MECHANISMS OF ANTIBACTERIAL ACTION

W. W. UMBREIT

Merck Institute for Therapeutic Research, Rahway, New Jersey

At the outset it is to be understood that the above title refers primarily to the antibacterial action of agents capable of being introduced into the animal body, which have the property of inhibiting or killing the bacteria therein without causing comparable harm to the host. It is apparent that, with this group, chemotherapy (the successful treatment of the infected individual) is possible. Among these agents are materials whose action is that of a competitive inhibitor of an important metabolite; the family of sulfonamides is the prime example of this class. Since the mechanism of action of sulfonamides has been intensively studied and has been adequately reviewed elsewhere (29, 99), this subject will not be touched upon here, except to restate that one of the mechanisms of antibacterial action is that of competitive antagonism.

The other group of antibacterial chemotherapeutic agents comprises the clinically successful antibiotics. It is the purpose of this review to consider their mechanism of antibacterial action. This subject too has been intensively reviewed (1, 4, 9, 13, 30, 32, 46, 66, 98) but there is not the degree of agreement nor the clarity of concept of action that characterizes the group of competitive inhibitors. The reasons for this lack of understanding are not only the usual lack of data but also a lack of consideration as to what constitutes an antibacterial action of therapeutic significance. This situation forces the development of certain principles which it shall be the function of this article to point out. This review is devoted largely to the mode of action of the following antibiotics: penicillin, streptomycin, aureomycin, terramycin and chloramphenicol. It is recognized that there are, of course, a considerable residuum of cases in which we have no indication of mode of action or in which the substance is toxic for the animal as well.

With respect to the mode of action of the five antibiotics listed, we wish to know the answers to the following two problems:

1. The vulnerable point which the antibiotic attacks in the biochemistry of the bacterial cell.
2. The reasons for the specificity, especially why it is possible to use them in the living animal.

With respect to the first problem, as knowledge of such agents progresses it is generally found that more than one biochemical reaction is influenced by the antibiotic. All of these are indeed actions of the antibiotic but some may be related more to the conditions of the experiment than to their chemotherapeutic effect. We therefore really wish to know which of the biochemical lesions observed is related to the chemotherapeutic effect. Three relatively simple criteria are at present in use; these may require amplification as more information becomes available, but they suffice at present. First, the effects observed must be obtained

with the antibiotically-active forms of the antibiotic but not with derivatives devoid of antibiotic activity. Second, the effects observed must be obtained with concentrations of the antibiotic comparable to or identical with those required to inhibit bacterial growth. Third, the biochemical reaction which is affected must be vital to the cell economy.

With respect to the second problem, we must sometimes be content to wait until the first problem has been solved before the second one can be approached, but at any rate, specificity should not be ignored since it represents the essence of the problem of chemotherapy. Many details of the biochemistry of the antibiotically-sensitive reactions may remain obscure without greatly affecting our understanding of the mode of action. But unless we know why it is that these substances affect a bacterial cell in one manner and an animal cell in another, we have not reached an adequate understanding of their action.

The attempts to obtain answers to the two basic problems of mode of action of antibiotics will be recorded subsequently, but it has recently become quite evident that one approach, which might seem reasonable, must be abandoned. This approach compares the metabolic properties of resistant bacterial strains with those of sensitive strains and attempts to determine, from what has happened to the strain which became resistant, what the action of the antibiotic was on the strain that is sensitive. There is, of course, a great likelihood that in a chain of relations of this complexity, logical fallacy may creep in unnoticed. Experimentally, it is found that not all resistant strains are alike in their metabolic properties, so that if one reasons from the resistant strain, the alleged action on the sensitive strain depends on which resistant strain one happens to choose for study. Indeed, more careful consideration of this approach shows that the only way in which one can study the action of any drug is to study it in the system in which it acts and not in the system in which it has no action. Proposals of mode of action based on this fallacious approach will therefore be omitted except in one case where this method affords the only information currently available.

In all the foregoing it has been assumed that the five antibiotics in question act by interfering in some unspecified manner with the metabolic reactions of the cell. It is, of course, possible to conceive of other mechanisms of action. Tyrocidin (22, 31) and subtilin (74), for example, appear to act as germicidal surface-active agents and cells treated with these substances show not only a rapid and permanent loss of metabolic activity but also a rapid leakage of cell constituents into the medium. Other physical effects might be postulated; but there is, indeed, no evidence for them, so the basic assumption made is that the antibiotic specifically interferes with some important metabolic reaction in the cell. But further, it is assumed that the action is not a general one on a variety of enzymes, but is relatively specific. Some cases of this are known. For example, antimycin (64) appears to inhibit but one step ("Slater factor") in a complex respiratory chain and presumably this is the cause of its marked toxicity. Perhaps bacillomycin B, forming a complex with cytochrome c (84), acts in a similar manner. In short, the supposition underlying the study of the clinically

active antibiotics is that their action is chemically "pin-pointed" to a particular reaction (or a relatively few related reactions) within the susceptible cell. Time alone will tell whether this is a valid supposition but available data do not contradict it and afford the assumption considerable support.

