undesirable physical, chemical, or biological properties, or

TABLE 1

Summary of Chemical and Biological Properties of Some of the More Important Antibiotic Substances

Substance	*Organism*	*Active Against Bacteria*	*Properties*
Actinomycin A	*A. antibioticus*	Selective quantitative action.	Soluble in ether and alcohol, not in petrol ether; orange colored; highly toxic; thermostable; nitrogen bearing ring compound.
Aspergillic acid	*A. flavus*	Active against both Gram-positive and Gram-negative bacteria.	Soluble in alcohol, ether, acetone, not in petroleum ether. Acid nature. m.p. about 96° C.; m.w. 224; about 13 per cent nitrogen.
Citrinin	*P. citrinum*	Non-selective.	Soluble in water and in alcohol, precipitated by acid; quinone.
Clavacin	*A. clavatus*	Active against Gram-negative and some Gram-positive bacteria. Highly bactericidal.	Soluble in ether, chloroform, alcohol and water. Toxic.
Flavicin	*A. flavus*	See penicillin.	Similar in all respects to penicillin.
Fumigacin	*A. fumigatus*	Largely active against Gram-positive bacteria.	White, needle-shaped crystals; m.p. 185°–187°; soluble in alcohol, limited solubility in water.
Gliotoxin	*Trichoderma, Gliocladium*	Non-selective; fungicidal and bactericidal.	Soluble in chloroform, benzol alcohol, sparingly in water; contains nitrogen and sulfur.
Gramicidin	*B. brevis*	Lytic to Gram-positive bacteria.	Soluble in ether and acetone; thermolabile; active *in vivo;* polypeptide.
Notatin	*P. notatum* and *P. chrysogenum*	On Gram-positive and Gram-negative bacteria.	Insoluble in organic solvents, soluble in water; acts, in presence of glucose.
Penicillic acid	*P. puberulum*	Active against Gram-positive and also upon Gram-negative bacteria.	Colorless; soluble in water.
Penicillin	*P. notatum* and *P. chrysogenum*	Acts largely upon various Gram-positive aerobic and anaerobic bacteria.	Soluble in alcohol and water; thermolabile; active *in vivo;* low toxicity.
Proactinomycin	*Pr. gardneri*	Acts primarily upon Gram-positive bacteria.	Soluble in ether, benzene and water. Toxic.
Pyocyanase	*Ps. aeruginosa*	Lytic action on many Gram-positive and Gram-negative bacteria.	Thermostable; lipoid; activity largely due to unsaturated fatty acids.
Pyocyanin	*Ps. aeruginosa*	Bactericidal action largely against Gram-positive bacteria.	Chloroform-soluble, blue pigment; thermostable.
Streptothricin	*A. lavendulae*	Active on various Gram-negative and some Gram-positive bacteria.	Soluble in water and in acid alcohol, not in ether; organic base; thermostable. Low toxicity; active *in vivo.*
Tyrocidine	*B. brevis*	Lytic to Gram-positive and Gram-negative bacteria.	Soluble in alcohol, not in ether; thermostable; polypeptide.

have not as yet been studied sufficiently.

Chemical nature and activity of antibiotic substances—The antibiotic substances do not comprise a single group of chemical compounds but vary greatly in composition. Since very few of them have so far been crystallized and their chemical nature established, any system of classification of these compounds on the basis of their chemical relationship can only be tentative (Table 1). The following seven groups may now be recognized:

1. Polypeptides and proteins, including tyrothricin, lysozyme, actinomycetin, and notatin
2. Sulfur-bearing compounds, comprising gliotoxin and several others
3. Pigments, which include pyocyanin, hemipyocyanin, prodigiosin, chlororaphin, toxoflavin, and actinomycin
4. Lipoids and lipoid-like bodies. (These comprise a number of compounds produced by different types of microörganisms; only some of which, such as pyocyanase, have been investigated.)
5. Quinones and quinone-bearing compounds, including citrinin, penicillic acid, and fumigatin

6. Organic bases, comprising streptothricin and proactinomycin
7. Agents which are known to be produced by various microörganisms but which have not been isolated or studied in detail

The methods for measuring the activity of antibiotic substances are based upon their bacteriostatic nature, their selective action against various bacteria and other microörganisms, and the mechanism or mode of their action. Some of the antibiotic substances are also fungistatic, and even fungicidal in nature, like gliotoxin, actinomycin, clavacin, hemipyocyanin, the *Bacillus simplex* factor, and others.

