CHEMOTHERAPY

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I. INTRODUCTION. By intoducing the name "Chemotherapy" Ehrlich intended to mark off, from the general body of Pharmacology, a particular type of investigation, having as its aim the discovery of chemical substances acting specifically on pathogenic infections. This attempt to erect a new boundary fence, to delimit a new plot in the scientific field, has not passed altogether without criticism. It could be urged, and not without some justice, that the application of specific remedies to combat particular infections was as old as the use of cinchona for the cure of malarial fevers, of ipecacuanha for that of tropical dysentery, and of mercury for that of syphilis; and that the study of the action of these remedies had long been included in the orthodox pharmacology. It can even today be truly stated that, with the exact knowledge now available concerning the protozoal parasites of malaria and dysentery, chemotherapy has made no real advance on the traditional remedies for these diseases. Such improvements as have been made have resulted from a more accurate knowledge and complete separation of their alkaloids—a type of investigation having certainly a closer relation to the conventional pharmacology than to the special methods of chemotherapy. On the other hand there was justification for Ehrlich's suggestion that pharmacology had devoted attention almost exclusively to a detailed analysis of the symptoms produced in higher animals by toxic doses, even of remedies, such as quinine, which owed their therapeutic reputation to specific cure of definite infections. Chemotherapy, as he conceived it, was to shift the focus of interest to the action on the parasites. Its aim must be the discovery of substances maximally toxic for the infecting parasite, and minimally toxic for the infected host. His lifelong study of the distribution of chemical substances, especially of dyestuffs, among the organs of the body into which they had been introduced, and the chemical terms in which he was accustomed to interpret such observations, provided a conception, diagrammatic in its simplicity, for the

mode of action of such substances. The substance, given the requisite toxic properties, would kill or injure only the cells to which it became fixed by reason of its chemical affinities. The aim of chemotherapeutic investigation, therefore, must be to find toxic substances which, having a strong affinity for the protoplasm of the parasite and a weak affinity for that of the cells of the host, could be administered in sufficient doses to kill the infecting organism and leave the host unscathed. The search must be for substances which are maximally "parisitotropic" and minimally "organotropic."

It will be found difficult, as yet, to form a just opinion as to the rôle which this simple conception has played in the development of chemotherapy during recent years. It is hardly likely that it will retain permanent status as an exact scientific theory. The knowledge yet available concerning the chemistry of the protoplasm has no point of contact with a conception of this kind. Such knowledge affords no suggestion as to the nature of the chemical differences between the protoplasm of the vertebrate and that of the unicellular parasite, and furnishes no basis for prediction, or even for surmise, with regard to their differential affinities for chemical substances of known constitution. When a certain substance is found to cause the disappearance of, say, a particular species of trypanosome from the blood of an infected mouse, without harming the mouse, and fails to remove a similar infection from the rat, when administered in doses which that animal can tolerate, these observations are taken to indicate that the ratio of its parasitotropic to its organotropic properties is more favorable in the mouse than in the rat. Essentially, however, this is a mere restatement of the observed fact, that the mouse can be cured but the rat cannot. The supposition that the result is determined by the distribution of the substance between the cells of the host and the parasites is not based on independent evidence; such evidence as exists, apart from the curative result, is, in fact, not favorable to the conception. For example, the presence in the host's blood of parasites having a preferential affinity for the toxic drug might be expected to exert an antitoxic action, by diverting its action from the tissues. Certainly if this relation were found to be the rule, it would be claimed as evidence in favor of the conception. The relation actually observed, however, is the opposite of this; it is the infected animal which is the less resistant. In animals of the same species, again, suffering from the same infection, the effect of a drug on the parasites should, on the simple distribution theory, become stronger in proportion as its action

on the host becomes weaker. In some cases where a relation between the intensities of the two effects, on the infecting parasite and on the host, has been observed, it is the reverse of this; the more sensitive individual is the more easily cured. Again, the observation that, in many cases, a remedy acting potently against a certain infection *in vivo* is practically without visible action on the parasites *in vitro*, is not, by itself, consistent with the simple conception that its curative action is due to its chemical affinity for the parasites. It is true that these various anomalies can be, and have been more or less successfully reconciled with the original theory attributing curative action to specific chemical affinity; but this can only be effected by the introduction of subsidiary hypotheses, which mostly involve considerations of the interaction between the drug and the tissues of the host. The original conception can thus be redeemed from inconsistency with the observed facts; but the possibility only suffices to show that it is not necessarily untrue. Positive, independent support for the basic assumption of preferential affinity is curiously difficult to find.

In these circumstances it is impossible to feel confidence as to the general and permanent validity of Ehrlich's method of interpreting the facts. On the other hand it cannot be doubted that his bold conception has played a part of enormous importance in stimulating investigation. If the truth of a theory could be judged by the practical results which have resulted from efforts to test and apply it, this one would indeed be firmly established. But though, in the process of opening up a new territory for research, the practical value of a theory may largely be determined by its power of stimulating and encouraging experiment, and even of ignoring difficulties which a more fundamental consideration of the problem might present as insuperable, there comes a time when it is necessary to enquire whether it has not served its purpose. In the case under consideration, Ehrlich's theory has tended to focus attention on the effect of a remedy on the parasite. It led undoubtedly to the exploitation of new methods, which made a much needed departure from those of pharmacological orthodoxy. But there are growing indications that the attempt to interpret the experimental results too exclusively in terms of direct parasiticidal action may eventually hamper progress, by throwing back investigation into an empiricism from which it was Ehrlich's aim to rescue it. The object of this review is to survey the position as it presents itself to the writer at the moment, and to consider, in particular, certain facts which are difficult to reconcile with the simple distribution hypothesis. No

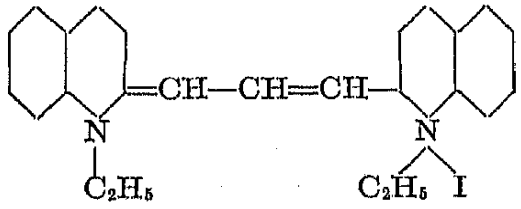
attempt will be made to mention, even by reference, the whole of the enormous wealth of detailed evidence. The aim will rather be so to present the salient and significant points as to show what part of the evidence is in conformity with the idea of direct and simple chemical action, and what part of it, on the other hand, indicates actions of much greater complexity, involving factors for the investigation of which the methods, even, are not yet available.

II. CHEMOTHERAPY OF BACTERIAL INFECTION. The attempts to combat bacterial infections by means of artificial chemical substances have been fewer in number and, on the whole, less fruitful of practical result than those directed to infections with animal parasites. Such as they are, the results are simpler and more easily interpreted in terms of direct action on the parasites. Bechhold and Ehrlich (1906, 1907), prepared a large series of phenol derivatives, introducing halogens and uniting phenolic groupings by bridges of various types, and in this way obtained compounds which far exceeded all previously known organic disinfectants in their lethal action on diphtheria bacilli growing in nutritive bouillon. The experiments were later extended to other bacilli and cocci. In therapeutic experiments, however, conducted on infected animals, none of these substances proved successful. The reason of their failure was more obvious when it was found that their disinfecting potency was enormously reduced by the presence of protein in the medium, as when the organisms were suspended for the test in blood serum instead of broth. Bechhold subsequently showed, by ultrafiltration, that the disinfectant had entered into combination with the proteins of the suspending medium, and had thus been prevented from reaching the bacteria. The observation is chiefly interesting, from its obvious significance in connection with the development of the theory of chemotherapeutic action. Further investigations by Bechhold (1909), concerned solely with disinfection outside the body, are of interest as showing a partial specificity of certain derivatives for certain organisms; thus, in the series formed by introducing successive halogen atoms into β. Naphthol, the optima for disinfecting action occur at different points for different bacterial species. Tribrom β. Naphthol, with a very high disinfecting power for pyogenic cocci and the diphtheria bacillus, had little for *B. pyocyaneus* and none for the tubercle bacillus.

This same specific liability of bacterial species to the disinfecting, or growth-inhibiting, action of chemical substances is, of course, the basis of the numerous differential or enriching media, in which the selection of one species, occurring in a mixture of many, is effected by

adding some substance—e.g., a dye, or a tellurium compound—which inhibits the growth of other organisms much more effectively than that of the one which is sought. The same selective action is clearly apparent in the results published, in recent years, by Browning, Cohen, Gaunt and Gulbransen (1922), and by Fairbrother and Renshaw (1922), on the relative disinfectant action of various dyes and related compounds on different bacteria.