It is further axiomatic that a drug must be absorbed by and react with the tissue it is to act upon, and antibiotics are no exception. Naturally, there are several factors, some perhaps of specificity, involved in the absorption process. A variety of studies (2, 17, 75) have shown several factors to be involved in the absorption of antibiotics; but these factors, while unquestionably involved in the action of antibiotics, are indeed similar to those of other drugs and have no particular bearing upon their mode of action. While some of the antibiotics may exist in the colloidal state (28) the bearing of this upon their mode of action seems quite remote.

The mode of action of penicillin is in one sense the best known since it has been studied more fully than that of any other antibiotic; but, in another sense, it is not precisely known and indeed it is subject to considerable controversy and confusion. In part this appears to be due to the types of measurements which may be made. A particular and singular reaction appears to be blocked by penicillin. When it is so inhibited one may measure the lack of end products of this reaction or the accumulation of intermediates whose further metabolism would normally proceed through this reaction. While at present the nature of this reaction is difficult to specify in detail, its existence may be inferred from the relatively specific adsorption of penicillin to susceptible cells. After some initial confusion in which claims were made for the absorption of penicillin by cell cytoplasm (12) in quantities of less than ten molecules per cell (72), separate groups of workers are now agreed that (40, 41, 70, 71) there is a specific reversible uptake of penicillin most probably responsible for its antibacterial activity and that the component responsible for the adsorption appears to be located in the cell wall (16). It appears that a similar specific adsorption component is necessary for penicillinase adaptation (63). Once this penicillin-binding component is inactivated, certain changes occur in the organism. These apparently have to do with the disorganization in the metabolism (both synthesis and breakdown) of nucleic acids, which in turn is related to the organism's ability to synthesize protein (20). The effects on amino acid assimilation (18, 21) and protein synthesis (33) are now thought to be reflections of the effect on nucleic acid synthesis (20, 47, 48). Further, several groups of workers have noted effects of penicillin (in relatively high concentrations) upon the nucleic acid metabolism of resting cells, and growing cells treated with penicillin show a relatively marked alteration in nucleic acid metabolism (24–27, 36, 42, 49). Furthermore, growing cells treated with penicillin show the accumulation of uridine-5-pyrophosphates (57–62) whose quantities and kinetic relationships are such that they appear to be on the pathway toward nucleic acids, rather than related solely to any coenzyme function they might possess. There thus appears to be a large area of agreement among investigators that penicillin acts by inhibiting an early stage of nucleic acid synthesis (ribose nucleic acid is the primary one affected), but it is as yet

impossible precisely to "pin-point" the site of action or to specify the exact mechanism of inhibition.

There remains a small group of observations on the action of penicillin which do not conform to the remainder of the studies. The relationship of glutathione to the action of penicillin (65) would apparently receive some support from the observation that penicillin competitively inhibits the breakdown of glutathione by liver preparations (6); but penicillin has no effect on the synthesis of glutathione by extracts of *E. coli* (20). The adaptive assimilation of nitrate by a coliform organism is inhibited by penicillin (19). The inhibiting effect of penicillin on the growth of *C. diphtheriae* and *Cl. Welchii* may be relieved by a factor from yeast extract (10). In a gram-negative organism penicillin inhibits the growth when glycine is supplied but not when leucylglycine is provided (76). The ultimate interpretation and the real significance of these effects are not yet clear; but they should serve to remind us that, in spite of the vast area of agreement and the data pointing to a single mode of action of penicillin, there are still phenomena sufficiently important that should not be ignored and for which in time an explanation should be sought.

Since there are only indirect methods of measuring the site of action of penicillin and these do not lend themselves to studies in animal tissues, there is no experimental approach to the larger problem that now looms ahead. That is, why can penicillin be used in the animal body without untoward effects? Certainly this is well established; but does not the body synthesize nucleic acids and if penicillin stops this process in the gram-positive and certain other bacteria, why does it not do so in the gram-negative bacteria or in the animal cell? A variety of hypotheses can be proposed, but to the best of our knowledge there is no experimental approach yet available for testing any of them.

