PHARMACOLOGY AND TOXICOLOGY OF ANTIBIOTICS

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INTRODUCTION

Whereas ten years ago tyrothricin (gramicidin) was the only antibiotic which had been investigated pharmacologically, a very large number of such agents have been discovered since then. Most of them proved too toxic for practical use; however, penicillin, streptomycin, aureomycin, and chloramphenicol have come to stay as some of the most effective weapons in the fight against infectious diseases. A very voluminous literature has accumulated on their use, as well as on that of other less prominent antibiotics. It is obvious that of the hundreds of published papers (*e.g.*, 1171 on streptomycin alone, according to Waksman's bibliography of 1948) only a limited number could be included in this review. Furthermore, it is almost certain that in spite of our desire to cover the domestic and foreign literature as thoroughly as possible, some papers have been missed which should have been included.

We have limited this review to such aspects of the studies of antibiotics as are of direct interest to pharmacologists and to investigators in the field of experimental therapeutics. Investigations dealing with the microbiological, chemical, pathological, and clinical aspects of antibiotic research have been either omitted or are referred to only to the extent necessary to round out the discussion of their pharmacology and toxicology.

In order to make this review more convenient to use, we have divided it into two parts. The first one is of a general nature; it is an attempt to summarize those aspects of the pharmacology of antibiotics which may be regarded as common and characteristic. The second part contains a detailed description of the four most prominent antibiotics: penicillin, streptomycin, Aureomycin, and chloramphenicol (Chloromycetin), plus an additional chapter on clinically less important agents which, however, have some pharmacologic interest. Each of the individual chapters is preceded by a very brief summary indicating the position of the particular agent in its field. Of necessity, such one- or two-sentence summaries are not only arbitrary, but also somewhat schematic and have to be interpreted accordingly.

The term "antibiotics," coined by Eaton Ward in 1899 but not generally used until 1944, when revived by Waksman in his book "Microbial Antagonisms and Antibiotic Substances," denotes a substance produced by microorganisms which has the capacity of inhibiting the growth and occasionally, even of destroying microorganisms (328, 329). However, the wide use of these agents in a multitude of infectious diseases heretofore treated with sulfonamides, arsenicals, gold and silver salts, azo dyes, etc., as well as the combination of antibiotics with other chemotherapeutic agents, has led to a somewhat loose usage of the term "antibiotic" and has made it appear as if it were interchangeable with "chemother-

apeutic," "germicidal," or "antiseptic." This, of course, is not the case. The definition of chemotherapy given in Funk & Wagnalls Standard Dictionary of the English language, is "the treatment of internal microbic diseases by injecting into the blood chemical substances that destroy the parasitic germs but are not poisonous to the human tissues. The principle is the same as that of serum therapy, but the material injected is a synthetic chemical compound, not a product of a living microorganism."

Our reason for pointing out these definitions at the beginning of this review is to draw attention to the one difference between chemotherapeutic agents and antibiotics which has a profound bearing on the analysis of their pharmacological properties: the origin from materials of varying, and often unknown composition. Whereas practically all other chemotherapeutic agents are chemically well defined and usually are made synthetically, antibiotics are, with but one exception (chloramphenicol), obtained through extraction and purification of microbial cultures. Frequently, as with penicillin, streptomycin or Aureomycin, their successful use in therapy antedates for a considerable time the analysis of their chemical structure; and in many instances, the use of an impure antibiotic is continued even after the chemists have succeeded in the preparation of the crystalline compound. Such a situation is bound to exert a pronounced influence on the reproducibility of experiments performed with these agents and on the conclusions based upon them: Experiments performed with a relatively impure material rarely permit broad generalizations.

Filtrates of microbial cultures and their concentrates are products of microbial metabolism which varies with such factors as composition of the medium, temperature, aeration, etc. In the early stages of production these conditions usually are not well standardized; yet, in general it is material of this type that is available for the initial pharmacological, toxicological, and clinical investigations. Furthermore, even after the technical procedures have become standardized, there is always the possibility of mutations of the parent strain. As long as these do not affect specific microbiological characteristics, they are likely not to be noticed even though they might profoundly alter the pharmacological and toxicological properties. The reason for this lies in the customary methods of standardization; since the "antibiotic" effect is the principal use of the agent, the biological standardization of antibiotics is based without exception, on their ability to inhibit or prevent bacterial growth. Such tests, however, fail to be affected by pharmacologic and toxicologic properties and hence will not reveal variations of this type. Some of the "safety" tests used in the standardization of antibiotics, such as an intravenous LD_{50} in mice, or the effects on blood pressure and body temperature, may serve as indicators of changed pharmacodynamic properties; however, they will not reveal changes which take place only after a somewhat prolonged administration or which occur in organ systems other than those under test (*e.g.*, neurotoxicity).

The bacteriologist or clinician examining a new antibiotic is usually content with a preparation which has been standardized for antibacterial potency and which is reasonably non-toxic. Hence, in clinical reports reference is usu-

ally made only to the number of biological units of the antibiotic administered to the patient. Side reactions as well as variations in tolerance are often ascribed to differences in patient sensitivity or to the development of a state of allergy. Only rarely is the specific lot number of the tested material recorded in the protocols; omissions of a reference to the type of solvent, to the concentration, and to the pH of the solution are not uncommon. Yet each of these factors plays a role at least as important as variations in the individual patient sensitivity.

The potential significance of the presence of impurities is best illustrated by a comparison of the unit potency of early samples of penicillin and streptomycin with that of the material now in use. The average purity of the antibiotics available for most of the initial clinical studies was only 14% in the case of penicillin and 26% in that of streptomycin. Thus, a patient who in 1944 received 1 million units of streptomycin was given, in addition to 1 gram of chemotherapeutically active substance, 3 grams of extraneous bulk. Some of this was probably inert, but some consisted of pharmacodynamically highly active material; furthermore, the low unit potency made it impossible to obtain certain therapeutic results which depend upon the administration of a large dose of the active material. Thus, whereas the favorable response of bacterial endocarditis to penicillin is firmly established, daily doses from 2 to 16 million units are required. With preparations averaging 225 units per mg., as was common in the early days of penicillin, this would have necessitated the administration of from 8 to 64 grams of solids. The difficulty of giving such large amounts, as well as the scarcity and high cost of penicillin in those days, prohibited the use of such doses. It is easy to understand, therefore, why early clinical reports stated that penicillin was ineffective in the treatment of bacterial endocarditis, while today its effectiveness is firmly established.

Bulk alone, aside from its possible irritating properties, is only a minor complicating factor in the investigation of low potency samples; a much greater difficulty arises from the lack of knowledge of the composition of this bulk. In penicillin it appears to consist primarily of pharmacodynamically inert material; for this reason, early pharmacological reports on penicillin do not substantially differ from those obtained with the pure fractions.

With streptomycin, however, the picture is entirely different. It has been shown that at least three of the toxic side effects of this agent are largely influenced by, or indeed entirely due to, impurities or degradation products of high pharmacodynamic activity. One of these is either histamine or a chemical closely related to it. Another impurity or degradation product is responsible for at least part of the neurotoxic effect; still another, not discovered by the standard methods of bioassay, appears to be responsible for the pain at the site of injection.

Another cause of changed pharmacodynamic properties may be a slight variation in the composition of the culture medium. Robinson *et al.* (256) have found that streptothricin, an antibiotic closely related to streptomycin, exerts a far more pronounced effect on the blood pressure when produced from a corn steep liquor medium than when obtained, by the same extraction method,

from a tryptone broth. More recently, it has been found in Waksman's laboratory that the use of still another medium yields streptothricin which has lost most of its local irritating properties although it still retains the delayed nephrotoxic effects. Since local irritation always follows the application of streptothricin obtained from tryptone broth, even if the pure crystalline material is used, Waksman's observation indicates that it may be possible to vary intrinsic pharmacodynamic properties of an antibiotic without altering its chemotherapeutic effects—a discovery of potentially far-reaching significance since it might permit the transformation of an effective but toxic antibiotic into one of therapeutic usefulness.

For the above reasons, each lot of a new antibiotic (unless available in chemically pure form) should be treated as an essentially new drug which has in common with the preceding lot only its origin, its specific antibiotic effect, and the name. Furthermore, since many of the side effects, particularly those related to pain or other highly subjective phenomena, are more likely to be observed in patients than in experimental animals, it is of utmost importance that the clinician and pharmacologist work in closest cooperation in the study of a new antibiotic.

Attention must also be paid to several other factors, always important in the evaluation of new drugs but of particular significance in the assay of antibiotics; the animal species used, the dosage regimen, the route of administration, and the physical and chemical properties of the salt used in the test. Thus, according to Welch (338, 339) the potassium and magnesium salts of penicillin exhibit an intravenous toxicity more than 4 times that of the sodium and lithium salts, probably due to the specific toxicity of the cation. Similarly, streptomycin in the form of its hydrochloride seems to cause more undesirable local effects than it does as the calcium chloride complex or sulfate.

Full awareness of the many variables involved in the evaluation of the pharmacodynamic properties of a new antibiotic will lead to greater caution in the formulation of claims and may prevent the discarding of a new agent which, although endowed with high antibiotic potency and other desirable features, might at first appear to be too toxic for clinical use. Since obviously it is undesirable to judge the pharmacological and toxicological properties of a new drug on the basis of results obtained with impure preparations, regular re-investigation is needed until it is possible to perform the pharmacological analysis with the pure agent. However, any re-investigation of pharmacodynamic properties has to be paralleled with that of antibiotic potency. There are numerous examples showing that the chemotherapeutic effects of early and cruder preparations differ from those of the later, more highly purified samples. This is due, not only to the introduction of variants with changing methods of production (for example, penicillins F, G, K, X), but also to the elimination of certain microbiologically inert materials which nevertheless may modify the antimicrobial effect. Good illustrations are the "enhancement factor" recently described by Welch *et al.* (341), and the factors reported by Hobby *et al.* (146, 150, 152) and Miller *et al.* (204).

Our knowledge of the pharmacological properties of antibiotics is limited, in general, to those relatively few agents which have found therapeutic use (penicillin, streptomycin, Aureomycin, and Chloromycetin), or which have at least appeared to be promising in the earlier stages of their investigation. This is regrettable because it is most likely that antibiotics exist which, on account of a low chemotherapeutic index, are not suitable for chemotherapeutic purposes but nevertheless possess pharmacodynamic properties which not only merit a thorough study but might even recommend them for use in fields other than the chemotherapy of infectious diseases. Streptothricin and actinomycin are examples of antibiotics which had been studied pharmacologically before they were discarded. The latter is so highly toxic that a single administration of 50 micrograms per kgm. to a mouse produces death within 10 days; its use for any form of systemic therapy is, therefore, out of the question. Toxicologically, however, actinomycin is unusually interesting because it produces a shrinkage of the spleen to one tenth of its original size, for reasons unknown and not studied. Streptothricin, a forerunner of streptomycin and closely related to it, has been more thoroughly investigated (206, 240, 255, 256) because, at the time of its discovery by Waksman and Woodruff in 1942 (335), it appeared to accomplish for the therapy of gram-negative infections what penicillin does in the gram-positive field. However, before streptothricin was ever tried in man, its pharmacological investigation was discontinued when streptomycin appeared and proved to be considerably less toxic. Fortunately, from the pharmacologist's point of view, the studies had already progressed far enough to establish certain most interesting toxicological properties which will be discussed later.

The number of antibiotics which have been pharmacologically investigated in spite of an unfavorable chemotherapeutic index is almost zero. As long as antibiotics are intended solely for the chemotherapy of infectious diseases, such an attitude is understandable. There is no reason to assume, however, that some members of this very large group of powerful agents should not possess pharmacological properties which might render them useful and safe for purposes other than the treatment of infectious diseases, regardless of whether the chemtherapeutic index is favorable or not. An example in point is streptomycin which has found a limited but highly successful therapeutic application in the treatment of Ménière's disease because of its property to paralyze selectively the vestibular centers (103).

The lack of pharmacological data on antibiotics which fail to possess a favorable chemotherapeutic index is readily understandable. The development of a new antibiotic is an extremely costly and time-consuming undertaking and it is obvious that some simple technic for separating the wheat from the chaff has to be followed. The method now generally adopted consists in determining, first, the presence of antibiotic activity *in vitro* against a limited number of microorganisms; and second, in establishing the maximum tolerated dose in mice (usually by intraperitoneal injection). If these tests give satisfactory results, the determination of *in vivo* activity in experimentally infected mice follows. Unless a new agent passes these preliminary requirements, it is not studied further. It

may be of interest to mention that out of many thousands of new antibiotic broths examined each year, not more than a few dozen are carried beyond the initial stage; only a few of these reach the stage of thorough pharmacological study in animals, and still fewer are carried to the next step, the clinical evaluation in man. How many of the discarded ones might possess valuable pharmacotherapeutic properties, no one can guess. It is safe to state, however, that the very method of selection excludes from a thorough study those which are pharmacodynamically most active.

The use of the chemotherapeutic index as principal criterion in the screening of antibiotics has a number of consequences which should be mentioned at this time. Firstly, the original culture broth may contain so little of the new antibiotic that this might lead to the discard of a potentially valuable agent only because the toxic properties of the broth prevented its trial at a level sufficiently high to be effective chemotherapeutically. To realize the enormous changes in the ratio of active to inactive (or toxic) material in a liquid culture medium one may compare the increases in the yields of penicillin broth at the time of Fleming's original discovery of *Penicillium notatum* up to the present, when new mutant strains of *Penicillium chrysogenum* are used. In 1941, one cc. of culture broth yielded 2 units of penicillin; in 1942, after the change from *P. notatum* to *P. chrysogenum*, this figure was 200 units; in 1944, with the introduction of a mutant strain, the yield per cc. had risen to 800 units; and at present certain strains produce as much as 2000 units per cc. or a thousand-fold increase in yield over Fleming's original culture.

Another consequence of the generally adopted technic of developing a new antibiotic through initial screening of broth concentrates and gradual purification, with pharmacological and clinical investigations performed at that intermediate stage, is a variation in the composition of the material used for a very large part of the pharmacological and clinical trials. After the introduction of a new antibiotic into practical therapy several years may elapse before its chemical composition and structure have been completely elucidated and the pharmacological properties of the pure material have been established. Thus, the first published reports of the pharmacologic properties of penicillin (1, 55, 102, 123, 153, 241, 254) were based on material varying in purity from 10 to 30%; and the first extensive pharmacological study of streptomycin (208, 259) employed material which ranged in purity from 10 to 90%, most of the experiments having been conducted with streptomycin of approximately 50% purity. In the case of penicillin, the presence of impurities was irrelevant; with streptomycin, however, the interference of toxic impurities and degradation products was already recognized at the time of the original study, but the lack of sufficient quantities of the pure antibiotic precluded the use of such a material for the pharmacological investigation. Furthermore, it was material of that relatively low grade of purity which was then in universal clinical use. Indeed, even to date, no thorough pharmacological studies of pure penicillin "G" or pure streptomycin have been published, although both antibiotics have been available in pure form for quite some time. Consequently, certain pharmacody-

namic effects of an impure material may continue to be ascribed to the antibiotic, although upon re-investigation it might be found that they were the result of impurities. How considerable the changes accompanying the increasing purity of an antibiotic can be, may be demonstrated by a comparison of the pharmacologic properties of the antibiotic streptomycin. We select this agent not only because it possesses sufficient intrinsic pharmacodynamic activity to permit the demonstration of qualitative changes with progressing purification, but also because the experimental data shown in table I were all obtained in the same laboratory and by the same investigators, thus eliminating variations due to differences in technic, animal strains, environmental and dietary conditions, etc. It will be noticed that the original streptomycin of approximately 10% purity differs from the pure material in so many aspects that, from a pharmacological

TABLE 1

CHANGE OF PHARMACOLOGIC PROPERTIES OF STREPTOMYCIN WITH INCREASING PURITY

PURITY (approx)	L.D. 50 I.V. Units/20gm.	L.D. 50 S.C. Units/20gm AVERAGE	MINIMUM CIRCULATORY EFFECT Units/Kg AVERAGE	RESPIRATORY EFFECTS Units/Kg AVERAGE	PYROGENIC EFFECTS	RENAL EFFECTS	HEPATIC EFFECTS	LOCAL EFFECTS	MINIMAL CENTRAL NERVOUS EFFECTS
10%	725 (670-890)	7000	100	5000	+	+	+	+++ AT 2000 U/cc	I.C. AT 500 U/Kg S.C. 13 DAYS AT 50,000 U/Kg
25%	2200 (1750-2600)	10,000	900	15,000	±	+	+	+++ AT 20,000 U/cc	I.C. AT 1100 U/Kg
50%	2500 (2000-2920)	10,000	4000	25,000	−	+	+	+ AT 133,000 U/cc	I.C. AT 1700 U/Kg S.C. 32 DAYS AT 50,000 U/Kg
95% TO 100%	4000 (3500-4640)	25,000	25,000	NONE AT 40,000	−	−	−	NONE AT 133,000 U/cc	I.C. AT 2500 U/Kg S.C. 43 DAYS AT 50,000 U/Kg

viewpoint, one almost might regard the two preparations as unrelated. Yet the chemotherapeutic activity of the same number of units of streptomycin is essentially the same regardless of the degree of purity. As the purity of streptomycin increases, there is a marked decrease in the intensity of certain pharmacodynamic effects, notably those upon the circulatory system, the body temperature and the renal and hepatic functions; there also is a certain reduction in the incidence of neurotoxic manifestations at comparable dose levels. It has been conclusively demonstrated (208, 290) that the marked circulatory effects of early samples of this antibiotic were caused by a histamine-like impurity which could be completely eliminated by further purification. The pyrogenic effects were likewise due to contamination with products of bacterial metabolism which could be removed. The actual cause of the hepato- and reno-toxic effects has not been definitely established, but it has been shown that these effects fail to appear with the pure material even with very large doses. A

similar decrease in the incidence of side reactions has been reported for other antibiotics, although no exact pharmacological data are available.