These selective actions *in vitro*, where there is no question of the participation of other living cells than the microörganisms under investigation, may be regarded as presenting the fundamental problem of chemotherapy in its simplest terms. Browning, Cohen and Gulbransen (1922) find, for example, that "sensitol red," a dye used in sensitising photographic plates to red rays, and having the constitution



"Sensitol red"

has a disinfectant action on *Staphylococcus aureus* more than 2,000 times as powerful as that which it exerts on *B coli*, when both are in peptone water. Their figures further indicate that the presence of serum much reduces its action on the staphylococcus, but somewhat increases its action on *B. coli*. On no system of hypothetical "tropic" properties of the dye for the coccus and the bacillus, on the one hand, and the serum proteins on the other, can these observations be intelligibly expressed. They must be accepted, for the present, simply as empirical facts. Yet the conditions are extremely simple compared with those which obtain in an experiment conducted on the living animal.

An attempt to study in partial isolation some of the factors concerned in the cure of bacterial infection by chemical agents has recently been made by Felton and Dougherty (1922 b). These authors measured, in a series of dyes, and in derivatives of the cinchona group of alkaloids, the toxicity for mice, the bactericidal action on *Staphylococcus aureus* in whole blood, and the inhibitory effect on the phagocytosis of staphylococci by leucocytes in serum. In a large series of dyes—in most of the triphenyl methane dyes tested, in safranines, phenazines and quinones, —and in that of the cinchona derivatives, they found that the inhibi-

tory action on leucocytes was exhibited in weaker dilutions than the bactericidal action. So far as these measurements can be taken to cover the factors at work in disinfection *in vivo*, they would be unfavorable to the efficacy of these substances under such conditions. A more favorable relation of bactericidal to leucotoxic properties was found in the case of some triphenylmethane dyes—p. Methoxy malachite green and ethyl violet—and in that of "proflavine" (diamino acridine), which, with the corresponding methyl ammonium derivative ("trypraflavine" or "acriflavine"), had already been found by Browning and his co-workers (1917, 1918) to have a powerful anti-bacterial action, augmented by the presence of serum, with a relatively low toxicity for the whole mammal or for its leucocytes. Another member of the same group, 2 ethoxy 6, 9 diamino acridine, was found by Morgenroth, Schnitzer and Rosenberg (1921) to give a very favorable ratio between disinfectant actions as determined, on the one hand, *in vitro*, and on the other in the tissues of a mouse, by local injection. Its hydrochloride has been introduced to commerce as "Rivanol," and has been used, by local injection, to treat cellulitis and erysipelas (Rosenstein, 1921). In spite of these favorable indications there is little, if any, evidence yet available to show that these acridine dyes can cure a bacterial septicemia. Their chief practical application has been in the local treatment of infected wounds or mucous surfaces. The action of trypaflavine on trypanosome infections will be mentioned later.

The nearest approach to the successful treatment of a bacterial septicemia by a chemical agent is found in the use, by Morgenroth (1911, 1912), of an artificial member of the cinchona series of alkaloids, ethylhydrocupreine ("optochin"), in pneumococcus septicemia. Since the alkaloid has very powerful and strongly specific inhibitory and lethal effects on pneumococci *in vitro*, its action in the infected living animal seems, at first sight, a peculiarly clear example of the action of a drug directly harmful to the parasite. Even in this case, however, there is suggestive evidence in favor of the view that the immune reaction of the host plays an important part in the cure of an infection. Thus Neufeld and Engwer (1912), Engwer (1913), and especially Moore have demonstrated the enhancement of the curative effect of a specific antipneumococcal serum by doses of optochin ineffective by themselves. The enhancement is much too great to be accounted for by a mere summation, and suggests that, short of actually killing the organisms, either the chemical or immunological antagonist may so alter them as greatly to weaken their resistance to the other.

Similarly incompatible with the conception of the action of such substances, on septicemia in animals, as a direct disinfection, was the phenomenon described by Felton and Dougherty (1922 a), working with some members of the large series of artificial derivatives of cinchona alkaloids produced by Jacobs and Heidelberger (1919 a, 1920, 1922). Felton and Dougherty found an optimum dose for the prevention of septicemia, with simultaneous injection of the drug and various multiples of the lethally infecting dose of pneumococci. If the dose of the alkaloid was increased beyond that optimum, but still well below the limit of the host's tolerance, the number of lethal infective doses which it would antagonize rapidly fell again, a number of pneumococci, which a smaller dose of the alkaloid completely suppressed, now producing a spreading infection and fatal septicemia. The authors regard this as showing that there is a reversal of relationship from bacteriotropism with the small doses to organotropism with the large. One could hardly find a better example of the desperate expedients which are necessitated by the effort to make the facts of therapeutic action fit into the rigid frame of the distribution-hypothesis. When once the coöperation of the host's defensive reaction is recognized as necessary, in accordance with the above-mentioned evidence, produced by Neufeld and Engwer and by Moore in the case of optochin, the necessity for such strained assumptions vanishes. With the lower doses, the direct antibacterial action is reinforced by the host's defensive reaction, the total effect, on the analogy of the action of optochin, being much more than a mere summation. With the higher doses, even within the tolerance of the healthy animal, the defensive reaction is impaired and suppressed, and the maximum direct antibacterial action obtainable with the alkaloid *in vivo* is inadequate, without this reinforcement, to deal with the infection.

Mention should also be made of another direction in which chemotherapeutic investigation has made contact with the treatment of bacterial infections. Chaulmoogra oil had a traditional reputation in the treatment of leprosy. The treatment was made more effective by the introduction by Heiser (1914), and improvement by Rogers (1916) of parenteral injections of the oil, or of soaps made from certain fractions (Rogers). Recently a further improvement (Macdonald and Dean, 1921) has been made by the use of artificial ethyl-esters of the separated chaulmoogric and hydnocarpic acids—peculiar fatty acids occurring in the oil, and shown by Power to be characterized by the presence of a closed carbon ring. A scientific, chemotherapeutic

basis seems to have been given to this treatment by the work of Walker and Sweeney (1920), who find that soaps of these acids, while they are harmless to the ordinary bacteria, have a pronounced antiseptic or inhibitory action on cultures of all "acid-fast" bacteria. This suggests a direct, toxic action of these fatty acids on such organisms, presumably associated with their possession of a waxy envelope. In view of somewhat similar, albeit less convincing, therapeutic claims made for the soaps of other oils, e.g., cod liver oil, which Walker and Sweeney found inert in their cultural experiments, the position cannot be said to be perfectly clear; but their results are at least highly suggestive. The difficulty of drawing any certain conclusions from a coincidence between inhibiting action *in vitro* and curative action *in vivo* is illustrated by Lewis's (1917) work on the tubercle bacillus, in which a very wide survey of dyes resulted in the discovery of several which selectively stained the bacilli, and strongly inhibited growth in culture, but produced no definite curative effects.

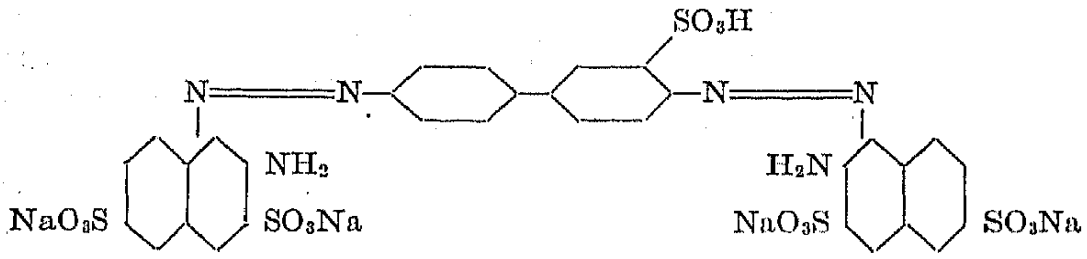
Another point of some theoretical importance, which emerges from the experiments on chemotherapy of bacterial infections, concerns the production of resistant strains. Morgenroth and Kauffmann (1912), by passage through mice treated with substerilizing doses of optochin, produced strains of pneumococci which, in the mice, were abnormally resistant to the treatment by this alkaloid. The possibility of free cultivation facilitated the study of this phenomenon of the acquisition of tolerance by bacteria under the simplest conditions. Marks (1910), by patient subculture into media containing increasing strengths of arsenious acid, produced an eight-fold rise in the resistance of a strain of hog-cholera bacillus to this substance. The treatment with arsenic raised the resistance of the organism to antimony, however, in even greater proportion—about forty-fold. The change in resistance was accompanied by modifications in the morphology and the fermentative reactions of the organism. Tugendreich and Russo (1913) similarly produced tolerance of pneumococci to optochin, and Shiga (1913) accustomed the cholera vibrio to dyes by serial cultivation *in vitro*. It is not perfectly clear whether tolerance so acquired is due to selection or to direct, heritable modification of survivors, but, in either case, the possibility of its production by the direct action of the drug, without the participation of a living host, has importance in connection with phenomena to be dealt with later.