Streptomycin represents something of a contrast to penicillin in that there are a variety of reactions inhibited by it. Streptomycin forms complexes with nucleic acids and nucleoproteins (11, 14, 25, 26, 73), which combination alters the surface charge of the bacteria (45); it inhibits in a somewhat specific manner diamine oxidase (55), an enzyme also inhibited by pyocyanine, streptothricin and chloramphenicol; it has been claimed to interfere with inositol metabolism (38, 56, 69) and pantothenate synthesis (37), and to inhibit an unknown reaction called the "oxalacetate-pyruvate" reaction (54, 86, 87, 93). Because of the multiplicity of these effects it has been necessary to attempt to distinguish between those which might be related to the inhibition of the growth of the organism and those which might be related to the chemical properties of the molecule rather than to the antibacterial effect *per se*. The status of each of the reactions mentioned and their relation to possible mode of action have been reviewed elsewhere (88). After application of the criteria mentioned at the start of this review, so far only one reaction survives as bearing a possible relation to the mode of bactericidal action. This is the "oxalacetate-pyruvate" reaction. Its nature remains somewhat obscure since it does not appear to be any of the known reactions of oxalacetate and pyruvate (94). Inasmuch as reactions of these substances have been intensively studied, it would seem unlikely that

further reactions involving them would be of quantitative significance. Yet this appears to be the case. Some progress has been made in clarifying the nature of the streptomycin-sensitive reaction with the discovery of a new intermediate in metabolism, 2-phospho-4-hydroxy-4-carboxy adipic acid. This compound is a seven-carbon phosphorylated tricarboxy acid, isolated from dog liver (67). It was shown to be an intermediate in the metabolism of the rat, by tracing the incorporation of radioactive phosphorus into it (90, 91). In *E. coli* it is formed apparently only when a dicarboxy acid and pyruvate are present, and its formation is inhibited by streptomycin (91) at levels comparable to those required to inhibit growth. However, it is not yet known what role this substance may play in metabolism. At any rate, the "oxalacetate-pyruvate" reaction is becoming less obscure and the action of streptomycin upon it is therefore becoming of more significance. The site of action of streptomycin is thus biochemically "pinpointed" and, as in the case of penicillin, it turns out to be a relatively singular reaction, essentially undetected by other methods of studying metabolism.

There remains, of course, a considerable residuum of effects of streptomycin which do not fit into the picture of the inhibition of a single reaction as the cause of streptomycin antibiosis, and the area of agreement on the mode of action of streptomycin is somewhat smaller than that for penicillin. Aside from several actions of streptomycin mentioned above (88) which are not considered as bearing directly upon its mode of action since they either occur only when the concentration of streptomycin is very high or occur also with derivatives of streptomycin which are not antibiotically active, there remain some which do fulfill the necessary criteria. One of these is the action of streptomycin on the avian tuberculosis organism (53) in which the oxalacetate-pyruvate reaction has not been demonstrated but in which a portion of the oxidation of certain of the higher fatty acids is specifically inhibited. Another stems from a report (3) that the dissimilation of pyruvate under anaerobic conditions is inhibited by streptomycin. In this case, however, the known reactions of pyruvate when separately tested are not sensitive to streptomycin and the inhibition occurs in the presence of bicarbonate and with an active oxalacetate decarboxylase present (92), so that it is possible that in this case one is dealing with yet another manifestation of the oxalacetate-pyruvate reaction. Bacterial strains resistant to streptomycin show a variety of alterations in metabolism which are indeed so inconsistent from strain to strain that it is evidently impossible to provide any general explanation of the reaction inhibited in the sensitive strain (82, 89).

The reasons why streptomycin may be used in the animal body are reasonably clear. The reaction sensitive to streptomycin occurs in animal tissues but a permeability barrier to streptomycin exists, not only at the cell wall but also at the surface of the mitochondria (95). Pharmacological studies (44, 51, 52) show that, while streptomycin does penetrate from the blood stream into the tissue, the amount so penetrating is very small, and more direct studies of such penetration show that the cell is protected by an additional permeability barrier at the surface of the mitochondria, which is the apparent site of the sensitive reaction.

This simple mechanism, that is, mere physical separation of streptomycin from the site of the sensitive reaction, seems to account for its ability to kill those bacteria which it can attack in the animal body. This means, however, that if the sensitive bacteria are protected from the streptomycin by themselves growing within the host cell, or walled off in other ways, they will not be attacked by the drug, which apparently explains the usual failure of streptomycin in brucellosis (35, 43). In human tuberculosis one may suspect that it is the extracellular organisms which are primarily attacked.

