DRUG RESISTANCE, WITH SPECIAL REFERENCE TO TRYPANOSOMIASIS*

BY

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In 1907, Franke and Roehl, working in Ehrlich's laboratory, recorded a very interesting observation (Ehrlich, 1907). They found that the feeding of nagana-infected mice with parafuchsin caused the disappearance of trypanosomes from the peripheral blood; after a week or two, however, the parasites reappeared, but further administration of the drug caused them to disappear again. The process could be repeated, but not indefinitely, as after a time it was noticed that the parafuchsin feeds began to produce less and less effect; after each successive administration of the drug the time of banishment of the parasites from the blood of the mouse became shorter and shorter, until, finally, a period was reached when the drug entirely failed to influence the parasites. On transference of the parasites to normal mice they were found to be still uninfluenced by parafuchsin, and it became evident that they had in fact acquired a heightened resistance to the drug. This fundamental observation was quickly followed by the discovery that strains of trypanosomes resistant to other drugs could be developed.

The practical significance of the capacity of trypanosomal infections to become drug-resistant, or drug-fast, for the treatment of the infections in man and stock was at once recognized by Ehrlich, and during the last twenty-five years the whole question, in its various aspects, has been the subject of an enormous literature and of a considerable amount of work. Nobody who attempts to orientate himself through the vast number of papers dealing with the subject of drug resistance can fail to be impressed with the many contradictory statements which have been made, with the unwarrantable and sweeping inferences which have been drawn from the most inadequate experimental data, and with the enormous superstructure of hypothesis which has been erected on a most insecure foundation of questionable facts.

This is the more interesting because the clinician, having grasped the fact that there are such things as drug-fast strains of trypanosomes, has hastened to explain his failure to cure many of his cases of human trypanosomiasis on the hypothesis that the parasite has become arsenic- or antimony-resistant. He has not paused to reflect that he has really obtained not the slightest evidence that the trypanosomes infecting his patient have actually developed resistance to the drug or drugs he has employed, or to ask himself whether there may not possibly be some other explanation for the failure of his treatment. As a matter of fact, although analogies suggest that drug resistance probably plays a very important part in the therapy of human trypanosomiasis, there is remarkably little direct evidence that drug-resistant strains do actually develop during the treatment of infected man. When we turn to other diseases such as malaria, syphilis, and leishmaniasis, we find similar explanations given of the failure to cure. Such statements are even less justifiable, as we have no satisfactory proof that *Plasmodium* sp. ever becomes resistant to quinine, or *S. pallida* to mercury and salvarsan, or *Leishmania donovani* to preparations of antimony.

It is now about five years since my colleagues and I commenced a serious study of chemotherapy, and almost

immediately we became so impressed with the importance of drug resistance that we have devoted almost all our time to this aspect of the subject.

The method of testing the therapeutic action of drugs by injecting them into infected laboratory animals, although an indispensable procedure for obtaining the vital information whether or not a drug is of any practical value for the treatment of the infection, is far from adequate if the object of the inquiry be to obtain information regarding the mechanism of the action of the drugs in question, and of the manner in which the infections become resistant to the action of these drugs. It fails to afford an unequivocal answer to such questions as the following: Does the drug owe its therapeutic value to a capacity to act directly upon the parasites, or does it become parasitocidal only after it has undergone some change in the body of the host; or, again, does the drug act by stimulating the body of the host to produce a parasitocidal substance? What happens when an infection becomes drug-resistant? Is the change primarily in the host or in the parasite; and what is the nature of the change? What are the factors which conduce to the production of a drug-resistant infection?

Considerations of this nature convinced us that much valuable information could be obtained from an examination of the trypanocidal power of drugs *in vitro*, as thereby all the unknown factors, of uncertain and possibly inconstant value, dependent on the vertebrate host, would be eliminated. Much work along these lines has of course been done in the past, but the results were contradictory and of little real value, because no satisfactory means had been devised for keeping trypanosomes alive *in vitro*.

Our first problem, then, was to discover a method whereby a suspension of trypanosomes could be kept alive, in undiminished numbers and in a condition of unlowered vitality, for a period of at least twenty-four hours at 37° C. As soon as we had developed a technique which would enable this to be done we were immediately in a position to make a considerable number of important observations.