Although generally the pharmacological properties of an antibiotic show fewer variations and frequently decrease in severity with increasing purity, the presence of impurities may in certain instances enhance the chemotherapeutic activity. Thus, Cornman (65) and Lewis (183) have shown that impure samples of penicillin possess a selective effect against rat and mouse sarcoma which can not be found with pure penicillin G. Dunham and Rake (76) observed that impure penicillin exerted a definite effect on the motility of *Treponema pallidum* which was absent with pure penicillin G. A similar observation was made in our laboratories by Graessle and Bugie (205) in testing the effect of penicillin against *Microfilaria immitis*. Smith, in 1946 (301), found that root growth and germination were retarded by impure, but not by pure penicillin and he stated that the indole-3-acetic acid and phenyl acetic acid present in impure penicillin were responsible for this difference. Welch, Randall and Price (341) and Hobby, Lenert and Hyman (150) demonstrated that the chemotherapeutic effect of impure penicillin was considerably greater than that of pure penicillin G. The addition of the impurities of crude penicillin to pure penicillin G resulted in a material which had the same biological activity as impure penicillin. Similar observations have been made for streptomycin when tested against *Eberthella typhosa* (149).

It has not been established whether the increased activity of impure penicillin is due to an "enhancement factor" or is primarily caused by the presence of several types of penicillin (X, G, F, K). It has been shown conclusively by Hobby *et al.* (146), Eagle *et al.* (80, 81, 82, 86) and others (60, 158, 185, 220, 323, 340) that the various penicillins present in amorphous penicillin vary considerably in chemotherapeutic activity. Assigning to penicillin G an activity index of 100, the differences were as much as 127, 100, 57, 40, respectively (146), or 260, 100, 50, 9, respectively (86), when *Streptococcus hemolyticus* was used as the test organism. With a strain of pneumococcus, the corresponding figures were 160, 100, 83, 19, respectively (81). Although any intensification of the chemotherapeutic effect is desirable, it would seem necessary to ascertain in each case that other, possibly objectionable, properties of the antibiotic are not enhanced at the same time. From a pharmacological point of view it would seem best to isolate and study each of the "enhancement" factors and then add them in known quantity to a known amount of the antibiotic (322a). This was done with the penicillin enhancement factor described by Welch; the result was disappointing in that no differences in therapeutic effectiveness were found in patients given penicillin together with the enhancement factor and those receiving penicillin G alone (145). That the various penicillins present in amorphous penicillin differ in pharmacological as well as chemotherapeutic activity is well known. Thus, Tompsett *et al.* (318) have shown that whereas only 47% of penicillin X is bound to the albumin component of the serum, as much as 91% of penicillin K may be bound; and Clowes and Keltch (61) have demonstrated that larger amounts of penicillin K than G are removed from solutions exposed in the

Warburg apparatus to various quantities of liver or muscle slices. Any single one of these factors, as well as their combined effect, is likely to influence the pharmacological and chemotherapeutic properties. With penicillin, however, the only differences likely to be noticed are those in antibiotic activity since all forms of penicillin are remarkably free from pharmacodynamic or toxic properties. This, however, is not the case with pharmacodynamically less "inert" antibiotics such as bacitracin or neomycin. The latter has already been shown to be composed of two distinct entities and possibly may contain even more (231). Hobby *et al.* (151) have recently demonstrated a much more favorable chemotherapeutic index for neomycin than that obtained by other investigators using the same parent strain but presumably different media and methods of extraction. These facts emphasize that extreme caution is needed when results obtained by one investigator with one lot of an antibiotic are generalized. It cannot be urged too often that each lot of an antibiotic, as long as it is impure, be regarded as an essentially new drug.

Absorption, excretion, and accumulation in the body are major factors in determining the pharmacodynamic properties and the efficacy of a drug. Marshall was one of the first to demonstrate the great clinical importance of the maintenance of a sufficiently high blood concentration of sulfonamides and to emphasize the advisability of using the concentration of a drug in plasma or blood rather than dosage by mouth as a measure of adequate medication (194). The concept of prolonged maintenance of a bacteriostatic concentration of the chemotherapeutic agent as a prerequisite to successful chemotherapy with sulfonamides became so firmly established that it was transferred to the field of antibiotics, without experimental foundation for its application to that type of drug. While it cannot be questioned that a certain minimal concentration of an antibiotic is needed to exert a chemotherapeutic effect and that a definite relationship exists between bacteriostatic or bactericidal concentration *in vitro*, the concentration in plasma and that in body tissues, it has more recently been shown that the conditions governing the therapeutic efficacy of an antibiotic are not the same as those for the sulfonamides. It must be kept in mind that sulfonamides are primarily bacteriostatic (54, 169) whereas antibiotics are (in low concentrations) bacteriostatic and (in high concentrations) bactericidal. For this reason as well as the fact that the sulfonamides are inactivated by pus and tissue extracts, mice infected with streptococcus cannot be cured by a single administration of a sulfonamide regardless of the size of the dose whereas they can be saved by a single dose of penicillin (114, 342) or streptomycin (259). Furthermore, the effect of sulfonamides upon bacterial metabolism is more readily reversible, than that of some antibiotics, including penicillin. It has been shown that organisms removed from exposure to penicillin fail to resume growth for several hours (225, 226, 227). Other factors relevant to this discussion are the type of assay method and the state (free or protein bound) in which the chemotherapeutic agent is present in the body. The customary assay for sulfonamides is chemical and is based on their ability to diazotize and produce a highly colored azo dye. Most antibiotics, on the other

hand, are routinely determined by microbiological assays even though chemical methods may be available. The chemical determination of sulfonamides reveals the amount of both free and protein bound drug, whereas microbiological assays only measure the quantity of free (effective) drug.

No one can question the fact that, in order to produce a chemotherapeutic effect, contact of the antibiotic with the microbial cell is needed in a certain concentration and for a minimum length of time (77). Many factors, however, influence these conditions which differ not only with each antibiotic, but also with such factors as the type of infecting organism, the site of infection and the size of inoculum. Thus, antibiotics which are rapidly absorbed and excreted, such as penicillin, may require more frequent administration than those which possess a slower rate of excretion, unless special chemical or pharmaceutical forms are devised to overcome these alleged shortcomings. Penicillin and streptomycin have been shown to affect primarily growing cells (54, 147, 154), and it may therefore be reasoned that under such circumstances interrupted administration of the antibiotic will be more effective than the maintenance of a constant blood concentration. Streptomycin and penicillin fail to penetrate the barriers surrounding certain foci of infection, *e.g.*, areas of caseation in tuberculous cavities or scar tissue in bacterial endocarditis, unless measures have been taken to provide the necessary contact. These may consist in the maintenance of dose levels far above those ordinarily required; in the combined administration of the antibiotic with another drug intended to provide better penetration, *e.g.*, heparin (186, 187), potassium iodide (350); or in mechanically applying the antibiotic as closely as possible to the site of infection, *e.g.*, streptomycin injected into tuberculous cavities (197, 315) or into the subdural or submeningeal space. Other factors which must be taken into account when determining the size and spacing of doses are the sensitivity and microbiological characteristics of the organism to be eliminated. With organisms such as streptococci, staphylococci, and pneumococci, which grow at a rapid rate and multiply in less than one hour, a more frequent administration is needed than with others, such as *M. tuberculosis* which has a life cycle of several days. Also, it is obvious that infections with a large inoculum require higher and more frequent administration, particularly if the infecting organism belongs to the rapidly multiplying group. Another major factor in determining the concentrations of an antibiotic required for bactericidal effect is the individual sensitivity of the infecting strain of microorganism. It is well known that this sensitivity varies greatly not only within the species belonging to the same genus, but also within the individual strains and among the cells of a given population. Whether the variation in a population is the consequence of selection of naturally occurring mutants or the result of an acquired resistance to the drug has not been completely elucidated and cannot be discussed here in detail. The reader is referred to review articles dealing with this topic (313, 351). It is certain, however, that regardless of the origin of the resistance, it greatly affects the dosage regimen and requires a discussion in this review since the prevention of drug resistance is one of the principal factors affecting the success of antibiotic therapy.

Several methods have been suggested to prevent or overcome development of drug resistance. One, suggested by Ehrlich in the therapy of trypanosome infections with arsenicals, is to administer a dose of the chemotherapeutic agent adequate to eliminate *all* pathogenic microorganisms. This will prevent the development of "acquired" resistance, whether due to a gradual adaptation of the microorganism to the drug or to a step-wise mutation which results in a bacterial flora consisting predominantly of resistant cells. Another measure, largely based on the finding of naturally resistant cells, consists in the combined administration of two or more chemotherapeutic agents (27, 48, 119, 166, 167, 302, 319, 322). This measure is based on differences in the mode of action of chemotherapeutic agents. It is now generally recognized that the most valuable property of an antibiotic, its ability to interfere with the development of the bacteria without inflicting severe damage to the cells of the host, resides in the inhibition of certain metabolic functions vital to the microbial system. The differential inhibition may be brought about in at least two ways. In the case of streptomycin, it has been found that the antibiotic interferes with the reaction between pyruvate and oxalacetate, sometimes called the "Krebs condensation" (217, 218, 299, 320, 321). This reaction constitutes the system whereby a wide variety of substances enter the terminal respiration system. The reaction is an important one, not only in microbial growth, but in animal tissues as well, in which it serves the same functions. However, in the animal there exists a permeability barrier at the cell wall and also at the mitochondria where this reaction is localized in the animal cell. This barrier prevents the streptomycin from penetrating in the animal to the site of the "Krebs condensation," even though it enters the bacterial cell where the reaction system is not so protected. The selective action of streptomycin upon the microbial cell and its lack of interference with the metabolism of the host is explainable in this manner, even though the reaction inhibited is an important one to both organisms.

In the case of penicillin, its action would appear to be based upon the specific inhibition of the synthesis of a particular type of nucleic acid, an enzyme involved in the transport of certain amino acids across the cell wall (109, 110). While direct data are not yet available it appears quite likely that this is a reaction vital to the bacteria involved but one which either does not exist or can be readily dispensed with in the animal. Thus both agents inhibit vital enzymatic processes in the bacteria. Their selective effect appears due in one case (streptomycin) to physico-chemical factors of permeability and in the other (penicillin) to a difference in the type of enzymatic systems involved.

If resistance is due to the ability of the microorganism to develop an alternate enzymatic pathway for the same metabolic process, then the simultaneous administration of two or more chemotherapeutic agents, each differing in the details of their (enzymatic) mode of action, is likely to minimize the possibility of such an adaptation. Such results have been reported for the combination of sulfonamides with antibiotics, of antibiotics with p-aminosalicylic acid (PAS) and of one antibiotic with another.

If resistance has developed in spite of the use of large initial doses of an antibiotic or the combined administration of several chemotherapeutic agents, it is in some instances possible to continue effective chemotherapy through an increase in the dose of the antibiotic. This course, however, can be taken only if the original sensitivity of the pathogen was low or if the antibiotic used is as nontoxic as penicillin. In these instances, the combined administration of two or more chemotherapeutic agents may be effective, particularly when the drugs act synergistically. Thus, Eagle and Fleischman (83) observed that the curative effect of a full dose of bacitracin or penicillin could be obtained when $\frac{1}{7}$ and $\frac{1}{40}$ of the doses of each antibiotic were combined. A similar effect was noted *in vitro* by Bachman (10). The advantage of combination therapy, however, is not limited to an additive or synergistic chemotherapeutic effect or to a reduction in toxicity. One of us (OEG) has shown that the addition of a chemotherapeutically ineffective dose of PAS to streptomycin delays or even prevents the development of resistance in the tubercle bacillus *in vitro* (119). Additional preliminary evidence has been presented by Karlson *et al.* on the advantages of the combined use of PAS and streptomycin, Promin and streptomycin, and Promin, PAS and streptomycin (165).

One last resort to circumvent an already developed resistance is the replacement of the antibiotic originally used by another chemotherapeutic agent active against the same pathogen. Since the antibiotics have been generally found to be less toxic and more effective than the older chemotherapeutic agents, this implies a continued search for new antibiotics possessing a bacteriological spectrum similar to those used at present but effective when resistance to another antibiotic has developed, or with a lesser tendency for resistance to develop against them.

PENICILLIN

Penicillin was first described by Fleming in 1929 as the active principle of the metabolic products of *Penicillium notatum*. It has now been shown that several forms of penicillin exist. They have in common a ring structure of alanine and beta-dimethylcysteine, and vary in the substituent acid coupled to the alanine amino group. The quantitative differences among the penicillins have not been of sufficient magnitude to warrant the large scale production of types other than penicillin G (benzyl penicillin) (fig. 1). This type is now available in crystalline form, is of standard potency and does not require refrigeration in the dry state. The penicillins are moderately strong acids whose water-soluble sodium, potassium, or other salts are used clinically. Penicillin is extremely active against gram-positive bacteria, certain pathogenic gram-negative cocci and *T. pallidum*, and to some extent against the virus of psittacosis. It is practically devoid of toxic properties and is pharmacodynamically almost inert. Systemic effects may be obtained by either parenteral or oral administration.

Before entering into a discussion of the pharmacological and toxicological properties of penicillin (of which there are only very few), it seems advisable to state that the term "penicillin," as originally used by Fleming (100),

covers a group of distinct chemotherapeutic substances, all derived from molds and chemically closely related, but differing from each other in certain pharmacological and biochemical properties. Among the many types of penicillins known the following are the most commonly obtained by fermentation procedures: F, G, K, X; the original impure penicillin probably consisted of a mixture of these. In such a mixture, the relative proportion of each depends upon the method of culture (*e.g.*, flask production favors either penicillin F or penicillin X and deep fermentation favors penicillin G and penicillin K), the medium, the extraction procedure, and the type of strain used. For a long time, the methods used to determine the exact amount of each type of penicillin present in a commercial preparation were none too satisfactory. For this rea-

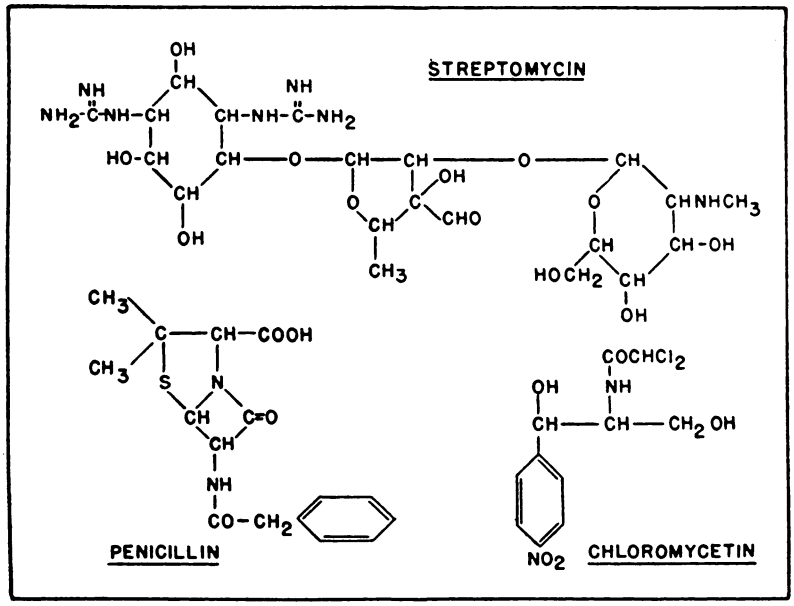

FIG. 1.

son pharmacological and clinical data based on dosage figures in terms of penicillin units are not strictly comparable, unless the penicillin preparation used consisted of one type only. Such preparations were not commercially available before 1944 and not in general use until sometime afterwards. Of the various types, to date only penicillin G (benzyl penicillin) is on the market and readily available.