If one compares the mode of action of penicillin and streptomycin one is struck by certain similarities in their action. For example, in both cases, they combine with a specific enzyme system in an irreversible manner, the reaction carried out is one not evident by other methods of study, and whenever resistance develops the reaction sensitive to the drug is lost (whatever else may happen to the resistant strain). They differ, however, in the nature of the reaction inhibited and in the apparent reason why they may be used in the animal. One might presume that those aspects of their action which they possess in common might be general characteristics of the actions of other antibiotics. It would therefore be of interest to learn the mode of action of a third antibiotic, to determine whether there are features common to a group, or whether each agent operates by different mechanisms. Three candidates are available for this consideration; chloramphenicol (Chloromycetin), aureomycin and terramycin.

With respect to chloramphenicol, it does not inhibit a wide array of reactions, including proteolytic enzymes (77, 81), but it does have a curious effect upon bacterial esterases (81) and on the crystalline liver esterase. Inhibition is observed at concentrations about 10-fold higher than those required to inhibit bacterial growth but within the physiologically effective range. In animal mitochondria, the esterase is not inhibited, which suggests a barrier, as with streptomycin, preventing the antibotic from reaching the site of the sensitive reaction. However, this is not quite a reasonable explanation since chloramphenicol does apparently penetrate the red blood cell (23) and also acts upon certain rickettsial infections in which the parasite is intracellular. It is, at the moment, difficult to relate this action of chloramphenicol on esterases to its mode of action in killing the organism, partly because of our lack of knowledge of the critical metabolic importance of esterases, but more important, because of the curious response of esterases to chloramphenicol, involving stimulation as well as inhibition.

A variety of enzyme systems acting on chloramphenicol itself have been described (15, 78–80), but these reactions do not seem to be pertinent to its mode of action inasmuch as alterations in the chloramphenicol molecule reduce or eliminate the antibiotic effect. The inhibition of *E. coli* and *L. casei* by chloramphenicol is decreased by phenylalanine, and to an extent by tyrosine and tryptophan (97). This antagonism is non-competitive, but is demonstrable over only a narrow range of concentration of the drug and only with minimally effective doses of chloramphenicol. This was, however, taken to mean that, since chloramphenicol is a naturally occurring analogue of phenylalanine, it might owe its antibacterial properties to interference with the action of phenyl-

alanine, and that, over a narrow range, more phenylalanine could compensate for the loss of certain reactions inhibited by chloramphenicol. Other workers (5, 85), however, feel that the antibiotic interferes with the early stages of tryptophan synthesis, especially the formation of indole from anthranilic acid (5). Further, a growth factor for *L. citrovorum*, not folinic acid but produced by incubation of folic acid with hemopoietic tissue, is claimed to reverse chloramphenicol inhibition (83). Finally, chloramphenicol has been shown (20) to inhibit protein synthesis in *Staph. aureus* without interference with glucose fermentation, extracellular peptide formation or nucleic acid synthesis. All these experimental results have not yet been integrated into any common explanation or any picture of a possible mode of action of chloramphenicol. They seem to point in somewhat opposite directions and it is apparent that the definitive experiments have yet to be conceived and executed.

If the status of knowledge of the mode of action of chloramphenicol is in a somewhat immature state, that of the action of aureomycin and terramycin is dominated by a singularly illogical conception, in that the majority of the studies have been done, not with susceptible bacteria, but with animal tissue, where one might suppose the problem is—why do these antibiotics not act on the animal? Aureomycin, applied to animal homogenates at relatively high concentrations, inhibits aerobic phosphorylation, possibly through blocking some part of the Krebs cycle (8, 39, 96). Terramycin apparently acts in the same manner (50), as indeed do dinitrophenol, quinacrine, gramicidin, usnic acid and barbiturates (7, 34). There appears to be some difference of opinion with regard to the similarity of the mode of action of aureomycin and terramycin (8, 39, 50, 68), but in view of their similarity in chemical structure we shall consider them as a single unit. At relatively high concentrations such phosphorylation "uncoupling" is evident with aureomycin in *Staph. aureus* (20), but it seems quite unlikely that this uncoupling reaction can explain the antibiotic activity even in *Staph. aureus* since growth and protein synthesis are sensitive to much smaller concentrations of the drug. Further, there is a difference in the action of aureomycin (which inhibits glutamate accumulation but not glucose fermentation) and terramycin (which inhibits both) at higher concentrations, but both agents inhibit protein synthesis at concentrations comparable to those required to inhibit growth (20).