It was found that the organic pentavalent arsenical and antimonial compounds are but slightly trypanocidal, a solution of 1 in 1,600 being required to destroy the parasites within twenty-four hours. The organic trivalent arsenical compounds are, however, extraordinarily trypanocidal, as even when diluted several hundred million times they killed the trypanosomes within twenty-four hours; this also applies in the case of the arsenobenzols—novarsenobillon and arsenophenylglycineamide—but arsenophenylglycine, although very active, is much less so than the other arsenobenzols, a solution of 1 in 3,200,000 being required to destroy the trypanosomes in twenty-four hours. Sodium arsenite and tartar emetic likewise displayed considerable action, the corresponding trypanocidal titres being respectively 1 in 3,200,000 and 1 in 6,400,000.

This work in conjunction with a parallel series of experiments on infected mice enabled us to reach the conclusion that the therapeutic action of the trivalent compounds of arsenic and antimony is dependent entirely on their amazing trypanocidal power. As the pentavalent compounds have practically no trypanocidal power, they must be reduced in the body of the host to their corresponding trivalent forms before they can exercise a trypanocidal action. In order to explain the mechanism of the therapeutic effect of the arsenical and antimonial compounds it is therefore unnecessary to assume some unknown and complicated co-operation on the part of the host. The host undoubtedly plays an important part in the therapeutic process; it is responsible for immune-body formation, and it determines the rate of excretion

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of the drugs and the rate of reduction of the pentavalent compounds; but we have no evidence that it assists in the therapeutic process by changing fundamentally the chemical constitution of the arsenical or antimonial compounds.

Strains of *T. rhodesiense* made resistant to atoxyl and acriflavine respectively were tested *in vitro* and *in vivo* against a whole series of drugs: (a) the aromatic compounds of arsenic—namely, atoxyl, tryparsamide, arsacetin, reduced tryparsamide, reduced arsacetin, halarsol, and novarsenobillon; (b) the aromatic compounds of antimony—namely, stibosan and stibenyl; (c) the non-aromatic compounds of arsenic and antimony—namely, sodium arsenite and tartar emetic; and (d) the non-metallic compounds, acriflavine and Bayer 205. It was found that the two strains—one made resistant to atoxyl and the other to acriflavine—behaved in exactly the same way. Both exhibited a pronounced degree of resistance to all the aromatic compounds of arsenic and antimony and to acriflavine, but both were just as sensitive to sodium arsenite, tartar emetic, and Bayer 205 as was the normal parent strain. We then prepared other strains resistant to tryparsamide, arsacetin, halarsol, and N.A.B. respectively, and found that each of these was identical with the two previous strains. Hence we reached the general conclusion that if a strain of trypanosomes becomes resistant to any of the aromatic compounds of arsenic or antimony, it likewise becomes resistant to all the other commonly employed aromatic compounds, but not to the non-aromatic compounds of arsenic and antimony. It is consequently misleading to refer to "arsenic resistance" or to "antimony resistance"; the resistance is really to the various substituted phenyl-radicals of the aromatic compounds.

It has frequently been said that drug resistance is not merely a character of the trypanosomes themselves, but that it is in some unexplained way dependent upon the host. This idea, which seems firmly fixed in the minds of almost all who have interested themselves in chemotherapy, is apparently based on observations made by Breinl and Nierenstein and by Mesnil and Brimont in 1908 and 1909. These authors recorded experiments from which they concluded that a strain of trypanosomes made resistant to atoxyl in one species of host (mouse) lost that resistance when transferred to another species (rat). We have repeated and extended these experiments and have failed to obtain the slightest evidence to warrant this inference. A strain which is made resistant to an aromatic arsenical in the mouse is likewise resistant when transferred to the rat or rabbit, and vice versa.

With the aid of our *in vitro* technique, we have been able to show that whereas a normal strain of *T. rhodesiense* is killed by a solution of something like 1 in 100,000,000 of reduced tryparsamide within twenty-four hours at 37° C., the resistant strain is able, under similar conditions, to withstand a solution of 1 in 400,000 of the drug. The resistance of our atoxyl-fast strain has remained unchanged for several years, during which it has been passaged through over 300 mice, and all attempts to lower its resistance by sodium thiosulphate, as described by Citron, have failed.

Drug resistance is therefore a stable character which is inherent in the trypanosomes themselves, and is in no way dependent on the particular host in which the parasite happens to find itself.

