Chemotherapy in Tropical Medicine.

A LECTURE DELIVERED BEFORE THE CHEMICAL SOCIETY ON FEBRUARY 17TH, 1944.

By A. J. EWINS, F.R.S.

THE outstanding successes achieved in recent years by the therapeutic employment of the sulphonamide group of drugs has, perhaps, tended to overshadow the wider aspects of chemotherapy and the service it has rendered to medicine in other fields. The sulphonamides are now employed in all parts of the world and, in the past few years, they have revolutionised the treatment of many diseases due to bacterial infections. In the same field, the even more amazing chemotherapeutic properties of the mould metabolism product, penicillin, which are now being brought to light, have still further emphasised this aspect of chemotherapeutic achievement.

Bacteria are, however, not the only infecting organisms, and virus, protozoal and parasitic infections are responsible for many diseases. In the tropics in particular, infection both of men and of animals by protozoa and by parasites has been one of the greatest handicaps to the full economic development of many areas, and the toll of life due to the ravages of such diseases has been and still is enormous. I propose, therefore, this afternoon briefly to review some of the successes which have been achieved in attempts to find chemotherapeutic agents which can be used safely and effectively, either to cure or to alleviate the effects of such infections. The diseases thus caused are generally referred to as " tropical diseases," but it must be remembered that bacterial infections are no less common in the tropics—that tuberculosis, pneumonia, meningitis and other ills are just as rife there as in more temperate zones, and often more devastating in their effects.

Early in the present century, the incidence of trypanosomiasis, or " sleeping sickness," in Central Africa had become a very urgent problem. In some districts its ravages were such that the native population was being rapidly and very considerably reduced in numbers. It is recorded that, in one district alone, during a severe epidemic, some 60% of the population lost their lives in the course of a few years. Even at the present time it is estimated that something like a million natives may be treated for this disease in a single year. Fortunately the white races are less susceptible and rarely become infected.

Although sleeping sickness had been recognised for several hundred years, it was not until the beginning of the present century that it was first shown to be due to infection with trypanosomes. Some years earlier, in 1880, *T. evansi* had been shown to be the cause of " *surra,*" a disease to which horses in India are subject, and in 1895 *T. brucei* was shown to be the causative agent of a similar disease, affecting both horses and cattle in South Africa.

The trypanosome responsible for sleeping sickness in man was first demonstrated by Ford and Dutton, in 1901—1902, and was named *T. gambiense.* In 1902 Castellani found, in both the blood and the cerebro-spinal fluid of five cases, a trypanosome which was finally identified as *T. gambiense.* These observations were confirmed in the following year by Bruce and Nabarro.

At this time, Ehrlich was carrying out researches into the mode of distribution of drugs in the body, with a view to putting the development of such products for therapeutic employment on a rational basis. In his investigation he was led to the study of the selective affinities of certain dyes for the cells of the blood and various organs of the body. The work of Laveran and Mesnil, who had succeeded in 1902 in producing experimental trypanosomiasis in rats and mice, afforded a ready means of observing the effect of such dyes on the living trypanosomes, and in 1904 Ehrlich and Shiga demonstrated that a new dye of the benzidine series, trypan-red, showed marked trypanocidal action in mice infected with *T. equinum.* A year or two later, in 1906, Nicolle and Mesnil examined a number of other dyes of the same series and found that trypan-blue was even more effective when tested in mice infected not only with *T. equinum,* but also with *T. brucei.* Moreover, they were able to show some general relationships between constitution and trypanocidal action in this series. None of these products proved to be of practical therapeutic value, but the work afforded a starting point for research which led ultimately, some years later, to the preparation of Bayer 205 (suramin), a drug which has been found to be of great value in the treatment of human trypanosomiasis.

Meanwhile, by 1905, the protozoacidal properties of atoxyl in a variety of animals infected with various pathogenic trypanosomes had been demonstrated at Liverpool by Thomas and Breinl. Ehrlich and his collaborators, however, having established that this product, which had been regarded originally as the anilide of phenylarsonic acid, is the sodium salt of *p*-aminophenylarsonic acid, proceeded to develop and investigate a series of organic compounds of arsenic, their work culminating in the discovery of salvarsan (arsphenamine) and, a little later, of its more soluble derivatives, neoarsphenamine and sulpharsphenamine.

As everyone knows, these products afforded a rational and successful means for the treatment of syphilis—one which endures to this day. It is perhaps less well known that neoarsphenamine, which is the product by far the most widely used, is equally effective in the treatment of yaws, a tropical disease resembling syphilis in some of its aspects and prevalent in many countries, especially among the natives of Central Africa. This drug is also successfully employed in the treatment of a group of tropical relapsing fevers which, like yaws, are caused by spirochætal infections.

It was this outstanding success which gave to research in the field of chemotherapy that impetus which has been so fruitful of results in recent years. Much of the early work was naturally directed to extending

our knowledge of the chemotherapeutic possibilities of the organic compounds of arsenic. Since Ehrlich's original discovery, research in this field has been phenomenal and there must be many hundreds of compounds which have been examined for trypanocidal and similar properties. Atoxyl has already been mentioned. For the treatment of sleeping sickness, however, it proved far from satisfactory, mainly owing to the high incidence of visual disturbance, and even of blindness on occasion, following its use.