The principal differences between penicillins F, G, K, and X are as follows:

(A): Microbiologically their *in vitro* activity differs considerably with regard to the quantity required for inhibition of various microorganisms (60, 80, 81, 82, 86, 146, 158, 185, 220). Results obtained by Eagle in the treatment of rabbit syphilis showed that penicillin K has approximately $\frac{1}{16}$ of the activity of penicillin G. In experimental pneumococcus infections in mice, an impure preparation

of penicillin K was $\frac{1}{6}$ as active as penicillin G and $\frac{1}{8}$ as active as penicillin X; in experimental streptococcus infection a purer preparation of penicillin K was $\frac{1}{11}$ as active as penicillin G and $\frac{1}{30}$ as active as penicillin X.

(B): Penicillin K disappears much more rapidly from the circulating blood than does penicillin G, F, or X (79) apparently due to inactivation by serum (78). The penicillins vary considerably in the degree to which they are bound to protein; this ratio approximately parallels their antibiotic activity *in vitro* when serum is added to the medium (318). Penicillin K has also been reported to be inactivated by tissues to a greater extent than other penicillins (61, 253, 316). Gardiner *et al.* (111) have examined the effect of a purified preparation of amorphous penicillin and of pure penicillins G, F, X, and K, respectively, on the vitreous humor of rabbit eyes. It was found that penicillin G and amorphous penicillin (probably due to its predominant content of penicillin G) were the least toxic and penicillin K was the most; penicillin K, in addition to causing loss of all retinal elements and pigment migration into the retina, produced extensive areas of gross destruction and atrophy of the retina with the formation of new vessels in the vitreous. There were no material differences in the diffusion rates of the various types of penicillin in the vitreous, aqueous, cornea, sclera and ciliary body.

Pedersen-Bjergaard and Tonnesen (232), investigating the influence of rat liver *in vivo* on penicillins F, G, K, and X, reported that, in the rat, lower concentrations of penicillin K were found after injection into the portal vein than after injection into the femoral vein. On perfusion through isolated rat liver, a more rapid fall in the concentration of penicillin K was noted than for the other penicillins. The authors concluded that penicillins F, G, and X are inactivated by the liver to a smaller extent, or at least at a slower rate, than penicillin K. Moreover, it was shown by Anderson and Brodersen (9) that bilaterally nephrectomized dogs eliminated penicillin at a rapid rate from their blood stream after an intravenous injection. The renal clearance of penicillin K in man appears to be $\frac{1}{4}$ to $\frac{1}{2}$ that of penicillin F, G, and X (88).

It is obvious that any one of the factors cited above can alter the outcome of pharmacological or clinical studies, if the penicillin preparations used consisted of a mixture of all four penicillins in varying proportions, and particularly if the investigator was not kept informed of changes in subsequent lots during the progress of his investigation. Unfortunately, this occurred during the war when penicillin preparations which differed widely in their relative penicillin G and K contents were supplied for clinical studies; as a consequence there were differences in the therapeutic results and considerable uncertainty with regard to the most effective dosage regimen.

Since the differences in the chemotherapeutic properties of the various penicillins are quantitative rather than qualitative and variations in efficacy can therefore be corrected by changes in dosage, it would seem preferable to use a pure preparation of one of the penicillins rather than an amorphous mixture containing four active components with widely differing absorption and excretion properties and different bacteriological spectra, and being present in varying and

unknown proportions. Penicillin F, G and X lend themselves equally well to clinical use, whereas penicillin K is definitely inferior for the reasons given above. For practical purposes, however, penicillin G is the agent of choice because of the ease and relatively low cost with which it can be produced on a commercial scale. There certainly is no clearcut experimental evidence in animals or man to justify the use of an amorphous mixture of four penicillins. The shift to pure benzyl penicillin (penicillin G) will also obviate the use of microbiological potency units and permit their replacement by terms of weight (mg. of pure benzyl penicillin).

The early studies with crude penicillin had already shown that this antibiotic possessed a very low toxicity in spite of its high antibacterial potency (100). Chain (55) confirmed Fleming's original observation that a penicillin-containing broth or a concentrate prepared therefrom was no more toxic than the broth itself. A number of pharmacologic and toxicologic studies have been reported with the impure penicillin (1, 102, 153, 254). These need not be discussed in detail since the slight local and systemic effects observed are now known to have been caused by impurities.

The first pharmacological investigation of pure penicillin G was that reported by Van Dyke (324). This study, conducted in a small number of animals (because of the scarcity of the material), essentially confirmed the predictions made by earlier workers with regard to the nontoxicity of pure penicillin. Doses as high as 9.8 million units per kgm. or 5.93 gm. of pure penicillin G per kgm. were injected intravenously in mice without lasting harmful effects. During the first two weeks following injection there was a transient loss of weight and transient anemia. Single doses of 1,700,000 units per kgm. were without apparent effect on behavior, total white and red blood cell counts, hemoglobin content, and electrocardiographic pattern. Toxicity studies of pure penicillin G extending over a prolonged period of time in different species and with large numbers of animals on each dose level have not been reported in the literature, probably due to the fact that even the impure penicillin was practically nontoxic and that pure material was not available at the time when a study of the toxicity of penicillin was in the center of interest. Recent experiments with mice show the intravenous LD_{50} of pure penicillin G to be 3,500,000 units per kgm. body weight (171).

Although penicillin possesses an exceptionally low toxicity, the earlier observations by Hamre *et al.* (123) and Heilman and Herrell (135) regarding a peculiar hypersensitivity of guinea pigs to penicillin have been confirmed with pure penicillin G (171, 245). There is at present no explanation for this; it is well known, however, that guinea pigs are hypersensitive to many drugs including other antibiotics such as Aureomycin (134), and it appears not unlikely that the low penicillin tolerance of this species belongs to the group of allergic phenomena.

Chronic toxicity: No animal studies have been reported in which pure penicillin G was administered over periods of several weeks to months. Van Dyke (324) gave a total dose of 1,320,000–1,870,000 units per kgm. of pure sodium penicillin G to mice during 7 days and found a transient loss of weight and a transient anemia. Kuna (171) administered to mice daily intravenous and sub-

cutaneous injections of 1,000,000 units and 2,500,000 units per kgm. respectively for six days. These doses were well tolerated; however, doses of 2,000,000 units administered intravenously or 5,000,000 units per kgm. administered subcutaneously for a period of 6 days caused a 40% mortality. That penicillin is extraordinarily well tolerated in man even in large doses and over a long period of time, is evident from numerous clinical reports in which the drug was given to patients with actinomycosis or bacterial endocarditis in extremely large doses. Up to 2 million units of penicillin per day for a period of 273 consecutive days were administered to patients with endocarditis (236), and a total of 644,480,000 units for a period of 67 consecutive days with daily doses of 30 to 40 million units in a case of abdominal actinomycosis has been reported by Sanford and Barnes (276).

Miscellaneous Pharmacological Effects: Circulatory System. High concentrations of pure penicillin G depress the amplitude of contraction of the frog heart (324). However, the concentrations required (100 mgm. %) are so hypertonic that it is possible that the changes observed may have been nonspecific. Anesthetized cats given single intravenous doses of 500,000 units per kgm. of pure penicillin G sodium show a moderate fall of carotid blood pressure and a slight stimulation of respiration (171).

Isolated Intestine. Addition of 5 to 100 mgm. of pure penicillin G sodium to a 20 cc. organ bath produces a slight to moderate relaxation of the isolated rabbit intestine (171). *Isolated Uterus.* Pure penicillin G sodium in a concentration of 1 part of penicillin in 546,000 parts of bath fluid caused a slight contraction of the isolated guinea pig's uterus (324). McCloskey and Smith (198), using amorphous penicillin, found that in guinea pigs 30,000 units had no appreciable effect on uterine activity. In guinea pigs sensitized with 300 to 1000 units and tested for uterine activity after one month's interval, some of the animals showed a positive response. In humans, it has been claimed that administration of penicillin during pregnancy may produce increased uterine activity and even abortion (174, 176). However, these results were obtained with impure penicillin and it is questionable whether the reported effects were due specifically to penicillin and not to a general hypersensitivity. *Neurotoxicity.* Johnson and Walker (163) have reported the occurrence of neurotoxic signs in man following intracisternal injection of 50,000 units of amorphous penicillin. The same group of workers (336) had shown that intracisternal, intraventricular or intracortical injection of 200 to 2000 units of penicillin in mice, cats and monkeys produces acute myoclonic convulsions and epileptiform seizures accompanied by changes in the electroencephalogram. These changes are not caused by the volume, concentration or hydrogen ion concentration of the injected penicillin solution and are not due to accidental impurities, since destruction of the antibiotic activity by penicillinase abolishes this effect. *Local Effects.* Fleming noted in his early studies that the amount of penicillin which completely inhibited the growth of staphylococci *in vitro* did not interfere with the function of the leukocytes. Reports by Abraham *et al.* (1) have shown that the leukocytes of man remain active for at least three hours in the presence of a 1:1000 dilution of penicillin. The experiments of

Medawar cited by the above authors indicate that crude penicillin at a concentration of 1:6000 is definitely toxic to fibroblasts of the embryonic chick heart; However, exposure to a concentration greater than 1:1600 for forty-eight hours was necessary before irreversible changes occurred. The studies reported by Herrell and Heilman (139) indicated that the presence of penicillin in amounts which would be antibacterial did not interfere with the migration or growth of tissue elements such as lymphocytes, fibroblasts or macrophages. Van Dyke (324) reported that crystalline sodium penicillin G was not hemolytic for rabbit erythrocytes suspended in isotonic sodium chloride solution; neither did a solution containing 3417 units per cc. (2.05 mgm.) interfere with the oxygen consumption of yeast cells, duck erythrocytes, or slices of rat liver.

Irritation and pain at the site of injection were common complaints following the administration of impure penicillin. Such material caused local irritation and even necrosis in animals (1, 142). These effects were largely due to impurities since more highly purified preparations produced appreciably fewer local reactions (117, 14). It is now commonly agreed that pure penicillin G sodium is practically free from such effects. As is to be expected, the degree of local irritation and pain depends to a large extent upon the concentration of solution and the speed of injection.

Absorption and Excretion. The initial studies on the absorption and excretion of penicillin were made by Abraham and his associates (1). The intravenous injection of penicillin immediately resulted in a serum concentration which inhibited bacterial growth; however, within two hours it fell to levels which could barely be detected. Penicillin could be demonstrated in the urine for approximately six hours. These studies were expanded by Rammelkamp and Keefer (248) and Dawson *et al.* (68), who observed that when penicillin in doses ranging from 5000 to 40,000 units per patient was injected intravenously, the concentration in the blood reached a high peak almost immediately after the injection. Thereafter the concentration decreased so rapidly that after several hours little, if any could be detected by the microbial assay. The peak concentration, as well as the duration of detectable levels, was found to depend upon the quantity of penicillin administered (84).

Upon intramuscular injection, the peak blood concentration is achieved more slowly and is somewhat longer sustained. An even lower and more sustained level follows subcutaneous injection. The rapid disappearance of penicillin from the plasma is coupled with its simultaneous appearance in the urine (245). Recently penicillin containing the radio-active isotope S^{35} has been prepared (264, 265) and with this material the fate of penicillin in the body has been followed. It was found that penicillin injected intramuscularly is not stored in the body but is quickly excreted, partly as penicillin and partly as penicillin-breakdown products (264). That penicillin is rapidly absorbed from the gastrointestinal tract was first shown in animals by Abraham (1) and in man by Rammelkamp and Keefer (248). Numerous investigators (12, 45, 67, 99, 262) have confirmed these results with purer preparations of amorphous penicillin as well as with pure penicillin G sodium: however, a considerable portion of penicillin is lost by the

oral route of administration, due to inactivation by the acidity of the stomach or by penicillinase-producing bacteria in the lower intestinal tract or by incomplete absorption. Using labelled penicillin, it was found that the amount of radioactivity in the gut could account for the penicillin not found in the urine (264). For these reasons it is generally agreed that to obtain blood levels by oral administration comparable to those following parenteral administration, it is necessary to give between five and six times the amount of penicillin. When penicillin was very scarce and expensive, its oral administration was not favored. There are now, however, no fundamental objections to oral penicillin therapy and the use of this mode of administration has recently gained greatly in favor (47, 85). The acid inactivation of orally administered penicillin can be reduced appreciably by simultaneous or preceding administration of alkaline buffers or certain proteinaceous substances such as skimmed milk powder, whey powder, etc. The quantity of penicillin excreted in the urine of dogs after repeated administration of such buffer agents was found to be 4 to 6 times greater than after a similar administration of penicillin in water (307).

Dosage Regimen and Pharmaceutical Forms: As pointed out earlier in this review, the maintenance of a constant bacteriostatic blood concentration of penicillin was regarded for a long time as one of the prerequisites of effective chemotherapy with this agent. As a result of numerous pharmacological and clinical investigations, however, some questions have arisen as to the scope of applicability of this concept (278). Although no one can deny that for effective chemotherapy it is necessary to obtain a certain minimal concentration of the agent in the blood and the tissues, no fixed minimum values for these concentrations can be set. Rather, they depend upon the type of infecting microorganism and the severity of the infection (virulence of microorganism; size of inoculum and site of infection). The desire to maintain high penicillin concentrations in the blood, together with the objection of patients and nursing staffs to repeated injections at short intervals, has led to numerous types of so-called "repository" forms which all have one objective in common: to release a more or less constant amount of penicillin from a large tissue depot into the general circulation. A very large number of such pharmaceutical forms has been developed, starting with the "Romansky-formula" (penicillin in peanut oil and wax emulsions (261)) and progressing to such preparations as procaine penicillin (268). Many of these preparations well serve the purpose for which they were designed, viz. the maintenance of a prolonged blood concentration with a single injection of the antibiotic. If these forms were as free from side reactions as is pure penicillin G sodium, their use would appear entirely justified. It has been found, however, that certain of these preparations are much more likely to produce local irritation as well as allergic reactions (178, 179) and the exertion of judgment in their use would seem advisable.

Another adjuvant to penicillin therapy which perhaps should be mentioned is the use of agents which compete with penicillin for the same renal exsecretory mechanism and, by thus retarding its excretion, prolong the presence in the blood. The best known among these are para-aminohippuric acid (26), Diodrast

(247), and Caronamide (4-carboxy-phenylmethanesulfonanilid) (23, 25). The last named substance has been used clinically with success (24, 288, 312). Although no major undesirable side effects follow its administration, the now plentiful supply of low-priced penicillin, as well as a certain reversal in the trend towards the necessity of maintenance of high blood concentrations, appears to have reduced the demand for agents of this type.

STREPTOMYCIN

Streptomycin, discovered in 1944 by Schatz, Bugie and Waksman (277, 330), is obtained from broth cultures of *Streptomyces griseus*. It is highly effective against gram-negative microorganisms and *Mycobacterium tuberculosis*. With the exception of its selective neurotoxic effect upon the eighth nerve, which imposes a definite limitation on the size of dose and length of treatment, it is devoid of serious toxic properties. It is poorly absorbed from the gastrointestinal tract; systemic effects are best obtained by parenteral administration.

Chemical and Physical Properties

Streptomycin is a tribasic compound with the formula $C_{21}H_{39}N_7O_{12}$ (Fig. 1). It contains two strongly basic guanido groups and a weakly basic methylamino group. Crystalline salts which have been prepared are the reineckate, the helianthate, the p-(2-hydroxy-1-naphthylazo)-benzenesulfonate, the sulfate, the hydrochloride and the calcium chloride complex. When heated with alkali, maltol is formed through a rearrangement mechanism and degradation.

On acid hydrolysis streptomycin yields streptidine and streptobiosamine. The structure of streptidine is that of inositol in which two hydroxyl groups are replaced by guanido groups. Streptobiosamine is a disaccharide consisting of N-methyl-l-glucosamine and a 4-methyl-l-tetrose in which the hydrogen at the 3 position is replaced with a formyl group.

Early lots of streptomycin contained varying impurities which considerably obscured the pharmacological and clinical analysis. Foremost among them was a substance closely related to histamine (208, 259), if not histamine itself (290), which in certain lots was present in amounts corresponding to 0.006 mgm. histamine hydrochloride per mgm. streptomycin base. It accounted for such side effects as nausea, vomiting, headache, flushing of the face, etc. Streptomycin of intermediate purity also was frequently contaminated with bacterial pyrogens and other still unidentified impurities which were responsible for a rise of body temperature occurring only after administration of several doses and therefore differing from the usual pyrogen reaction. Varying signs and symptoms of allergic nature, such as pain in the joints and skin rashes were also observed as well as disturbances of hepatic and renal function (267); the latter consisted of proteinuria, cylindruria, hemoglobinuria and hematuria. With the exception of the effect caused by hypersensitivity to streptomycin itself, these side effects have largely disappeared with the use of pure streptomycin preparations and need not be considered further.