Apart from the light which it may throw on the meaning of phenomena, which are encountered in connection with the treatment of infections

by obligatory parasites, the chemotherapy of infection by the true bacteria cannot be said to have achieved, as yet, anything of really practical importance. The use of the acridine dyes (Browning) and of the higher homologues of optochin (Morgenroth, 1917), in the local treatment of infected wounds or mucous membranes must be classed with measures of external disinfections rather than with chemotherapy in the proper sense. It is to the infections due to animal parasites that we must turn for the most characteristic and successful examples of its application, both in experiment and in practice. The spirochaets, though a strong case has been made, from the independent biological point of view, for their affinity with the vegetable bacteria, from the point of view of their infective action, and the means suited to cope with it, which is our present concern, must be ranked rather with the animal parasites.

III. Chemotherapy of trypanosome infections. A very large part of the systematic investigation of the possibility of obtaining specific chemical remedies has been directed to the cure of infections with trypanosomes. The organisms of this genus, responsible for several well-known tropical diseases of man and lower animals, are peculiarly well adapted for this type of enquiry. They are easily transmitted to suitable laboratory animals, and, when thoroughly adapted by passage, they produce an extraordinarily uniform type of infection, with remarkably little variation in the time of its progress to a fatal issue, in the absence of curative treatment. Progress has been made along two distinct routes, and the investigation in each direction has recently reached what appears to be an important summit of practical success, though there are doubtless higher peaks for future attainment. We have, on the one hand, the line of investigation of which the starting point was the trial of various dyestuffs, and of which the most recent, and apparently the most successful development, has been the introduction of the substance which, though uncolored, has important points of structural similarity with certain dyes, and which is known, as yet, only as "Bayer 205." On the other hand we have the line of investigation which started from the observation of a partial curative action produced by arsenious acid, has passed through a series of organic arsenical derivatives, and at the time appears to have reached a relative optimum in the introduction of the substance known as "tryparsamide." It will be convenient, in the first place, to sketch the general course of progress along the two routes separately.

1. The action of dyes and analogous compounds. Ehrlich's early interest in the distribution of dyes in the organs of the living animal, and his study of their specific affinities for different cell-structures, naturally led to the attempt to discover substances of this type which, by their property of becoming selectively fixed by the parasitic protoplasm, would have a parasiticidal action in doses which the host would tolerate. The first germ of the idea is seen in his observations with Guttmann, published as long ago as 1891, in which the staining of the malarial parasite by methylene blue is made the basis of an investigation of the possibility of curing a malarial infection by administering this dye. A distinct effect was obtained, but the results were not sufficiently impressive to make the dye a serious rival to quinine. Later when, with Shiga, Ehrlich began a systematic investigation of dyes as curative agents, he had at his disposal the technique of transmitting a trypanosome infection through a series of mice, as developed by Laveran and Mesnil (1902). After preliminary trials of several members of the benzopurpurin series of dyes, chosen, apparently, chiefly on account of their demonstrably long persistence in the body of a mouse into which they had been injected, the substance known as Trypan-red was obtained, through the coöperation of the dye-manufacturing firm of Cassella (Ehrlich and Shiga, 1904).

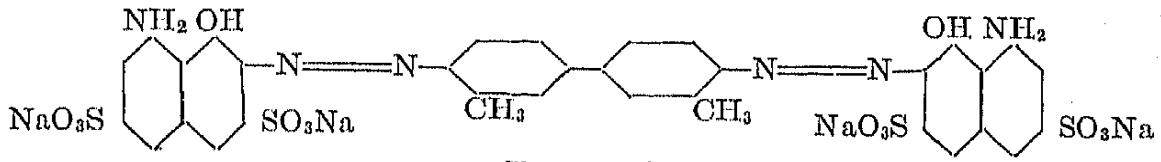


Trypan-red

This proved to have a satisfactory curative and prophylactic value for the infection of mice with the trypanosome of Mal de Caderas (*T. equinum*). It is to be noted, however, that it was relatively ineffective against the same organism when infecting the rat, and against other trypanosomes (e.g., *T. brucei*) in the mouse. It was further observed by Ehrlich and Shiga that the dye was practically innocuous to any trypanosomes, when applied to them *in vitro* even in relatively strong solutions. This chemotherapeutic paradox is one which we shall meet repeatedly. In the case of some therapeutically active substances, such as the arsenicals, we shall see that there is reasonable ground for attributing the efficacy *in vivo*, of a drug which is relatively inert *in vitro*,

to a chemical alteration into a directly active derivative taking place in the host's body. There is no ground, save that of doubtfully justifiable anology, for assuming that such a change takes place in the case of trypan-red, or, indeed, in that of any member of this series of curative dyes and analogous compounds.

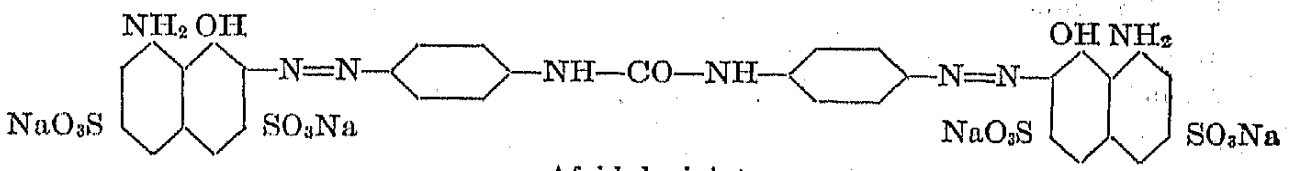
A more practically successful member of the same group was the substance introduced into chemotherapeutic investigation by Mesnil and Nicolle (1906), and subsequently known as Trypan-blue. This substance, a derivative of tetrazotized tolidine, has an obvious similarity in its general structure to trypan-red.



Trypan-blue

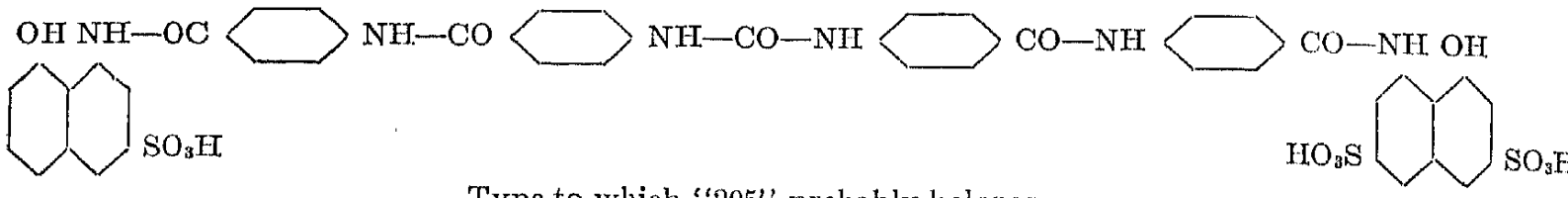
It was found to be an effective curative or prophylactic agent against *T. brucei* in mice, but its practical application has been found in dealing with another type of infection, viz., that due to intracorpuscular parasites of the genus *Piroplasma* in dogs and cattle (Nuttall and Hadwen, 1909).

Another similar substance, with which promising results were obtained by Mesnil and Nicolle, was prepared by condensing tetrazotized diamino-diphenyl urea with naphthylamin-disulphonic acid.



Afridol-violet

This dye, "afridol violet," has not itself been practically applied; its greatest interest to-day lies in the fact that it presents a point of structure, viz., the presence of an amide or urea linkage, which entitles it to be regarded as the forerunner of a series of compounds prepared in recent years by the Bayer Company, one of which, known as yet only by its serial number, "205," shows promise of much greater importance than any of the immense number of dyes which have been tested for therapeutic properties. The exact constitution of "205" is still kept secret, but it is a colorless substance, with properties which indicate a high probability, as pointed out by H. King, that it belongs to a series, patented by the Bayer Company, and having a formula of the general type:



Type to which "205" probably belongs

If this formula be compared with that of "afridol violet," it will be seen that the most important difference is the absence of the diazo-linkages by which the dye-properties are conferred. It is an interesting commentary on the validity of the theoretical conceptions with which this type of investigation may be said to have originated that, starting with a survey of dyes, chosen for trial on account of their visible fixation by the protoplasm of parasites, among other cells, it should have arrived at the discovery of a substance, more powerfully curative of typanosome infections than any dye yet tested, but itself wholly devoid of dyeing properties, and even of color. So far as they have been investigated the therapeutic properties of "205" appear to be remarkable. (Händel and Joetten (1920), Mayer and Zeiss (1920), Muhlens and Menk (1921), Wenyon (1921).) Like its colored forerunners it has no visible action on trypanosomes *in vitro*, though this absence of effect is less significant in view of the long period of about two days before its curative action begins to be manifest after its introduction into an infected animal. The ratio between the curative and tolerated dose has been assigned rather widely different values by different observers, but it is in any case large, and therefore favorable to the use of the substance as a practical remedy. Its most remarkable property is the prolonged persistence of its effect on the resistance to infection shown by the animal into which it has been injected. A single injection appears to be capable not merely of curing definitely an infection already existing in a laboratory animal, but of rendering the animal insusceptible to infection by trypanosomes for a period of weeks or even months.

