SCIENCE

VOL. 91 FRIDAY, APRIL 12, 1940 No. 2363

SCIENCE: A Weekly Journal devoted to the Advancement of Science, edited by J. MCKEEN CATTELL and published every Friday by

THE SCIENCE PRESS

New York City: Grand Central Terminal

Lancaster, Pa. Garrison, N. Y.

Annual Subscription, $6.00 Single Copies, 15 Cts.

SCIENCE is the official organ of the American Association for the Advancement of Science. Information regarding membership in the Association may be secured from the office of the permanent secretary in the Smithsonian Institution Building, Washington, D. C.

THE PRESENT STATUS AND PROBLEMS OF BACTERIAL CHEMOTHERAPY[1]

By Dr. E. K. MARSHALL, Jr.

DEPARTMENT OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, THE JOHNS HOPKINS UNIVERSITY

SUCCESSFUL chemotherapy of bacterial diseases is an innovation of the last four years only; in the history of medical progress it is a newborn babe—a lusty infant with powerful lungs and an incredible capacity for growth. Have its shoutings and precocity been justified and have its growth and development been directed along the right paths?

Chemotherapy, in the case of protozoan infections, can be considered to be of comparatively ancient origin. The sixteenth-century use of mercury in syphilis and the seventeenth-century use of cinchona bark in remittent fevers and of ipecac in dysentery are examples of a specific form of therapy which was later designated "chemotherapy." All three of these drugs are old and popular remedies and were used without a knowledge of the etiology of the disease or of the mode of action of the remedial agent. Although Koch in 1881 reported unsuccessful attempts at bacterial chemotherapy in anthrax infections of guinea pigs, real experimental chemotherapy began with the very significant experiments of Ehrlich and Shiga in 1904. Their report of the cure of an otherwise fatal trypanosome infection in mice by one injection of the dye trypan red marks the beginning of a new epoch, despite the fact that animals other than the mouse were not cured and that the drug was of no practical use. This work led in 1910 to the well-known discovery of the therapeutic effect of organic arsenic com-

[1] This paper was given before the Society of the American Bacteriologists, New Haven, December 28, 1939.

pounds in spirochaetal diseases. Arsphenamine and neo-arsphenamine were the first synthetic organic compounds to be used successfully in chemotherapy.

Thirty years have elapsed since Ehrlich's momentous discovery and we can inquire what advances have been made. Although we now have drugs of quite different chemical constitution—dyes of the trypan red group, acriflavine, styryl-quinoline compounds, organic arsenic compounds, compounds of antimony and bismuth, and certain colorless non-metallic compounds favorably influencing experimental trypanosome infections, the synthetics plasmoquin and atebrin as substitutes for quinine in malaria, and such drugs as carbarsone and chiniofon for amebic dysentery—I think it is fair to say that progress in the field of protozoan chemotherapy has been quite slow. The organic arsenicals are still the sheet-anchor for the treatment of syphilis. These drugs are given by injection and no leads toward finding non-metallic compounds effective in syphilis appear to have been discovered. Moreover, there is as yet no completely satisfactory and generally accepted explanation of the mode of action of any chemotherapeutic drug in any protozoan disease.

The bacterial diseases were considered until recently to be resistant to chemotherapy; the bacteria were thought too primitive in structure to be influenced by chemicals which were not highly toxic to the host. Many attempts had been made to cure bacterial infections in animals by the use of drugs, but these all met with little or no success with the exception of Morgenroth and Levy's finding in 1911 that ethylhydrocuprein would cure mice of a pneumococcus infection. The effective dose of this compound, however, was very near the lethal one, and the experiments were of no real clinical service.