It is quite evident that studies on the mode of action of antibiotics are not sufficiently advanced to permit any generalizations as to their actions or to formulate with any precision a positive statement of any common feature of their action. It seems clear, however, that as a group they are not competitive analogues of metabolites. A great deal of further study will be necessary before definite progress beyond this stage is possible.

REFERENCES

1. Bailey, J. H. and Cavallito, C. J.: Antibiotics. Ann. Rev. Microbiol., 2: 143-182, 1948.
2. Bailey, J. H. and Cavallito, C. J.: The effect of aliphatic acids on the activity of certain antibacterial agents. J. Bact., 60: 269-274, 1950.
3. Barkulis, J. L.: Inhibition of anaerobic pyruvate dissimilation in *Escherichia coli* by dihydrostreptomycin. J. Bact., 61: 375, 1951.

4. Benedict, R. G. and Langlykke, A. F.: Antibiotics. Ann. Rev. Microbiol., 1: 193–236, 1947.
5. Bergmann, E. O. and Sicher, S.: Mode of action of chloramphenicol. Nature, 170: 931, 1952.
6. Binkley, F. and Olson, C. K.: Metabolism of glutathione. IV. Activators and inhibitors of the hydrolysis of glutathione. J. Biol. Chem., 188: 451–457, 1951.
7. Brody, T. M. and Bain, J. A.: Effect of barbiturates on oxidative phosphorylation. Proc. Soc. Exper. Biol. & Med., 77: 50–53, 1951.
8. Brody, T. M. and Bain, J. A.: The effect of aureomycin and terramycin on oxidative phosphorylation. J. Pharmacol. & Exper. Therap., 103: 338, 1951.
9. Brownlee, G.: Antibiotics with particular reference to mode of action. Ann. Rev. Microbiology, 5: 197–208, 1951.
10. Chattaway, F. W., Hall, D. A., Happold, F. C. and Holdsworth, E. S.: Reversal of penicillin inhibition by a yeast extract. Nature, 164: 314–315, 1949.
11. Cohen, S. S.: Streptomycin and desoxyribonuclease in the study of variations in the properties of a bacterial virus. J. Biol. Chem., 168: 511–526, 1947.
12. Cooper, P. D. and Rowley, D.: Location of radioactive penicillin in *Staphylococcus aureus* after contact with the drug. Nature, 164: 842–843, 1949.
13. Cutting, W. C.: Actions of antibiotics *in vivo*. Ann. Rev. Microbiol., 3: 137–158, 1949.
14. DiMarco, A. and Boretti, G.: On the complexes formed by streptomycin and basic dyes with ribonucleic acid. Interference of salts. Enzymologia, 14: 141–152, 1950.
15. Egami, F., Ebata, M. and Sato, R.: Reduction of Chloromycetin by a cell free bacteria extract and its relation to nitrite reduction. Nature, 167: 118–119, 1951.
16. Few, A. V., Cooper, P. D. and Rowley, D.: Reaction of penicillin with the Staphylococcal cell wall. Nature, 169: 283–284, 1952.
17. Fischer, R. and Seidenberg, S.: Homologous mechanism of bactericidal action and gram staining. Science, 114: 265–266, 1951.
18. Gale, E. F.: The nitrogen metabolism of gram-positive bacteria. Bull. Johns Hopkins Hosp., 83: 119–175, 1948.
19. Gale, E. F.: Nitratase adaptation: An apparent effect of penicillin which can be reversed by salt. Brit. J. Exper. Path., 30: 356–364, 1949.
20. Gale, E. F.: Points of interference by antibiotics in the assimilation of amino acids by bacteria. Symposium on Mode of Action of Antibiotics, 2nd Int. Cong. Biochem., Paris, 5–20, 1952.