Various more or less plausible hypotheses can be made to account for these anomalies in the action of "205," but there is no method yet obvious by which any one of them can be put to the test of experiment. The substance may be fixed with great tenacity by some of the tissues. The behavior of related compounds, such as trypan red and trypan blue, which are recognizable by their color, is in favor of such a supposition. The gradual and long persistent staining of the tissues of the host by these dyes is a familiar incident of their therapeutic application. (Franke (1905), Bouffard (1906).) The supposition of the more con-

tinued presence of the substance in the body, however, is not by itself sufficient to account for the curative effect or for the artificial insusceptibility to infection, since evidence of direct lethal action on the parasites is wanting in this case, as in that of the dyes. Recourse must, therefore, be had to one or more hypotheses; but these, since they have as yet received very little support from independent evidence, cannot be regarded as much more than convenient resting-places for the mind. We may suppose, for example, that the drug only produces its effect by long-continued action, and that the relatively brief period during which its action can be observed under the microscope does not suffice for it to become visible. If that were the case an important factor in the curative efficacy would be the property of becoming fixed to the host's tissues, and liberated therefrom into the body fluids in small concentration, but over an extended period. Or it may be suggested, as has been done, that the essential curative action depends not on the direct killing of the parasites, as observed by their loss of motility and rapid degeneration under the microscope, but on destruction of their power of multiplication. This is in conformity with the observation that trypanosomes treated with a dye outside the body may lose their infectivity without visible changes in their motility or their structure. It is stated also (Busck), that trypan red will stop the multiplication of free-swimming ciliates without impairment of their other functions. We shall meet this suggestion in other connections, but the positive evidence in its favor is hardly sufficient to warrant its adoption as a general chemotherapeutic principle, or its facile invocation to explain any discrepancy between actions outside and inside the body. If it is really the secret of the paradox which immediately concerns us, it is obvious that fixation by the host's tissues, and the resulting mild, persistent action on the parasites of the gradually liberated drug, must be a factor in the effect. Another possibility is that the curative substance is not directly hurtful to the parasites, but that some parasiticidal derivative is formed from it by interaction with the host's tissues. We shall find that there is evidence in favor of such a view in the case of certain arsenical compounds, from which derivatives having a powerful, directly lethal action *in vitro* can be produced. No such evidence is available in the case of the substances which we are now considering, or indeed in that of any of the curative dyes or non-metallic compounds; the suggestion is possible, but no more. The same is true of another suggestion, namely, that the direct action on the parasites may not be due either to the substance as

administered, or to any derivative thereform, but to something produced by the tissues of the hosts in response to an action on their cells. Again, there is no evidence in favor of such a view: it is simply a convenient assumption to explain a difficulty.

It will be clear that all these possibilities, and indeed any which can be invoked to explain the curative effect in the host, of substances not obviously hurtful to the parasites outside the body, almost of necessity involve a participation of the host's organs in the curative action. Dyes were made the starting point of this line of investigation, because of the ease with which their distribution between tissues and parasites could be followed by the eye. Yet in this group the evidence so obtained is in favor of a strong affinity for the host's tissues rather than for the protoplasm of the parasites; these dyes can be seen to be strongly "organotropic," while the evidence for their directly "parasitotropic" properties is, at best, not strong.

If we take the known facts concerning the action of such a substance as "205," without reference to any particular theoretical conception, we can say that this substance, having no demonstrable direct effect on the parasites, causes their complete disappearance from the body of an animal infected with them; that for a long period after a single injection of "205" the animal is resistant to further infection by trypanosomes, and that during that period its body fluids contain something which possesses the property of curing infection; but that there is no clear evidence as to whether that something is a remnant of "205" itself, or some derivative thereof, or some antagonist produced by the host itself in response to the stimulation of its tissues by "205." The analogy of the dyes, with their demonstrable long persistence in the host's tissues, suggests, but by no means proves, that "205" is itself the substance at work.

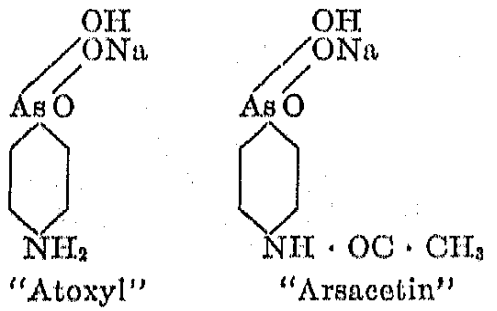
The successful experiments on the cure of infected animals by "205" have been followed by a series of apparently complete cures of well-advanced infection in man with *T. gambiense* and *rhodesiense*. A single injection has not proved adequate for the treatment of the human patient, but a course of eight or more injections has succeeded in producing what appear to be permanent cures, in a number of cases which, a short while ago, would have been regarded as quite beyond the reach of any treatment. The toxic effect of the drug itself on such patients has been a temporary and apparently not serious nephritis. (Mühlens and Menk (1921), Yorke (1921), Low and Manson-Bahr (1923).)

Another group of dyes of which the curative action in trypanosome infections has been investigated is that of the triphenyl methane dyes.

The results with these have been, on the whole, of theoretical rather than practical importance. Wendelstadt and Fellmer (1906) experimented with Malachite Green, and obtained evidence of curative action, but associated with so much local injury of the host's tissues as to make its practical application impossible. Ehrlich and his co-workers tried a number of dyes of this group and obtained promising results with Parafuchsine, though the local action on the tissues was still undesirably powerful with this substance. A chlorinated derivative of Parafuchsine, called "Tryparosan" (Roehl, 1909), was found to be more powerfully curative and at the same time much less toxic.

Another series of dyes examined by Ehrlich (1909a) and his co-workers was that containing pyronine, acridine derivatives, and related oxazines and selenazines. Of these the substance first known as "Trypaflavin" (diamino-methyl-acridinium chloride) gave curative results in mice infected with trypanosomes which, in view of its low toxicity, seemed highly promising of practical value. Its main practical use, however, has proved to be as a bacterial antiseptic, and the importance of this group of dyes, as a whole, is due more to the highly interesting results which they have given in connection with the production of tolerant strains, considered in a later section, than to their practical therapeutic value.

2. The action of compounds containing arsenic. Lingard (1899) had treated Surra in horses successfully with arsenious acid, but Laveran and Mesnil (1902), confirming Bruce's (1897) earlier observations in Africa, found that it produced only a temporary disappearance from the peripheral blood of the trypanosomes of Nagana (*T. brucei*). The application, by Thomas and Breinl (1905), of the organic arsenical derivative, which had for some time been used in treating skin diseases under the name "atoxyl," registered a definite advance. Its efficacy was soon confirmed by a number of workers, but the next step of importance was the elucidation by Ehrlich and Bertheim (1907) of the true structure of "Atoxyl" which, since it proved to be a para-amino phenyl arsenic acid, with a reactive free amino-group, formed a favorable starting point for a series of derivatives.

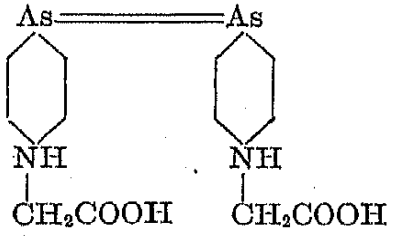


"Atoxyl" "Arsacetin"

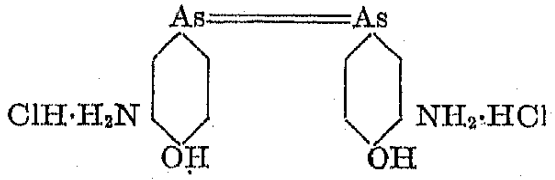
The first of these derivatives showing a more favorable experimental ratio between the tolerated dose and the dose curative of trypanosome infection, was produced by acetylating the amino-group, and named "Arsacetin." Neither Atoxyl nor Arsacetin has fully justified the early hopes of its value as a remedy in human trypanosomiasis, both having been found, in practice, to produce permanent blindness in a serious proportion of the patients treated. The study of their action, however, had results of great theoretical importance. Like the benzidin dyes, above-mentioned, these arsenical derivatives had no action on trypanosomes *in vitro* which could explain their curative action. Uhlenhuth, Hubener and Woithe (1908) reject the view that its action in the body is due to liberation of the arsenic in inorganic form—which would, indeed, be difficult to reconcile with the comparative inefficacy of the inorganic compounds when directly injected—and attribute the curative effect to the action of Atoxyl on the body cells, causing an increased formation of protective substances. The experiments of Levaditi, Brimont and Yamanouchi (1908, 1909) gave greater precision to such a conception, and suggested a participation of Atoxyl itself or some derivative of it, in the composition of the protective substance. They found that when a solution of atoxyl is mixed with an emulsion of liver, a product is obtained which is directly toxic to trypanosomes. The possibility of precipitating this toxic substance with alcohol and its instability to heat led Levaditi (1909) to conclude that it was an arsenic-protein complex, to which he gave the name "trypanotoxyl." He regarded the mechanism as a reduction of Atoxyl by the liver substance, and a combination of reduction-product with protein, the latter acting as the link for anchorage to the trypanosome. Ehrlich and Roehl (1909 b, c), however, had already shown that reduction of Atoxyl, by any ordinary reducing agent, produced, in para-amino phenyl arsenious oxide, a substance having a very intense direct toxicity for trypanosomes. According to Roehl's experiments, the function of the liver emulsion in Levaditi's was simply to reduce Atoxyl to arsenious acid, the subsequent combination of this with protein merely weakening its action. On the whole the weight of evidence seems in favor of this simpler view, that the activity of Atoxyl in the body is due to its reduction to the arsenious form, with a trivalent arsenic, by the action of the tissues. The reduced product is not only toxic for trypanosomes, but much more toxic than Atoxyl for the host; and it was in harmony with Ehrlich's view, that mice which were easily poisoned by Atoxyl were likewise more easily freed by it from trypano-

