III

DRUG-RESISTANCE *

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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 recognised 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 super-

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structure 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 *Sp. pallida* to mercury and salvarsan, or *Leishmania donovani* to preparations of antimony.

It is now about five years since my colleague 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 parasiticidal only after it has undergone some change in the

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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 : 1,600 being required to destroy the parasites within twenty-four hours. The organic trivalent arsenical compounds are, however, extraordinary 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 : 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 : 3,200,000 and 1 : 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 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, viz., atoxyl, tryparsamide, arsacetin, reduced tryparsamide, reduced arsacetin, halarsol, and novarsenobillon; (b) the aromatic compounds of antimony, viz., stibosan and stibenyl; (c) the non-aromatic compounds of arsenic and antimony, viz., sodium arsenite and tartar emetic; and (d) the non-metallic compounds, viz., 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 to Bayer 205 as was the normal parent strain. We then prepared other strains resistant to tryparsamide, arsacetin, halarsol, and NAB 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 or antimony. It is consequently misleading to refer to “arsenic-resist-

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ance" or to "antimony-resistance"; the resistance is really to the various substituted phenyl-radicals of the aromatic compounds.

CHARACTERS OF DRUG-RESISTANCE

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 : 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 : 400,000 of the drug. The resistance of our atoxyl-fast strain has remained unchanged for several years, during which it has passed through over 300 mice, and all attempts to lower its resistance by sodium thiosulphate, as described by Citron, have failed.

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 chemo-receptors of the resistant parasites for the drug. That theory has more or less fallen into disrepute. It has been criticised by Sir Henry Dale, and by the American pharmacologist, Voegtlin, who writes that it was just the realisation of the total inadequacy of this theory which

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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 be 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 : 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*, *i.e.*, 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*, *i.e.*, 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.

Drug-resistance, therefore, is a stable character which is inherent in the trypanosomes themselves, and is in no

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way dependent on the particular host in which the parasite happens to find itself. Whilst the resistant parasites exhibit no perceptible morphological differences from the normal parasites, they owe their resistance to the fact that they are no longer able to absorb the drug, although this is present in concentrations which are immediately fatal to the normal strain. This remarkable change in the parasites is mainly due to a process of mutation.

EFFECT OF PASSAGE THROUGH GLOSSINA ON ARSENIC-RESISTANCE OF A TRYPANOSOME

Does drug-resistance in trypanosomes survive the cyclical passage of the parasite through its natural intermediate host, the tsetse fly? That this question is one of great scientific interest and of fundamental practical importance was recognised by Ehrlich so long ago as 1911. At Ehrlich's instigation Werbitzki and Gonder set themselves to inquire whether an arsenophenylglycine-fast strain of *T. lewisi* (the common rat trypanosome) would develop in the rat louse, *Hæmatopinus spinulosus*, and, if so, whether it would remain arsenic-fast. As the result of their experiments, these authors concluded that the passage of the resistant strain through the louse causes the disappearance of its resistant character.

Ehrlich (1911) in commenting on these observations, stated that not only were they of great scientific interest in so far as they touched the question of the transmission of acquired characters and the theory of mutation, but that they were also of great practical significance. On the one hand, the chemotherapeutic treatment of infectious diseases was becoming ever wider and of greater importance, and, on the other, the development of drug-resistant strains had been observed among the various pathogenic organisms as a consequence of such treatment. These facts had given rise to an uneasy feeling that the various diseases might undergo a fundamental change of an unfavourable nature whereby, finally, drug-resistant strains of parasites would be produced and propagated; thus infections in the future would prove intractable to chemicals which previously had shown themselves to be very active. In his opinion, these observations of Gonder, that acquired characters are entirely or partly lost through

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the processes of fertilisation and insect transmission, were well calculated to allay this disquietude.

There can be no doubt that these remarks of Ehrlich have exerted a profound influence, not only on scientific thought, but also on the practical measures adopted for the combat of sleeping sickness epidemics—for example, the French method of widespread atoxylisation of the sick in the bush.