21. Gale, E. F. and Paine, T. F.: Assimilation of amino acis by bacteria. XII. Action of inhibitors and antibiotics on the accumulation of free glutamic acid and the formation of combined glutamate in *Staphylococcus aureus*. Biochem. J., 48: 298–301, 1950.
22. Gale, E. F. and Taylor, E. S.: Action of tyrocidin and detergents in liberating amino acids from bacterial cells. Nature, 157: 549–551, 1946.
23. Glazko, A. J., Wolf, L. M. and Dill, W. A.: Distribution of chloramphenicol (Chloromycetin) and its metabolic products between human red cells and plasma. Proc. Soc. Exper. Biol. & Med., 72: 602–604, 1949.
24. Gros, F. and Macheboeuf, M.: Aspects biochemiques mode d'action de la penicilline. Symposium on Mode of Action of Antibiotics, 2nd Int. Cong. Biochem., Paris, 101–123, 1952.
25. Gros, F., Macheboeuf, M. and Jeulin, S.: Recherches sur le mode d'action biochimique de la streptomycine dans le metabolisme d'une bacterie *Clostridium sporogenes*. Ann. Inst. Pasteur., 75: 242–254, 1948.
26. Gros, F., Macheboeuf, M., Rybak, B. and Lacaille, P.: Action de la streptomycine sur les bacteries nonproliferantes. I. Agglutination des bacteries par la streptomycine. Ann. Inst. Pasteur., 77: 246–263, 1949.
27. Gros, F. and Rybak, B.: Action de la penicilline et de la streptomycine sur le catabolisme de l'acide ribonucleique. Helv. Chim. Acta, 31: 1855–1863, 1948.
28. Hauser, E. A.: The colloidal nature of antibiotics. Ann. New York Acad. Sc., 53: 18–26, 1950.
29. Henry, R. J.: The mode of action of sulfonamides. Bact. Rev., 7: 175–262, 1943.
30. Herrell, W. E.: Newer antibiotics. Ann. Rev. Microbiol., 4: 101–128, 1950.
31. Hotchkiss, R. D.: Gramicidin, tyrocidine, and tyrothricin. Adv. Enzymology, 4: 153–199, 1944.
32. Hotchkiss, R. D.: The mode of action of chemotherapeutic agents. Ann. Rev. Microbiol., 2: 183–214, 1948.
33. Hotchkiss, R. D.: The effect of penicillin upon protein synthesis by bacteria. Ann. New York Acad. Sc., 53: 13–17, 1950.
34. Johnson, R. B., Feldott, G. and Lardy, H. A.: The mode of action of the antibiotic, usnic acid. Arch. Biochem. 28: 317–323, 1950.
35. Kornegay, G. B., Forgacs, J. and Henley, T. F.: Studies on streptomycin blood levels and urinary excretion in man and animals. J. Lab. & Clin. Med., 31: 523–534, 1946.
36. Krampitz, L. O. and Werkman, C. H.: The mode of action of penicillin. Arch. Biochem., 12: 57–67, 1949.
37. Lichstein, H. C. and Gilfillan, R. F.: Inhibition of pantothenate synthesis by streptomycin. Proc. Soc. Exper. Biol. & Med., 77: 459–461, 1951.
38. Loo, Y. H., Carter, H. E., Kehm, N., Anderlik, B.: The effect of streptomycin on a varient of *Torula utilis*. Arch. Biochem., 26: 144–150, 1950.
39. Loomis, W. F.: On the mechanism of action of aureomycin. Science, 111: 474, 1950.
40. Maass, E. A. and Johnson, M. J.: Penicillin uptake by bacterial cells. J. Bact., 57: 415–422, 1949.
41. Maass, E. A. and Johnson, M. J.: The relations between bound penicillin and growth in *Staphylococcus aureus*. J. Bact., 58: 361–366, 1949.
42. Macheboeuf, M.: Recherches biochimiques sur le mode d'action des antibiotics: penicilline, streptomycine, tyrothricine. Bull. Soc. Chim. Biol., 30: 161–184, 1948.