some infections than those which were more resistant to its action. Reference must be made later to some difficulties arising in connection with the tolerant strains. Meanwhile, we may note that Ehrlich's view of the action of Atoxyl departs from his original chemotherapeutic conception, in that the efficacy of Atoxyl is attributed to an immediately organotropic property. It is the reduced arsenious compound, formed in virtue of the original organotropism, which is now held to be parasitotropic.

The point of greatest importance, however, was the establishment of the superior curative activity of the compound with trivalent as compared with that containing pentavalent arsenic. This led to the production of a series of compounds containing trivalent arsenic, of which the important ones were arsenophenylglycine and dihydroxydiamino-arsenobenzene, which, in the form of its hydrochloride, has achieved world-wide celebrity under the serial number "606," and the names "Salvarsan," Arsenobenzene, Arsphenamine, etc.



Arsenophenylglycine



"Salvarsan" (606)

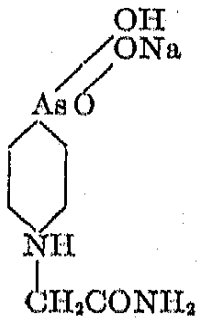
Both these substances are reduced beyond the arsenious-oxide to the arseno-condition, with a double molecule, formed by the linkage of the free valencies of the two arsenic atoms. Arsenophenylglycine gave highly promising results as a curative agent in experimental trypanosomiasis, but it failed to fulfil this promise when applied to the treatment of sleeping-sickness in man. In the case of salvarsan, which was produced by Ehrlich and Bertheim (1912), in the course of Ehrlich and Hata's (1910) search for a remedy for spirochetal infections, the powerful curative action in trypanosome infections has been somewhat overshadowed by the rapid and successful development of its use in the spirochaetal infections. Here we may note that, just as in the case of the fully oxidized derivatives of arsenic acid, we find again, in these fully reduced arseno-compounds the paradox of intense curative action on trypanosome (or spirochaet) infections *in vivo*, with no direct action *in vitro* to explain it. And again, in the case of salvarsan, we find, in the corresponding partially oxidized derivative, p-hydroxy m-amino phenyl arsenious oxide, a substance with an intense lethal action on

trypanosomes (or spirochaets) outside the body. There have again, as in the case of atoxyl, been suggestions of the formation in the body of some trypanocidal or spirocheticidal complex between the proteins of the blood or the tissue cells and salvarsan or some derivative thereof; but again the facts appear to be satisfied by the simpler theory that the parasiticidal substance is the partially oxidized arsenious-oxide derivative. This is very clearly indicated in recent papers by Voegtlin and Smith (1920) who, using a method first adopted by Morgenroth and Rosenthal (1911) for measuring the rate of disappearance of trypanosomes from the blood after curative injections, found that the effect began immediately in the case of the arsenoxid, after a definite latent period in the case of the arseno-compound, and after a longer latent period in the case of arsenic acid derivatives. These latent periods correspond with the greater liability of the arseno-compound to oxidation than of the arsenic-acid form to reduction, and the conclusion is drawn that, in either case, a preliminary to the curative effect is oxidation or reduction to the arsenoxide.

This view gives a satisfactory explanation of the therapeutic efficacy in the body of the arsenical compounds which are practically inactive on the parasites outside it. It leaves still to be explained, however, the choice for therapeutic purposes of substances which, only after injection, give rise to the directly parasiticidal derivatives, rather than these latter themselves. It is true that the arsenoxides have a much greater toxicity, not only for the parasite, but also for the host. But this alone will not explain their unsuitability for therapeutic use; for, tested on mice infected with trypanosomes, they show a high ratio between the tolerated dose and that needed to free the blood from trypanosomes, though the absolute dosage is in both cases on a lower level. The explanation must, I think, be sought by considering a factor, in the efficient treatment of infections with trypanosomes or spirochaets, which has hitherto hardly received adequate attention, namely, the factor of persistence of action. If the ready-formed arsenoxide is injected, there will momentarily be present in the blood a concentration of the direct parasiticide higher than is ever produced by its slow formation from either the more reduced or more oxidized compound; but the arsenoxide is so toxic for the host that the concentration cannot in any case be raised to a high level with safety, and the dose given at one injection must be small. To obtain the optimum effect, by administration of the arsenoxide itself, it would probably be necessary to maintain for hours or days a continuous infusion of the substance in very high dilution. This slow, persistent supply of the arsenoxide can be maintained for relatively long periods by

injecting the arsenic-acid or the arseno-derivative. In the case of salvarsan the persistence is aided by the insolubility of the free base, which must, at the reaction of the blood and tissues, be liberated and largely deposited.

This factor, of the liberation of the directly parasiticidal product of partial oxidation or reduction, is probably of importance in the action of all members of this group. And, in default of any clear knowledge of the factors which determine the rate of excretion of the parent substance, or of the rate of its change into the arsenoxide form, either of which may be affected by small changes in the nature and position of the groups attached to the benzene nucleus, there is clearly no warrant for the assumption that a change in structure, which increases the therapeutic effect, does this by increasing the preferential affinity for the protoplasm of the parasite, or by introducing an affinity for a new type of "chemoreceptor." The curative process is too complicated to be expressed in such simple terms, and involves the coöperation of the host's tissues, that is to say, some degree of "organotropic" property. It is highly probable that, in the treatment of different types of infection, the optimum rate and most favorable site for the production of the directly active substance, will in one case be provided by use of an arsenic acid derivative, in another by that of an arseno-derivative. Both have their special drawbacks. The arseno-derivatives are unstable, sensitive to exposure to air, and very difficult to obtain pure. Slight differences in the constitution, especially of the derivatives with sulphur-containing radicles attached to the amino-groups, may cause wide variations in toxicity and in therapeutic potency. Nevertheless, the arseno-derivatives are the most effective of the arsenical compounds yet obtained in the treatment of spirochaetal infections. On the other hand, the most successful arsenical compound yet tried in the treatment of human sleeping-sickness (due to *T. gambiense*) appears to be an arsenic-acid derivative, the substance known as "tryparsamide," an amide of the arsonic-acid compound corresponding to arsenophenyl glycine.



"Tryparsamide"

This substance, prepared by Jacobs and Heidelberger (1919 b, c), and selected by Brown and Pearce (1919) from a large series as the most favorable in its curative action, has now been used with highly promising results in the treatment of human sleeping-sickness. (Pearce (1921), Chesterman (1923).) It shares with "Bayer 205" the responsibility for the much more hopeful position which has recently developed with regard to the treatment of that disease. Like its parent, atoxyl, "tryparsamide" is not free from the liability to produce blindness.

3. Antimony, bismuth and other metals. Cushny appears to have made the first suggestion of a trial of compounds of antimony and bismuth, on account of their close chemical relationship to arsenic. Certain antimony compounds have been found effective in infections by trypanosomes, but not with equal certainty those of bismuth, while the reverse, as we shall see, is true of spirochetal infections. In neither case have the compounds used been aromatic complexes, with the metal attached to a benzine ring, as in the case of the arsenicals. The antimonyl—and bismuthyl—tartrates have presumably been chosen chiefly on account of their convenient solubility. Tartar emetic and its sodium analogue were used with success by Plimmer, with Thompson and Bateman (1908), and simultaneously by Mesnil and Brimont (1908 a). There are many later records of their experimental and clinical use. The action on the trypanosomes is apparently a simple and direct one, the parasites being killed outside the body by high dilutions of tartar emetic (Mesnil and Brimont). Metallic antimony suspended in oil and injected intramuscularly is apparently as effective as the antimonyl tartrate (Plimmer and Bateman), and antimony trioxide is stated to be even more efficacious. (Kolle, Hartoch, Rothermundt and Schürmann, 1913.) There appears to be no need for a more complicated assumption than that of a directly lethal action on the parasites, which must be much more sensitive to the antimony compound than are the tissues of the host. The contrast between this simple type of action and that of substances like "Bayer 205" and "tryparsamide" is the more interesting in view of the fact that, up to the time of the introduction of these latter, tartar emetic and the simple compounds of trivalent antimony, such as the trioxide, were among the more effective of the therapeutic agents tried in human trypanosomiasis. It emphasizes the possibility that the special efficacy of a complex arsenical remedy, at any rate, is due rather to the favorable rate and locality of the production from it of some simpler, directly trypanocidal substance, than to any specific affinity for the protoplasm of the trypanosome due to its molecular configuration.