Unfortunately, apart from other considerations, there is one fundamental criticism of Werbitzki and Gonder's work, and that is that the flea and not the louse is the normal intermediate host of *T. lewisi*. Many years later, Reichenow and Regendanz repeated Werbitzki and Gonder's work, but used the flea instead of the louse: they reached the conclusion that passage through the flea exerted no influence one way or the other on the arsenic resistance of the trypanosome. The chief difficulty in reaching any definite conclusions from Reichenow and Regendanz's work is that there is no evidence that their so-called resistant strain of *T. lewisi* was substantially more resistant than was their normal strain.

Duke (1927) is the only worker who has investigated the problem with one of the African pathogenic trypanosomes and the tsetse fly. His experiments suffer from the same defect as those of Reichenow and Regendanz, namely, that it is very doubtful whether his resistant strain was any more resistant than his normal strain.

In view of the great scientific and practical importance of the problem and of the very unsatisfactory nature of previous attempts to solve it, we decided to reinvestigate the subject: with this object in view we asked Dr. Duke of Uganda and Dr. Corson of Tanganyika to be good enough to send us by air mail regular supplies of the pupæ of *Glossina palpalis* and *Glossina morsitans* respectively. During the past eight months we have received about 4,000 *G. palpalis* pupæ collected on the shores of Lake Victoria and about 3,000 *G. morsitans* pupæ from Kikori, Tanganyika.

With the flies hatched from these pupæ in our laboratories at Liverpool, we have been able to transmit not only a normal strain of *T. brucei*, but also a branch of this strain which we had made completely resistant to tryparsamide. Both strains passed through their cyclical development in the tsetse fly completely unchanged, *i.e.*,

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the normal strain was, after transmission, just as sensitive to the aromatic arsenical as it was before transmission, and the resistant variety just as resistant to aromatic arsenicals as it was previously. I should add that we have just succeeded in passing the resistant variety a second time through *Glossina*, and that it still preserves its resistant character unimpaired.

These facts show what an astonishingly fixed character arsenic resistance is. When once a strain of trypanosomes becomes arsenic-fast it continues to manifest this character for prolonged periods when passed through laboratory animals by means of the syringe, and two cyclical transmissions of the parasite through its normal insect intermediate host in no way modifies its acquired character of arsenic resistance. Apart from its academic and scientific interest, this discovery has an obvious bearing on the question of treating sleeping sickness patients in the presence of tsetse fly, and particularly on the prophylactic measure much favoured by the French, of wholesale atoxylisation of patients in their villages by means of itinerant medical missions. In short, it seems clear that Ehrlich's premonitions of twenty years ago have proved to be not ill-founded.

Considerations of this nature indicate the paramount importance of avoiding in the treatment of trypanosomiasis the production of drug-resistant strains of trypanosomes, and lead immediately to the question: 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

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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 ease. If when a relapse occurs in a rabbit which has received a number of small subcurative 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.

Much more work is required before we shall understand all the conditions which favour the production of drug-

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resistance, but our investigations indicate clearly that the type of compound and dosage, especially the size and spacing of the doses and the length of the course of treatment, are factors of great importance.

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 strain has attained to a certain degree of resistance it is hopeless to attempt to cure the patient by further administration of any aromatic arsenical or antimonial compounds; and, still more important even than this, we must remember that tsetses infected from a patient harbouring a resistant strain of trypanosomes will hand on the strain unchanged to other victims. In this connection, I might mention that in the course of examining the sensitiveness to arsenicals of various strains of trypanosomes recently obtained from sleeping sickness cases I came across one strain from an untreated patient which was completely resistant to the aromatic arsenicals.

In concluding this aspect of the subject, it cannot be too strongly emphasised 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

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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.

SPIROCHÆTES

In contrast with the trypanosomes, remarkably little is known regarding the capacity of spirochætes to develop drug-resistance. Kritchewski (1927) examined seven strains of *Sp. recurrentis*; he found that whereas mice infected with certain of the strains could be sterilised by a single maximum tolerated dose of salvarsan, mice infected with other strains could not. Similar differences have been observed clinically. Although novarsenobillon is generally recognised as a specific for relapsing fever and there are numerous records of cure with it, yet failures to cure have been recorded by van den Branden and van Hoof (1922), by Shah (1924), and by Gray (1928), and others.

