THE CHEMOTHERAPY OF PROTOZOA
AND BACTERIAL INFECTIONS

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The term Chemotherapy in its more restricted sense is employed to designate that branch of investigation which deals with the discovery of chemical agents which act specifically on infectious diseases and with the study of their mode of action. The introduction of this term we owe to Ehrlich and the great progress which has been made in this field within the comparatively short space of two decades is due largely to the brilliant achievements and leadership of this worker. The subject is now so vast that in the short time which is allotted to us we cannot do more than discuss certain phases of what has been learned. Our treatment of the subject must naturally fall into two divisions, that dealing with the chemotherapy of bacterial infections and finally, that in which animal parasites have been made the objective. We shall deal with the former first, since efforts to influence experimental bacterial infections with chemical compounds antedate similar studies with animal parasites.

Not long after it became known that there are substances which would kill micro-organisms in the test tube, attempts were made to apply such germicides to disinfection within the organism. All of these experiments postulated a direct action of the chemical upon the micro-organism within the host such as occurs in disinfection outside of the body. Among the very first investigations in this direction were those of Koch who attempted to sterilize animals which had been infected with anthrax bacillus by the use of bichloride of mercury.

Although he was able to inject this substance into the blood in such an amount as to achieve a concentration many times that which was necessary to kill the micro-organisms \textit{in vitro}, all
the attempts proved fruitless. Koch attributed the discrepancy between the in vitro and in vivo results to the fact that mercuric chloride forms a chemical union with the serum proteins, as evidenced by the formation of a precipitate and was therefore rendered ineffective.

In later work, particularly that of von Behring, we see numerous other attempts to achieve internal disinfection. Occasionally indications were obtained with the use of certain inorganic substances but such results proved of no practical value because of their great uncertainty and irregularity and the great toxicity for the animal shown by the amounts of chemical required. Although occasional efforts in this direction continued, this type of investigation came to be regarded as futile and almost impossible of achievement. We can see in the words of von Behring an expression of this gloomy outlook. After ten years of persistent attempts he finally concludes:

"It can be regarded almost as a law that the tissue cells of man and animal are many times more susceptible to the poisonous effects of disinfectants than any bacteria known at present. Therefore, before the antiseptic has a chance either to kill or to inhibit the growth of the bacteria in the blood or in the organs of the body, the infected animal itself will be killed. The pessimism of him who declared that disinfection in the living body is for all time impossible appears to be only too justified."

But with the more recent highly successful application of synthetic drugs to the treatment of protozoan diseases and because of the failure in so many instances of the more rational immunological methods, interest again centered in the possibility of the therapeutic application of bactericidal agents. The problem has proven extremely difficult to approach by any other means. It has been particularly difficult because of the lack of an empirical background such as had placed the qualities of arsenic, quinine and mercury at the disposal of the worker with protozoan infections. It has seemed to some that the only method short of the mere empirical testing of all sorts of substances in the experimental animal was to employ the
bactericidal test at least as a means of initial orientation, even though it appear the crudest makeshift.

The first extensive experiments in this direction were made by Bechhold and Ehrlich, who found in the group of halogenated phenols a number of substances which exhibited in vitro a high order of inhibiting action on the growth, especially of the diphtheria bacillus. At the same time it was possible to follow certain influences which chemical constitution seemed to exert on antiseptic action, such as the exalting effect, up to certain limits, of the multiplication of halogen groups or the addition of methyl groups to the molecule. But what is more important was the observation that none of these substances would sterilize laboratory animals infected with the diphtheria bacillus, and this, in spite of the fact that the toxicity of some of the substances was low enough to permit the achievement of a concentration in the blood 100 times that required to kill this bacillus in the test tube. A possible explanation for this failure was again found in the greater affinity of these disinfectants for the blood proteins than for the bacteria. Although no visible precipitate was caused on mixing their solutions with serum, as in the case of mercuric chloride, their bactericidal action in vitro was greatly reduced by the process. In this work we find for the first time the expression "partial specificity" to designate a type of selective antiseptic action which a chemical may display towards a particular micro-organism.

Compatibility with serum and partial specificity are factors upon which the subsequent workers with antiseptics have come to lay particular emphasis and their observations have furnished us with not a few examples of the latter. In the tendency toward specificity was found a grain of hope, since it seemed a step in the right direction—in the direction of the specificity displayed by immunity principles. Many instances of partial specificity have been observed especially among the organic dyes. A number of these observations have been used as the basis of methods for the differentiation of bacteria in culture, such as that which employs gentian violet in media to restrain the growth of Gram-positive strains, or the use of brilliant green
to separate the typhoid from the colon bacillus. A study of the bactericidal properties of the quaternary salts of hexamethyl-
lenetetramine has brought out instances of substances which displayed a decided preference for the streptococcus. In not
a few cases, substances were found which killed the streptococcus in dilutions which were 20-30 times greater than those required
to sterilize suspensions of meningococcus and gonococcus. A
most unusual example of specificity has come to light in the case of the toxicity of the alkaloid optochin for the pneumococcus,
but to this we shall return later. Quite recently Browning, Cohen and Gulbransen, have uncovered perhaps a still more
remarkable instance in the case of the cyanine dye, sensitol red.
The lethal effect which it displays against staphylococcus is 2000
times that which it exhibits against the colon bacillus in peptone
water. I am, however, not aware of any experiments which have
been performed with this substance in infected animals.

