THE BACTERICIDAL PROPERTIES OF THE QUATERNARY SALTS OF HEXAMETHYLENETETRAMINE.

I. The Problem of the Chemotherapy of Experimental Bacterial Infections.

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(Received for publication, March 1, 1916.)

The researches of Ehrlich and his coworkers during the past decade, which led to the discovery of salvarsan, have created a new procedure in the search for remedies for the control of infectious diseases. The discovery of this drug was the result of a logically conducted development of the chemistry of organic arsenic compounds in the directions indicated by their biological properties. This new procedure may be regarded in a way as a revival of the one in use during the time preceding the period of purely biological method, for at that time the therapeutic values of quinine, mercury, arsenic, and salicylic acid were first discovered. The resemblance of the old to the new consists essentially in the fact that in each case chemical substances were used as the therapeutic agents, with the difference, however, that the finding of the old chemical remedies in no way involved the use of synthetic organic chemistry, controlled by constant biological cooperation, the essential feature of the new chemotherapeutic method. This new departure must not be confused with the older synthetic pharmacology, the aim of which was quite different.

A few workers, and the number is constantly increasing, have been attracted to the new field, and the occasional report of promising results, taken with the drugs already proven of therapeutic value, affords ample justification for greater expectations. But to each worker the first and perhaps greatest problem which presents itself is the finding of leads. Salvarsan, optochin, and the trypan dyes, the chief products of the new chemotherapy, all owe their discovery to the development of leads which had been furnished by clinical observations in the

field. Arsenic, mercury, quinine, and certain dyes are perhaps the only substances which up to now have been regarded with assurance as leads for chemotherapeutic development. And of these, with the exception of optochin and perhaps of salicylic acid, all have been indicated for protozoan infections only, and there is promise that by the proper development of these substances the problem of the control of most protozoan infections will be successfully solved.

When we turn, however, to the cure of infections of purely bacterial origin the problem which confronts us is found to be more difficult of attack. Here not only the difficulty of procuring a suitable experimental infection as a test object, but the absence of clinical observations which might be regarded in the nature of leads which could form the basis for a rational chemotherapeutic procedure, makes it imperative from the start to seek for such leads. The successful chemical development of quinine which led to the discovery of ethylhydrocuprein already indicates that resource may be had eventually to the above mentioned protozoan leads. And it is a fact that perhaps with the exception of salicylic acid this drug is the first substance to have been used successfully in an experimental bacterial infection. This one fact creates the hope that chemotherapy may find a wider application in the control of bacterial infections. It is conceivable, however, that ultimate success may depend upon the finding of leads other than those which have been successfully used against protozoan infections.

It would seem that a wealth of material should lie ready among the numerous classes of organic substances which have been found to exert powerful bactericidal effects *in vitro*. But the failure of many of these when tried in experimental infections has led to the realization that besides a bacteria-killing property the fulfillment of certain other requirements is essential for the achievement of an internal antisepsis. Some workers believe that no indication of the probable effect of a substance *in vivo* can be discerned from its action *in vitro*, and that the successful control of an infection by chemical agents can be attained only by indirect means. This might occur, on the one hand, as perhaps in the case of atoxyl, by the chemical transformation *in vivo* of the injected drug into some active form. On the other hand, the substance might act through the protective mechanism of

the host either by increasing phagocytosis or by stimulating the production of immunity principles. It is, of course, probable that in some instances these phenomena might play a part and that a possible scheme of chemotherapeutic attack could be developed along these lines.

Owing to the ease of handling bacteria *in vitro* and the simplicity of the bactericidal tests it would be unwise because of former failures to condemn the *in vitro* method, at least as a means of initial orientation. If it were accompanied by certain parallel studies the *in vitro* method should do more than afford only orientating data. As pointed out by Bechhold and Ehrlich[1] and others in the past, besides a mere bactericidal power other conditions must be fulfilled before a substance may be considered even a possibility as a therapeutic agent, provided of course a direct action by the drug itself is in question.

Aside from the obvious conditions of solubility and relative nontoxicity, the drug once in the circulation, whether by direct intravenous injection or by absorption, must be maintained therein for a sufficient length of time and in sufficient concentration in order to unfold its *in vitro* effect. In other words, its free access to the foci of infection should not be completely obstructed.

To accomplish this it must not enter too rapidly into chemical or physical combination with the constituents of the tissues, of which the blood is a fair representative. It must not be too speedily eliminated. And, finally, it must not be too rapidly altered in any way by metabolic processes which would nullify its bactericidal character. There may be still other and less definite factors which separate the *in vitro* from the desired *in vivo* result. If, however, the *in vitro* bactericidal tests could be complemented by a parallel study of those properties of substances which would decide whether they could satisfy the above requirements *in vivo*, our choice of substances for the *in vivo* experiments could be in great measure controlled. A system, though somewhat arbitrary, would be substituted in an undertaking which would otherwise be directed by a haphazard and entirely opportunistic policy.