In addition to side effects caused by impurities chemically unrelated to strep-

tomycin, some pharmacodynamic effects generally regarded as specific for the streptomycin molecule were exhibited to a larger and more varying degree by the earlier samples of low and intermediate potency. Foremost among them were the acute and delayed effects upon the vestibular and auditory centers. It has now been established that the acute neurotoxicity, produced by intracisternal or intrathecal injection, is due to the guanidine content of the streptomycin molecule (172). Since streptomycin of low and intermediate purity may contain a higher amount of guanidine in the form of degradation products, the sum total of the bound and the free guanidine in material of that type determines the ultimate degree of acute neurotoxicity. Guanidine, however, is not responsible for the delayed neurotoxic effects which are encountered upon prolonged administration of the antibiotic. This type of neurotoxicity is caused by the streptidine portion of the streptomycin molecule (210). Since streptidine possesses practically no antibiotic activity and cannot be detected by the microbiological and chemical assays now generally used, it is easily understandable that streptomycin lots containing varying amounts of streptidine do not necessarily produce the same degree of clinical neurotoxicity, even though the same doses of streptomycin base may have been administered. It has been found (209) that the acute and the delayed neurotoxic effects of streptomycin samples of intermediate purity run practically parallel; any lot which exhibits a high degree of acute neurotoxicity is therefore likely to be undesirable for clinical use.

The now general use of pure streptomycin in the form of its hydrochloride, sulfate, and calcium chloride complex has largely eliminated lot to lot variations; differences in toxic side effects now observed must be attributed to variations in patient sensitivity. However, in contrast to penicillin, even pure streptomycin possesses marked pharmacodynamic properties which will be described in the following.

Acute Toxicity: The acute toxicity of streptomycin is most pronounced with intravenous and intracisternal injection; next in order is the toxicity exhibited after intramuscular and subcutaneous injections; and least, due to poor absorption from the gastrointestinal tract, is the oral toxicity. Intracisternal injections elicit clonic-tonic convulsions (215); these acute neurotoxic manifestations are not observed when other routes of injection are employed. Death following the injection of a lethal dose of streptomycin is due to respiratory paralysis since the heart continues to beat for some time after respiration has come to a standstill. Otherwise fatal doses may be survived if artificial respiration is instituted and is maintained long enough to permit the elimination of a substantial portion of the injected streptomycin. The approximate ratio of intravenous to subcutaneous to oral toxicity is 17:4:1. The acute toxicity following intracisternal injection is much higher, the LD_{50} being only about one tenth that of the intravenous LD_{50}. Frogs, not depending solely upon pulmonary respiration, tolerate doses of streptomycin injected in the abdominal lymph sac about 15 times larger than those tolerated by mice, provided they are kept partially submerged in frequently changed water. During the state of poisoning they exhibit complete motor paralysis and cessation of all respiratory movements.

The oral toxicity of streptomycin is very low, due to poor absorption from the gastrointestinal tract. Nevertheless, and contrary to statements found in the literature, it is possible to produce the typical neurotoxic signs of streptomycin poisoning as well as death by administration of excessively large doses. Thus, of 4 cats fed daily doses of 1 gm. per kgm. of streptomycin calcium chloride complex, 2 developed signs of neurotoxicity on the 9th and 14th day, respectively. Pathological examination revealed the presence of multiple ulcers in the stomach Microscopically the ulcerations were found to extend to, and occasionally penetrate, the muscularis mucosae. The kidneys were markedly congested at the corticomedullary junction and contained hyaline casts in the collecting tubules. Two cats fed 2 gm. per kgm. developed neurotoxic signs on the 12th and 19th day, respectively (171). Neither animal showed gross pathologic changes in the tissues or organs.

After the intravenous or intraperitoneal injection of a lethal dose of streptomycin, death usually occurs within 5 minutes. The interval between injection and appearance of toxic signs is longer with the intramuscular and subcutaneous routes, but even here it rarely exceeds 30 minutes. On the basis of an exhaustive statistical analysis it was found (221) that of 10,000 mice surviving the initial 30 minutes after intravenous injection of a median lethal dose, only 10 died during the following two days. No deaths occurred among a randomly selected group of 2500 of these surviving mice which were observed for 10 days after the injection. The intravenous LD_{50} varies within very narrow limits, provided that uniform test conditions are maintained and, more particularly, that the speed of the injection is kept constant. However, any direct relationship of the intravenous LD_{50} of a particular streptomycin lot to the degree of its clinical tolerance is questionable. There are indications, nevertheless that many streptomycin lots of intermediate purity which have a high intravenous toxicity are less well tolerated by patients (207). For this reason the determination of the intravenous toxicity in mice, as required by the Food and Drug Administration, seems justified, at least for material not having the highest degree of purity.

The sensitivity of different animal species to an intravenous injection of streptomycin varies considerably. Frogs appear to be the least sensitive and monkeys and dogs the most sensitive, with cats, rabbits, guinea pigs, rats and mice ranging in between. Cats and monkeys are most susceptible to the neurotoxic effects. The sensitivity of man cannot be stated, for lack of experimental data. There appear to be considerable individual variations; generally, on a body weight ratio, man seems to be more sensitive than any of the experimental animals (89, 104).

In animals, the signs of acute streptomycin poisoning consist of restlessness, impairment of respiration, loss of balance and, occasionally, convulsions and coma. Larger animals, such as monkeys, dogs and cats, may also exhibit nausea and vomiting. Pigeons injected with 100 to 300 mgm. of streptomycin base per kgm. body weight exhibit stupor and flaccid paralysis, occasionally preceded by brief convulsions. The birds may fully recover the following day (127).

Chronic Toxicity: Determination of the chronic toxicity is of far greater prac-

tical importance than that of the acute toxicity because the clinical use of streptomycin usually extends over a period of many weeks, particularly in the treatment of tuberculosis. The only long range toxicity studies carried out with pure streptomycin are those of Mushett and Silber (216). All other chronic toxicity studies reported in the literature were carried out with streptomycin of intermediate purity and the interpretation of these experiments is therefore subject to the limitations arising from the use of such material.

Doses up to 100 mgm. streptomycin base (in the form of an impure preparation of the hydrochloride) per kgm. body weight were injected daily into rats for several weeks without other untoward effects than irritation at the site of injection. Doses of 200 mgm. of streptomycin base (in the form of the pure calcium chloride complex) per kgm. body weight injected for 42 days failed to elicit signs of chronic neurotoxicity. However, 600 mgm. of the same material per kgm. body weight caused neurotoxic signs within an average of 17 days (171). Up to 300 mgm. of streptomycin base (in the form of an impure hydrochloride) per kgm. body weight, incorporated in the diet, were fed for 3 months to rats without adverse effects upon the health and gain of weight. Dogs and monkeys tolerated repeated daily injections of 300 mgm. streptomycin base (in the form of the pure calcium chloride complex) per kgm. body weight without development of the biochemical and pathologic changes which had been previously observed by the same authors when they used even smaller doses of a somewhat impure preparation of streptomycin hydrochloride (215, 290). In the course of neurotoxicity studies, cats were given daily injections of 100 mgm. of streptomycin base (in the form of its pure calcium chloride complex) per kgm. body weight for 2 months without development of pathologic signs other than those of the typical neurotoxicity. In monkeys, the biochemical and pathologic changes in kidneys and liver resulting from prolonged administration of impure streptomycin preparations are reversible if the drug is discontinued before irreparable anatomical damage has been inflicted (215, 290). Pigeons injected intramuscularly with 100 and 200 mgm. of streptomycin base (in the form of the pure calcium chloride complex) per kgm. body weight develop after 24 and 19 days, respectively, a slight tremor of the head, unsteady gait, and loss of ability to perch on a rod and to fly (127).

Circulatory Effects: The histamine-like effects of the early impure streptomycin preparations were due solely to a contaminant of histamine-like nature (208). Even pure streptomycin, however, causes a gradual fall of the arterial blood pressure when given in large doses (100 to 200 mgm. streptomycin base per kgm. body weight). With doses of 200 to 400 mgm. per kgm. this effect usually is irreversible. The blood pressure drops as low as 10 to 15 mm. Hg; if artificial respiration is maintained until the excess of streptomycin has been excreted, the blood pressure remains at this low level for a long time and gradually returns to normal. If death occurs despite artificial respiration, it is probably caused by a paralysis of the vasomotor centers which then fail to respond to stimulation with picrotoxin, metrazol, diparcol or increased carbon dioxide tension. Electrocardiograms taken at frequent intervals following the injection of very large doses of streptomycin show no significant changes.

In the isolated frog heart, 1.25 mgm. of streptomycin calcium chloride complex per cc. of Ringer solution moderately decreases the amplitude of the auricular and ventricular contraction. The rate of perfusion through the isolated frog leg and the isolated rabbit's ear is not influenced by 0.1 cc. of a 10% streptomycin solution injected into the tubing.

Respiratory Effects: Small doses of pure streptomycin (10 to 20 mgm. base per kgm. cat) may increase the rate and amplitude of respiration; large doses (150 mgm. per kgm.), when injected intravenously, depress the respiration to the point of complete paralysis. Respiratory depression is one of the first signs of streptomycin poisoning and is the immediate cause of death in acute toxicity experiments. Animals can be revived through artificial respiration.

Renal and Hepatic Effects: As mentioned earlier, the renotoxic and hepatotoxic effects observed with impure preparations of streptomycin fail to occur when pure material is used. It may therefore be concluded that pure streptomycin is essentially free from this type of toxicity. Repeated subcutaneous injection of pure streptomycin produces acidophilic colloid-like droplets in the cytoplasm of the renal tubular epithelium in dogs and rats (214). Such animals, however, show no abnormalities in renal function and these cytoplasmic inclusions may therefore be regarded as an interesting histochemical phenomenon without clinical significance. These droplets appear within one week after the daily injection of 400 mgm. streptomycin base per kgm. body weight and gradually decrease in size, after cessation of administration, to disappear completely within 4 weeks.

Neurotropic Effects: The neurotropic effects of streptomycin, first described by Hinshaw and Feldman (143) in man and by Molitor *et al.* (208) in animals, constitute the most characteristic pharmacodynamic property of this antibiotic. Since they occur with even the purest material they must be regarded as an intrinsic property of streptomycin. These effects may be produced in animals by repeated subcutaneous, intramuscular or intravenous injections of from 50 to 400 mgm. of streptomycin base per kgm. body weight. In cats they have been observed even after administration by mouth; the doses required to produce neurotoxic signs by this route, however, were extremely large (1 to 2 gm. per kgm. for 2 to 3 weeks). The time needed to develop the characteristic neurotoxic disturbance varies with the size of the individual daily dose, with the total amount given over the entire experiment, with the conditions of the test (high temperature and humidity appear to shorten the time) and, perhaps most important, with the species of animal. Cats, dogs and monkeys appear to be the most sensitive; rabbits, guinea pigs, rats and mice, the least. The apparent differences in sensitivity to the neurotropic effects may be due, in part, to the habits of locomotion of the species used, which renders the effects more easily detectable in some species than in others. Thus, it has been reported that in mice and rats which normally fail to exhibit vestibular disturbances, these can be made manifest through certain experimental conditions such as swimming (53) or walking a tight-rope (192). In animals, the signs of characteristic streptomycin poisoning consist of changes in gait and posture; ataxia, at first of the hindlimbs but later also of the forelimbs; and a progressive loss of rotational nystagmus, affecting first the post-rotational and later the pre-rotational response (128, 130).

In man, the inability to keep the eyes focused on a given spot causes difficulties in reading. This symptom was first erroneously interpreted as a toxic effect of streptomycin upon the optic nerve. It is now generally recognized, however, that streptomycin does not directly affect the optic nerve and that any impairment of vision is due indirectly to the vestibular disturbance. In addition to the peripheral vestibular functions, streptomycin also affects certain closely related central cerebellar and oculomotor functions. The ataxia and other manifestations of vestibular dysfunction gradually disappear after withdrawal of the drug (326). However, recovery may be incomplete and the time required for full recovery is as long as 12 to 18 months.

The dose required to affect the hearing in animals and man is considerably larger than that needed to produce the vestibular disturbance. First affected is perception of the high tonal frequencies; the intermediate frequencies are affected later. The ability to perceive speech thus may still be normal at a time when a serious disturbance in the other frequency ranges already exists. Application of exact audiometric tests thus becomes a necessity if the early stages of the toxic effects of streptomycin upon auditory function are not to be missed.

Guinea pigs receiving daily for approximately 2½ months 300 mgm. streptomycin base per kgm. body weight by subcutaneous injection (266) showed loss of hearing in the high frequencies when tested by Preyer's reflex, an impairment of postural reflexes and a loss of postrotational nystagmus. Histological examination of the inner ear, labyrinth and central nervous system failed to reveal damage of the organ of Corti, the nerve fibers and the peripheral parts of the vestibular apparatus, but showed very distinct changes in the triangular nucleus and, to a lesser degree, in the ventral nucleus of the eighth nerve. In mice, the cause of vestibular trouble is a change in the sensory statokinetic terminals. The possibility of a simultaneous central lesion cannot be excluded, but it may be the effect of secondary degeneration (52).

Stevenson *et al.* (311) observed in dogs treated for 28 days with 170 mgm. of streptomycin per kgm. a liquefaction necrosis in the ventral cochlear nuclei and a clumping of Nissl-like material in the neurons of these nuclei. These authors found similar changes in tuberculous patients who had died after prolonged streptomycin treatment. Using microspectrographical methods, Floberg *et al.* (101) found in guinea pigs injected with 75 mgm. of streptomycin per kgm., given daily in three equally divided doses up to 28 days, that the nucleoprotein content in the cytoplasm of the nerve cells of the vestibular ganglion and Deiter's nucleus decreased and that this phenomenon was correlated with the cessation of vestibular function. The effect was confined to the neurons of the vestibular pathway; no changes were observed in the Purkinje cells of the cerebellum, in the cells belonging to the hypoglossal nucleus, or in those of the inferior olivary body.

Miscellaneous Effects: Smooth muscle: In concentrations of 25 to 50 mgm. % streptomycin produces a slight relaxation of the isolated uterine and intestinal muscle.

Gastric secretion: Pure streptomycin counteracts the stimulating effects of

histamine on the secretion of hydrochloric acid. It does not significantly affect the secretion of bile and pancreatic juice (66).

Local effects: The local effects of streptomycin greatly depend upon the purity of the preparation and the concentration of the solution; to a lesser extent they seem to be affected by the type of salt used. Pure streptomycin is relatively free from local irritation when injected in concentrations not exceeding 20%. Heilman (131) and Bucher (43) found that streptomycin possesses a rather low degree of cytotoxicity although it exceeds that of penicillin. Howes (159) investigated the effect of streptomycin on wound healing in animals and in patients. The epithelialization was retarded by streptomycin concentrations in excess of 0.4%; a concentration of 0.2%, however, proved to be of definite value in promoting the healing of contaminated wounds. Experiments performed by Mushett (213) with streptomycin of much greater purity not only confirmed these results, but indicated an even accelerated rate of wound healing when a concentration of 0.75% of pure streptomycin was applied.

Effect of Hematopoietic System: In animals no significant changes of the red cell, white cell, and eosinophil counts and the blood picture have been reported, even after prolonged administration of large doses of streptomycin, except for a slight normocytic anemia. In man, however, numerous authors (5, 50, 69, 289) have observed the development of eosinophilia, occasionally as high as 50% but usually below 15%. The eosinophilia may disappear after administration of antihistaminics and in general the eosinophil count returns to normal after cessation of therapy. It would thus appear that the eosinophilia is part of an allergic response to streptomycin since many of these patients also developed other allergic signs such as skin rashes, maculopapular erruptions and, in isolated instances, exfoliative dermatitis (15).

Skin reactions also occur not infrequently as an "occupational disease" in personnel handling streptomycin, such as physicians, pharmacists, nurses, operators in pharmaceutical plants, etc. The testing of such people at regular intervals for intradermal sensitivity to 0.1 mgm. streptomycin has been recommended since a positive skin test precedes by some time the appearance of gross signs of streptomycin sensitization (325). Side reactions such as swelling and pain in the joints, more frequently observed with the less pure lots of streptomycin, may also be regarded as allergic phenomena.

The reports on the effect of streptomycin on blood coagulation are contradictory. Macht (191) states that "next to the chemotherapeutic properties of penicillin and streptomycin and their low toxicity, the most important pharmacologic finding is their thromboplastic activity. . . ." Overman and Wright (222) on the other hand, report that streptomycin prolongs the prothrombin and the coagulation times. Since despite the wide use of streptomycin no other reports have appeared in the literature, it seems justifiable to assume that the effects on blood coagulation are not sufficiently marked to cause serious concern.