In the quality of serum compatibility we have presented to
us in its simplest form the idea that a substance must display
a greater affinity for the micro-organism than for the tissues
of the infected animal in order to be germicidally efficacious.
But few substances have been found which retain their full
activity in the presence of serum. A few favorable instances
have been observed among the quaternary salts of hexamethyl-
lenetetramine which were prepared from certain chloracetyl-
amino compounds. A number of these exhibit a bactericidal
effect in serum and protein solution which is very little less than
that found in salt solutions, but their application to internal dis-
infection was without result. Optochin maintains toward the
pneumococcus a high degree of bactericidal efficiency in serum.
The low concentration of 1:20,000 in serum and even that of
1:8000 in oxalated blood have proved sufficient to kill this micro-
organism in 2 hours and in much higher dilutions it exhibits a
definite growth restraining influence. However, this is con-
siderably less than what is observed in ordinary salt solution,
since in the latter medium a dilution of 1 to 500,000 or more
is all that is required. Much more favorable relationships have
been observed in the case of acridine compounds. Browning
and his co-workers have found that diaminoacridine and its methochlorides, or proflavine and flavine respectively, actually exert a more potent action on the staphylococcus and colon bacillus in serum than in ordinary saline. This very unique quality and the unusually potent bactericidal properties of these substances led to their application in the local treatment of wound infections.

Still more difficult than to find substances which will resist the diverting influence of serum constituents is to find antiseptics which will maintain a high bactericidal effect in blood. In experiments in our laboratories, Doctor Felton made a number of observations in regard to this point, and was able to find but few instances of substances which retain a high order of antiseptic potency in this medium. Mention may be made of malachite green and a few of its derivatives and especially the flavines and certain dyes of the rosinduline group. The observed values were, however, lower than those shown in ordinary saline.

There are still other properties which may be considered among the desiderata for the ideal internal disinfectant. One of these is speed of bactericidal action. It has been very difficult indeed to find substances which act rapidly under the conditions which obtain in the circulation. In order to kill the pneumococcus in 15 minutes, optochin must be used in a concentration of 1:1000 in whole blood. Even flavine in the same concentration fails to sterilize in one hour, although in two hours it may act in a dilution of 1 to 32,000. Taken alone, speed of action may not be essential, especially if a substance can be maintained sufficiently long in the circulation to develop slowly its action or to exercise merely a growth restraining or bacteriostatic effect. But here again we must postulate other conditions in order to obtain such a result. Repeated observations with optochin in animals and in man have shown that it is possible to render the blood antiseptic for pneumococcus but the observations of Moore and Chesney have shown that this may be complicated by the development of fastness. Browning and Gulbransen have demonstrated that the blood of rabbits may
display a degree of inhibiting action for staphylococcus and the colon bacillus for several hours after the administration of diaminodiacridine, but this alone was apparently inadequate.

In the control of certain infectious processes the difficult requirements of proper distribution and tissue penetrability of the antiseptic must be taken into consideration. In a former lecture before this Society, Lewis, in describing his experimental studies on the chemotherapy of infections produced by the tubercle bacillus, has stressed the importance of combining in a substance a degree of germicidal action with specific tissue penetrating power.

Finally, there is another group of requirements which must be discussed in connection with the \textit{in vivo} operation of bactericidal substances. Not only must a substance be of sufficiently low general toxicity, but it must prove comparatively harmless to the general powers of resistance and to the normal protective mechanism of the infected animal. In this connection we may have to deal with quite an extensive and perhaps less tangible group of factors which can be incorporated into systematic study only with the greatest difficulty. It may be just at this point that the real difficulty lies and we shall have occasion to refer to this later.

In this connection, observations have been made to determine the effect on phagocytosis which might be exhibited by bactericidal substances. The experiments of Felton\textsuperscript{8} have recently shown that not a few highly bactericidal dyes and cinchona derivatives markedly reduce the phagoecytic index in dilutions in which they might exhibit a bactericidal effect in blood. Exceptions in this regard may perhaps be the flavines, which, according to Browning, are not appreciably injurious in dilutions above 1:500.

But if we consider in retrospect all of the conditions which may separate the observed \textit{in vitro} properties of an antiseptic from its successful operation in an infected animal, it is apparent that we have presented a very difficult case for the idea that the control of bacterial infections may be approached from the standpoint of internal disinfection. This has certainly been
very generally borne out in attempts to apply such substances in actual infections. We shall later see that even in the more successful application of compounds to the control of protozoan infections, there have appeared repeated discrepancies between the action of substances on the parasite in the test tube and in the animal. It would seem, therefore, that for the time at least, we are thrown back on the empirical testing of substances in the infected animal as the only safe procedure.

The first striking instance of the successful application of a chemical to the treatment of a bacterial infection in the laboratory animal we owe to the observations of Morgenroth and his co-workers. Previous studies of Neufeld and others had shown a certain biological similarity between trypanosomes, spirilla, and the pneumococcus in the susceptibility of their envelopes to the dissolving action of bile salts. While engaged in a study of the effect of quinine derivatives in experimental trypanosome infections in small animals, Morgenroth and Halberstadter were able to demonstrate a certain curative effect of these substances. Then partly because of the possible biological similarity between the pneumococcus and the trypanosome and because of certain clinical observations on the applicability of quinine in the treatment of pneumonia, he was induced to make similar studies in experimental infections with the pneumococcus. Morgenroth and Levy found that a pneumococcus septicemia induced in mice was but little affected by injections of quinine. On passing to hydroquinine, which differs from it by but two hydrogen atoms, a more definite influence was to be seen in delaying the death of the animals. An optimal effect, however, greatly exceeding that of the former was furnished by ethylhydrocupreine, or optochin, in which the methoxyl group of hydroquinine has been changed to ethoxyl. It was found possible to save a good percentage of the infected animals by repeated injections particularly of a solution of the drug in oil. Unfortunately the clinical application of the drug has been limited by its untoward toxic properties, especially the tendency to produce amblyopia. Moore and Chesney, from extensive observations on patients suffering from acute lobar pneumonia, have
concluded that the use of optochin in the treatment of this condition cannot be recommended, since it is impossible to administer a sufficient amount to produce an effective concentration in the bloodstream without exposing the patient to the danger of toxic effects. During the use of the necessarily subeffective doses the pneumococci were observed even to become fast to the drug.