This situation has been radically altered by the discovery of the sulfonamide derivatives as bacterial chemotherapeutic agents. The known scope of effective treatment with these compounds is widening rapidly, and brilliant success has already been achieved, but so much ground remains to be explored that the ultimate consequences of the discovery are beyond prediction. The history of this recent development of bacterial chemotherapy is too well known to need repetition—the landmarks are Gelmo's synthesis of sulfanilamide in 1908, Mietzsch and Klarer's patent of the azo dye "prontosil" in 1932, Domagk's report on the curative effects of "prontosil" in streptococcus infection in mice in 1935, and the announcement by the Tréfouels, Nitti and Bovet, later in that year of the activity of the simple organic compound, sulfanilamide.

Although sulfanilamide (and allied drugs) were first thought to have a specific action on the streptococcus alone and to be incapable of influencing other infections, it has now been definitely proved that these drugs are effective in a wide variety of experimental infections in animals and a number of infectious diseases in man. Thus the therapeutic effect of sulfanilamide (or allied compounds) is excellent in experimental mouse infections due to the β-hemolytic streptococcus, meningococcus and pneumococcus. It is still good, but less satisfactory in mouse infections produced by strains of gonococcus and staphylococcus; Proteus, colon, typhoid and paratyphoid organisms; the Sonne strain of the dysentery bacillus; a strain of Listerella; Hemophilus influenzae, the Welch bacillus, and certain members of the Pasteurella group, including the plague bacillus. Prolongation of life, with few or no survivals, is reported for infections produced by strains of Salmonella typhimurium, Friedländer's bacillus; Pasteurella pseudotuberculosis and the anthrax bacillus. A definite inhibitory effect on the development of experimental tuberculosis in the guinea pig and rabbit, an alteration of the natural course of experimental Brucella infections in guinea pigs and Bacterium necrophorum infection in rabbits, and the remarkable curative effect in certain human urinary tract infections also attest to the wide-spread antibacterial powers of the sulfonamide group of drugs. In protozoan infections, the only conclusive evidence of effectiveness is that reported for malarial infection of monkeys. In virus infections the results so far obtained are negative or inconclusive, with the exception of lymphogranuloma venereum and trachoma. In both of these cases, there is some doubt if the infecting agent can be classed as a true virus.

The above attempt to classify roughly the efficiency of sulfonamide compounds in various infections may be subject to considerable revision when more accurate experiments are available. We know now that very marked differences in susceptibility between different strains of the same organism occur and usually only one strain of the particular organism has been used in the investigations upon which the above classification is based. Also, the low virulence for mice of certain organisms and the necessity of using mucin to enhance this virulence in certain experiments may cause errors in comparisons with other experiments where organisms of very high virulence serve as the infecting agent. The use of different methods of dosage of the drugs is another important factor that may disturb such comparisons as the above (this will be referred to more fully later). Lastly, the possibility of the favorable results being due to the cure of some secondary infection and not the primary one must be considered; the favorable therapeutic results of these drugs reported in elephantiasis of filarial origin and in smallpox are probably due to an action on the secondary streptococcal infections.

These almost pan-antibacterial properties of sulfanilamide and allied compounds represent, indeed, an enormous advance in the field of bacterial chemotherapy, but no end of problems still need investigation. It appears that in general α-hemolytic streptococci and anerobic streptococci are much more resistant to these drugs than the β-hemolytic class; within the class of β-hemolytic streptococci, group D appears very resistant and evidence exists that certain strains of other groups are very resistant to the action of these drugs. The therapeutic efficiency of this group of drugs has been tested against various types of pneumococci, but it now appears that as much or more difference may exist between strains of the same type as between types. Similar relations probably obtain in other genera of bacteria which are susceptible to these drugs.

Another question of prime importance is that of the specificity of these drugs in bacterial infections. In the case of the chemotherapy of protozoan diseases, we know that quinine and atebrin are active in malaria, the organical arsenicals and bismuth in syphilis, germanin and tryparsamide in trypanosomiasis and emetin and carbarsone in amebic dysentery, but that interchange of drugs and diseases can not be made. Are the sulfonamide drugs active in some degree on all bacteria and do the more potent drugs simply act on a larger range of infections than the less potent ones? Do these drugs have some degree of activity in all infections where death results from rapid multiplication of bacteria, and fail only in infections where a small number of bacteria can form a toxin sufficiently potent to kill the animal, as in tetanus and diphtheria? Or is there a real specificity of the drugs for different bacteria, as in protozoan chemotherapy? Accurate data are needed to answer these questions before the best use of these new chemical weapons or the quickest evolution of new ones can be attained.