Prolonged oral administration of streptomycin might interfere with the synthesis of vitamins in the intestinal tract, particularly that of vitamin K. Smith and Robinson (297) have shown that the addition of streptomycin to the diet of

mice, in amounts equivalent to a daily intake of 30 to 300 mgm. of streptomycin base per kgm. body weight, reduced the coliform count within 24 hours from a normal of approximately 100,000 to one of 100 per 3 mgm. of feces, and that this low level was maintained throughout the period of treatment. A significant reduction also occurred in the number of nonlactose fermenting organisms. Although no signs of an overt vitamin deficiency were observed in these animals when they were on a nutritionally adequate diet, Emerson and Smith (93) found signs of a vitamin deficiency similar to that of biotin deficiency in rats on a purified diet containing streptomycin equivalent to a daily intake of from 580 to 875 mgm. of streptomycin base per kgm. body weight. No changes in prothrombin time were noted, however. A slight increase in prothrombin time has been observed in rats after two to three weeks of daily oral administration of 2 gm. per kgm. of streptomycin calcium chloride complex. Although this effect may have been due principally to the inhibition of the intestinal microflora which synthesizes vitamin K, the possibility exists that sufficient drug was absorbed through the damaged gastrointestinal tract to exert a more direct effect upon prothrombin. Ravdin *et al.* (250) observed that patients on a diet low in vitamin K and given streptomycin by mouth develop, within 4 to 5 days, a definite prolongation of prothrombin time which returned to normal after discontinuation of streptomycin therapy and which could be counteracted by the administration of vitamin K or by blood transfusion.

Absorption and Excretion

Methods of determination: Several methods are available to determine the concentration of streptomycin in body fluids and tissues; their advantages and disadvantages are briefly discussed in the following section.

A. Microbiological methods: These methods are based on the inhibition of standard strains of test organisms (the most frequently used are *Staphylococcus aureus* (308) and *Klebsiella pneumoniae* (6, 71)) in solid or liquid media. The degree of inhibitory activity is estimated in the case of solid media by the diameter of the growth free zone around a paper disc saturated with the antibiotic (190), or around a cup filled with a solution of the antibiotic (327). In liquid media, the degree of turbidity caused by growth of the test organism in serial dilutions of the antibiotic is measured photoelectrically. Both technics are about equally sensitive, permitting the detection of one microgram per cc. with an accuracy of approximately $\pm$ 10%. There are, however, many factors which influence the outcome of a microbiological assay and unless all conditions are strictly standardized, far greater variations may occur (occasionally unknown to the investigator).

B. Chemical methods: The first procedure described for the chemical determination of streptomycin was that by Scudi *et al.* (284). This method gave reasonably good values with preparations of high purity, but with material of intermediate purity the potency values were too high, at times exceeding 2 to 4 times those obtained by microbiological assay. The first satisfactory method devised was that by Boxer *et al.* (32) which was based upon alkaline degradation

of the streptomycin molecule to maltol followed by colorimetric determination with ferric ammonium sulfate. This method is specific for streptomycin but not sufficiently sensitive for use in most body fluids. Another much more sensitive method is that of Boxer and Jelinek (30) based on the formation of a fluorescent hydrazone of streptomycin with a 9-hydrazine-acridine hydrochloride. Another colorimetric method was described by Marshall (196) which is based on the formation of a colored semi-carbazone by reaction of streptomycin with a highly colored semicarbazide. Both methods have the same degree of specificity, the fluorometric method being about 5 to 10 times more sensitive, while the colorimetric method, although less sensitive, is more rapid and does not require a fluorophotometer. Generally, chemical assay methods are preferable to microbiological ones because of greater accuracy and specificity; they require, however, more elaborate equipment. The chemical methods of assay were not available during the first years of streptomycin research when many of the studies quoted below were carried out; even today, microbiological assay methods are still widely used despite their lesser accuracy and greater variability. In the case of dihydrostreptomycin, these methods have to be resorted to, since no chemical method for its determination is as yet available. Rake and Donovick (238) have drawn attention to the wide divergence in published statements on the urinary recovery of streptomycin in man. Much of this is undoubtedly due to differences in the assay methods and technics. The accuracy depends largely on the number of replicate assays, the quality of the technic, the accuracy of the standard, the type of sample assayed and the test organism used (it should be a bacterium not affected by serum or other body fluids (282)).

It is also important to know whether the figures obtained in a particular investigation refer to values on whole blood or in serum. Streptomycin does not penetrate the erythrocytes (30, 195) so that concentration in the serum usually is about twice that in the whole blood. Following the parenteral injection, the drug concentration in the serum reaches a peak shortly after the injection and thereafter decreases at a uniform and relatively rapid rate (2, 44, 132, 252, 255, 306, 359). With large doses the concentration remains for a longer time at high levels; the rate of decrease is proportional to the concentration of the drug in the blood (33). Within the dose range of 4 to 20 mgm. per kgm. the concentrations were found to be proportional to the dose per kgm. body weight; below this range, the blood levels were erratic and unpredictable. High blood concentrations are obtained most rapidly by the intravenous route; next in order are the intramuscular and subcutaneous modes of administration. Marshall has shown that in dogs as well as in man the plasma concentration obtained within 1 hour after intramuscular injection of streptomycin closely approximates that obtained from intravenous injection of the same dose, indicating a rapid absorption from the muscles. Administration by continuous intravenous or intramuscular drip had been recommended in the early days of the clinical use of streptomycin; this method, however, is no longer used, not only because of the inconvenience to the patient but primarily because it is now realized that a constant high blood concentration is unnecessary (360), particularly for the treatment of tuberculous

infections. If desired, however, it is possible to maintain therapeutic blood concentrations by repeated parenteral administration. In man, the injection of 2 to 3 grams per patient results in blood concentrations between 20 and 60 micrograms per cc. (359). There are considerable individual variations in the rapidity with which high blood concentrations may be built up, depending largely upon the function of the excretory organs (4, 44).

The absorption of streptomycin from the gastrointestinal tract is much slower, but even with this mode of administration the blood concentrations produced are sufficient to protect animals against systemic infections as well as to elicit the typical neurotoxic signs. Very large oral doses are fatal, but there is some doubt as to the specificity of the cause of death under such conditions. The dosage ratio for comparable therapeutic results parenterally and orally is approximately 1 to 20. In view of the limited and erratic absorption from the gastrointestinal tract, oral administration has not been considered a practical means of streptomycin therapy, except when it is desired to obtain high concentrations in the gastrointestinal tract for the purpose of reducing the number of pathogenic bacteria. The effectiveness of streptomycin under these conditions has been demonstrated in animals (93, 297) as well as in man (250, 267).

Edison and Kuna (173) have observed considerable differences in the rate of absorption of the various salts of streptomycin and dihydrostreptomycin from the gastrointestinal tract, the sulfates being much more slowly absorbed than either the hydrochloride or the calcium chloride complex. In mice, the LD_{50} after oral administration of various streptomycin salts is directly proportional to the rate of absorption and the concentrations demonstrable in the blood. Absorption after rectal administration is even less than after administration by mouth.

Administration of streptomycin by inhalation through nebulization in an aerosol has been tried (13, 132) but this method has generally been abandoned. Local irritation as well as a high incidence of allergic reactions were observed, and the therapeutic results, especially in pulmonary diseases and tuberculous infections of the nasopharynx and larynx, were unsatisfactory unless streptomycin was given simultaneously by the parenteral route. Several clinicians, particularly in Europe, have recommended the instillation or injection of streptomycin into tuberculous cavities, cysts, and similar foci of infection which, because of mechanical obstacles (scar tissue, etc.), are not readily accessible to parenterally injected streptomycin. Various technics have been developed, such as the direct transpleural injection (314) and instillation through a catheter (197). In all cases, the local application of streptomycin is only an adjunct to systemic therapy and its principal objective is the sterilization of a tuberculous focus of infection as a preliminary step to thoracic surgery.

Streptomycin penetrates the blood-brain barrier to a very limited degree. With doses of 1 to 3 gm. per patient per day, given by repeated intramuscular injection, the streptomycin concentration in the cerebrospinal fluid ranged between 1 to 5 micrograms per cc., while in the same patients the blood concentrations were 12 to 27 micrograms per cc. (359). Heilman *et al.* (132) state that in the presence of meningitis large doses given by the usual parenteral routes re-

sulted in cerebrospinal fluid concentrations approximately one fifth of those in the blood serum of the same patients. However, in patients not suffering from meningitis the diffustion of streptomycin into the cerebrospinal fluid does not take place readily.

In cats, Hawkins *et al.* (130) reported the following average concentrations in blood and cerebrospinal fluid after parenteral injection of 400 mgm. per kgm.: After 2 hours: in the plasma, 517 to 874 micrograms per cc.; in the cerebrospinal fluid, from traces to 5 micrograms per cc.; after 24 hours: in the plasma, from 21 to 116 micrograms per cc.; in the cerebrospinal fluid, from traces to 35 micrograms per cc. If chemotherapeutically effective streptomycin concentrations are to be obtained in the cerebrospinal fluid, it is evident that the antibiotic must be injected intrathecally, intracisternally or by puncture of the suboccipital space. If given in this manner, high concentrations which persist for a considerable time may be attained (44). Pure streptomycin given intrathecally in therapeutic doses is tolerated without serious side effects, but preparations of intermediate purity have frequently been reported to cause marked irritation of the meninges, which occasionally leads to mechanical obstruction of the circulation and to the development of acute hydrocephalus (11, 28, 46, 95, 211, 224).

Streptomycin enters the fetal circulation and amniotic fluid (132, 344, 359). The concentration of streptomycin in the blood of the umbilical cord varies, depending upon the dose and the time which elapses between the last injection and birth. Watson and Stow (337) reported that daily administration of 2 gm. of streptomycin for over 90 days to 2 patients in the second trimester of pregnancy, who suffered from rapidly progressing far-advanced hematogenous pulmonary tuberculosis, had no ill effect on the infants; in particular, they developed no signs of neurotoxicity.

Distribution in body tissues: The distribution of streptomycin in body tissues and fluids has been determined by numerous investigators using the microbial and the chemical methods of assay. Most of these studies have been performed with the microbial method of assay and only relatively few observations are on record in which the more accurate chemical methods of determination were employed (33, 195). Adcock and Hettig (2) tested tissues from two patients who had died from tuberculous meningitis and found the highest concentrations of streptomycin in the kidneys (20 to 94 units per gm.), much lower concentrations in the lung and heart muscle (1 to 6 units per gm.) and none in brain and liver. In another postmortem study in man, Pulaski and Sprinz (237) reported significant amounts in the kidney, liver, muscle and thyroid, but none in lymph nodes, spleen, testes, lung or brain; pus taken from abscesses was also negative. Thirty minutes after intramuscular injection of 10 mgm. streptomycin base per kgm. rabbit, detectable levels were found in the conjunctiva, the extra-ocular muscle and the sclera (177). In the chorioretinal layers, the optic nerve and the cornea, low concentrations were found 2 hours after an injection of 100 mgm. per kgm.; the lens showed no streptomycin even after this large dose. The same authors studied in rabbits the penetration of streptomycin into the aqueous and vitreous humor after parenteral administration. Following an intravenous injec-

tion of 10 to 100 mgm. per kgm., streptomycin appeared in the aqueous humor within 5 minutes and was detectable up to 5 hours after injection, depending upon the dose; only low levels, however, were obtained in the vitreous humor. These findings were confirmed in patients with glaucoma: intravenous injection of 600 mgm. streptomycin base per patient resulted in concentrations of 3 to 19 micrograms per cc. in the aqueous humor, at blood levels ranging from 40 to 75 micrograms per cc.

Streptomycin diffuses readily from the blood into the peritoneal fluid, particularly when pathologic processes are present (44, 212, 359). The penetration into the pleural fluid is slower, but eventually reaches concentrations one fourth to one half of those in the serum. Hawkins, Boxer and Jelinek (129) used the hydrazine method to determine the streptomycin concentrations of blood, cerebrospinal fluid, brain, liver, lung and spleen in cats sacrificed 24, 48 and 72 hours, respectively, after a single dose of 400 mgm. streptomycin per kgm. At 24 hours, the blood concentration was too high for the tissue levels to be meaningful. At 48 and 72 hours, when the blood and cerebrospinal fluid values had fallen to 1 to 2 micrograms per cc., the concentration in the viscera was from 9 to 38 micrograms per gm. In a second series, cats were given 100 mgm. per kgm. for 7 to 28 days and sacrificed 24 hours after the last dose. The following concentrations were found: lung, 7 to 32 micrograms per gm.; liver, 10 to 41 micrograms per gm.; spleen, 12 to 22 micrograms per gm.; kidney, 119 to 234 micrograms per gm.; urine, 21 to 360 micrograms per cc.; plasma, 1 to 3 micrograms per cc.; cerebrospinal fluid, 1 to 3 micrograms per cc.; tissues of the central nervous system, 0 to 2 micrograms per gm. With the exception of one, all animals had developed marked signs of neurotoxicity. From these data it is evident that the neurotoxic manifestations are not due to an accumulation of the drug in the tissues of the central nervous system.

Excretion: The largest part of streptomycin administered by the parenteral route is excreted through the kidneys. The values reported by various authors in animals (32, 95, 132, 195) and in man 2, 44), obtained by the use of either the microbiological or chemical methods of assay, are in fair agreement. In the dog, up to 80% is excreted in the urine; in man, up to 90%, with most values reported between 50 and 60%. Renal clearance in both dog and man is approximately 70% of the simultaneously determined glomerular filtration rate (33), a fact which indicated that protein binding may render a substantial part of the streptomycin present in the plasma unavailable for filtration through the glomerulus. Boxer, Jelinek and Edison (31) investigated this possibility in dogs, using two different approaches: (a) measurement of the distribution of streptomycin on both sides of a cellophane membrane after dialysis of plasma against a Ringer solution containing varying amounts of the antibiotic; and (b) determination of the streptomycin concentration in the ultrafiltrate of plasma containing varying amounts of the drug. The values obtained by both procedures were in good agreement and indicated that 33 to 35% of streptomycin is bound to serum protein (albumins and globulins). Wide variations in urine flow and in the concen-

tration of the drug in the plasma did not influence renal clearance, thus making tubular reabsorption unlikely. The reported values of clearance per cc. of plasma per minute varied from 34 to 59 in the dog and from 30 to 80 in man (2, 195). The rate of disappearance of streptomycin from the blood is similar in anesthetized and non-anesthetized dogs (120). Small amounts of streptomycin are excreted in the feces following parenteral administration (92, 252). The question regarding the occasional low recovery values of streptomycin from the urine has not yet been answered satisfactorily. There is no evidence that the antibiotic is destroyed or inactivated in the body; it does accumulate in certain body tissues particularly in the kidneys, but it seems doubtful whether the amount of streptomycin thus bound could be high enough to account for the differences between administered and excreted drug. After peroral administration, streptomycin is primarily excreted through the feces and only small amounts, representing the absorbed portion, are found in the urine. Considerable amounts of parenterally administered streptomycin are excreted in the bile of animals (122, 306) and man (237, 356, 357, 358).

MANNOSIDO STREPTOMYCIN (STREPTOMYCIN B)

The evaluation of results obtained with streptomycin preparations of intermediate purity is still further complicated by the fact that such material may contain another entity of streptomycin with a microbiological spectrum differing from that of streptomycin. Fried and Titus (106, 107) have isolated from streptomycin concentrates a fraction which they named streptomycin B (mannosido streptomycin) and which differs chemically from streptomycin A, the entity commonly referred to as streptomycin. When tested *in vitro* in a yeast-beef broth, streptomycin B had only 14 to 93% of the chemotherapeutic activity of streptomycin A, depending upon the test organism used (243). A similar difference in activity was found in experimental infections in mice. Hobby and Lenert (148) have reported that certain residues obtained in the preparation of crystalline salts of streptomycin are from 2 to 5 times more active than highly purified salts of streptomycin sulfate. Since streptomycin B is not precipitated as a calcium double salt and may not be accounted for in the assay procedures, it may be present in such residues as described by Hobby and Lenert and may thus account for the reportedly greater activity of a streptomycin concentrate over that of either pure streptomycin A or streptomycin B. The unitage of mannosido streptomycin as determined by chemical assay is approximately 4 times greater than when determined microbiologically. The pharmacological and toxicological properties of streptomycin B are very similar to those of streptomycin A. Streptomycin B does not cause delayed deaths in animals and hence is not related to streptothricin.

Confirming the findings of Rake *et al.* (242) on the pharmacological and toxicological properties of mannosido streptomycin, Kuna and Cuchie (171a) have shown that the toxicity and other pharmacological properties of streptomycin B correspond to the streptomycin potency determined by the chemical

assay, whereas the delayed neurotoxic effects are comparable to those of an equal dose of streptomycin A when administration is made on the basis of microbiological units.