It would appear that Morgenroth in his first experiments was unaware of the potent germicidal action of this substance on the pneumococcus, so that a knowledge of this property played no part in its initial chemotherapeutic application. We owe to Wright the recognition of this quality as well as that of the fact that optochin kills the pneumococcus in high dilution in the presence of serum. The confirmation of these observations led Morgenroth to attribute the curative action of optochin in the animal to its direct bactericidal effect and in his later studies he used the test tube as a guide. This later work developed the interesting influence which the chemical structure of the cinchona alkaloids exhibited on their specific antiseptic action.

On passing from quinine, through hydroquinine to optochin, a sudden jump in the action upon the pneumococcus occurs to fall again on passing higher up the series of alkyl compounds. However, with the stage of the isoamyl ether a bactericidal effectiveness towards streptococcus and staphylococcus becomes evident which is augmented on passing to the heptyl and isooctyl compounds and then drops again. These observations while interesting in themselves had to do rather with external disinfection and proved inapplicable to the treatment of systemic infections.

It would seem that the powerful bactericidal properties of optochin lend support to the belief that its curative effect in mice is a result of its direct bactericidal action. But certain observations of Neufeld and Engwer, and later by Moore, have suggested the interposition of the host in the process. Moore found that a single small dose of optochin in oil which in itself was quite ineffective in the treatment of an experimental pneumococcus septicemia in the mouse, when combined in the
treatment with an amount of homologous anti-pneumococcus serum which was also far under the effective dose was capable of increasing the threshold value of this serum at least fifty times. This result indicates that the combined action of the two therapeutic agents was many times that of a simple summation of their protective effects. Whether a similar synergism or rather fortification occurs between the drug and certain undetermined protective factors when the drug is used alone in a so-called curative dose has not been definitely ascertained, but Moore’s observations would seem to weaken somewhat the view that the bactericidal effect is the only factor.

Taking all that we have considered with reference to optochin and its near relatives, as well as the ancient reputation which quinine has always had, we cannot escape the enticing suggestion that perhaps there is something inherent in the molecule of these alkaloids which, like the elements arsenic and mercury, has conferred upon them certain potentialities as specific etiological remedies and that this property might be turned to wider usefulness if we could only find the way.

A number of years ago we turned our attention to this problem in the laboratories of the Rockefeller Institute at the suggestion of Doctor Flexner and also of Doctor Cole who was particularly interested in the possibility of finding new therapeutic agents for the treatment of infections produced by the pneumococcus. In this work Doctor Heidelberger and I were extremely fortunate in the enjoyment of the skilful biological coöperation of Doctor Wollstein and later of Doctor Felton.

The first problem was purely chemical. We have already seen what a pronounced change in biological properties has followed but slight chemical alterations in the quinine molecule. Would it be possible to find other means of altering the cinchona molecule in a systematic manner, but in a way to maintain its general integrity, and perhaps permit us to follow the influence of chemical modification on biological action? The naturally occurring members of the group, as we have seen, had been thoroughly studied and also their hydro-derivatives and the homologous ethers of hydroquinine as well. A successful solu-
tion of the chemical problem was found by making use of the fact that these alkaloids as tertiary bases react with alkylhalides to form quaternary salts. In one direction in particular—by the use of chloroacetylamino compounds as alkylhalides it was possible to develop if necessary a practically unlimited number of new derivatives, and, in a way, to leave the original alkaloid molecule intact. Many substances were prepared into which a thorough selection of the usual organic groups had been introduced. Then again a similar opportunity was offered in the preparation of a series of hydroxyazo dyes from hydrocupreine, the phenolic compound obtained by demethylating hydroquinine. By coupling hydrocupreine with the diazonium salt of any aromatic amino compound it was possible to make a logical series of substances in which any chemical change could be featured at will. Still other series of a similar character were prepared, so that in a way it might be said that the opportunity of testing the influence of chemical changes on the action of these alkaloids had been fairly completely presented. While realizing from all that was said before that the measure of bactericidal power was of doubtful importance, nevertheless, such studies were made because of their possible statistical value and since no other criterion short of the animal test was available. Within the groups of quaternary salts and azo dyes numerous instances of marked bactericidal power for the pneumococcus were noted and in several cases were found to equal even that of optochin. However, in the majority of instances, this activity was found to be greatly diminished in the presence of serum. I shall not burden you with an extensive account of these observations, but shall rather summarize the results obtained in the use of these substances in the experimental pneumococcus infection. Such tests made on substances, irrespective of bactericidal potency, showed a few which seemed to exhibit a small measure of therapeutic effect. The quaternary salts of hydroquinine with chloroacetyl-\(p\)-aminophenol and chloracetyl-\(p\)-anisidine, in particular, if given not too long after inoculation of the mice or if not in too large amounts, often, though irregularly, cured the animals. An interesting and not infrequent observation which
was made with these and similar substances was the effect of doses above a certain threshold, even though much under the lethal dose. In such cases, instead of the apparent cure observed with smaller doses, an actual acceleration of the disease was noted. An explanation of this curious paradox is perhaps not difficult to find, and may lie in the possibility that amounts of drug which are not frankly toxic may be sufficient to injure the animal’s natural powers of resistance in such a way as to make it more susceptible to the infection.