One difficulty in answering definitely the above questions is that the data available in the reports of various investigations give more of a qualitative than a real quantitative comparison of different drugs or of the same drug under different conditions. Some of the reasons for this appear to be as follows. Relatively simple methods suffice for detecting anti-bacterial activity of drugs *in vivo,* but a more elaborate procedure is required to evaluate such activity quantitatively. Data on the effectiveness of drugs have been obtained in most cases by administration of doses according to schedules which have differed with each investigator. Extreme variations of drug concentrations in the blood must have resulted from the various dosage schedules employed, as well as from differences in absorption, excretion and distribution of different drugs. With many drugs administration at intervals greater than six hours has resulted in high concentrations of short duration followed by periods when little or no drug was present in the blood. The lack of a suitable therapeutic response as an end-point and of a standard for comparison, as well as complete ignorance of the accuracy of the comparison, has invalidated quantitative conclusions from many experiments.

A paper has just appeared from our laboratory on the experimental basis for a method for the quantitative evaluation of the effectiveness of chemotherapeutic agents against streptococcus infection in mice. A considerable amount of as yet unpublished data obtained with this method would indicate that it gives a more or less absolute comparison of the effectiveness of different drugs or of the same drug under different conditions in both streptococcus and pneumococcus infections in mice. The method in brief is as follows. A more or less constant blood concentration of drug during the period of therapy is maintained by using food in which the drug has been incorporated. By treating mice in individual cages the daily drug intake of each mouse can be determined. Drug diets are so selected that one may expect to obtain with different drug intakes survival percentages greater and less than fifty. The diets are fed for one or more days prior to and for the desired period after infection. Irrespective of the percentage of drug in any diet, the average daily drug intakes (per mouse) can be arranged in groups and correlated with percentage survivals. The dosage-survival curve is now computed and the *Median Survival Dose* ($S.D._{50}$) with its standard error obtained. This can be converted into the *Median Survival Blood Concentration* ($S.B.C._{50}$) by a factor which relates blood concentration to daily drug intake of the drug being tested. By using a standard, one obtains a comparative value for the S. B. $C._{50}$'s which may be nearly absolute, even though the S. B. $C._{50}$'s themselves are variable. It is hoped that the use of this method or some improvement along the same lines will yield data of sufficient accuracy to give unequivocal evidence for or against ideas which are now mostly a matter of opinion or guesswork.

Nearly all research directed toward bacterial chemotherapy of experimental infections has utilized the mouse as the experimental animal. The reasons for this choice are not difficult to surmise: the susceptibility of the mouse to certain strains of streptococci, pneumococci and many other bacteria of human origin, the availability of more or less pure strains, the ease with which large numbers of these animals can be handled and their relatively low cost are probably mainly responsible for the use of these rodents. Very few investigations have been concerned with the therapeutic effect of the sulfonamide type of compounds in experimental infections in animals other than the mouse. In practically all cases where one of these

drugs has been introduced into clinical use as a bacterial chemotherapeutic agent, experimental therapeutic studies in the mouse have been transferred directly to man. Fortunately, the transfer from the lowly mouse to man has been justified in most instances, but the history of protozoan chemotherapy should lead us to fear that this will not always be so. The rapidly fatal infection in the mouse is obviously not similar to infection with the same organism in human beings, and, I think, there is a great need of developing satisfactory methods for studying experimental infections in other animals which may resemble the human more nearly as regards size and phylogenetic relationship. Another reason for the use of animals other than the mouse lies in the fact that such comparative therapeutic research may give valuable information as to the importance of the host factor in the mechanism of action of these drugs. Infection of animals of different species with the identical organism and quantitative study of the effects of therapy with the same drug should yield information of value. The reason that these drugs are ineffective against a strain of streptococcus of low mouse virulence has been stated to be due to the large number of organisms necessary for a fatal infection. In support of this is the observation that when mucin is used to reduce the lethal dose, effective therapy with sulfanilamide is obtained. When it is remembered, however, that fatal infections produced by the injection of large numbers of staphylococci and other bacteria can be successfully treated, it would appear that more investigation is needed. It is possible that the reason for the difference between infections of low and high virulence may be found in experiments on different species of animals.