What is the nature of the remarkable change which a drug-fast strain of trypanosomes has undergone—a change which enables it to withstand about 250 times as much reduced tryparsamide as does the original strain? Ehrlich, in his side-chain theory, explained drug resistance by postulating a diminution in the affinity of the chemoreceptors of the resistant parasites for the drug. That

theory has more or less fallen into disrepute. It has been criticized by Sir Henry Dale, and by the American pharmacologist Voegtlin, who writes that it was just the realization of the total inadequacy of this theory which led him to take up the study of chemotherapy. Voegtlin himself has devised a very ingenious theory which postulates that trypanosomes resistant to arsenicals owe their resistance to the development of an excess of sulph-hydryl compounds which enables them to take up relatively large quantities of arsenicals without harm to themselves.

Our experiments, however, have shown that drug-resistant trypanosomes owe their resistance to the fact that they do not absorb aromatic arsenicals even though these are present in concentrations rapidly fatal to normal strains. When, for example, a large number of normal trypanosomes are suspended for one hour at 37° C. in a nutrient medium containing 1 in 12,800,000 of reduced tryparsamide, and the parasites are subsequently removed by the centrifuge, all the drug is found to have disappeared from the medium. When, however, a resistant strain of trypanosomes is used none of the drug is removed. These results, which have recently been confirmed by v. Jancso in experiments of quite a different nature, appear to render Voegtlin's hypothesis untenable, and are in harmony with Ehrlich's hypothesis, or with any hypothesis which postulates that drug resistance is due to an impermeability of the parasites.

Incidentally it might be mentioned that by the aid of our *in vitro* technique we have been enabled to investigate the very interesting question whether the development of drug resistance by a strain of trypanosomes is the result of a process of selection—that is, the weeding out of the more sensitive individuals, and survival of those naturally resistant which tend always to reproduce their like; or whether it is due to a process of mutation—that is, a gradual change in all, or in certain, individuals resulting from the stimulus of frequent exposures of the strain to suitable concentrations of the drug, thus giving rise to the acquisition of a new character which is transmitted through innumerable subsequent generations. We believe that our experiments indicate that the development of drug resistance by a trypanosome is fundamentally the result of a process of mutation, although we are unable to exclude the possibility that under certain circumstances the process of mutation may be aided by one of selection.

What factors are especially conducive to the production of a drug-resistant strain of trypanosomes? We have devoted much time to the investigation of this important problem, and have found that the rapidity with which resistance is produced not only varies greatly with different drugs, but also with the manner in which they are administered. Generally speaking, it is more easy to produce a strain resistant to the aromatic arsenicals and antimonials by such drugs as atoxyl, tryparsamide, arsacetin, and stibenyl, than by halarsol and novarsenobillon. The production of a strain resistant to Bayer 205 is a most tedious and lengthy matter; and so far we have not succeeded in making a normal strain resistant to tartar emetic, although—and this is probably a matter of practical importance—it is exceedingly easy to achieve this end by the indirect method of first making the strain resistant to atoxyl. Then again, the rapidity with which resistance to a drug is produced depends on the size and spacing of the doses. The importance of these factors in the production of drug resistance was clearly shown by a number of experiments in which we produced strains of trypanosomes highly resistant to the aromatic arsenicals by a series of short *in vitro* exposures of the parasites to reduced tryparsamide. The speed with which resistance developed, and the degree of its development, depended on the concentration of the solution of reduced

tryparsamide to which the parasites were exposed. The optimum concentration of drug for this purpose was found to be the highest employed which failed to destroy all the trypanosomes in the suspension exposed to the drug. By this means a strain of trypanosomes was quickly produced which was at least 500 times as resistant to reduced tryparsamide as was the original strain. The use of lower concentrations of drug likewise sufficed for the production of a resistant strain of trypanosomes, but the process was much slower and the degree of resistance resulting was much less.

We have examined a considerable number of methods of making a strain of trypanosomes drug-resistant in the mouse, and found that the quickest and most certain method is to give daily such doses of the drug as just fail to clear the peripheral blood of parasites or at most do so for only a day or two. This is in accordance with the conclusions reached from the *in vitro* experiments—namely, that the optimum conditions for the production of drug resistance are attained when the trypanosomes are repeatedly exposed to the highest concentration of drug which just fails to destroy them all.

Although such a method cannot be adopted in the case of animals, like rabbits, in which trypanosomiasis is essentially an infection of the tissues, nevertheless the production of highly resistant strains in these animals is a matter of the greatest use. If, when a relapse occurs in a rabbit which has received a number of small sub-curative doses of atoxyl or tryparsamide the trypanosome is transferred to, and tested in, mice, it is almost certain to show a definite degree of resistance; and the resistance can rapidly be enhanced by continuing to treat the rabbit with rather larger doses of the drug.