In this connection, we have made also a survey of the various groups of organic dyes in regard to their curative effect on pneumococcus infections in laboratory animals, but with similar unpromising results. Even flavine with its high bactericidal power in blood and serum for the pneumococcus gave but the slightest indication of influencing the infection. Observations made with quite a variety of other classes of substances were of a similar negative character.

To sum up—while the experience of workers has brought to light not a few groups of bactericidal compounds, very few individual substances have displayed even a trace of specific therapeutic activity. These few substances have proved to be unique within the group to which they belonged, since closely related compounds, or those differing from the active substances by relatively insignificant chemical modifications have proved to be quite useless. I shall not keep you longer with this portion of the story except a word perhaps as to the general outlook for the chemotherapy of bacterial infections. At present the data available seem quite barren of suggestion. But I do not wish to imitate here quite the gloomy view which was quoted at the beginning of this paper. The mere instance of optochin and of the therapeutic value of chaulmoogra oil and the more recently used esters of chaulmoogric and hydnocarpic acids obtained from this oil in the treatment of leprosy are indications that the control of bacterial infections by drugs is by no means impossible of achievement. The real difficulty lies in the necessarily opportunistic experimental method and the lack of a rational scientific means of approach. The problem is essentially a search for a
substance of unknown nature but which must yet perform a service by a mechanism of which we are equally uncertain. It may be possible to inquire successfully into what biological properties such a substance must possess, or under what biological conditions it will have to operate; but such information will not point to the substance itself. In the case of bacterial infections, experience has presented us with too few points of contact between our knowledge of chemical structure and whatever biological properties may be required in the exhibition of a therapeutic effect to present a rational basis for the search for curative substances. Perhaps the real solution of the problem may be ultimately found by gaining an insight into the chemical nature and mode of operation of the substances which are normally elaborated by the organism in its fight against infection.

If we turn now to the application of chemical agents to the treatment of protozoan diseases, we are confronted by a far more encouraging state of affairs. We have already alluded to the traditional virtue of quinine and mercury and that these were purely empirical acquisitions. However, with the recognition of the etiological factors of the diseases which these substances influenced, the role which they occupied as real curative agents was likewise recognized. During the past twenty years considerably more has been added to our chemical equipment in the treatment of this group of diseases as a result of the application of laboratory methods of both chemistry and biology. In the present paper I can, however, touch upon only certain phases of this development. The appearance in 1902 of the classical experiments of Laveran and Mesnil,21 was the beginning. By the use of sodium arsenite these workers succeeded in temporarily clearing the blood of mice infected with the parasite responsible for the disease of cattle called "Nagana." Then there followed within a few years a succession of observations in which substances belonging to a number of different groups were found to possess a similar efficacy in experimental infections produced by the same or similar animal parasites. These substances fall naturally into several groups which it will serve our purpose best to treat separately. Soon after the observations
of Laveran and Mesnil, Ehrlich and Shiga, demonstrated that mice infected with trypanosoma equinum could be permanently cured by a single injection of trypan red, a tetrazo dye prepared by coupling tetrazotized benzidinesulfonic acid with a naphthylaminedisulfonic acid. This is noteworthy as the first instance of the cure of an experimental trypanosome infection with a single dose of a synthetic chemical. However, the value of the substance itself seems to have been limited, since it failed to exert a similar therapeutic effect in other animals or with other types of trypanosomes.

We meet here for the first time a peculiar paradox which was destined to appear repeatedly in later work. Although trypan red proved to be active in vivo, it was inactive in the test tube. At the time, these workers noted the staining of the tissues of the animal by the dye after injection. They considered that its action was possibly due to the persistent effect of small amounts of the drug or of some active alteration product of it upon the parasites. The production of an active immunity in the animal by the rapid destruction of the parasites by the drug was considered also as a contributing factor. Observations of a similar character with other dyes became the basis for much subsequent discussion and inquiry as to the mechanism involved in the successful removal of trypanosome infections by such substances. In these discussions, which I cannot present at length, attempts were made to explain this paradoxical action also by the assumption of a direct injury produced by the dye on the trypanosome of a character which did not affect their motility but prevented their multiplication. The suggestion that a chemical alteration of these substances in the body to a more active form, while not so obviously applicable to this class of substances, has been more successfully applied, as we shall see, in explaining the action of arsenic compounds. Subsequently, Nicolle and Mesnil, found, from the study of a large number of similar substances, a benzidine dye trypan blue which proved still more efficacious in that it cured mice infected with the more resistant trypanosomes of Nagana and Surra. Dyes of all kinds were then investigated and, in succession, members
of the triphenylmethane group and a number of orthoquinoid dyes such as the pyronines, oxazines, thiazines and acridines were successfully brought into the field of investigation. But the practical application of the dyes, it would seem, has been on the whole rather restricted. The discovery of the therapeutic properties of the individual members of the different groups appears to have been for the most part the outcome of a rather opportunistic selection of substances. There have been attempts to correlate the therapeutic properties of some of them to their chemical constitution and to their selective staining property for the parasite. But, on the surface at least, it would seem that but few facts have emerged which could be applied to a systematic attempt at their further chemical development, or in explanation of whatever degree of therapeutic value they may have shown.