This discussion of experimental therapy can not be closed without a word about the important matter of dosage. It has frequently been stated that it takes ten to twenty times as much sulfanilamide (or allied drug) to cure an experimental streptococcus infection in the mouse as to cure an infection with the same organism in man. Such statements still occur in the current literature. This idea is based on the amount of drug given per kilogram of body weight. As an actual fact, experimental infections in the mouse respond to blood concentrations of these drugs which are certainly not higher but probably lower than those effective in infections with the same organism in man. The erroneous conceptions of dosage are due to several facts, namely, that different schedules are used by mouth, that dosage between mouse and man is not directly related to body weight, and that the mouse absorbs and excretes many of these drugs much more rapidly than man. It takes more than ten times the daily dose, on the basis of body weight, to give the same blood concentration in the mouse as in man. I do not believe that any real scientific justification exists for the *size* of the doses employed at present in patients; what has been developed is empirical but probably the best that can be done at present. Until more is known in a quantitative way about strain susceptibility and resistance of various organisms to these drugs, one is not justified in experimenting with human dosage in serious infections. It is important to realize that this group of drugs is the only one in all therapeutics where the dose is based upon blood concentration, where sound experimental observations justify the *schedule* of dosage, and where a maintenance of a constant blood concentration over several days is attempted. This is probably owing to the fact that these drugs are given in such quantities that it has been fairly easy to devise accurate and simple methods for determining their concentrations in the blood.

We come now to a consideration of an equally important side of the subject of bacterial chemotherapy, namely, the effects of the drug on the host. This involves a determination of toxicity, of the action of the drug on individual organs and tissues, of the absorption, excretion and distribution of the drug and of the fate of the drug or changes undergone by it in passing through the host. These points are all considered in orthodox pharmacology in the investigation of any new drug, but I think that certain departures from the orthodox methods are necessary in studying these newer antibacterial drugs.

In connection with toxicity, it seems obvious that both acute and chronic toxicity should be determined on several species of animals. In addition, for the subacute or chronic toxicity, we should know the blood concentration which, when maintained for several days, produces toxic symptoms—the same method being used to determine the toxicity as when the drug is administered for its therapeutic effect. There is good evidence to believe that a blood concentration which is innocuous if maintained for only a few hours may prove quite toxic if maintained hour after hour for several days. One must also remember that toxicity is not altogether a matter of life and death; the limiting factor in the use of these drugs is generally some toxic manifestation which does not kill the patient. We need more determinations of this kind of toxicity in animals, the relative effect of these drugs on the blood, cerebral cortex, vomiting center, kidneys, liver, etc., and also the development of methods for assessing on animals the ability of the drugs to produce reactions which in men are due to hypersensitiveness and idiosyncrasy.

In determining toxicity by giving drugs by mouth, there is a pitfall into which many have fallen—the reporting of a substance to be of low toxicity, when this is due to its poor absorption from the gastrointestinal tract. In my opinion, we are no more justified in considering such a substance as non-toxic than we

would be in the following instance. Barium sulfate given by mouth is not absorbed into the body; it remains *outside* of the body and hence is not toxic. To conclude from this experiment that barium is nontoxic is about the same as concluding that potassium cyanide is not toxic because I can carry a well-stoppered bottle of several hundred grams in the pocket of my coat without experiencing any ill-effects. We need to know the effects of these drugs after they have gained access to the blood and tissues; we should base toxicity on the relative blood concentrations which produce equal responses.