DIHYDROSTREPTOMYCIN

Dihydrostreptomycin is obtained by catalytic hydrogenation of streptomycin (19, 108, 230). It is not inactivated by cysteine, hydroxylamine, semicarbazide or treatment with mild alkali and is therefore more stable than streptomycin. Its bacteriological spectrum is very similar to that of streptomycin. Bacterial strains which have developed resistance to streptomycin are also resistant to dihydrostreptomycin and vice versa. The biological properties of dihydrostreptomycin closely resemble those of the parent compound. Only with *in vitro* tests (particularly with members of the Salmonella group) have any significant differences been found between the two antibiotics. *In vivo*, both compounds appear to have the same order of antibacterial activity. By the use of a highly standardized tuberculosis infection in mice, no significant differences were found by Rake *et al.* (244) when the variations between dose increments were 50%. Feldman and his associates (96) found dihydrostreptomycin as effective as streptomycin in experimental tuberculosis in guinea pigs. Clinical investigations comparing the efficacy of the two antibiotics in tuberculous patients have yielded generally comparable results (155, 144).

The acutely toxic dose of dihydrostreptomycin in mice was found to be 5 to 5.5 mgm. per mouse compared to 4 to 4.5 mgm. for streptomycin. Shock began to appear at a dose level of 3 mgm. of dihydrostreptomycin, compared to 2.5 mgm. of streptomycin. All deaths occurred within one hour after injection, and the weight gain and food consumption of the treated animals which survived revealed no difference between both groups during the ensuing 21 days. There were no pathological findings at autopsy in either group (244). Dogs and monkeys receiving subcutaneously 200 mgm. streptomycin base per kgm. daily, divided into two equal doses, for 18 days (dogs) and 5 days (monkeys), showed no biochemical, hematological or pathological changes during the treatment, or at autopsy performed 12 days after the last injection (172). The only significant difference between streptomycin and dihydrostreptomycin is in their relative neurotoxicities. While qualitatively indistinguishable from each other, the neurotoxicity of dihydrostreptomycin when tested by the effect on the vestibular system is quantitatively approximately half of that of streptomycin. Numerous clinical reports have confirmed in man the markedly lower neurotoxicity of dihydrostreptomycin (70, 144, 155, 287). Recently Romansky (260) has reported a delayed neurotoxic effect of dihydrostreptomycin on the auditory system of patients, which in several instances was stated to have taken place several weeks after treatment had been discontinued; in a number of patients the neurotoxic effect was reported to have resulted in severe to complete loss of hearing. Apart from the observation of Allison, Volk and Vitagliano (5a), these results have not been confirmed by other reports.

Hawkins (126) in a careful investigation has failed to demonstrate in cats a

greater neurotoxicity of dihydrostreptomycin upon the auditory system. On the contrary, dihydrostreptomycin appeared in his experiments to affect the auditory system to a lesser degree than did streptomycin; this result parallels his findings concerning the neurotoxicity of dihydrostreptomycin for the vestibular system.

The clinical dosage schedule recommended for dihydrostreptomycin is very similar to that for streptomycin. Parenteral administration is essential if effective concentrations in blood and tissues are to be obtained. Dihydrostreptomycin is absorbed from the gastrointestinal tract to a lesser degree than streptomycin, and differences in the absorption of different salts of dihydrostreptomycin are far more pronounced than in the case of streptomycin (173). Thus, rats receiving 725 mgm. of dihydrostreptomycin base per kgm. by mouth showed within 1 to 2 hours a peak blood concentration of 2700 units per cc. in the case of the hydrochloride, but only 175 units per cc. in the case of the sulfate; with similar amounts of streptomycin hydrochloride the maximum blood level was 4000 units per cc. while with the sulfate it was only 200 units per cc. Although the doses given were very large they were still far below the toxic range (peroral LD_{50} in mice: 31.5 gram dihydrostreptomycin base per kgm.).

The distribution of dihydrostreptomycin in various body fluids (180) and its excretion are very similar to that of streptomycin. After intramuscular injection it is rapidly absorbed into the blood where it reaches it greatest concentration approximately 1 hour after injection, and remains in detectable concentrations up to 24 hours. It passes the placental membrane and is found in the fetal blood. It is present in significant amounts in the pleural fluid and in the cerebrospinal fluid of patients. Dihydrostreptomycin is excreted primarily through the kidney, as is streptomycin. Clinical reports seem to indicate a lower incidence of allergic reactions with dihydrostreptomycin (155). Particularly, the development of eosinophilia is less frequent (144).

AUREOMYCIN

Aureomycin, discovered in 1947 by Duggar (75), is obtained from *Streptomyces aureofaciens*. It is highly effective against rickettsial and certain gram-positive and gram-negative infections. It has also been reported to be active against lymphogranuloma and the virus of atypical pneumonia. With the exception of frequent gastrointestinal disturbances, no serious toxic effects have been reported. The preferred mode of administration is by mouth, although it may also be injected intravenously.

Chemical and Physical Properties

Aureomycin (Duomycin) is an antibiotic derived from a strain of *Streptomyces aureofaciens* (75). Upon concentration and purification, the crystalline hydrochloride, a yellow powder, is obtained. This substance is a weakly basic compound, soluble in distilled water or 5% glucose solution to the extent of 14 mgm. per cc. In physiological sodium chloride solution the antibiotic precipitates at concentrations greater than 1%. A saturated aqueous solution of the hydro-

chloride is acid and has a pH of 2.9. When neutralized or made alkaline the antibiotic deteriorates rapidly (56, 235) at room or at incubator temperature. Aureomycin also decreases in activity when it is permitted to stand in plasma at 37°C (97) but not at a temperature of −20°C (125). As a dry powder in sealed ampules it was found to maintain its potency for at least 7 months. If the solution is kept at temperatures below 4°C, potency can be maintained for a considerable period (97). At a concentration of 500 mgm. per cc. in a phosphate buffer (M/20, pH 7.3), the antibiotic exhibited a 32, 64 and 128 fold loss of activity at 37°C after 24, 48 and 72 hours, respectively (125).

Antibiotic Activity

Aureomycin is active not only against infections due to certain gram-positive and gram-negative bacteria, (35, 41, 42, 63, 97, 137, 219, 280, 303, 348, 355) but also against those caused by rickettsiae and the virus of lymphogranuloma and psittacosis (35, 41, 62, 72, 175, 263, 279, 280, 352, 353). It has also been reported to be active in man against primary atypical pneumonia (35, 98, 168, 201, 280, 281). Its activity appears to be about equal to that of penicillin for the gram-positive cocci and to that of streptomycin for the gram-negative bacilli. Although its activity against *M. tuberculosis* appeared encouraging *in vitro*, it has been reported to be of no clinical value against this disease (280, 310, 309, 239, 233).

Toxicity

The lethal toxicity of Aureomycin is relatively low, the intravenous LD_{50} in mice and rats being 134 mgm. and 118 mgm. per kgm., respectively (124). Bryer *et al.* (41) found a somewhat higher acute toxicity in mice (LD_{50}: 100 mgm. per kgm.). The majority of mice survived single subcutaneous injections of 3 gm. per kgm.; all died after 5 gm. per kgm. Rats tolerated subcutaneous injections of 50 mgm. per kgm. daily for 8 days with only slight loss of weight and mild inflammatory reactions at the site of injection. Dogs developed transient hyperpnea, weakness and anorexia after a rapid intravenous injection of 50 and 100 mgm. per kgm.; injection of 150 mgm. per kgm. was followed by respiratory distress, generalized paresis and somnolence. Death occurred 6 hours after administration of this dose. At autopsy, the bladder contained a bloody urine, due possibly to the acidity of the 10% solution of Aureomycin hydrochloride used (40, 41). A dog which received intramuscularly 20 mgm. per kgm. of Aureomycin hydrochloride in 1% procaine solution daily for 9 days, developed anorexia and loss of weight. Harned (124) gave dogs a total of 270 mgm. per kgm. of Aureomycin hydrochloride over a period of 8 days; no toxic signs other than irritation at the site of injection, lameness and temporary loss of weight were observed. Blood counts, clotting time, liver and kidney function and electrocardiogram were not affected.

Oral administration to rats and mice of 100 mgm. per kgm. daily for 17 days, followed by 100 mgm. per kgm. twice daily for a total of 14 weeks, produced no unfavorable effects on general appearance, growth rate, hemoglobin concentration and blood picture. The blood sugar values of the tested and the control

animals were the same, and the two groups did not show significant differences in the mean arterial blood pressure at the end of the experiment. No pathological changes attributable to Aureomycin were found either by gross or microscopic examination.

Dogs given daily oral doses of 100 mgm. per kgm. of Aureomycin for periods of 9 to 15 weeks appeared to be in excellent condition. No gross or microscopic changes were observed when the animals were sacrificed and the tissues examined. Heilman (134) has found that guinea pigs are particularly susceptible to Aureomycin; doses of approximately 1 mgm. per kgm. daily, which in other species are tolerated without untoward reactions, injected into guinea pigs caused death within 7 to 10 days.

The effect of Aureomycin upon the blood pressure was studied in dogs under ether and pentobarbital anesthesia. The rapid injection of Aureomycin hydrochloride (10 mgm. per kgm., pH 2.5) produced a fall in blood pressure which was almost identical with that produced by the injection of a similar quantity of hydrochloric acid. The vasodepressor effect depended to a considerable extent upon the speed of injection. At a rate of 5 to 10 mgm. per kgm. per minute in doses up to a total of 50 mgm. per kgm., no effect upon the heart was found, as measured by electrocardiogram. Above that dose, "minor and temporary effects" have been noted (124).

In dogs and cats anesthetized with ether, pentobarbital or Dial, Aureomycin doses of 20 to 100 mgm. per kgm. did not alter the response to epinephrine, histamine, acetylcholine or faradic stimulation of the vagus.

The effect of Aureomycin upon the respiration of dogs, cats and rabbits was quite similar to that of an equivalent injection of acid or base of a comparable pH. A mild diuretic action was observed when Aureomycin was fed to rats. This effect was less than half that of caffeine under identical conditions. Following doses of 50 to 100 mgm. per kgm. in dogs, traces of albumin appeared temporarily in the urine. Albumin was occasionally noted after the administration of 50 mgm. per kgm. to rabbits.

No effect on the central nervous system of rats, guinea pigs, rabbits and dogs could be demonstrated after intravenous injection of 50 mgm. per kgm. at a rate of 10 mgm. per kgm. per minute.

Aureomycin has no effect on the isolated rabbit intestine or guinea pig uterus. It has no antipyretic effect in rabbits made pyretic with typhoid vaccine. In guinea pigs it does not alter the response to the inhalation of a standard histamine spray.

In man, no serious toxic effects have been observed following single or repeated doses of Aureomycin. Among the most frequent and disturbing side effects are gastrointestinal upsets, chiefly nausea, vomiting and diarrhea, which sometimes occur even after the first dose but more often are noted only after repeated administration (98, 219, 281). It would seem that, in addition to differences in patient sensitivity, considerable lot to lot variation exists and it has been suggested that the gastrointestinal side effects may be due in part to varying amounts of unknown impurities occurring in the present commercial material.

The side effects have been reduced by withholding the drug for several days (219), by reducing the dose to one half, or by simultaneous administration of antacids (42, 281). In view of the effect of Aureomycin on the bacterial flora of the intestinal tract, the administration of vitamin supplements has been recommended (219) when Aureomycin is given over a prolonged period of time. Spink and associates (303) have reported the frequent occurrence of a temperature rise accompanied by a shock-like condition, tachycardia, and a fall in blood pressure in patients receiving the initial oral dose of 0.5 gm. Aureomycin; no serious consequences followed this reaction. Schoenbach and Bryer (281) reported that patients may complain of mild drowsiness without other neurologic abnormalities such as vertigo, tinnitus, nystagmus or auditory disturbances.

Local Effects: The initial pharmacologic investigators have recorded severe local irritation following intraperitoneal, intramuscular, subcutaneous or intracutaneous injection of Aureomycin, regardless of the pH (2.5 to 8.5) (124); a similar irritation occurred upon intravenous injection both in animals and in man. For this reason some investigators have abandoned the administration of Aureomycin by intravenous drip which reduced substantially nausea, vomiting and gastrointestinal disturbances (275); intermittent intravenous injection was better tolerated than continuous drip, but nevertheless produced venous irritation. Collins *et al.* (63) found that the intramuscular injection of Aureomycin in distilled water was very irritating; this irritation could be partly overcome by the use of buffers or by procaine in concentrations of 0.5 to 1%. Because of the high frequency of adverse local reactions, oral administration appears at present to be the method of choice (189).

Distribution and Excretion: Studies of the absorption, distribution and excretion of Aureomycin are greatly handicapped by the fact that this antibiotic is rather unstable under the conditions required for microbiological assays. Herrell and Heilman (140) and Brainerd *et al.* (34) have recently improved the microbiological assay technic and have applied it to studies of the absorption and distribution of Aureomycin in man. A chemical method for the assay of Aureomycin has recently been described, but its applicability to the determination of the antibiotic in body fluids has not been evaluated (181). Only a limited amount of research in animals has been reported on the blood concentrations following parenteral or oral administration (124). When three doses of 20 mgm. Aureomycin hydrochloride were injected intravenously at 2-hour intervals in dogs, the concentration in the serum was 40 micrograms per cc. and in the cerebrospinal fluid 0.8 microgram per cc., approximately 2 hours after the last dose. Herrell and Heilman have reported that in man Aureomycin traverses the blood-brain barrier in amounts sufficient to produce chemotherapeutic results. On an average, the concentration in the cerebrospinal fluid was one fourth that in the serum of the same patients. Bryer *et al.* (40) reported that a concentration of 1.25 microgram per cc. of serum was obtained 15 minutes to 1 hour after intramuscular injection of 20 mgm. of Aureomycin per kgm. in rabbits and 40 mgm. Aureomycin per kgm. in dogs. In man, plasma levels of approximately 2 micrograms per cc. could be detected after the oral administration of 1 gm.

every 6 hours (97). After intravenous injection the serum level falls rapidly during the first hour and then more slowly for 12 hours after a single injection of 0.5 gm. of Aureomycin. The serum content of the patients averaged between 2 to 4 micrograms per cc. (140). Concentrations of 2 micrograms per cc. or less are usually demonstrable in the serum after the intramuscular injection of 50 to 200 milligrams (34, 77, 200). Somewhat higher values (peak levels of 4 to 8 micrograms per cc. of serum, 6 to 12 hours after repeated administration of 0.75 gm.) were reported by Herrell and Heilman (140), possibly due to a different method of assay. Aureomycin appears to be concentrated in the liver and excreted in the bile. In patients in whom cholecystectomy had been performed and the common duct and the remaining ducts were normal, the concentration of Aureomycin in the bile was 8 to 16 times greater than in the serum. Aureomycin appears in appreciable concentration in the urine of animals or man during the first hour, and in high concentrations between the second and eighth hour (63); excretion continues for more than 24 hours and has been reported to last as long as 3 to 4 days (223). Large amounts of Aureomycin are constantly excreted in the urine of patients who receive multiple oral doses of 0.5 to 1 gram every 6 hours. Aureomycin diffuses readily through the placenta and is present in the fetal circulation. If therapeutically effective amounts are present in the serum, diffusion occurs readily into the pleural fluid (140).

CHLORAMPHENICOL (CHLOROMYCETIN)

Chloramphenicol (Chloromycetin) is obtained from *Streptomyces venezuelae*. It is the first, and at present the only, antibiotic prepared by chemical synthesis. It is highly effective against rickettsial and certain gram-negative and gram-positive bacterial infections and has been reported to be effective against the virus of atypical pneumonia. Chloramphenicol appears to be free from serious toxic effects. Its preferred route of administration is by mouth, but it may also be injected parenterally.

Chemical and Physical Properties

Chloramphenicol (Chloromycetin), an antibiotic produced by *Streptomyces venezuelae* was discovered by Ehrlich and his associates (90, 91) and independently by Carter *et al.* (49, 118). It has been isolated in crystalline form, its structure has been determined (17, 18) and synthesis has been accomplished (64). It is an aromatic nitro compound (17) of the formula D-threo-1-nitrophenyl-2-dichloracetamido 1,3-propanediol (251) Fig. 1. It has been synthesized by several methods (64, 188). A chemical method for the determination of chloramphenicol in biological material has been devised (115).

Under experimental conditions chloramphenicol has been shown to be active against certain gram-negative (90, 112, 300) and gram-positive bacteria (300), some rickettsia (291, 292, 293, 294) and viruses (291, 292, 294). It possesses only a low degree of activity against the human strain of *Mycobacterium tuberculosis* (354). The literature covering its *in vitro* and *in vivo* activity has been reviewed by McLean, Schwab, Hillegas and Schlingman (199). Preliminary

studies by these investigators indicate that increased resistance of certain bacterial species can be induced *in vitro*. Tests designed to detect the development of resistance in *Rickettsia prowazekii* have been negative after 13 passages in chick embryos.