An exception to this, perhaps, is the rather striking property which was found by Ehrlich and his associates to accompany the presence of the orthoquinoid structure of certain dyes. These dyes quickly produced tolerant or fast strains of trypanosomes which were also quite fast to arsenic. Paraquinoid dyes did not show this effect. Again Nicolle and Mesnil, in a thorough survey of the tetrazobilin dye group concluded that in order to develop therapeutic qualities the coupler used must be a naphthalene derivative containing at least one amino group and two sulfonic acid groups. You will see from the chart that this condition is satisfied by both trypan red and trypan blue. And it is possible that this may have been made the starting point for the preparation of a substance known as Bayer-205, which has recently come to offer considerable promise. The exact nature of this substance has been carefully withheld but there is a suspicion that it belongs to a group of compounds which the Bayer Company patented some time ago. I have on the chart formulæ which suggest the possible relationship of this substance to trypan red and trypan blue.

Preliminary observations of Händel and Joetten and Mayer and Zeiss have shown that this substance possesses remarkable curative properties in experimental trypanosomiasis. In small
doses which were often far below the toxic dose for the particular animal, the drug was found to cure the ordinary laboratory animals which had been infected with different strains of trypanosomes. Here again we find the curious circumstances that the substance itself displayed no marked action on the trypanosomes in vitro and microscopic studies seemed to suggest that the drug may act by preventing multiplication of the parasites. Later, therapeutic experiments in dourine in horses pointed to its usefulness as a prophylactic and curative agent but more important was its application to human trypanosomiasis. We shall return to a discussion of this in another connection. Although we can only infer the probable nature of this remedy, it would seem that an advance has been made by applying chemical knowledge to the problem of varying a group of substances which had already given indications of therapeutic values. Thus, the dyes from which this substance has emerged have come to share again, for the time at least, a prominence which was taken from them very early in chemotherapeutic studies by the advance in the application of arsenic compounds.

If we turn now to the arsenic group we find that the organic arsenicals have proved a most profitable direction for the experimental studies which led to the finding of synthetic etiological remedies. This is attributable in part to the fact that we are dealing with substances in which the therapeutic quality is apparently inherent in the arsenic itself irrespective of the actual form or by what mechanism its action may come into play. This has made chemical reasoning simpler in attempting to refer biological action to chemical constitution, for it is far more difficult to interpret what should be the point of reference when a large organic molecule is considered with regard to any inherent biological activity.

The impetus to investigation in this direction came with the report of Thomas,27 that a proprietary arsenic preparation called atoxyl displayed a definite therapeutic effect in animals infected with different strains of trypanosomes. The rapid confirmation of this observation soon led to its employment in African sleep-
ing sickness, and it has proved helpful in the treatment of the early stages of this disease.

But a step of the most far-reaching importance was Ehrlich's brilliant recognition of the true nature of atoxyl as the sodium salt of p-aminophenylarsonic acid or arsanilic acid. In analogy with sulfanilic acid, it is formed by the fusion of aniline with arsenic acid. In its essentials, the chemistry of organic arsenic compounds had been pretty thoroughly studied by Michaelis and his co-workers, but the methods at the disposal of these chemists were, in a sense, limited. The discovery that arsenic could be introduced into the molecule by direct arsenation opened to Ehrlich and his associates a means for a most thorough development of the chemistry of this group of substances. The immediate suggestion which the structure of p-aminophenylarsonic acid presented was detoxification by acetylation of the amino group. But the resulting product arsacetin, though possessed of a degree of therapeutic power, was soon discarded because in its clinical application, like atoxyl, it not infrequently produced blindness and other toxic manifestations. Here again the peculiar paradox appears that the pentavalent compounds are without appreciable action on the trypanosomes in vitro. But Ehrlich's fertile imagination soon found a possible solution for this discrepancy. The fact was noted that individual animals treated with certain pentavalent compounds would show a varying tolerance for the drug and those which exhibited the lower tolerance could be cured by smaller doses. This observation was coupled with the well known ability of the tissues to exhibit a reducing action and with the fact that certain trivalent forms of arsenic are far more toxic than the pentavalent forms. Ehrlich concluded that the pentavalent arsenicals are reduced by the tissues and unfold their activity in the trivalent form. This view was supported by the direct comparison of the relative toxicities of the three stages of oxidation of arsanilic acid, p-aminophenylarsinoxide is 75 times more toxic for the mouse than arsanilic acid, while the arseno compound is 30 times more toxic. Similar toxicity relationships have been repeatedly observed.

But more important still is the contrast in the trypanocidal
The effect of the oxide and the arsenic acid in vitro. In a dilution of 1:100,000 the former kills the microorganisms immediately, whereas atoxyl fails to act even in a 5 per cent. solution. In some cases the arseno-compounds have shown a similar potent action in the test tube but this has not always appeared. Mention may be made of observations of the failure of salvarsan to act directly upon the relapsing fever organism or on the treponema pallidum in the test tube although the organisms thus treated were observed to lose appreciably in virulence as shown by subsequent injection into animals. Ehrlich and Roehl placed particular emphasis on the oxide stage which may be formed either by reduction of the pentavalent compound or by oxidation of the arseno-derivative as the form in which the biological manifestations eventually appear. It is perhaps important to mention this point, since recent statements have appeared which seem to overlook this fact.

The conception of the biological potency of trivalent arsenic was at once applied to further synthetic attempts to find new curative agents in the arsenic group. The great toxicity of the arsenoxides, however, and the possibilities which the arseno compounds came to afford was the reason for the main interest which seemed to attach to the latter group. This work, as you all know, culminated in the brilliant discovery of the value of salvarsan and its derivatives in the treatment of certain spirochaete diseases.