The bismuth analogue has been tried in experimental trypanosomiasis with only partial (Frouin and Guillaumie, 1921) or no success (Adler, 1921). We shall see, on the other hand, that it is highly efficacious as a remedy for spirochaetal infections. Frouin and Guillaumie obtained somewhat weaker actions on trypanosome infections of mice, with cerium, yttrium and rhodium salts, but none with those of niobium.

Compounds of other metals have chiefly been used for combined treatment, together with those containing arsenic or antimony. Moore, Nierenstein and Todd (1907) advocated a combined use of atoxyl and mercury salts, but Plimmer and Thomson (1908) found that, in dosage sufficient to free the blood from trypanosomes, such treatment produced fatal lesions. Loose chemical combinations of salvarsan with other metals, such as gold or copper, have been found effective in treating experimental infections with trypanosomes, but the only compound of this kind, the "silver salvarsan" to attain practical importance has found its use in the treatment of spirochetal infections.

4. Resistant strains. One of the most interesting and theoretical important chapters in the chemotherapy of trypanosomiasis is that dealing with the development of resistant strains. When an inadequate dose of a curative agent has been given, so that a relapse of the infection occurs, further treatment is often found to be less effective, and the strain, transferred to another animal of the same species, retains its resistance and continues to do so through an indefinite number of passages. By carefully graded treatment it is possible to raise the resistance to a high level, at which it is maintained during further passages without treatment. (See Ehrlich (1907, and Browning (1907, 1908).) The phenomenon was first observed in the case of atoxyl, but was soon found to apply to other curative agents, such as the dyes. A strain resistant to trypan red was normally sensitive to atoxyl, and *vice versa*, but several such specific resistances could be developed in the same strain by the appropriate treatments.

The phenomenon at first seemed to give strong support to the conception of specific affinities for special groups in the protoplasm of the trypanosome. If the chemoreceptors for atoxyl disappeared, or were greatly reduced, the strain became proportionately resistant to the effect of this substance. When a strain resistant to atoxyl was found to be still sensitive to arsenophenylglycine, this indicated that the latter was attached to a separate "acetico-ceptor," which the treatment with atoxyl had not removed. The problem, however, has proved

to be by no means simple. In the case of an agent acting simply and directly, like tartar emetic, it can indeed be shown that the trypanosomes of a strain resistant to tartar emetic will tolerate *in vitro* a higher concentration of the drug than the original, sensitive strain (Mesnil and Brimont, 1908 b). On the other hand the resistance to tartar emetic was best produced by treatment of the infected animal with arsenious acid, while resistance to the latter itself was only obtained by very prolonged and careful treatment (Gonder, 1912). In some cases treatment with arsacetin or arsenophenylglycine has produced strains resistant not only to these substances but to tartar emetic. A still greater complication is introduced by the behavior of a resistant strain when transferred to a host of another species. Mesnil and Brimont (1908 b) produced a strain resistant to atoxyl in the mouse, and found that, when it was transferred to the rat, the sensitiveness disappeared and the strain remained normally sensitive during 40 passages through this host-species, to regain its resistance immediately when retransferred, without further treatment, to the mouse. In the dog, on the other hand, the resistance acquired in the mouse was retained. There could be no clearer evidence of the coöperation of the host's tissues in the curative action of atoxyl; on the other hand, it becomes very difficult even to speculate on the mode of their intervention. If their action were merely to reduce the atoxyl to the arsenious condition, we should have to suppose that the resistance of the parasite was to the arsenoxide; but this gives no explanation of the fact that the resistance is a property of the strain of trypanosomes and not of the hosts. Moreover there is a perplexing observation recorded by Ehrlich (1909 a) in which a strain rendered resistant, in mice, to partially oxidized arsenophenylglycine was found to be *more* sensitive to this compound *in vitro* than the normal strain. He interprets this in accordance with his view that the lethal effect visible *in vitro* is not the same as that which is effective in the body, attributing the latter to stoppage of multiplication. But such an explanation fails entirely to touch the problem presented by a resistance exhibited in one host species and not in another. Levaditi (1909), on his theory of a complex arsenic-protein poison, would regard the resistance in the mouse as specific for mouse "trypanotoxyl," leaving the strain normal in its reaction to rat "trypanotoxyl;" but again it is difficult to suppose that a specific difference, so sharp as to discriminate between mouse and rat proteins, should not exist between mouse and dog proteins. It is evident that no satisfactory solution of these difficulties is yet available, and we must be content to note the

indication which they give of the complicated nature of the chemotherapeutic process, and of the essential coöperation therein of the host's tissues.

One other highly interesting series of phenomena must receive passing mention. The resistance to drugs appeared at first to be highly specific. Later work of Ehrlich's pupils (Kudicke (1911), Gonder (1912)) showed an extraordinary reciprocal relation in the development of resistance between certain dyes, on the one hand—pyronines, acridine derivatives, oxazines, selenazines, all possessing, as pointed out by Ehrlich, an orthochinoid structure—and the arsenicals, such as atoxyl, on the other. Resistance to either of these groups seemed to confer resistance to the other; and, indeed, the most rapid and effective method of producing an atoxyl-resistant strain was found to be the injection into an infected animal of a non-sterilizing dose of a dye, such as trypaflavin. To state that the chemoreceptor for arsenic and for orthochinoid dyes must be identical, is to offer no explanation for a wholly mysterious phenomenon; it is merely to state the fact of the reciprocal action in producing resistance, in other words. Nor is it rendered, as yet, more comprehensible by the extraordinarily interesting observation of Werbitzki (1910), confirmed and extended by Gonder (1912), that trypanosomes of a strain rendered resistant to one of these dyes, though otherwise apparently normal, have lost the kinetonucleus or blepharoplast, which, according to Laveran and Roudsky (1911), can be seen to become selectively stained, and then to disintegrate under the action of the dye. Resistance to arsenicals, produced directly by ineffective treatment with these, has no effect on the morphology of the trypanosomes.

IV. Spirochaetal infections. Interest in the chemotherapy of these has, until recently, centered almost wholly round salvarsan and its soluble derivatives (neosalvarsan, sulfarsenol, sulpharsphenamine, etc.). It is beyond the purpose of this review to discuss the enormous literature on their action. We may be content to note the greater efficacy of the original "606," thrown out of true solution at the reaction of the blood, as compared with that of the derivatives soluble at neutral reaction, when both are given intravenously. It may be suggested that the factor of persistence of action is here at work, the active arsenoxide being slowly liberated over a long period from the insoluble base deposited in the tissues; and this suggestion receives support from the observation that the more soluble and readily excreted derivatives acquire an enhanced efficacy with hypodermic

injection. Some of these preparations, indeed, owe their popularity to the possibility of giving them hypodermically without causing excessive pain and induration. The increased curative action obtained by associating a silver compound with salvarsan, producing the so-called silver-salvarsan, is not yet adequately understood. It was supposed that a silver salt (nitrate or sulphate) formed an addition-compound with salvarsan, the silver and arsenic combining in virtue of residual affinities. Recent work seems to have supported the simpler, if less precise, conception, that the compound consists of silver chloride or oxide in colloidal solution, protected from aggregation by the emulsoid salvarsan. It must be left an open question whether the silver as such participates in the spirocheticidal action, or whether its association with the salvarsan favors the liberation of the directly therapeutic arsenoxide in the body.

Of fresher interest is the experimental demonstration by Levaditi and Sazerac (1921, 1922), of the curative action in syphilis, and in the closely analogous natural infection of rabbits with *Spirochaeta coniculi*, of the bismuthyl tartrate of potassium and sodium—a bismuth analogue of tartar emetic. Fournier and Guenot (1921), (1922) have used it extensively and with success in treating human syphilis. The sodium salt had already been used by Robert and Sauton (1916) for experimental treatment of spirochetosis in fowls. It will be noted that again, when arsenic is replaced by one of its chemical relatives, antimony or bismuth, the necessity for employing a complex and unstable organic derivative seems to disappear, and a relatively simple salt of convenient solubility seems to have an adequate therapeutic effect. Later experience has tended to emphasize the view that it is bismuth, administered in any form which will secure adequate absorption, which is the essential chemotherapeutic agent in this case. On the other hand, in the first observation of the action of bismuth compounds on a spirochaetal infection, in which Robert and Sauton demonstrated its curative effect in infections with *Sp. gallinarium*, these authors record that the sodium bismuthyl tartrate was without action on the parasites *in vitro*.