In the clinic, chloramphenicol has shown promise in the treatment of typhoid fever (349), urinary tract infections caused by gram-negative bacteria (59), brucellosis (346) and, particularly, certain rickettsial and viral diseases such as typhus, Q fever, Rocky Mountain spotted fever, lymphogranuloma venereum and atypical pneumonia (228, 229, 234, 295, 296, 347).

Chloramphenicol occupies a unique place among the antibiotics in that the pure compound was available from the very early stages of the investigation; it was thus possible to conduct pharmacological and clinical studies with a minimum of variables due to impurities. As is to be expected, the synthetic antibiotic is in every respect identical with that obtained from natural sources (121, 294).

Chemical and Physical Properties: Chloramphenicol has a very low solubility in water, acid or alkali; it is only slightly soluble in chloroform, ether, methyl alcohol and ethyl alcohol. It is quite stable (17) in aqueous solution and shows no loss of activity when heated at 100°C. for 4 hours or at 30°C. for 1 month.

Toxicity: Mice: The LD_{50} of chloramphenicol in propylene glycol solution is approximately 245 mgm. per kgm.; 200 mgm. per kgm. are tolerated (300). However, Gottlieb *et al.* (118) reported a tolerance of approximately 500 mgm. per kgm. for their independently isolated crystalline material. Oral administration of 1 gm. per kgm. in gum acacia produces in some animals depression from which they recover in less than 24 hours; 1.25 gm. per kgm. given in the same manner causes tremors and prostration but is followed with complete recovery. Subcutaneous injection of 100 mgm. per kgm., in two divided doses daily for 15 consecutive days, caused only a slight retardation of growth. Divided subcutaneous doses of 200 mgm. per kgm. per day were tolerated at least for 11 days while doses above 400 mgm. per kgm. per day produced ataxia, weight loss and death within a few days. When the antibiotic was administered in the diet, normal weight gain occurred at 0.25% concentration (360 mgm. Chloromycetin per kgm. per day); on a 1% drug diet (1290 mgm. per kgm. per day) there were no deaths but the mice lost an average of 15% in body weight. *Rabbits:* 100 mgm. per kgm. per day, in two divided daily doses, injected subcutaneously were tolerated for at least 8 days (300). *Dogs:* A single intravenous injection of 150 mgm. per kgm. given at the rate of 450 mgm. per minute caused sudden death as a result of respiratory failure and fall in blood pressure. Single doses of 25, 50 and 100 mgm. per kgm. injected at the rate of 100 mgm. per minute resulted in progressively larger declines of blood pressure from which the animals recovered; 12.5 mgm. per kgm. injected at the same rate were without effect. Three dogs injected with 72 to 88 mgm. per kgm. per day gained slightly in body weight but developed anemia of varying degrees during the treatment period. One dog given orally 143 mgm. per kgm. per day lost 0.3 kgm. in body weight during the 24-day treatment period, but showed no alteration of red cell count or hemoglobin

values. None of the 4 dogs showed significant changes in total white cell or differential counts, blood non-protein nitrogen, blood sugar and liver or kidney function; no changes in behavior were observed. A similar absence of toxic reactions was noted by Gruhzit *et al.* (121) in dogs maintained up to 133 days on daily oral doses of chloramphenicol of 50 and 100 mgm. per kgm. (317). No serious toxic effects have been observed in man. Two volunteers received an initial dose of 1 gm. followed for 10 days by daily total doses of 1 gm. (0.2 gm. every 4 hours). Another subject was given an initial dose of 2 gm. followed in 8 hours by a single dose of 0.5 gm. No symptoms or signs attributable to the drug were observed either during or after the period of treatment in these subjects (184). Occasionally, nausea has been observed after ingestion of the drug. This could be overcome by giving smaller doses more frequently. Intramuscular injection of a 1 gm. dose of Chloromycetin in a peanut oil suspension produced local irritation and swelling at the site of injection. Doses of 100 to 300 mgm. in 2 cc. of 70% propylene glycol caused considerable pain at the time of injection. There was no swelling at the site of injection nor was tissue injury found at autopsy which was performed 7 days later (300).

Absorption, Distribution and Excretion: The absorption, distribution and excretion of chloramphenicol can be studied by either a microbiological or a chemical method of assay. The latter is not specific for the active antibiotic since inactive degradation products still containing the nitro group are also included in the determination. Reliable estimates of active chloramphenicol are therefore best made by microbiological assay procedures and the differences between the chemical and microbiological values represent inactive degradation products. The specificity of the chemical method can be increased by a solvent extraction procedure (116).

Chloramphenicol is rapidly absorbed when given by mouth, and is rapidly excreted or inactivated. Intravenous injection in dogs of 19 mgm. per kgm. resulted in a peak blood concentration of 17 micrograms per cc. during the first 15 minutes, followed by a gradual decrease during the next 4 hours. Upon intramuscular injection in dogs of 101 mgm. Chloromycetin per kgm. the antibiotic could be detected in the serum after 1 hour (5 micrograms per cc.), reaching a peak in two hours (7 micrograms per cc.); 8 hours after injection it could no longer be detected (300). In the rat, it has been shown (116) that the distribution in the tissues is not uniform; the greatest concentration was observed in the kidneys and liver and low values were found in the brain and spinal fluid. The maximum serum levels after oral administration appear to be proportional to the size of the dose. Approximately 10% of the orally given antibiotic is recovered as such from the urine during the first 24 hours, while the bulk is found in the form of inactive nitro compounds. Renal plasma clearance figures indicate that Chloromycetin is largely excreted by glomerular filtration, while the inactive metabolic products are excreted by the tubules. Upon oral administration to man, a limited portion is found in the bile. The concentrations observed are considerably lower than those found in the bile of experimental animals (dogs, guinea pigs, rats), particularly of the rat in which as much as three fourths of

the administered dose are excreted through the bile in 8 to 12 hours (116). The major route of excretion in man is by way of the kidneys, with the urinary excretion accounting for approximately 90% of the administered dose in 24 hours.

TYROTHRICIN

Tyrothricin has primarily historical interest because it was the first in the series of modern antibiotics. It was isolated in 1939 by Dubos (73, 74) from a culture of *Bacillus brevis*, a gram-positive soil bacillus. Subsequent work showed that it was composed of two distinct chemical entities (156, 157), gramicidin and tyrocidin, present in the ratio of approximately 1:5. Of these, gramicidin is about 25 to 50 times more active against gram-positive microorganisms than is tyrothricin, while tyrocidin possesses some *in vitro* activity against gram-negative organisms. Tyrothricin and its fractions are not readily soluble in any of the common solvents; however, suspensions of these agents can be prepared for experimental purposes by first dissolving them in ethanol and then slowly mixing this solution with physiological sodium chloride solution. Gramicidin is the least soluble of the tyrothricin fractions and for this reason appears to be less active toxicologically and pharmacologically than either tyrocidin or tyrothricin. Several attempts have been made to increase the solubility of tyrothricin. Lewis (182) has prepared a formaldehyde derivative which shows reduced hemolytic properties in animals and some loss of toxicity, although it retains at least 50% of its antibiotic activity. Fraenkel-Conrat *et al.* (105) prepared a succinyl derivative and a succinyl-methylated derivative of gramicidin. These preparations are water-soluble, only slightly hemolytic and about one-fifth as active chemotherapeutically as gramicidin or tyrothricin. An antibiotic microbiologically closely related to tyrothricin but differing from it in chemical composition was described by Gauss and Brazhnikova (113).

Toxicity: The toxicity of the non-fractionated antibiotic, tyrothricin, was first studied by MacLeod, Mirick and Curnen (193). Robinson and Molitor (258) investigated the pharmacological and toxicological properties of pure gramicidin and tyrocidin and compared them with the non-fractionated antibiotic (tyrothricin). The acute toxicities of tyrothricin, gramicidin and tyrocidin were determined in mice and rats by intravenous, intraperitoneal and peroral administration. None of the fractions was toxic upon oral administration even in excessive doses (10 gm. per kgm.), due apparently to lack of absorption from the gastrointestinal tract. Intravenously and intraperitoneally, however, all fractions were quite toxic, gramicidin and tyrothricin much more so than tyrocidin. With all preparations the toxicity appeared markedly greater when observations were extended over a 7-day period. With gramicidin death occurred usually during the first 24 hours; however, with tyrothricin and tyrocidin, a large number of the animals died 3 to 4 days after injection. Toxic signs, consisting of restlessness followed by depression, appeared 1 to 2 hours after parenteral administration of a lethal dose. Death appeared to be due to respiratory failure since the heart continued to beat for some time after breathing had ceased. In dogs daily intravenous doses of 2 mgm. per kgm. of gramicidin in the form of a 5% glucose sus-

pension caused death within 2 to 8 days. All animals developed anorexia and loss of weight. During and following each injection most animals secreted excessive amounts of saliva and showed a slight rise in body temperature. No significant changes in heart and respiratory rate were noted until shortly before death, when the heart rate became slow and the respiration shallow and irregular. All dogs developed marked leucocytosis and those which tolerated more than 10 consecutive doses became anemic, with erythrocyte counts ranging from 2 to 4 million per cubic millimeter. In one dog, in which drug administration had been discontinued, the blood picture returned to normal within 2 months. The hemolytic action of gramicidin, which is one of its principle toxicologic properties, has been studied by Heilman and Herrell (133).

Miscellaneous Pharmacologic Effects: Tyrocidin in doses of 64 mgm. per 100 cc. produced a marked contraction of the isolated rabbit intestine and a decrease in rate and amplitude of the isolated frog heart; gramicidin, due to its insolubility, was practically without effect while tyrothricin occupied an intermediate position. Tyrocidin and tyrothricin lowered the blood pressure and depressed the respiration in cats (258).

Local Effects: Suspensions of gramicidin and tyrothricin one half of one per cent in sodium chloride solution produced no irritation when instilled in the conjunctival sac of rabbits; application of the dry material by dusting, however, caused marked conjunctival irritation and a long-persisting opaqueness of the cornea. The subcutaneous and intracutaneous injection produced local nodules which persisted for 5 to 6 weeks (258). Herrell and his associates have studied the cytotoxicity of tyrothricin and gramicidin. When the toxicity of the fractions of tyrothricin was studied by their inhibiting effect upon the migration of macrophages in tissue cultures, gramicidin was most toxic, tyrothricin was next in order and tyrocidin was least toxic (141). In a comparison of the cytotoxicity of gramicidin, penicillin and several of the more common antiseptics, gramicidin produced less tissue toxicity than the other germicides, with the exception of penicillin (139).

Pathology: Animals which died acutely exhibited marked congestion of the lungs and abdominal viscera with petechial hemorrhages in the heart, lungs and kidneys; the livers showed central necrosis. There were diffuse hemorrhages in the spleen with pronounced phagocytosis of erythrocytes. Animals which survived several doses developed ascites, fatty degeneration of the liver and degenerative changes in all organs (258).

In view of its marked systemic toxicity, tyrothricin should not be used parenterally. It has, however, been applied locally with success (136, 138, 170, 246, 249) in the treatment of empyema and infected ulcers and wounds. It is still being widely used in veterinary medicine for the treatment of infectious mastitis (22).

STREPTOTHRICIN

Streptothricin, first described by Waksman and Woodruff (335), is an antibiotic produced by *Streptomyces lavendulae*. In many ways it may be regarded

as a forerunner of streptomycin with which it compares rather closely in its bacteriological spectrum, excepting that it possesses a rather marked fungicidal activity which is not found in streptomycin. Its systemic toxicity, however, is such that it does not appear safe for parenteral administration. Although its acute toxic effects closely parallel those of streptomycin, streptothricin differs from it by a rather interesting delayed toxic property. Animals which have recovered from the first impact of a single large dose relapse after 1 to 2 days of apparent well-being. Their fur becomes ruffled, they breathe with increasing difficulty, the urinary output decreases and they die within several days after the reappearance of the toxic signs (240, 256, 257). The immediate lethal effect of streptothricin preparations appears to be independent of their antibacterial potency and is likely caused by (histamine-like) impurities which are chemically unrelated to the antibiotic. The delayed mortality is proportional to the number of antibacterial units administered and therefore must be regarded as an intrinsic property of the antibiotic, particularly since an almost pure preparation produces the same effects as preparations of intermediate purity. A similarly delayed effect was observed upon topical application of streptothricin. Instillations into the conjunctival sac of a rabbit of a buffered solution containing 500 to 1000 units per cc. caused an immediate slight irritation which completely disappeared after 12 to 24 hours. However, after an interval of 24 to 48 hours a second and much more severe reaction appeared, although no new application of streptothricin had been made and the conjunctival sac had been thoroughly washed with sodium chloride solution immediately after the original application. This second reaction was characterized by an intense hyperemia and the formation of a thin white membrane which soon covered the entire cornea. Permanent loss of vision is the usual outcome of a single application of streptothricin to the eye. A similarly delayed toxic reaction is observed upon topical application to mucous membranes. No explanation is yet available for this rather interesting phenomenon.

Repeated administration of smaller doses of streptothricin to mice, rats, guinea pigs, dogs, and monkeys caused a progressive loss of body weight and muscle tone, hematuria, a steady decrease of urinary output and eventual death from renal insufficiency. The impairment of renal function seems to be closely related to the total amount of drug given. Dogs and monkeys dying from repeated administration of streptothricin show hepatic damage and renal changes predominantly in the tubules, congestion and extensive hemorrhages in the intestinal tract, and ulcerations with marked suppuration in the tongue, lips and buccal mucosa. Mice dying from a single injection of streptothricin showed extensive tubular damage with many hyaline and granular casts. Mice surviving for more than 1 month developed alopecia and showed signs of polyneuritis. Since bacteriological examination of the intestinal flora of these animals revealed a marked decrease in the number of lactose-fermenting bacteria (297) large doses of vitamins (thiamine, riboflavin, pyridoxine, pantothenic acid, biotin, vitamin K) were tried, but without success. With the exception of the delayed effects on the kidneys and the delayed local effects, pure preparations of streptothricin produced no marked pharmacodynamic effects when tested on isolated organs

or in acute experiments. Streptothricin is rapidly absorbed after parenteral injection and is excreted primarily through the kidney; approximately 10% is excreted in the bile.

As has been stated earlier in this review, it has been possible to modify some of the pharmacodynamic properties of streptothricin without changing its bacterial spectrum, by growing *Streptomyces lavendulae* on a medium of different composition. However, while such a modified preparation has largely lost its local irritating properties, it still possesses the nephrotoxic ones and for this reason the systemic use of streptothricin appears to be unsafe.

NEOMYCIN

Neomycin was discovered by Waksman and Lechevalier (333) and is produced by *Streptomyces fradiae*. It possesses a bacteriological spectrum closely resembling that of streptomycin. Neomycin is of great potential therapeutic interest because bacterial resistance develops at a much slower rate than it does to streptomycin, and because microorganisms which have become completely resistant to streptomycin still are very sensitive to neomycin (331, 332, 334). Furthermore, the antibacterial activity of neomycin against a large number of gram-positive and gram-negative microorganisms is, on a unit basis, significantly greater than that of streptomycin.

It has already been established that neomycin is chemically unrelated to streptomycin. It is composed of at least two, and possibly more, distinct fractions (331, 151), one of which has been isolated by Peck *et al.* (231) and designated as neomycin A. Little is known as yet about the pharmacodynamic properties of this antibiotic, except that partially purified neomycin sulfate and chemically pure neomycin A possess a relatively low acute toxicity. Partially purified neomycin sulfate (180 to 220 units per mgm.) has an acute intravenous toxicity (LD_{50}) of approximately 10,000 to 12,000 units per kgm. Death occurs immediately following higher dosages. Daily subcutaneous injection for 5 days of 1,200 units per mouse in two divided doses was well tolerated (151); however, if impure neomycin was given in a dose of 1,000 units per day per mouse for a longer period, some mice lost weight and died after 14 days. Pathological examination of the kidneys revealed focal necrosis and desquamation of the tubular epithelium particularly in the convoluted segments (213). Sections stained with Sudan IV failed to reveal significant amounts of lipid in the kidney. Since the renal necrosis due to neomycin is focal in distribution and neither fatty changes nor glomerular damage have been observed, the histopathologic picture differs from that noted after the injection of streptothricin (213). There is some indication that purification of the antibiotic will reduce its toxicity. A single large dose of neomycin does not produce delayed toxic effects such as observed after streptothricin. Nothing is known as yet with regard to the possible neurotoxicity of neomycin.

SUBTILIN

The antibiotic subtilin produced by *Bacillus subtilis* was described by Jansen and Hirschmann (160). Subtilin is a basic peptide of low molecular weight (7,000 to 10,000). It is stable in dry form, especially if acidified before drying. Light has

a destructive action on it, particularly in aqueous or alcoholic solutions. Subtilin has a solubility in water of approximately 10% at pH of 2.0 to 6.0, only 0.5% at pH 7.0, and 0.2% at pH 8.5. In alkaline solutions the antibiotic is rapidly inactivated. Subtilin is precipitated at low salt concentrations and therefore has a limited solubility in physiological fluids. It has also been shown to have surface-tension lowering properties. Subtilin is soluble in 70% ethanol, methanol, propylene glycol and acetic acid, but not in ether, acetone or chloroform (8).