In his theoretical considerations to explain the action of chemotherapeutic agents, Ehrlich assumed a direct action of the substance or its alteration product on the parasites. He conceived this action as being brought about by the avidity of certain groups or chemoceptors in the cell of the parasite for certain groups contained in the chemical. By assuming these discrete affinities within the cell the attempt was made to give more visible expression to certain phenomena which were observed in the reaction of microorganisms to chemicals containing certain groups in the molecule. But, on the whole, workers no longer agree that such precise definition can be given to the relationship between chemical constitution and therapeutic action.
The efficacy of salvarsan was attributed in part to its orthoaminophenol group which was supposed to direct the drug at the parasite in a way to permit the lethal action of the arsenic to develop. The analogy was drawn with trypan blue which likewise possesses an amino and hydroxyl group in positions ortho to one another. But there are many cases where such analogies do not hold. It is said, for instance, that the isomers of salvarsan containing this configuration are all much less active than salvarsan itself. Again particular stress has been laid on the dystherapeutic effect of the methyl group but numerous instances have arisen in which substances containing the methyl group have actually exceeded in effectiveness the unmethylated compound. In this respect, trypanflavine is more efficacious as a trypanocide than the unmethylated proflavine. Quinine, which is methoxychinonidine is more efficacious in malaria than cinchonidine or the phenol, cupreine. The sulfuric group is still another well known example. In many combinations it may destroy therapeutic efficiency. Morphine or quinine when converted into the sulfuric esters, although again easily recoverable by saponification, are physiologically practically inert. On the other hand, several sulfuric acid radicals are contained in the molecule of trypan red, trypan blue and probably Bayer-205. The conclusion is forced, therefore, that we may attribute only the bare possibility of certain effects to certain groups in the molecule and in any event, the molecule as a whole certainly must be considered.

But the striking changes in biological properties that often follow very minute changes in structure and the occasional appearance of regularity of biological response to such changes have always been a fascination and a source of temptation to the worker to try to utilize such general tendencies in the synthesis of drugs. Several years ago, a group of us at the Rockefeller Institute, Doctors Brown and Pearce and Heidelberger and myself were tempted to take up a phase of this question. In this work the attempt was made to obtain some information from a systematic biological study of the toxic and therapeutic properties of certain types of arsenic compounds which might
be applied to the finding of new remedies. This work which at first embraced a study of the treatment of experimental trypanosomiasis was later extended to include that of the experimental infections produced by the relapsing fever organism and experimental syphilis in the rabbit.

We have already described the emphasis which has been placed on trivalent arsenicals. Since the biological activity was an exhibition of a property of some form of trivalent arsenic, it was undoubtedly a logical step, if considered alone, to use this group of compounds directly. But we may regard this question from another angle. There seems fair evidence at hand that the greater tolerance for pentavalent arsenicals as a group is not alone a function of their greater biological inertness as compared with the trivalent-compounds but also to the speed with which the greater portion is excreted before the reducing action of the tissues can manifest itself. This is but a manifestation of the fact that the arsonic acids which form neutral salts possess a relatively greater portability in the blood stream and body fluids than the trivalent forms as well as a greater penetrability and may readily and quickly diffuse through the tissues. The arsenoxides possess this to a much less degree and because of their degree of unsaturation are chemically very reactive and while perhaps initially portable, readily become bound by and denaturize such functional or tissue elements as may be determined by the general character of their molecule before the same degree of distribution can be achieved. This rapid fixation finds expression in the rapid exhibition of potent toxic manifestations. The arseno compounds as a group possess double the molecular weight and, although perhaps also quite reactive, are the least readily transported and the least diffusible of all. Where there is no special acid salt forming group, substances of this class may be held in solution for a short time only, aided perhaps by the protective serum colloids, but may be readily deposited as such along the route before complete distribution is possible and then more slowly unfold their toxic effects through oxidation to the oxides. If we were dealing with an infection which is confined to the blood stream alone it would seem, therefore,
admissible to place reliance upon the direct quick action of the trivalent compounds and the simple formula of the ratio of parasitotropic to organotropic properties of a substance. But when the more usual tissue infection is to be influenced the question of distribution and diffusibility becomes a very important factor to consider. It is quite conceivable that certain advantages in this regard may result by the use of the pentavalent form which may compensate for what it may seem to lose in immediate direct parasiticidal properties. By more complete distribution and tissue penetration it might be possible to reach localities which may harbor such residues of an infection as may again become generally active. In such localities the reducing action of the tissues may supply a small but sufficient amount of therapeutically active material.

In spite of Ehrlich's somewhat orthodox adherence to the trivalent idea we can see evidence of his occasional return to the pentavalent compounds. In his Schlussbetrachtungen, the possibility is pointed out of the applicability of hydroxyaminophenylarsenic acid, the pentavalent stage of salvarsan, for local therapy where the arseno compound circulating in the blood cannot reach the affected tissues in sufficient amount, as, for instance, in local eye involvement. Hata's experiments showed several pentavalent compounds to exhibit a good ratio of curative to toxic action but we obtain the impression that these workers were perhaps prejudiced against the group because of a neurotropic tendency which a number of these substances displayed. This exhibited itself in the development of incoordination in the so-called "dancing-mice."