While the effects on the host of sulfanilamide and sulfapyridine have been investigated in some detail, the effects of other drugs of this group which are used clinically are not known in any detail. Such a state of affairs is unfortunate and needs correction if real scientific advance is to continue. To cite one point only, the rapid diffusion and penetration of sulfanilamide to all parts of the body is undoubtedly one of the factors which makes the substance a successful chemotherapeutic agent. This does not occur with the same readiness with all drugs of this group: accurate data on those to be used in the patient are important.

The intelligent use of any drug demands that its mechanism of action shall, if possible, be understood, and the logical method of attacking the problem of substitutes for use in place of sulfanilamide or in infections in which this drug fails would appear to be the discovery of how sulfanilamide acts. It is not surprising that in the very short time that intensive work on sulfonamide derivatives has been pursued no satisfactory explanation of the mechanism of action has been found, for in the chemotherapy of no protozoan diseases is there as yet any complete and generally accepted explanation of the mode of action of the drug. The subject of the mechanism of action is obviously very important and justifies intensive work: There is reason to believe that the attack upon this problem may be more fruitful of results in the case of bacterial than in that of protozoan chemotherapy.

Time will not permit, nor do I think it worth while to attempt to trace in detail the numerous studies on the mechanism of action of these drugs; the subject has been frequently reviewed and will be the topic of our round table discussion to-night. All observers seem to agree that sulfanilamide inhibits the growth of the invading organisms and that this alone may be sufficient to control the infection but not to eradicate it without the cooperation of the defense mechanism of the host. Many chemical compounds are bacteriostatic or bactericidal in high dilution *in vitro,* but have no action on infections *in vivo* at all comparable to that of the sulfanilamide group of drugs. More than high bactericidal properties *in vitro* is necessary to make a drug an effective chemotherapeutic agent: Low toxicity to the defense mechanisms of the host and penetration of all tissues and organs may be quite as important as bactericidal properties. I should like to point out the need for accurate and quantitative data upon which to base and by which to test out theories of mode of action. Such data should cover the many ramifications of this field; such as *in vitro* studies, the nature of the curative process in infected animals, the relation of *in vivo* activity of various compounds, correlation of the metabolism and biochemical behavior of various organisms with their resistance or susceptibility to these drugs, the curative effect of the drugs on the same organism in different hosts, the question of changes of the drug by the host or infecting organism, and the effect of changes in chemical constitution on the activity of a drug. It is only when a broad outlook on the whole problem is taken that the true mechanism of action will be revealed. Methods are lacking at present for a quantitative study of many of these problems.

The situation referred to above is well illustrated by the present status of *in vitro* studies. The data so far published are discordant and contradictory; different workers have reported that concentrations of sulfanilamide of 10 to 20 mgm. per cent. may result in experiments on streptococci in no effect, in bacteriostasis and in bactericidal effects. Obviously, fundamental studies of the variables encountered in *in vitro* experiments must be undertaken. As a result of such studies we know now that the strain of streptococcus, the size of the initial inoculum, the composition of the medium, and the temperature at which the test is performed may have a marked effect on the result obtained. More carefully controlled and quantitative work *in vitro* is needed under different conditions before the true *in vitro* activity of these drugs can be defined.

Another very important problem is the relation of chemical constitution to antibacterial action. This is simply a part of the broader problem of the relation of chemical constitution to pharmacological action, one of the important problems of pharmacology, but one about which comparatively little is known. Although over a thousand chemical compounds of the sulfonamide type have been prepared and tested for their therapeutic effect in infected mice, only qualitative conclusions can in general be drawn because few comparisons of active substances have been made in a really quantitative manner. Neither the sulfonamide group nor sulfur in the molecule is necessary for activity. With few exceptions, all the compounds which are active contain a nitro, amino or substituted amino group in the para position on the benzene ring. The exceptions need to be examined more carefully.