There is thus some evidence that in nature there exist different strains of *Sp. recurrentis* exhibiting different degrees of sensitiveness (or resistance) to salvarsan.

ATTEMPTS TO PRODUCE DRUG-RESISTANT STRAINS OF SPIROCHÆTES

Margulies (1910) endeavoured to produce a resistant strain of *Treponema anserinum* by treating a series of infected hens with subcurative doses of salvarsan: to commence with it was found that the curative dose of the drug was 3.5 mgm. per kilo. of hen, and finally a point was reached after forty-six passages when double this dose, *i.e.*, 7.0 mgm. per kilo. failed to cause the spirochætes to disappear from the blood; this dose, however,

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is far below the maximum dose tolerated by hens, viz., 200 mgm. per kilo. An attempt to produce a resistant strain of *Treponema recurrentis* in mice was abandoned after experiments lasting seven months.

Rothermundt and Dale (1911) gave up an attempt to produce a resistant strain of *Treponema anserinum* after experiments lasting two and a half months.

Gonder (1912) worked with a Russian strain of *Treponema recurrentis* in mice, for which at the beginning of his experiments the minimum curative dose of salvarsan was 1.25 mgm. per 20 gm. of mouse. After prolonged experiments involving 100 passages he eventually succeeded in producing a strain which withstood the maximum dose of salvarsan tolerated by mice, i.e., 4 to 5 mgm. per 20 gm. of mouse. With *Treponema anserinum* the process proved more difficult, but eventually after experiments involving 190 passages the resistance of the spirochaetes to salvarsan was raised ten times.

Feldt (1932) succeeded in making a strain of *Treponema recurrentis* in mice, which was originally sensitive to doses of salvarsan equal to 1 mgm. per 20 gm. of mouse, resistant to the maximum tolerated dose for mice, viz., 5 mgm. per 20 gm. In the first series of experiments this was achieved after forty passages, and in the second after fifty passages. He likewise succeeded in producing a solganol resistant strain. It is recorded that the salvarsan-fast strain had lost its resistance after a further passage through untreated mice during a period of ten weeks, but that the solganol-fast strain was still resistant after passage through normal mice during a period of nineteen months.

Akasawa (1932), using a strain of *Spirillum minus* obtained from man, made it resistant to salvarsan in mice. He states that the strain preserved its resistance after 120 passages through untreated mice during a period of two years, and that it exhibited its resistance when transferred to rats.

SYPHILIS

Whilst a great deal has been written on the question of drug-resistant syphilis, next to nothing exists in the way of precise knowledge of this subject. In certain German clinics especially the impression prevails that in early syphilis treatment now is not as efficacious as in former

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years. Silberstein (1924) of the Königsberg clinic found that it has been much more difficult to render the Wassermann reaction of the blood negative in recent years than formerly, but his conclusions have been criticised by Moore and Robinson (1930), who, working at the Johns Hopkins Hospital, found that there was no significant difference in serological results in recent as compared with early years.

Experimental attempts to produce a resistant strain of *Treponema pallidum* have given very unconvincing results. Margulies (1910) failed to produce arsenobenzene-fast strains in rabbit syphilis, but Launoy and Levaditi (1912), Klauder (1924) and Feldt (1932) all claim to have succeeded in enhancing the resistance of *Treponema pallidum* by treating a series of infected animals with various drugs. Critical examination of their papers shows, however, that the increased resistance was comparatively slight. Akatsu and Noguchi (1917) record experiments in which they claim to have made strains of *Treponema pallidum* and *Treponema refringens* resistant to salvarsan and various other compounds *in vitro* by gradually increasing the concentration of the drugs in the culture media in which the spirochætes were growing.

Summing up the position, we find that although there is some evidence that it is possible to produce drug-resistant strains of the blood spirochætes, and possibly even of *Spirochæta pallida*, it is a much more difficult matter than in the case of trypanosomes. The clinical evidence that drug-resistance plays any serious part in the therapy of syphilis is still very unsatisfactory. The whole subject, however, is of very great importance and obviously calls for much more work.