Considerations of a practical character have arisen in connection with the arseno compounds caused by certain inherent difficulties which are associated with their chemical and physical properties. They are unsaturated, highly reactive substances. Slight modifications in the method or conditions of preparation may have a great influence on their general toxic and also therapeutic behavior. It is not possible to obviate this readily by the subsequent use of the ordinary methods of purification such as recrystallization but very careful control of preparative con-
ditions is required which can be only empirically ascertained. It has been necessary, therefore, to maintain a constant biological control of those substances of the group which are clinically employed. Further, the development of this class of substances is in a sense limited because the arseno compounds depend for solubility on the presence of salt-forming groups. The pentavalent compounds possess much more favorable chemical and physical properties. The arsonic acids as a rule are perfectly stable, crystalline compounds which can be readily purified. They form stable salts and therefore do not require the presence of other salt-forming groups for solubility.

It appeared to us, therefore, that the development of pentavalent compounds was certainly worthy of further trial. In the selection of the chemical material for our studies such groups of substances were chosen which would combine a sufficient degree of accessibility with the opportunity for ample and logical chemical development in such direction as the biological result might indicate. From this material it was hoped to accumulate statistical data regarding the influence of chemical constitution on biological action which could be utilized in further work. An idea of the chemistry of these groups of substances is best obtained by reference to the chart. The studies were not alone confined to the pentavalent group but in certain instances where the presence of a solubilizing group would permit, reduction to the trivalent stage was tried to test the value of such a chemical transformation.

Over 230 substances were prepared. Their action as regards toxicity and therapeutic properties in one or more laboratory animals infected with the different animal parasites was studied. The observations were too extensive to attempt to do more here than very briefly and superficially summarize the results. Substances were observed which varied all the way from those possessing a very high toxicity to such which were easily tolerated and at the same time exhibited varying degrees of therapeutic power. But the observations with these substances brought out the fact that in the biological measure of each compound so many different factors had to be considered that it became ex-
tremely difficult to find any sharp regularities with regard to the influence of structure on action. But in some cases, it was possible to note a few very general tendencies which were utilized to advantage in the work.

The most important group and that which was most fully studied was that of the amide and substituted amides of phenylglycine-p-parsonic acid. This type of compound was chosen partly for the reason that the glycine amide NHCH$_2$CONH group not only offered an excellent chance to add practically any chemical group to the molecule which was desired, but because the glycine amide group itself is a normal constituent of proteins and should be inherently compatible. These substances were in general prepared by the interaction of the amino group in arsanilic acid or its analogues with chloroacetyl derivatives of amines in which members of both the aliphatic and aromatic series were given ample representation. Changes in both therapeutic and toxic effects were observed in response to chemical alterations in the molecule often of a decided character which depended not only upon the animal used but also the type of infecting organism. Only in certain instances was it possible to follow any general tendencies. To begin with, the parent substance, phenylglycine-p-parsonic acid, we have met a near relative before in the form of arsenophenylglycine, which is obtained from the arsonic acid by reduction. Contrary, however, to the potent therapeutic properties exhibited by arsenophenylglycine in experimental trypanosomiasis, the pentavalent form, while it can be tolerated by laboratory animals in large amounts, is practically devoid of therapeutic effect. This was at variance with instances noted in other groups of compounds and like all such irregularities, which have made prediction impossible, there is no explanation at hand. In general, it has become evident that the tendencies which are observed in the apparent influence of constitution on biological action are confined pretty narrowly to substances which are built on the same general plan.
On passing to the amide or ureides of the compounds containing these carboxyl groups, no matter where they were situated in the molecule, there was in general an appearance of therapeutic effect in spite of a tendency to increased toxicity. This therapeutic effect of course varied greatly with the particular compound, the animal and the infection used. A number of substances of the series from the biological standpoint proved to be of outstanding interest. In an extensive series of biological tests, Doctors Brown and Pearce\(^3\) definitely established that one of these in particular possesses unusually favorable biological properties. This substance, the sodium salt of phenylglycinamide-p-arsonic acid, or tryparsamide, as it has since been named, has now come to be of definite, practical, therapeutic importance.

Tryparsamide is a white, crystalline, stable substance which is extremely soluble in water with the formation of a practically neutral solution and is very easily prepared by the interaction of sodium arsanilate and chloroacetamide. As regards the biological properties of this substance, I cannot do better than quote from Doctor Pearce a sort of summary:\(^4\)

"Toxicological experiments demonstrated that the reaction of different species of laboratory animals to the drug was of a favorable character. The substance lends itself well to almost any method of administration and can be given to animals in large doses and the toxic effects were confined to doses relatively close to the minimum lethal dose. The recovery of animals from sublethal intoxication was remarkably rapid and complete, thus making possible the repetition of large doses at comparatively short intervals of time. The therapeutic activity of the drug in experimental trypanosomiasis was particularly evidenced by the relative speed and sharpness of action in the acute blood infections of mice and rats and by the potency and duration of action in the subacute and chronic tissue infections of guinea pigs and rabbits. The accomplishment of a permanent cure was obtained in the experimental infections produced by five strains of pathogenic trypanosomes. \textit{Tr. brucei}, \textit{Tr. gambiense}, \textit{Tr.}
evansi, *Tr. equiperdum* and *Tr. equinum*. Comparative experiments in laboratory animals with drugs which had been previously used, such as atoxyl, arsacetin, arsenophenylglycine, and the salvarsan derivatives, all of which had been previously used in the treatment of human trypanosomiasis, showed that tryparsamide was in many respects superior."