Subtilin has been shown to be active *in vitro* against a variety of gram-positive bacteria, certain actinomyces, *M. tuberculosis*, and relatively few gram-negative pathogens (270, 345). It exerts a high degree of activity in animals infected with *D. pneumoniae* (271), *B. anthracis* (269, 272), *Strep. pyogenes* (269, 272), and *S. aureus* (269). The methylated, ethylated and glycolated derivatives of subtilin were studied for their bacteriostatic action on tubercle bacilli (57). The methylated subtilin derivatives were twice as active as the original samples against *M. tuberculosis*. Preliminary studies have indicated that subtilin has a suppressive effect on experimental tuberculosis in guinea pigs (269, 273). In tuberculous infections in Syrian hamsters, no beneficial influence of the antibiotic could be demonstrated (8).

The acute intravenous toxic dose of subtilin (1% solution) in mice was 60 mgm. per kgm. (LD_{50}); on subcutaneous injection, the LD_{50} was 670 mgm. per kgm.; when administered orally, 5.0 gm. per kgm. proved to be lethal (7). The high intravenous toxicity of subtilin was believed to be due to the flocculation or precipitation of subtilin by the electrolytes present in serum (8). By using physiological sodium chloride solution rather than aqueous solutions of subtilin, the intravenous LD_{50} in mice was found to be reduced from 100 mgm. per kgm. to 140 mgm. per kgm. (58). Following the intravenous injection of 400 mgm. per kgm. of subtilin in sodium chloride solution, death occurred immediately, whereas it required but 200 mgm. per kgm. to obtain the same effect with aqueous solutions. The influence of physiological sodium chloride solution upon the toxicity of subtilin was also determined in rabbits by intracisternal injection. The administration of 0.6 mgm. per kgm. of subtilin in aqueous solution produced convulsions and death in 50% of the animals, whereas the same amount of subtilin in sodium chloride solution produced only tremors but no convulsions or deaths.

Following the subcutaneous or intramuscular injection of strong aqueous solutions of subtilin, only very low concentrations of the antibiotic appear in the blood. The electrolytes in tissue fluids apparently precipitate subtilin at the site of injection and thus absorption is exceedingly slow (343).

Subtilin was found to be non-irritating when instilled as a 1% solution into rabbit eyes (8). When compared to tyrothricin for possible hemolytic action upon red cells subtilin was found to be less toxic. It had no immediate effect, but caused hemolysis after an exposure of 24 hours at 4°C (269). The toxicity of subtilin to living embryonic chick heart tissue cultivated *in vitro* has been determined (274). Under the conditions of the test the antibiotic was approximately 20 times more toxic to *Staphylococcus aureus* than to chick heart tissue.

In crude form, subtilin was found to possess a marked capacity for sensitizing

guinea pigs. It was noted that 60 mgm. per kgm. brought about regular sensitization of these animals and produced anaphylactic deaths in 8 of 14 animals (8).

In man, the effectiveness of subtilin was determined in 8 patients with laryngeal or endobronchial tuberculosis by topical application of nebulized solutions. It was concluded that in its present form subtilin has no place in the treatment of tuberculosis except for purely investigative purposes (94).

The maximum dose administered to one patient was 600 mgm. daily for 6 weeks. No evidence of toxicity to the kidneys, liver, or bone marrow was encountered. A mild irritation of the respiratory tract, mild dyspnea, and increase in the cough and sputum, and a mild pharyngitis occurred during the first few weeks of treatment and then disappeared. Recurrent headaches were common among patients receiving the drug. The lack of serious toxicity may have been due to the mode of administration (topical application) which results in a negligible systemic absorption of subtilin (94).

POLYMYXIN

Polymyxin was discovered independently and almost simultaneously by Benedict and Langlykke (21), Stansly, Shepherd and White (305) and by Ainsworth, Brown and Brownlee (3). It is the generic name for a number of antibiotics derived from different strains of *Bacillus polymyxa* and designated by the letters A, B, C, D, etc. (304). The known polymyxins may be characterized as basic polypeptides, the salts of which are water-soluble, with a specific antibiotic activity against gram-negative bacteria. The known members have as common molecular constituents, α,γ, diaminobutyric acid, threonine and a branched C_8 fatty acid (20, 51). Polymyxin A (formerly called Aerosporin and discovered by Ainsworth, Brown and Brownlee (3)) contains D-leucine in addition; polymyxin B has as additional constituents both leucine and phenylalanine (164); polymycin C contains as additional amino acids only phenylalanine; and polymyxin D (the material formerly called "polymyxin" and first described by Stansly, Shepherd and White (305)) contains D-leucine and D-serine in addition to the common constituents. Polymyxins A, B and D have been examined systematically for their chemotherapeutic properties; all are potent antibiotics, selectively active against gram-negative pathogens. In experimental infections due to *Hemophilus pertussis*, polymyxin A is superior to polymyxin B and both are superior to polymyxin D. In very acute infections due to *E. typhosa* and *K. pneumoniae*, polymyxin A is more effective than polymyxin B and polymyxin D.

Pharmacologic Properties: The pharmacologic properties of the known polymyxins are, with one important exception, very similar and the differences are mainly quantitative. Polymyxin A and polymyxin B have about the same order of toxicity, while polymyxin D is about half as toxic as either A or B. The subcutaneous toxicity is approximately 10 to 12 times lower than the intravenous or intraperitoneal toxicity, and the onset of toxic manifestations occurs much later. Injected intracisternally in rabbits, the average toxic dose is approximately 0.6 mgm. per kgm. The mean LD_{50} for mice is 6.9 mgm. per kgm. for poly-

myxin A; 6.1 mgm. per kgm. for polymyxin B; and 11.9 mgm. per kgm. for polymyxin D. When administered subcutaneously, the mean LD_{50} in mice is 87.5 mgm. for polymyxin A; 82.5 mgm. for polymyxin B; and 160 mgm. per kgm. for polymyxin D (36). In the experiments reported by Bryer *et al.* (39), generally larger doses (approximately double those of polymyxin D) appeared to be tolerated, due possibly to the use of a different strain of mice. Qualitatively, the findings of both groups of investigators were in excellent agreement.

With lethal doses of polymyxin A and B, death occurs from respiratory failure in less than 2 minutes. Near lethal doses produce vasoconstriction, muscular incoordination and respiratory distress, followed by clonic convulsions and later flaccid paralysis. The mice recover from these signs within approximately 15 minutes and subsequently remain well. Dogs survived single rapid intravenous injections of 10 and 15 mgm. per kgm. of polymyxin D. Injection of 25 mgm. per kgm. was followed within 5 seconds by general convulsions, ascending paralysis, incontinence and coma; 7 minutes after the injection respiration became irregular and eventually ceased, although the heart continued to beat. Death occurred in 20 minutes. Doses of 5 and 10 mgm. per kgm. given intramuscularly twice daily for 7 days were well tolerated by dogs; no weight losses were observed during the week following the last injection. Intracisternal injection into rabbits of sublethal doses of polymyxin A (0.3 to 0.5 mgm. per kgm.) failed to produce marked vestibular dysfunction. A temporary rotatory nystagmus was seen during the first hour; thereafter, and for a subsequent observation period of 12 days, nystagmus after rotation in the lateral plane remained normal. The average lethal dose upon intracisternal injection in rabbits was 0.6 mgm. per kgm.

Nephrotoxic Effects: Polymyxin A, polymyxin B and polymyxin D differ from each other primarily and significantly in their effects upon the kidneys. These are very marked with polymyxin A and D and practically absent with polymyxin B. In rats, guinea pigs, rabbits and dogs, as well as in man, polymyxin A and polymyxin D cause proteinuria and the appearance of epithelial cells and cellular casts. In the rat, these are observed within 24 hours after the first injection of a dose of 20 mgm. per kgm. of polymyxin D. Brownlee and Bushby (37) found that the nephrotoxic properties of polymyxin A were not directly related to its antibiotic potency, and the possibility exists, therefore, that the renotoxic principle is not identical with the antibiotic. However, since even the purest batches of polymyxin A continued to be nephrotoxic, whereas polymyxin B of much lower purity was essentially free from that effect, it is obvious that the latter antibiotic is the one of choice for clinical use.

On autopsy, mice, rats and dogs injected with polymyxin A or D exhibit severe damage to the tubular epithelium; the secretory epithelium shows all stages of disintgegration and large numbers of granular, hyaline and cellular casts are seen. In contrast to this, the kidneys of dogs treated with polymyxin B display only a mild hyaline droplet degeneration of the epithelium of the convoluted tubules. Brownlee and Short (38) found that substances which antagonize the nephrotoxic effect of D-serine also antagonize the nephrotoxic activity of poly-

myxin A. The most effective protective agent was DL-methionine, followed by methylcysteine. Animal protein hydrolysate was also found to be effective if given by mouth. Protection by methionine is complete in dogs; 10 mgm., injected once, protects against the nephrotoxic effect of 1 mgm. polymyxin A given 4 times daily for 3 days. In man, however, no such complete protection is seen.

Absorption and Excretion: All polymyxins appear to be readily absorbed when injected parenterally. Absorption from the gastrointestinal tract is slower and less complete. Neither the nephrotoxic factor nor the antibacterial activity of polymyxin A or D is affected by digestion with trypsin, pepsin or papain. They disappear slowly from the serum and tend to accumulate when repeatedly injected. Dogs given subcutaneous injections of polymyxin A excrete large amounts in the urine during as well as for several days after treatment. The greater the kidney damage, the higher the concentration of the antibiotic which appears in the urine. The concentration of polymyxin B in the urine is small when compared to that of polymyxin A, but is still sufficient to inhibit gram-negative organisms. Polymyxin does not diffuse into the cerebrospinal fluid when injected parenterally. It was found in the blood, however, after intrathecal injection of 5 to 10 mgm. per kgm. In patients, 3 to 7 mgm. per kgm. per day of polymyxin D given intramuscularly produced blood concentrations of 1 to 10 micrograms per cc. Twelve hours after institution of treatment, the antibiotic appeared in the urine and reached concentrations varying between 10 to 160 micrograms per cc.

BACITRACIN

Bacitracin is an antibiotic produced by the Tracey strain of *Bacillus subtilis* (161) and is active against certain gram-positive bacteria and *Treponema pallidum* (87). The original material grown by flask culture in shallow layers of tryptone, beef infusion or similar media, was stated to possess only slight systemic toxicity. However, when larger quantities were needed and production had to be undertaken on a semi-commercial scale a deep fermentation process was developed in which a soya bean medium was used. This greatly increased the yields but resulted in a more toxic material. Subsequent improvements in the manufacturing process, resulting in a highly purified material as well as the establishment of rigid specifications by the Food and Drug Administration, seem to have materially decreased the systemic toxicity. Bacitracin of this improved type has been used without serious adverse reactions in a large number of patients (203). In mice, no significant differences in nephrotoxicity were found, when bacitracin grown by surface and deep-tank culture were compared (298). Until pure bacitracin is procurable in the large quantities required for a complete pharmacological and clinical study, the question of the absolute toxicity of this antibiotic cannot be answered satisfactorily. Because of the lot to lot variations it is difficult at present to evaluate and compare results obtained by different investigators, particularly when no reference is made to the source of the material or the lot number.

Chemical and Physical Properties: Bacitracin appears to be a polypeptide (16). It is readily soluble in water, ethanol and methanol; the dry material is quite

stable at temperatures up to 37°C., but aqueous solutions are much less stable (29). It is not destroyed by trypsin or pepsin at body temperature; incubation with normal gastric juice at 37°C. for 4 to 6 hours does not cause a loss of potency (162).

Toxicity: The acute intravenous and intraperitoneal LD_{50} in mice varies within a very wide range (283) and appears to be independent of the antibiotic potency of the sample, since complete inactivation of the material by incubation for 8 days at 37°C. at pH 7 does not materially alter the results. Signs of acute toxicity were similar regardless of the lot of bacitracin used, and consisted in hyperexcitability and clonic convulsions, followed by severe depression. The cause of death appeared to be respiratory failure. No abnormalities in blood morphology were observed. After oral administration or subcutaneous injection for 5 days, no toxic effects other than apathy were noted. Mice injected subcutaneously for 5 days with 10 to 25 times the therapeutic dose remained normal and continued to gain weight. Similar results were obtained in rats treated daily for 5 consecutive weeks. Dogs and monkeys injected intramuscularly for 24 and 37 days, respectively, with 3,000 units per kgm. per day in three divided doses exhibited no serious pathologic signs during this period. There were no changes in blood sugar and non-protein nitrogen. The red cell count remained unchanged while there was a rise in the percentage of polymorphonuclear leucocytes and an occasional moderate leucocytosis in the dogs. Monkeys developed a definite fluctuating eosinophilia during the period of administration. No changes in behavior and body weight were noted in either species (286). The monkeys developed a transient albuminuria and glycosuria. In man, certain bacitracin lots have been reported to produce upon parenteral injection definite renotoxic effects (202). However, lots of low toxicity, meeting the Food and Drug Administration specifications of an LD_{50} of not less than 500 units per 20 gram mouse, have been used systemically with satisfactory results in 100 cases of surgical infections (203).

Miscellaneous Pharmacological Effects: Fifteen units of bacitracin in a 5% solution containing 1,500 units per cc. did not alter the amplitude or rate of contraction of the isolated frog heart; higher doses caused diminished contractility and progressive slowing. In dogs anesthetized with pentobarbital, the intravenous injection of bacitracin produced a transient fall of the arterial blood pressure followed by a rise. Antihistaminics did not influence the depressor effect. Following a rapid intravenous injection of more concentrated solutions (3,000 units per cc.), signs of central nervous disturbance were noted, consisting of salivation, nausea, spastic hindquarters and scoliosis due to muscle spasm (285). One twentieth to 0.1 cc. of an aqueous solution containing 6,000 units per cc. was injected intradermally into the shaved abdominal skin of rabbits without reaction within the ensuing 4 days. Instillation of a bacitracin solution containing 1,200 units per cc. into the conjunctival sac produced only slight and transient reddening. In guinea pigs sensitized by a subcutaneous injection of 150 units of bacitracin, a challenging dose of 300 units, injected intracardially after an interval of one month, failed to elicit anaphylactic phenomena.

Absorption, Distribution and Excretion: Scudi, Clift, and Krueger (285) reported appreciable concentrations of bacitracin in the blood of dogs as long as 7 or 8 hours after a single subcutaneous injection of 3,000 or 6,000 units per kgm. Peak concentrations were considerably lower than those following intramuscular or intravenous injection.

The blood levels and renal clearance of bacitracin have been studied by Eagle *et al.* (88) in both rabbits and man. Bacitracin disappears from the blood less rapidly than does penicillin. In rabbits the intramuscular injection of equal gravimetric doses of bacitracin and penicillin showed, one hour after the injection, serum levels of bacitracin 8 to 20 times greater than those of penicillin; two hours after the injection the difference was 20 to 100 fold. Bactericidal levels of bacitracin were maintained 6 to 7 times longer with bacitracin than with penicillin.

The intramuscular injection of bacitracin into man also produced higher and more prolonged blood levels than were observed after penicillin administration.

The marked difference in blood levels afforded by penicillin and bacitracin has been shown to be due to their different rates of urinary excretion. In contrast to the excretory mechanism for penicillin the crude preparations of bacitracin used were cleared by the kidney at a rate approximating that of glomerular filtration.

Pathology: High to lethal doses of bacitracin in mice cause severe renal damage, particularly in the form of tubular necrosis. It has, however, been concluded that the mouse is an unsatisfactory test animal (298). In contrast to the striking lesions found in the kidney of the mouse, the kidney of bacitracin-treated rats and dogs did not vary appreciably from those of normal controls. Also, the renal lesions in the monkey were insignificant in comparison with those in the mouse. Monkeys receiving very large doses approximating the LD_{50} developed renal tubular damage. There was no evidence of cellular damage in the liver of dogs and mice receiving 3,000 units per kgm. for approximately 5 weeks. The bone marrow of both species was found hyperplastic. There were no gross anatomical changes except local induration at the site of injection.

The position of bacitracin among the clincally important antibiotics has not yet been firmly established, largely because of the occasional occurrence of lots with a high degree of nephrotoxicity. However, it has already proved markedly effective upon topical application in infections caused by gram-positive microorganisms, including those resistant to penicillin. Furthermore, it has been shown to exert a pronounced synergistic effect with penicillin in experimental rabbit syphilis (87) and *in vitro* against several strains of *Streptococcus viridans* (10).

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