REFERENCES

- AKASAWA (1932): *Centralbl. f. Bakt. Originale*, **126**, 261.
 AKATSU and NOGUCHI (1917): *Journ. Exp. Med.*, **25**, 361.
 BREINL, A., and NIERENSTEIN, M. (1908): *Deut. med. Woch.*, **34**, 1181.
 BREINL, A., and NIERENSTEIN, M. (1909): *Ann. Trop. Med. and Parasit.*, **3**, 395.
 CITRON, H. (1930): *Zt. f. Immunitäts.*, **69**, 464.
 DALE, H. H. (1923): *Physiological Reviews*, **3**, 359.
 DUKE, H. L. (1927): Interim Report, League of Nations International Committee on Human Trypanosomiasis, 24.
 EHRLICH, P. (1907): *Berl. klin. Woch.*, **44**, 1313.

DRUG-RESISTANCE

- EHRlich, P. (1908) : *Verhand. d. deutschen. dermatol. Gesell. X. Kongress*, 52.
- EHRlich, P. (1908) : *Berichte der deutschen chemische Gesellschaft*, **42**, 1.
- EHRlich, P. (1911) : *Centralb. f. Bakt. Referate I. Beihefte* 94.
- FELDT, A. (1932) : *Klin. Woch.*, **11**, 1378.
- GONDER, R. (1911) : *Centralb. f. Bakt. Originale*, **61**, 102.
- GONDER, R. (1912) : *Ibid.*, **62**, 168.
- GRAY, G. D. A. (1928) : *Ann. Trop. Med. and Parasit.*, **22**, 273.
- KLAUDER, J. V. (1924) : *Arch. Derm. and Syph.*, **9**, 446.
- KRITSCHESKI, J. L. (1927) : *Klin. Woch.*, **6**, 441.
- LAUNOY and LEVADITI (1912) : *C. R. Soc. Biol.*, Paris, **72**, 653.
- MARGULIES (1910) : *Deut. med. Woch.*, No. 41.
- MESNIL, F., and BRIMONT, E. (1908 (a)) : *C. R. Soc. Biol.*, **64**, 637.
- MESNIL, F., and BRIMONT, E. (1908 (b)) : *Ann. Inst. Past.*, **22**, 856.
- MOORE and ROBINSON (1930) : *Arch. Derm. and Syph. Journ.*, **21**, 32.
- REICHENOW, E., and REGENDANZ, P. (1927) : *Abhandlungen aus dem Gebiete der Auslandstunde, Hamburgische Universität.*, Bd. 26. Reihe D. Medizin Bd. 2 (Festschrift Nocht), 446.
- ROTHERMUNDT and DALE (1911) : *Deut. med. Woch.*, No. 39.
- SHAH, W. (1924) : *Kenya Med. Journ.*, **1**, 148.
- SILBERSTEIN, M. G. (1930) : *Arch. Derm. and Syph.*, **21**, 166.
- VAN DEN BRANDEN and VAN HOOFF (1922) : *Bull. Soc. Path. Exot.*, **4**, 220.
- VOEGTLIN, C., DYER, H. A., and MILLER, D. W. (1924) : *Journ. Pharm. and Exp. Therap.*, **23**, 55.
- YORKE, W. (1932) : *Brit. Med. Journ.*, October 8th, 668.
- YORKE, W., ADAMS, A. R. D., and MURGATROYD, F. (1929) : *Ann. Trop. Med. and Parasit.*, **23**, 501.
- YORKE, W., ADAMS, A. R. D., and MURGATROYD, F. (1930) : *Ibid.*, **24**, 115.
- YORKE, W., and MURGATROYD, F. (1930) : *Ibid.*, **24**, 449.
- YORKE, W., MURGATROYD, F., and HAWKING, F. (1931) : *Ibid.*, **25**, 313, 351, 521.
- YORKE, W., MURGATROYD, F., and HAWKING, F. (1932) : *Ibid.*, **26**, 215, 577, 587.
- YORKE, W., MURGATROYD, F., and HAWKING, F. (1933) : *Brit. Med. Journ.*, February 4th, 176.