On the basis of their toxicity to animals, the antibiotic substances can be divided into three groups: (1) Substances non-toxic to higher animals or possessing but little toxicity, when 0.5 gm. or more per kg. weight of animal is tolerated; here belong penicillin, citrinin, pyocyanase, streptothricin, actinomycetin, and fumigacin. (2) Substances that are fairly toxic to animals, including tyrothricin, notatin, and gliotoxin; although limited information precludes establishment of a toxic zone for these, a range of 0.1 to 0.5 gm. per kg. can be suggested tentatively. (3) Highly toxic compounds, such as actinomycin, clavacin and aspergillic acid; these are usually more toxic than 0.1 gm. per kg.; for many, such as actinomycin, as little as 0.15 mg. per kg. weight is toxic.

Of the various antibiotic agents, penicillin is the only one that has so far found an extensive chemotherapeutic application.[9] It is effective against hemolytic streptococcus and pneumococcus infections, as well as various staphylococcal infections; it is used in controlling local lesions of the eye caused by *Staphylococcus aureus;* however, it has no effect upon infections caused by *Mycobacterium tuberculosis, Trypanosoma equiperdum,* and influenza virus. It is effective against sulfonamide-resistant strains of pneumococci, although pneumococcus cultures can build up resistance against penicillin. In order to overcome the instability of penicillin and its rapid excretion, esters have been prepared which are said to be more stable and even offer promise of use by oral administration. Next to penicillin in chemotherapeutic value is tyrothricin which is being utilized for the control of mastitis in cattle and certain local infections. Pyocyanase was extensively used at one time for the treatment of various infections, but because of its instability, the variability of the organisms producing it, and insufficient knowledge concerning the conditions governing its formation, its use has been discontinued or been very greatly limited.

Mode of action of antibiotic substances—The mode of action of antibiotic substances upon bacteria consists largely in interfering with cell multiplication. This may be accompanied by a marked effect upon certain essential metabolic processes. On the basis of the limited information now available, the following mechanisms may be tentatively presented:

1. The antibiotic substance interferes with bacterial cell division, thus preventing further growth of the organism; the cell, unable to divide gradually dies. It has been shown by the use of the manometric method, that certain bactericidal agents in bacteriostatic concentrations may have no effect on the metabolic rates of bacteria; however, they inhibit cell multiplication.
2. The antibiotic substance interferes with the metabolic processes of the microbial cells, by substituting for one of the essential nutrients.
3. The antibiotic substance may interfere with the vitamin utilization of the organism; the staling effect of a medium, frequently spoken of in the case of protozoa as "biological conditioning" of the organism, may serve as an illustration; such effects have been overcome by the addition of a mixture of thiamin, riboflavin and nicotinamide.
4. The antibiotic agents bring about the

oxidation of a metabolic substance which must be reduced in the process of bacterial nutrition, or otherwise modifies the intermediary metabolism of the bacterial cell.

5. The agent combines with the substrate or with one of its constituents, which is thereby rendered inactive for bacterial utilization.

6. The agent competes for an enzyme required by the bacteria in order to carry out an essential metabolic process.

7. The agent interferes with various enzymatic systems, such as the respiratory mechanisms of the bacterial cells, especially the hydrogenase system; penicillin was shown capable of inhibiting the activity of urease.

8. The antibiotic substance may inhibit directly cellular oxidations, particularly those involving nitrogenous compounds, similar to the action of propamidine.

9. The antibiotic substance acts as an enzyme system and produces, in the medium, oxidation products, such as peroxides, injurious to the bacterial cell. The glucose oxidase produced by *P. notatum* catalyzes the following reaction:

$$\text{Glucose} + O_2 \rightarrow \text{Gluconic acid} + H_2O_2$$

10. The antibiotic substance favors certain lytic mechanisms in the cell, whereby the latter is destroyed; this mechanism may be either secondary or primary in nature.

11. The antibiotic substance affects the surface tension of the bacteria, acting as a detergent.

12. Bacteria subjected to the action of an antibiotic substance may develop mechanisms that render them resistant to the action of the substance.

13. Some bacteria may produce an enzyme, such as penicillinase, that brings about the destruction of the antibiotic substance.

SUMMARY

The utilization of antagonistic microorganisms, directly or through the production of antibiotic substances, offers three distinct potentialities to help man in overcoming his ills as well as those of his animals and of his crops:

1. Domestication of microörganisms for disease control.

2. Isolation of new chemotherapeutic agents for combating human and animal diseases.

3. Utilization of the activities of microorganisms for combating various plant diseases.

We are faced with a new field of research, which overlaps the fields of biology and chemistry and unites medicine with soil science. Many secrets of nature not sufficiently understood heretofore, will thereby be unraveled and new agents that may help man in combating diseases and epidemics discovered. This new field offers further great possibilities for the study of the nature and physiology of the bacterial cell, which may lead to a better understanding of the way of life of the smallest and, in many respects, the most interesting of the living systems.

REFERENCES

1. Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., and Florey, H. W. Further Observations on Penicillin. *Lancet*, 241:177–189; *Nature*, 148: 758–759; 149: 356, 1941–1942.