43. Hagoffin, R. L. and Spink, W. W.: The protection of intracellular brucella against streptomycin alone and in combination with other antibiotics. J. Lab. & Clin. Med., 37: 924-930, 1951.
44. Marshall, E. K.: The absorption, distribution and excretion of streptomycin. J. Pharmacol. & Exper. Therap., 92: 43-48, 1948.
45. McQuillen, K.: The bacterial surface. IV. Effect of streptomycin on the electrophoretic mobility of *Escherichia coli* and *Staphylococcus aureus*. Biochem. & Biophys. Acta, 7: 54-60, 1951.
46. Miller, C. P. and Bohnhoff, M.: The development of bacterial resistance to chemotherapeutic agents. Ann. Rev. Microbiol., 4: 201-222, 1950.
47. Mitchell, P.: Some observations on the mode of action of penicillin. Nature, 164: 259-262, 1949.
48. Mitchell, P. D. and Moyle, J.: Occurrence of a phosphoric ester in certain bacteria—its relation to gram staining penicillin sensitivity. Nature, 166: 218-220, 1950.
49. Mitchell, P. and Moyle, J.: Relationships between cell growth, surface properties and nucleic acid production in normal and penicillin-treated *Micrococcus pyogenes*. J. Gen. Microbiol., 5: 421-438, 1951.
50. Miura, H., Nakamura, Y., Matsudaira, H. and Komeiji, T.: The mode of action of terramycin. Antibiotics, 3: 153-158, 1952.
51. Molitor, H. and Graessle, O. E.: Pharmacology and toxicology of antibiotics. J. Pharmacol. & Exper. Therap., 98: Part II, 1-60, 1950.
52. Nelson, W. E., Forgacs, J. and Kucera, J. L.: Alterations of the distribution and excretion of streptomycin. Proc. Soc. Exper. Biol. & Med., 64: 20-21, 1947.
53. Oginsky, E. L., Smith, P. H. and Solotorovsky, M.: The action of streptomycin. IV. Fatty acid oxidation by *Mycobacterium tuberculosis*, avian type. J. Bact., 59: 29-44, 1950.
54. Oginsky, E. L., Smith, P. H. and Umbreit, W. W.: The action of streptomycin, I. The nature of the reaction inhibited. J. Bact., 58: 747-759, 1949.
55. Owen, C. A., Karlson, A. G. and Zeller, E. A.: Enzymology of tubercle bacilli and other mycobacteria. V. Influence of streptomycin and other basic substances on the diamine oxidase of various bacteria. J. Bact., 62: 53-62, 1951.
56. Paine, T. F. and Lipmann, F.: No antistreptomycin activity shown by inositol phospholipids. J. Bact., 58: 547, 1949.
57. Park, J. T.: The uridine-5'-pyrophosphate compounds found in penicillin-treated *Staphylococcus aureus* cells. A Symposium on Phosphorus Metabolism, Baltimore, Md. (Johns Hopkins Press), 1: 93-98, 1951.
58. Park, J. T.: Isolation and structure of the uridine-5'-pyrophosphate derivatives which accumulate in *Staphylococcus aureus* when grown in the presence of penicillin. Symposium on Mode of Action of Antibiotics, 2nd Int. Cong. Biochem., Paris, 31-39, 1952.
59. Park, J. T.: Uridine-5'-pyrophosphate derivatives. I. Isolation from *Staphylococcus aureus*. J. Biol. Chem., 194: 877-884, 1952.
60. Park, J. T.: Uridine-5'-pyrophosphate derivatives. II. A structure common to three derivatives. J. Biol. Chem., 194: 885-895, 1952.
61. Park, J. T.: Uridine-5'-pyrophosphate derivatives. III. Amino Acid containing derivatives. J. Biol. Chem., 194: 897-904, 1952.
62. Park, J. T. and Johnson, M. J.: Accumulation of labile phosphate in *Staphylococcus aureus* grown in the presence of penicillin. J. Biol. Chem., 179: 585-592, 1949
63. Pollock, M. R. and Perret, C. J.: The relation between fixation of penicillin sulfur and penicillinase adaptation in *B. cereus*. Brit. J. Exper. Path., 32: 387-396, 1951.
64. Potter, V. R. and Reif, A. E.: Inhibition of an electron transport component by antimycin. J. Biol. Chem., 194: 287-297, 1952.
65. Pratt, R. and Dufrenoy, J.: Evidence of the involvement of glutathione in the mechanism of penicillin action. J. Am. Chem. Soc., 70: 1671, 1948.
66. Pratt, R. and Dufrenoy, J.: Cytochemical interpretation of the mechanism of penicillin action. Bact. Rev., 12: 79-103, 1948.
67. Rapoport, S. and Wagner, R. H.: A phosphate ester of a tricarboxylic acid in liver. Nature, 168: 295-296, 1951.
68. Regna, P. P.: Chemical structure of terramycin in relation to mode of action. Trans. New York Acad. Sc., 15: II, 12-17, 1952.
69. Rhymer, I., Wallace, G. I., Byers, L. W. and Carter, H. E.: The antistreptomycin activity of lipositol. J. Biol. Chem., 169: 457-458, 1947.
70. Rowlands, S., Rowley, D., and Smith, E. L.: Studies with radioactive penicillin. J. Chem. Soc., 5: 405-407, 1949.
71. Rowley, D., Cooper, P. D. and Roberts, P. W.: The site of action of penicillin. I. Uptake of penicillin on bacteria. Biochem. J., 46: 157-161, 1950.
72. Rowley, D., Miller, J., Rowlands, S. and Smith, E. L.: Studies with radioactive penicillin. Nature, 161: 1009-1010, 1948.
73. Rybak, B., and Gros, F.: Quelques proprietes basiques de la streptomycine. Experientia, 4: 396-398, 1948.
74. Sacks, L. E.: Subtilin considered as a germicidal surface active agent. Antibiotics, 2: 79-85, 1952.
75. Schwietzer, C. H.: Chemical structure and secondary binding forces as causes of bacteriostatic action. J. Path. & Bact., 63: 402-407, 1951.
76. Simmonds, S. and Fruton, J. S.: Action of penicillin on bacterial utilization of amino acids and peptides. Science, 111: 329-331, 1950.
77. Smith, G. N. and Worrel, C. S.: Studies on the action of chloramphenicol (Chloromycetin) on enzymatic systems. I. Effect of chloramphenicol on the activity of proteolytic enzymes. Arch. Biochem., 23: 341-346, 1949.