In her clinical observations made in the Belgian Congo several years ago, Doctor Pearce, was able to substantiate the favorable indications which the experimental results with tryparsamide had given. It was shown that the drug was well tolerated and gives rise to no untoward symptoms except an occasional tendency, following too intensive a use of the drug, to produce visual disturbance in certain advanced cases. This, however, was usually transitory. The therapeutic results obtained in the preliminary use of the drug in 77 cases of sleeping sickness produced by *Trypanosoma gambiense* were very encouraging. More recently, Doctor Chesterman in the Belgian Congo has definitely confirmed Doctor Pearce’s general observations. From observations on the intravenous use of the drug he concludes that the maximum tolerated dose (which he believes should not exceed 4 grams per week for the full-sized adult) if given regularly for a period of about eight weeks is capable of completely removing trypanosomes from and rendering normal, the cell content of the cerebrospinal fluid of even the most advanced cases. This change is accompanied by a very marked clinical improvement which was observed to persist for practically a year, which was the longest time which had elapsed since his last use of the drug. Improvement was hardly less marked in cases which had resisted previous treatment with the drugs such as atoxyl. By a careful check on the patient it was possible to avoid the danger of any appreciable degree of visual disturbance. Up to the present, we see no reason to change our estimates of the value of the drug.

Doctor Smillie has recently had the opportunity to extend the studies with tryparsamide in another direction, to the treatment of mal de caderas which has become one of the biggest eco-
nomic problems of the vast Paraguay Valley in South America, since this disease is causing the yearly loss of thousands of horses. In a preliminary communication he reports that the drug has been found to be definitely efficacious in the treatment of the early stages of the disease when the blood of the horse or mule is swarming with trypanosomes. This stage is from all practical considerations of the greatest importance to control.

But to return to human trypanosomiasis, it is seen that the two main directions which chemical efforts have taken to produce remedies for this disease have almost simultaneously yielded results which now give a more encouraging outlook with regard to its control—on the one hand, Bayer-205, most probably closely related to the benzidine dye group, and tryparsamide, a pentavalent arsenic compound. At the present time, it is perhaps too early to compare the relative efficacy of Bayer-205 and tryparsamide. There have been reports already with regard to Bayer-205 which indicate that the drug, although of definite value as a prophylactic, or in the early stages of the disease, has failed to give results in the more advanced cases. Tryparsamide, on the other hand, as Pearce and Chesterman have shown, has been the cause of definite improvement in patients even in the more advanced stages of the disease. Bayer-205 has been reported to exhibit a definite toxic behavior from which the patients or animals only slowly recover. This takes the form occasionally of an albuminuria. A general tonic effect has characterized the behavior of tryparsamide and perhaps the only untoward action has been the occasional visual disturbance which has been noted to follow too intensive a use of the drug. But, as before stated, this is in most cases of a temporary character. We shall perhaps have to wait a number of years before these drugs will have reached their final evaluation.

There is a temptation perhaps to speculate with regard to the chance that the more successful of the arsenical remedies which have been used for the treatment of human trypanosomiasis have been pentavalent compounds. In the case of
tryparsamide, there seems every reason to attribute its efficacy, not only to its powerful stimulating action on the animal economy and on the animal resistance, but to its great tissue penetrability and to its affinity for the tissues of the central nervous system which are involved in the more advanced stages of the human disease. This property has been demonstrated again in the behavior of the drug in certain types of neurosyphilis. In neurosyphilis there is a distribution of organisms in the central nervous system which is, in a sense, comparable to that which occurs in trypanosomiasis. This has in the past suggested the possibility that perhaps even at the time when the etiological relationship of paresis to syphilis was merely suspected, drugs which have proved efficacious in the treatment of human trypanosomiasis might find a usefulness in the treatment of paresis. Years ago, atoxyl, for instance, was repeatedly tried in this connection, but in all these attempts, it has proved quite inadequate. It was, however, a natural thought to consider tryparsamide in this connection, although its treponemacidal properties are not very marked. Nevertheless, in experiments in rabbits it had been shown to possess a marked action on the experimental lesions produced by the spirochetes. This was evidenced by their complete resolution and healing even in the presence of actively motile spirochetes which seemed to show little or no tendency to cause a recurrence.

A few years ago, Doctors Lorenz, Loevenhart, Bleckwenn, and Hodges,\textsuperscript{37} in order to test the applicability of tryparsamide in this connection undertook a series of observations on a group of patients afflicted with paresis. They have concluded that tryparsamide on intravenous injection is definitely efficacious in the treatment of early paresis and certain other forms of neurosyphilis. Later observations have essentially confirmed this. It is perhaps too early to make a more precise statement with regard to the clinical use in all of its phases of tryparsamide in this disease, but sufficient is already known to demonstrate that it should have a field of usefulness in certain conditions for which there has been, heretofore, no efficacious mode of treatment.
If time would permit, still other phases of the progress which has been made in the application of chemical agents to the specific treatment of infectious diseases might be reviewed. But sufficient has been brought before you tonight to show that the results achieved have been far in excess of what was needed to justify the initiation of this type of investigation. In no sense, however, can any result such as the arsphenamines, Bayer-205 or tryparsamide be considered the summit of possible attainment and there is every reason to expect the ultimate discovery of more powerful agents. And as time goes on, new groups of substances and an ever-widening field for their therapeutic application may well be expected. There is perhaps no more hopeful direction for the continuation of effort. But those who engage in such undertaking must carry with them a certain measure of opportunism, since in spite of the constantly increasing number of positive observations, there is still lacking a general theory as to the chemical and physical factors which underlie specific therapeutic action. In this respect we are perhaps less informed than in the case of certain rules which seem to govern pharmacodynamic action. But I think that it is in this direction that further effort is required. We should not alone strive for the attainment of practical results, but continued attempts should be made to ascertain all of the theoretical factors which may contribute to chemotherapeutic action.

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