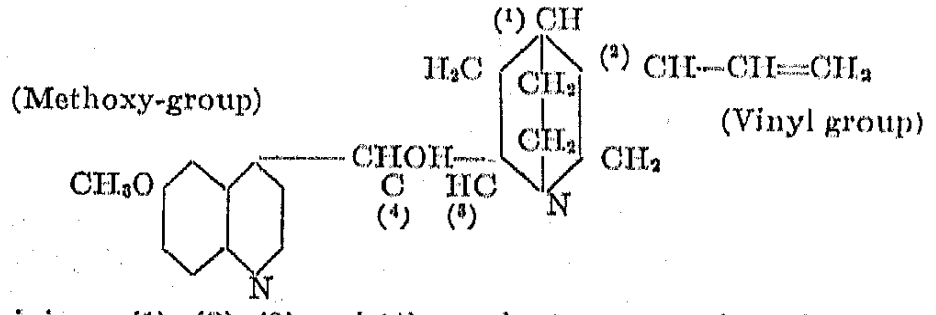
Recently Fournier, Levaditi and Schwartz (1922) have examined compounds of niobium, tantalum and vanadium. The last alone showed any effect on experimental or clinical syphilis, but these effects of vanadium were comparable to those obtained with the analogous bismuth salts. Pröscher, Seil and Stillians had already (1917) shown the effect of vanadium on syphilis.

We may note that, in their long series of experiments, which led ultimately to salvarsan, Ehrlich and Hata tried a number of dyes in spirochetal infections, without any results of value. "Bayer 205" which, though not a dye itself, may be regarded as the most successful achievement, up to the present, of chemotherapeutic investigation on compounds of this type, appears to be without effect on spirochetal infections. Up to a certain point, and especially in connection with the arsenical remedies, trypanosomes and spirochaets appear to show a certain community of response to therapeutic agents; thereafter divergence is apparent, the lines of investigation, which at the moment show the greatest promise for new development, being, in the one case, in the direction of complex organic substances related to the dyes, in the other case, in the direction of relatively simple salts of the metals related to arsenic and antimony.

V. Leishmaniasis. The successful treatment of Leishmania infections has hitherto been practically limited to the intravenous injection of tartar emetic and its sodium analogue. The use of these salts appears to have resulted from empirical trial by different workers (Vianna (1913), Carini (1914), Cristina and Caronia (1915), Rogers (1915)), working merely on the broad analogy of its previous use in other protozoal infections. It was remarkably successful, though the arsenical remedies had been relatively valueless. At the time of writing the first news is published of the successful treatment with "Bayer 205" of a case of kala-azar, which had resisted treatment by tartar emetic (Yorke, 1923). Simultaneously there is published by Lindenberg (1923) an account of the treatment by "Bayer 205" of the cutaneous Leishmaniasis of Brazil. Cases can apparently be cured by hypodermic injection, but local application of "205" to the sore is without effect: a most significant observation, if confirmed. This is but one example, out of a number, which will present themselves, of the lack of correspondence between the facts of chemotherapy and the accepted zoölogical affinities. "Bayer 205" is highly effective in certain trypanosome infections, quite ineffective in others, but apparently effective again in Leishmaniasis. The Leishmania parasite has, indeed, some zoölogical affinity with the trypanosomes, but hardly so close as that of different species of trypanosome for one another. We may note, in the same connection, the common liability of trypanosomes and spirochaets to the action of arsenical remedies such as salvarsan, which Leishmania does not fully share. No theory as to the systematic position of the spirochaets can suggest for them a closer biological affinity to the trypanosomes than that of Leishmania.

VI. Malaria. For the treatment of malaria the modern development of chemotherapy has effected practically nothing. Since cinchona bark was brought to Europe in the 17th century, real improvements in the treatment of malaria have been almost limited to improvements in the administration of this drug and its alkaloids, guided in the more recent period by the recognition of the malarial parasites and more accurate knowledge of the chemistry of the cinchona alkaloids. Ehrlich and Guttmann's (1891) trial and recommendation of methylene blue has been mentioned. Later observations have not supported its claim to rivalry with quinine, nor have any of the newer remedies for other protozoal infections, such as salvarsan, acquired an importance in the treatment of malaria at all comparable with that of quinine. The special reputation of quinine, as compared with the other natually occurring cinchona alkaloids would appear to be largely artificial. It apparently arose at a time when quinine and cinchonine were the well-known and characterized alkaloids, the latter contrasting unfavorably with quinine on account of its toxicity for man. In recent years, with improving knowledge of the cinchona alkaloids, others have been tried, with results which seem to indicate that some of them —hydroquinine, quinidine, cinchonidine—are at least as effective in the treatment of malaria, and not noticeably more toxic. (McGilchrist (1915), Giemsa and Werner (1914), Acton (1920).)

The point has practical importance since the whole policy of the artificial cultivation of cinchona has hitherto been governed, both in selection of soil and climate, and the selective breeding of the trees, by the supposed necessity of securing a maximum yield of quinine in proportion to the other alkaloids. Theoretically it is interesting as showing that neither the vinyl group, which is reduced to an ethyl group in hydroquinine, not the methoxy-group, which is absent in cinchonidine, nor the precise stereometric configuration of the quinine molecule, since quinidine differs in its arrangement in relation to at least one of the four asymmetric carbon atoms, is essential to the therapeutic action of quinine in malaria.



Quinine. (1), (2), (3) and (4) are the 4 asymmetric carbon atoms.

With the exception of hydroquinine, none of the many artificial derivatives which have now been made from these cinchona alkaloids have proved to be even as good as the natural ones in treating malaria: they have found their practical uses in other directions, in in the treatment of bacterial infections. The main question of chemotherapeutic interest, therefore, is still that of the manner in which quinine and its similarly acting relatives produce their antimalarial effect. It is a curious item in the history of the subject that Binz, as long ago as 1867, should have correctly predicted that the cause of malaria would prove to be a protozoön, on the quite inadequate ground of his observation that quinine, with its traditional value in malaria, had a lethal action on free-living protozoa (paramecium etc.) in relatively high dilutions. It would have been as logical, from the failure of quinine to cure tropical dysentery or sleeping sickness, to conclude that the infective agents in these diseases were probably *not* protozoa. After the discovery of the malarial plasmodia various attempts were made to observe the effect of quinine on the parasites. Many such observations were directed to following the degenerative changes in the parasites during the treatment of the patient with quinine; these, of course, leave quite open the mode of action of the remedy, whether directly on the parasite, or indirectly, by stimulating the defensive response to the host. Observations on the effect of quinine added to malarial blood *in vitro* have not produced any conclusive evidence in in favor of a direct parasiticidal action of the alkaloid. (Laveran (1891), Dock (1891), Marchiafava and Celli (1886), LoMonaco and Panichi (1900).) The last named authors appeared to regard the effect of quinine, in medicinal doses, as a stimulation of the young parasites, leading them to leave the red corpuscles and so to become exposed to the unfavorable conditions of the blood-plasma. This observation has an obvious relation to the interesting theory of quinine action much more recently put forward by Morgenroth (1918). This observer, as the result of tests made by physiological and bacteriological methods, came to the conclusion that, when quinine is added to shed blood, a very high percentage of it becomes extracted by the corpuscles, the serum or plasma being left almost free from quinine. On the basis of these observations Morgenroth puts forward the suggestion that quinine owes its curative action in malaria to its *organotropic* properties, especially its affinity for the red corpuscles. He leaves it an open question whether the quinine, so located, acts in relatively concentrated form on the already intracorpuscular parasites, or whether, as

he thinks more probable, the quinine, being concentrated on, or in, the surface layer of the corpuscle, repels the merozoites resulting from the asexual reproductive cycle, so that, being denied access to the place of their further growth, they perish in the plasma. While it is of interest to find Ehrlich's former colleague thus frankly adopting a view, which attributes the curative action of quinine to an organotropic and not a parasitotropic property, it must be borne in mind that none of the numerous determinations, of the distribution of quinine between corpuscles and plasma, which have been made by chemical means, give any support to Morgenroth's deduction from his biological tests. Ramsden, Lipkin and Whitley (1918) found a ration of 2 or 3 to 1 in favor of the concentration in the plasma, while later determinations, by Acton and King (1921), show a practically equal distribution throughout the blood mass. The discrepancy is obviously one which demands further investigation. For our present purpose it is sufficient to note that no simple conception, of directly lethal action on the malarial parasites, will fit the knowledge yet available as to the nature of the curative action of quinine in malaria.