Active compounds related to sulfanilamide can be divided into two classes: the first where there is substitution in the amino group, and the second where there is substitution in the sulfonamide group. To the first class belong such compounds as prontosil, neoprontosil, and N^4-benzylsulfanilamide; to the second, such substances as sulfapyridine, sulfathiazole and N^1-acetylsulfanilamide (Albucid). It is probable that substances of the first class owe their activity to their decomposition to sulfanilamide. Although it has been shown definitely that sulfanilamide is formed from these substances in the animal body, it has not been proved that their activity is entirely explained by this. Quantitative comparison of the effectiveness of the same blood concentration and duration of sulfanilamide as obtained from these substances with that obtained by giving sulfanilamide itself is necessary to solve the problem. The sulfanilamide derivatives of the second class are apparently not decomposed in the body and owe their activity to the compound as such. Although not proved, it is probable that nitro compounds act through the amines known to be formed from them.

Numerous other problems might be discussed if time permitted. To mention only a few. Can we obtain a substitute for sulfanilamide with the same activity but less toxicity to the host, or are activity and toxicity intimately linked together? Sulfapyridine, although highly effective, would not seem to be the final answer to the pneumococcus problem. More active drugs against viridans, staphylococcus, tuberculous and other infections are needed, and the whole question of the action of this group of drugs on virus infections needs careful investigation. The relative value of drug and serum and drug alone may be of importance in different types of infection. The marked susceptibility of fungi *in vitro* suggests the trial of these drugs in mycotic infections, while their definite bactericidal action *in vitro* reopens the question of local use.

In conclusion, there is no doubt that bacterial chemotherapy already occupies an important role in the therapy of infectious diseases, and with the solution of more and more of its problems will probably increase in importance. This lusty young infant in therapeutics can hold its own against its father—protozoan chemotherapy—and can even possibly aid it. Ehrlich's aim in chemotherapy was to make a success of the method of *Therapia magna sterilisans,* to destroy the invading parasites within one or two days by a single dose of a drug. Although a certain amount of success with this method was achieved in certain experimental infections in animals, it has not been successful in human diseases of protozoan origin. The alternative was adopted of administering repeated doses separated at intervals. These intervals were frequently sufficiently long to allow the previous dose to disappear from the body. In bacterial chemotherapy, it is now known that maintenance of a more or less constant concentration of drug in the blood and tissues day and night is necessary for the most effective therapy: Both an effective blood concentration and a sufficient duration of this concentration are necessary. To accomplish such a course of therapy, dosage must be thought of in terms of blood concentration and duration rather than in terms of the amount of drug administered. This method should be considered in protozoan chemotherapy; the results already reported in the arsenotherapy of syphilis by the continuous intravenous drip method are encouraging. The method of dosage introduced by bacterial chemotherapy may after all be considered a return to Ehrlich's *Therapia magna sterilisans* with a maintained blood concentration of drug replacing the single massive dose.

SCIENTIFIC EVENTS

THE ENGINEERING SOCIETIES LIBRARY[1]

THE Engineering Societies Library has made available at cost not only photostats, but also microfilm copies of material contained in the 160,000 volumes and thousands of periodicals in its collection. Regardless of residence, any engineer, library or company may order an 11×14-inch white-on-black (negative) photostat print on bromide paper at 30 cents each, which charge includes ordinary postage to any part of the world. Black-on-white (positive) prints are supplied by copying a negative print, which makes the cost 30 cents additional. Microfilm copies on 35-mm film are available at a cost of four cents per exposure (usually one page), with a minimum charge of $1.25 per volume.

Each photostat print contains one or two pages of the original article depending on its size. Reductions to approximately one half or enlargements to twice the original size can be made, if desired, without any extra charge. Unless an enlargement or reduction is specified, prints are made the same size as the original.

For the benefit of those who can come to the library in person, a trained staff stands ready to assist them in their problems. Despite the fact that it is the official library of national engineering societies, all

[1] The library is a joint cooperative enterprise of the American Society of Civil Engineers, the American Institute of Mining and Metallurgical Engineers, the American Society of Mechanical Engineers and the American Institute of Electrical Engineers.