As I have already pointed out, we do not yet know whether the failure to obtain cures in certain cases of human trypanosomiasis by atoxyl, tryparsamide, etc., is due to the fact that the treatment administered has resulted in the production of a resistant infection, or whether there is some other cause for the failure. Man is for obvious reasons a very unsatisfactory experimental animal, and in human trypanosomiasis the number of parasites in the peripheral blood is usually very small. These facts make it almost impossible to decide, by direct observation on man, whether or not the failure to cure in any particular case is due to development of drug resistance. From the analogy of our experiments in rabbits, it is highly probable that the production of a resistant strain in man is of frequent occurrence in practice, but, at the moment, that is as far as one is justified in going. However, the question is probably easy of solution by the procedure we adopted in the case of the rabbits—namely, by transferring the parasites to mice and testing their resistance to aromatic arsenicals in these animals.

If, as seems probable, a considerable number of cases of human trypanosomiasis treated with aromatic arsenicals do develop resistant infections, the fact is one of the greatest practical importance, because, when once a trypanosome strain has become drug-resistant, it retains the character apparently unimpaired for years, and because resistance to one of the aromatic arsenicals implies, broadly speaking, a resistance to all the aromatic compounds of arsenic and antimony.

Our experimental work has convinced us that when once an infection has developed a definite degree of resistance, it is hopeless to attempt to cure the animal by further administration of the aromatic arsenical or antimonial compounds; in fact, prolongation of treatment merely results in enhanced resistance. It is therefore a matter of great importance to ascertain beyond all doubt what part the development of drug resistance plays in the results of therapy of human trypanosomiasis, and the

particular factors which conduce to its development. Our experimental experience suggests that the development of drug resistance is slow to begin with—that is, there is no obvious change in the resistance of the trypanosomes in response to the first few doses of the drug, but when once resistance has begun to manifest itself it develops with great rapidity until a condition of complete resistance is reached, when further doses of the drug exercise not the slightest effect on the infection.

Furthermore, if human beings do, as the result of treatment, develop drug-resistant infection, the question whether the trypanosomes can retain that resistance in their passage through glossina becomes one of the utmost importance. It must, however, be borne in mind that because a patient is not cured by a course of treatment it does not necessarily follow that his infection has become drug-resistant. This is clearly shown by the fact that Chesterman and others record cures with tryparsamide in patients whom they had previously unsuccessfully attempted to cure with atoxyl. The failure to cure such cases with atoxyl could not have been due to the fact that the infections had become atoxyl-resistant, because atoxyl resistance implies also tryparsamide resistance. A reasonable explanation appears to be that in these cases there were foci of the infection which could be reached by tryparsamide, but not by atoxyl; and this hypothesis is supported by Chesterman's findings that cases which were not cured by the first course of tryparsamide cannot be cured by further dosage of that drug.

RECURRENT BOILS*

SOME POINTS RELATING TO AETIOLOGY, COMPLICATIONS, AND TREATMENT

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There is a singular disposition to regard the individual suffering from a boil with a certain degree of levity, yet we have it on no less authority than the Bible that "Satan went forth from the presence of the Lord and smote Job with sore boils from the sole of his foot unto his crown." Those who have endured the extreme pain of a boil in the external auditory meatus must indeed be tempted to suppose that the same master hand was responsible for their affliction.

To Pasteur is due the honour of demonstrating that the boil is caused by the action of the *Staphylococcus aureus*—a micro-organism which he found also in pus from carbuncles and osteomyelitis—but we are well aware that mere contact between the skin and this organism is not necessarily followed by the development of a boil or carbuncle. The number, the virulence of the cocci, and the degree of resistance of the individual are the determining factors, as in the case of many other infections. Some investigators, amongst them Goldsmith¹ and Ferguson Smith,² hold that *Staphylococcus aureus* is not a normal habitant of the skin; others state that every man is a carrier of pyogenic cocci. Investigations of this nature must necessarily be incomplete, for it is impossible to include in the examination the whole skin surface or the contents of the follicles; neither is there at present a satisfactory means of distinguishing between virulent and non-virulent bacteria of the same species. We may, however, assume that most individuals, if not all, are frequent carriers of *Staphylococcus aureus*. The relative number will be influenced by such factors as personal cleanliness and occupation. The personnel of a hospital,

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