2. Chain, E., Florey, H. W., Gardner, A. D., Heatley, N. G., Jennings, M. A., Orr-Ewing, J., and Sanders, A. G. Penicillin as a Chemotherapeutic Agent. *Lancet*, 239:226–228, 1940.

3. Clutterbuck, P. W., Lovell, R., and Raistrick, H. Studies on the Biochemistry of Microorganisms. XXVI. The Formation from Glucose by Members of the *Penicillium chrysogenum* Series of a Pigment, an Alkali-soluble Protein and Penicillin—the Antibacterial Substance of Fleming. *Biochem. J.*, 26: 1907–1918, 1932.

4. Dubos, R. J. Bactericidal Effect of an Extract of a Soil Bacillus on Gram-positive Cocci. *Proc. Soc. Exper. Biol. & Med.*, 40:311–312; *J. Exper. Med.*, 70:1–10, 11–17, 1939.

5. Dubos, R. J. The Effect of Specific Agents Extracted from Soil Microorganisms upon Experimental Bacterial Infections. *Ann. Int. Med.*, 13:2025–2037, 1940.

6. Fleming, A. On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to Their Use in the Isolation of *B. influenzae*. *Brit. J. Exper. Path.*, 10:226–236, 1929.

7. Florey, M. E., and Florey, H. W. General and Local Administration of Penicillin. *Lancet*, 244: 387–397, 1943.

8. Freudenreich, E. de. De l'antagonisme des bactéries et de l'immunité qu'il confère aux milieux de culture. *Ann. Inst. Pasteur*, 2:200–206; *Ann. Micrographie (Jahresber. path. Mikroorg.*, 5:530), 1888–1889.

9. Keefer, C. S., Blake, F. G., Marshall, E. K., Jr., Lockwood, J. S., and Wood, W. B., Jr. Penicillin in the Treatment of Infections. A Report of 500 Cases. *J.A.M.A.*, 122:1217–1224, 1943.

10. Schiller, I. Über "erzwungene" Antagonisten. *Centralbl. f. Bakt.*, 1, Or., 91:68–72; 92:124–129; 94:64–66; 96:54–56; 103:304–314; *Compt. rend. Soc. de biol.*, 105:423–425; 550–552, 1927, 1930.

11. Vaudremer, A. Action de l'extrait filtre d'*Aspergillus fumigatus* sur les bacilles tuberculeux. *Compt. rend. Soc. de biol.*, 74:278–280, 752–754, 1913.

12. Waksman, S. A., and Horning, E. Distribution of Antagonistic Fungi in Nature and Their Antibiotic Action. *Mycologia,* 35:47–65, 1943.
13. Waksman, S. A., and Woodruff, H. B. The Soil as a Source of Microorganisms Antagonistic to Disease-producing Bacteria. *J. Bact.,* 40:581–600, 1940.
14. Waksman, S. A., and Woodruff, H. B. Streptothricin, a New Selective Bacteriostatic and Bactericidal Agent, Particularly Active Against Gram-negative Bacteria. *Proc. Soc. Exper. Biol. & Med.,* 49:207–210, 1942.
15. Ward, H. M. Symbiosis. *Ann. Bot.,* 13:549–562, 1899.

UNRRA's Public Health Plans

James A. Crabtree, M.D., acting chief of the Health Division of the United Nations Relief and Rehabilitation Administration, Washington, announced on February 21 the plans that are being made to deal with public health and nutritional problems in the occupied countries. Although it will be difficult to predict exactly what the conditions will be, Dr. Crabtree said that it was possible to make some intelligent guesses on what the conditions under a variety of premises might be, including what might be found under a completely scorched-earth policy.

Dr. Crabtree believed that the number one problem would be starvation, with all its relationships to public health, and he believed that increased death rates from maternal and infant causes, from malaria, and typhus, and from tuberculosis would have to be dealt with.

Dr. Crabtree felt that it was safe to predict that the breakdown of sanitary safeguards, together with crowding, lack of shelter, lack of clothing, fuel, soap, and the great movements of population would certainly result in epidemics. He said that some progress was being made on the assembling of personnel for these purposes. At present the UNRRA supply problem is more advanced than the plans for personnel, but UNRRA is fully aware that medical supplies would be worth little without medical service. However, a few teams of physicians, nurses, engineers, and other health specialists have been organized and the plan will be to send into each country as it is liberated a relatively small, well rounded team of experts in various specialties of public health to consult with and to assist national governments in building up and strengthening their own medical care services and facilities. In addition special teams will have to be organized to deal with certain special problems such as epidemics and diseases that have been introduced into the country for the first time. Dr. Crabtree said that, while some of the occupied countries would need only the help of such general and special teams of workers, others which had been "scorched" of professional personnel might need a substantial number of doctors and nurses to come in and actually to help take care of the sick.