These considerations have convinced us that the procedure in the search for leads in the chemotherapy of bacterial infections may be

[1] Bechhold, H., and Ehrlich, P., *Ztschr. f. physiol. Chem.*, 1906, xlvii, 173.

logically systematized as follows: Substances which either by their general structure or by the possession of characteristic atomic groups are representative of as many types of organic substances as possible should be systematically selected for bacteriological and biological testing. Such facts as the bactericidal power and partial specificity for certain types, compatibility with tissue constituents (serum), and resistance to profound and rapid metabolic alteration should be noted and considered in the final interpretation of what in the chemical constitution of the substances is responsible for the observed biological behavior.

With organic substances there will be considerable difficulty in satisfying the last requirement. In the case of arsenicals and mercurials it is immaterial whether metabolization should occur, for the therapeutic characteristics of such compounds are elements. Their value may partly depend upon such metabolization. It does not seem improbable, however, that bactericidal substances may be found which, even though to a less degree than the arsenicals and mercurials, may be sufficiently resistant to metabolic changes to enable them to produce a sterilizing effect before they are disposed of by the host. The large number of pharmacologically active preparations must all persist long enough after administration to produce their physiological actions.

From the representative substances which have been found to possess the required biological properties, two classes of leads might be obtained: first, those substances which, like quinine, owe their bactericidal action to the general structure of the molecule; and, second, those which, like phenol, are bactericidal principally because of the possession of a certain atomic group. Once in the possession of *bactericidogenic*,[2] tissue-compatible molecules or side-chains, the same systematic development so successfully employed in the development of organic arsenicals by the alteration or addition of groups to the molecule might be here repeated in order to augment the specific bactericidal action, to detoxify it, or in some other way

[2] The word *bactericidogenic*, of obvious derivation, is employed in this and the following articles as a convenient term to express the property of certain chemical groups, when introduced into an organic molecule, of imparting bactericidal properties to that molecule.

furnish it with biologically desirable properties. In this way substances could be obtained which would form a rational basis for chemotherapeutic investigations.

The problem of the chemotherapy of bacterial infections and a possible scheme for its systematic attack have been discussed above in some detail with the purpose of affording a basis for a better understanding of the material which will be presented in the following papers.

From its nature this material will touch on but one phase of the above scheme and no claim is made of its complete realization. We shall present the results obtained in a systematic attempt to alter chemically the molecule of hexamethylenetetramine with the object of obtaining a class of bactericidal substances which could be employed in experimental infections. The use of this drug was inspired by the interest felt by Dr. Flexner in the possible application of some of its derivatives in the treatment of experimental poliomyelitis, and the material which will here be presented is but a part of a larger undertaking executed with it.

We shall attempt to show how, by the selection of a certain molecular group, namely hexamethylenetetramine, it has been possible to demonstrate its general bactericidogenic character. By the combination of this substance in the form of quaternary salts, in the manner to be described later, with a great variety of other molecular groupings a new class of bactericidal substances has been prepared[3] in which the bactericidal nature was principally attributable to the hexamethylenetetramine nucleus. On the other hand, the degree of this action was determined by the nature of the molecular groups added to hexamethylenetetramine. These added groups were likewise responsible for the partial specificity of certain of the preparations for particular bacterial species. This partial specificity did not favor one species alone, but all the species tested were found to be separately and specifically susceptible to some particular type of hexamethylenetetramine derivative. We must therefore conclude that the bactericidogenic character of hexamethylenetetramine ex-

[3] For the chemistry of these substances and the references to those prepared by others see Jacobs, W. A., and Heidelberger, M., *Jour. Biol. Chem.*, 1915, xx, 659, 685; 1915, xxi, 103, 145, 403, 439, 455, 465.

hibited in its quaternary salts is not specific but general in character. The specificity, however, is furnished by the proper choice of the molecular grouping added.

It will also be shown that a few of the hexamethylenetetraminium compounds which were tested were either not at all or but slightly inhibited by serum. A few, on the other hand, were found to be greatly inhibited by serum. The fact, however, that any one of the hexamethylenetetraminium salts is compatible with serum is enough to demonstrate the serum compatibility of the bactericidogenic hexamethylenetetramine portion of the molecule itself. We have here, therefore, a bactericidogenic, serum-compatible group. The remainder of the molecule determines the serum incompatibility of those substances the action of which was found to be inhibited by serum.

In the same way the toxicity relationships were found to be determined by the groups contained in that portion of the molecule added to the hexamethylenetetramine nucleus.

We can regard the material here presented merely as a beginning, but we feel that such a treatment of the problem as here presented may ultimately result in an accumulation of data which will be of value in the systematic search for substances which may be used in the control of experimental bacterial infections. Before passing judgment, however, on the chances offered by the further development of the quaternary salts of hexamethylenetetramine, the behavior of these substances in the animal organism should be studied in order to determine whether the bactericidogenic group in itself is sufficiently resistant towards metabolic changes. Otherwise these compounds as a class would be bactericidally inert *in vivo*.