78. SMITH, G. N. AND WORREL, C. S.: Ensymatic reduction of chloramphenicol. Arch. Biochem., 24: 216–223, 1949.
79. SMITH, G. N. AND WORREL, C. S.: The decomposition of Chloromycetin (chloramphenicol) by microorganisms. Arch. Biochem., 28: 232–241, 1950.
80. SMITH, G. N., WORREL, C. S. AND LILLIGREN, D. L.: The ensymatic hydrolysis of chloramphenicol. Science, 110: 297–298, 1949.
81. SMITH, G. N., WORREL, C. S. AND SWANSON, A. L.: Inhibition of bacterial esterases by chloramphenicol (Chloromycetin). J. Bact., 58: 803–809, 1949.
82. SMITH, P. H., OGINSKY, E. L. AND UMBREIT, W. W : The action of streptomycin. II. The metabolic properties of resistant and dependent strains. J. Bact., 58: 761–767, 1949.
83. SWENDSEID, M. E., WRIGHT, P. D., AND BETHELL, F. H.: A growth factor for *L. citrovorum* synthesized by hemopoietic tissue reversing Aminopterin and Chloromycetin inhibition. Proc. Soc. Exper. Biol. & Med., 80: 689–690, 1952.
84. TINT, H. AND REISS, W.: Some properties of a bacillomycin-B-cytochrome-c complex. J. Biol. Chem., 190: 133–148, 1951.
85. TRUHAUT, R., LAMBIN, S. AND BOYER, M.: Mechanism of the action of Chloromycetin on *Eberthella typhi*: Role of tryptophan. Bull. Soc. Chim Biol., 33: 387–393, 1951.
86. UMBREIT, W. W.: A site of action of streptomycin. J. Biol. Chem., 177: 703–714, 1949.
87. UMBREIT, W. W.: The metabolic action of streptomycin. Ann. New York Acad. Sc., 53: 6–12, 1950.
88. UMBREIT, W W.: Chemical structure of antibiotics in relation to mode of action. Streptomycin. Trans. New York Acad. Sc., Series II, 15: 8–11, 1952.
89. UMBREIT, W. W.: The mode of action of streptomycin. Symposium on Mode of Action of Antibiotics. 2nd Int. Cong. Biochem., Paris, 63–77, 1952.
90. UMBREIT, W. W.: Respiratory cycles. J. Cell. Comp. Physiol., 41: 39–66, 1953.
91. UMBREIT, W. W.: The action of streptomycin. VI. A new metabolic intermediate. J. Bact., 66: 74–81 (1953).
92. UMBREIT, W. W.: Unpublished experiments.
93. UMBREIT, W. W. AND OGINSKY, E. L.: Mode of action of antibiotics: Penicillin and streptomycin. J. Mt. Sinai Hosp., 19: 175–184, 1952.
94. UMBREIT, W. W., SMITH, P. H. AND OGINSKY, E. L.: The action of streptomycin. V. The formation of citrate. J. Bact., 61: 595–604, 1951.
95. UMBREIT, W. W. AND TONHAZY, N. E.: The action of streptomycin. III. The action of streptomycin in tissue homogenates. J. Bact., 58: 769–776, 1949.
96. VANMETER, J. C. AND OLESON, J. J.: Effect of aureomycin on the respiration of normal rat liver homogenates. Science, 113: 273, 1951.
97. WOOLLEY, D. W.: A study of non-competitive antagonism with Chloromycetin and related analogues of phenylalanine. J. Biol. Chem., 185: 293–305, 1950.
98. WORK, T. S.: The biochemistry of antibiotics. Ann. Rev. Biochem., 21: 431–458, 1952.
99. WORK, T. S. AND WORK, E.: The basis of chemotherapy. Interscience Publishers, N. Y., 1948.