VII. Amoebiasis. As in the case of malaria, the only drug which, even to-day, can be stated to have an indisputable and specific action on tropical dysentery, was introduced to Europe from South America in the 17th century for the treatment of that disease, more than two centuries before the organism responsible for it was discovered. The alkaloids of ipecacuanha root like those of Cinchona bark, have now been isolated and characterized; and again we find among them, in emetine and cephaeline, principles having an antiamebic action more potent and specific than that of any other known substance, including such artificial derivatives of emetine as have hitherto been prepared. The *Entamoeba histolytica* is an organism much more readily accessible for direct observation of effects *in vitro* than the malaria parasite; and until a few years ago the evidence seemed to point distinctly to the simplest possible mechanism for the curative action of emetine in amebic infections, namely, a directly lethal action on the amebae. Vedder (1911) showed that the alkaloids, in relatively high concentration (1 in 100,000) would kill free-living amebae, though Lyons (1912), who made similar experiments, was doubtful as to the adequacy of the results to explain the therapeutic action. Rogers' (1912) experiment, which indicated that *Entamoeba histolytica*, fresh from the human intestine, was killed in a few minutes by emetine in a dilution of 1 in 100,000, suggested that there was no need to look further than simple,

direct action on the parasite for an explanation of the therapeutic action in the patient.

Repetition of such observations, and their extension to the other alkaloids of ipecacuanha, to artificial derivatives therefrom, and to other substances, have made this simple view quite untenable. Kuenen and Swellengrebel (1913) observed a lethal action of emetine on *E. histolytica*, but it needed from 1 in 10,000 to 1 in 5,000 to produce the effect, and periods up to many hours for its development. The experiments of Kolmer and Smith (1916), and of Walters, Baker and Koch (1917) also showed a much lower order of activity for emetine on parasitic or free-living amebae. Dale and Dobell (1917) studied the effects of a number of these alkaloids on the fresh entamebae in the test-tube, obtaining the material mostly from cats infected from human cases of dysentery. Some of the same substances have been clinically tested on man by G. C. Low. A very curious contrast is revealed. While Dale and Dobell did not find emetine without effect on the isolated amebae, both it and cephaeline were far less active than Rogers' statement had suggested, and less so than other alkaloids, such as quinine and harmaline, which have no curative action in an amoebic infection. This has, in essence, been recently confirmed by Allen (1920), using amebae direct from human patients. On the other hand, one of the minor natural alkaloids from ipecacuanha, methylpsychotrine, and several artificial derivatives, such as demethoxyemetine, were found by Dale and Dobell to exhibit a more powerful toxic action for the isolated amebae and a relatively very low toxicity for the mammal. These, when tested on human sufferers from dysentery, all failed entirely to justify this promise of therapeutic value; so far as results at present available warrant a conclusion, it would be that the therapeutic efficacy of these alkaloids, in dysentery, has no relation to their toxicity for *Entamoeba histolytica* outside the body, but is, roughly, in direct proportion to their toxicities in man.

Another curious fact which emerged was that a strain of Entamoeba which was readily susceptible to treatment by emetine in the human patient, was completely resistant to emetine in kittens, to which it had been transferred from man before the treatment was applied. The close connection of the curative action of emetine with the behavior of the organism as a tissue-parasite is made clear by the fact that treatment with emetine eliminates infection with *E. histolytica* and leaves undisturbed the saprophytic entamebae and allied forms (*Entameba coli, Endolimax nana*) living in the contents of the colon. We are,

once more, almost compelled to suppose that the tissues of the host play an essential part in the curative action which the drug initiates. It is, of course, just conceivable that emetine, while not visibly affecting the motility and vitality of the amebae, when applied in high dilutions, has nevertheless a powerful inhibiting action on their reproductive activity, which, since artificial culture has not proved possible, cannot be detected outside the body. So far as experiments on free-living amebae in culture can provide sound evidence on this point, it is not in favor of such a view. Pyman and Wenyon (1917) found, indeed, an inhibiting action produced by emetine and cephaeline and none by psychotrine, which has no curative action. They observed, however, equally good inhibition of multiplication by N. methylemetine, the curative effect of which is, at best, very slight. It would be rather a forced assumption, moreover, to suppose that alkaloids nearly related to emetine, and having a more pronounced immediate and visible action, should be devoid of the inhibiting action on reproduction; and the assumption could not, in any case, explain why emetine inhibits the reproduction of the entameba in man, but not that of the same strain in the cat. To suggest, as an explanation, coöperation of the host's tissues, in a manner concerning which the knowledge yet available affords not even a hint, is unsatisfactory; but it seems at present the only alternative to a completely agnostic attitude.

VIII. Helminthiasis. Strictly speaking we ought to include under the heading of "chemotherapy" the removal of parasitic worms from the alimentary canal, by administering, by the mouth, substances which kill or paralyze the worm, without producing serious symptoms of poisoning in the host. The conditions, however, are so different from those which govern the treatment of infections by parasites of the blood and tissues, and the action involved is so obviously and beyond question a direct poisoning of the parasite, that the inclusion of this type of treatment in this review would be misleading rather than illuminating. The case is different with infections by worms of the blood and tissues: here the problems of treatment closely resemble those encountered in dealing with protistal infections. Such success as has been attained has resulted from the empirical trial of remedies which have been found effective in infections with protozoa or spirochaets. Infection with the guinea-worm can, apparently, be cured by intravenous administration of salvarsan or tartar emetic. (Jeanselme (1919), Montpellier and Ardoin (1919), Macfie (1920).) But the most striking and best authenticated success has been attained in treating

infections with Bilharzia (Schistosoma) by tartar emetic, injected intravenously. This was tried by Christopherson (1918), (1919), (1920) in 1918, who was induced to make the attempt by his observation of its success in Leishmaniasis. Before this successful trial, a chemotherapeutic attack on Bilharzia infections had been regarded as an almost hopeless enterprise, since the symptoms constituting the essential disease are produced, not by the adult worms in the circulation, but by the innumerable, irritating and highly resistant ova, finding their way to the exterior through the tissues of the bladder and the rectum. It is still a matter for surprise that the eggs should be killed by the antimony treatment, and that, with their death, the irritating action should cease. The evidence as to the mode of action is still incomplete. Christopherson describes experiments, which he regards as proving a direct lethal action of the tartar emetic on the ova and the free-swimming embryos (miracidia). It cannot be said that, having regard to the concentrations employed, they carry a strong conviction of the correctness of his view.

This theory of directly lethal action is rendered more difficult by the fact that a number of observers (Diamantis (1917), Mayer (1918), Bonne (1919), Debbas (1920), Erian (1919), Tsykalas (1921)) have made very similar claims for emetine, administered hypodermically or intravenously, as a cure for bilharziasis of the bladder or the rectum. On the other hand, it is at least extremely doubtful (Low and Gregg, Macfie) whether tartar emetic has, on Filaria infections, the curative effect which Rogers had suggested.

CONCLUSION

In the foregoing pages an attempt has been made at an impartial summary of the facts which must be covered by any general theory of the mode of action of specific chemical remedies for infections. Already in the early days of this type of investigation, when attention was focussed on the effects of certain chemicals on infection with trypanosomes, facts were constantly presenting themselves which could only with difficulty, and with aid of subsidiary hypotheses, be reconciled with the idea that the curative substance acted in virtue of its differential affinity for the parasitic protoplasm. The contrasts between effects *in vitro* and those *in vivo;* the phenomena of acquired resistance, specific for the infected host as well as for the infecting strain of parasites, and showing, at the same time, discrimination between nearly related remedies and community as regards others having no chemical

similarity; these phenomena were, and still are, difficult to bring within the scope of any simple type of explanation. The difficulty has greatly increased during recent years, as the methods of chemotherapy have spread to the treatment of other types of infection. When we are dealing with organisms of one genus, or of a few closely allied genera, the postulation of different chemoreceptors, corresponding to the different kinds of compound found therapeutically effective, though it does not convey more real information than the record of the results observed, does not involve any apparent absurdity. When, however, we try to apply the terminology to the more recent extensions of chemotherapy, and find it necessary to suppose, for example, that the *Bilharzia* worm has antimony-receptors in common with trypanosomes or *Leishmania*, and emetine-receptors in common with the dysentery ameba, it becomes increasingly difficult to use the nomenclature with complete solemnity. Ehrlich's theory will always deserve the credit of having provided a vigorous stimulus to the investigation of problems which, without some kind of working hypothesis, might well have seemed beyond the reach of an experimental attack. That being admitted, it is necessary, on the other hand, to admit that few of the successful results hitherto obtained have been obtained by a consistent application of the theory. Some of them seem, indeed, to be the result of experiments which a serious acceptance of the theory would have discouraged. As new successful applications have become more frequent, their basis has become increasingly empirical. It is difficult to resist the conclusion that a new theoretical foundation is required for further orderly building, and that this will have to take fuller account of the great complexity of the therapeutic process, and especially of the coöperation therein of the infected host. And, if this should mean some measure of reunion between "chemotherapy" and the parent pharmacology, from whose rather unenterprising tradition it claimed to be free, the result can only be to the advantage of therapeutic science.

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