In 1919 Jacobs and Heidelberger first prepared tryparsamide (sodium *N*-phenylglycinamido-*p*-arsonate), and on the basis of the experimental demonstration of its trypanocidal properties against a variety of trypanosomes, Brown and Pearce suggested its use in the treatment of sleeping sickness. Although not a remarkably powerful protozoacidal compound, it has the advantage that it penetrates into the cerebro-spinal fluid and is consequently effective in the later stages of the disease in which the central nervous system is involved. In conjunction with Bayer 205, to which I shall refer again, tryparsamide is now employed as the standard method for the treatment of sleeping sickness in British territories. In French Equatorial Africa orsanine (sodium *o*-hydroxy-*p*-acetamidophenylarsonic acid), prepared and studied by Fourneau in 1923, has been largely used in place of tryparsamide and is hence sometimes misleadingly referred to as the French "equivalent" of the latter. It is useful in all stages of the disease and, according to Hawking, Hennely, and Quastel, is, when injected intravenously, at least equal if not superior to tryparsamide in its ability to render the cerebro-spinal fluid trypanocidal.

Many attempts have been made to improve upon these products and within recent years neocryl (sodium succinamidomethylamido-*p*-arsonate), first prepared by Morgan and Walton in 1931, has been the subject of clinical trial in African sleeping sickness. It has, however, proved somewhat less effective than tryparsamide and it is not free from the disadvantage from which the latter suffers, namely, the risk of amblyopia following its employment.

More recently, Friedheim has prepared and reported on the clinical trials of two new organo-arsenic compounds, 4197 and 4289, which it is claimed show considerable advantages over earlier discoveries. The more promising of these appears to be Friedheim 4197, obtained by coupling arsphenamine with a naphtholdisulphonic acid. According to Friedheim the strongly acidic group present in such products facilitates their passage into the cerebro-spinal fluid. Unfortunately war conditions have interrupted the clinical trial of this compound.

I have already referred to Bayer 205. This product, which has been variously known as moranyl, antrypol and germanin and is now officially termed suramin, was originally introduced by the great German firm which the original designation indicates, and it was mentioned in the literature in Germany in 1919. Its constitution was not disclosed, however, until 1924, when Fourneau showed it to be the symmetrical urea of sodium *m*-benzamido-*p*-methylbenzoyl-1-aminonaphthalene-4 : 6 : 8-trisulphonate. As I have already mentioned, this important compound was the outcome of research based on the discovery by Ehrlich and Shiga in 1904 of the trypanocidal activity of trypan-red and by Mesnil and Nicolle a year or two later of that of afridol-violet. Experimentally the compound was shown to be of high trypanocidal activity and clinically it has proved to be an extremely valuable drug for the treatment of sleeping sickness in its early stages. It has little effect, however, on the later stages of the disease. For the treatment of African trypanosomiasis preliminary treatment by Bayer 205, followed by one or more courses of tryparsamide, gives the most satisfactory results, effecting an eventual cure in all but the most advanced cases.

Towards the end of 1937, King, Lourie, and Yorke published a paper demonstrating the trypanocidal activity of a number of symmetrical aliphatic diamines, di*iso*thioureas, diguanidines and diamidines and thus opened up a new line of research for the possible development of a series of chemotherapeutic agents in this field. The origin of this discovery is of considerable interest, for it was based on what appeared to be a sound hypothesis which proved on investigation to be fallacious.

From 1911 onwards, the importance of an adequate supply of glucose for the maintenance of the life of pathogenic trypanosomes *in vitro* had been observed by a number of workers, and in 1929 in the course of elaborating their method of maintaining living pathogenic trypanosomes *in vitro* at 37°, Yorke, Adams, and Murgatroyd found that the presence of a relatively large amount of glucose in the nutrient medium was essential, an observation since confirmed by a number of other workers. Jancsó and Jancsó, who had been concerned with the mode of action of Bayer 205, concluded that it acted by interfering with the carbohydrate metabolism of the trypanosome and, in 1935, proceeded to examine in experimental trypanosomiasis a number of guanidine derivatives known to possess hypoglycæmia-producing properties. One of these, synthalin, decamethylene diguanidine dihydrochloride, which at one time had been suggested as a possible substitute for insulin, was found to exhibit trypanocidal action in mice infected with *T. brucei*, and Jancsó and Jancso concluded that this was probably due to its hypoglycæmic action thus depriving the trypanosomes of a sufficiency of glucose for their metabolic needs.

In the same year, Schern and Artagaveyta-Allende showed that synthalin had a definite therapeutic effect on rats infected with *T. equinum* and came to much the same conclusion concerning its mode of action.

Not altogether satisfied that this explanation was correct, Warrington Yorke re-examined the whole question and, in collaboration with Lourie, showed that synthalin exerts a marked trypanocidal action *in vitro*, even in a concentration of 1 in 200 million—a concentration quite insufficient to produce any appreciable degree of hypoglycæmia in the normal animal. Moreover, they demonstrated that insulin itself has no

trypanocidal action either *in vitro* or *in vivo*, thus proving that the action of synthalin on the trypanosome is a direct lethal effect.

Following up this observation, King prepared a series of compounds of similar type, varying the constitution in the manner which I have already mentioned. These substances were likewise shown to possess trypanocidal properties in varying degree, both *in vitro* and *in vivo*. The most active compounds were found in the diamidine series, that exhibiting the greatest activity being undecane-1 : 11-diamidine. It was found, however, to be too toxic to be of practical value.

We, in our laboratories, set out to develop this new field in the hope of obtaining compounds which might find practical employment as chemotherapeutic agents. An account of this work was published in the *Journal* of this Society early in 1942. It was concerned mainly with a comparative evaluation of the chemotherapeutic properties of a number of symmetrically disubstituted aromatic diamidines.

The first compound prepared and examined was 4 : 4′-diamidinodiphenylmethane. It was chosen quite empirically as being of approximately the same molecular complexity as King's undecane diamidine. As was expected, it proved to be definitely trypanocidal when injected into mice infected with *T. equiperdum*, but was less active than undecane diamidine. Nevertheless the result was encouraging and it was decided to extend the investigation. Several methods of so doing suggested themselves: these included the introduction of substituents into the aromatic rings, increasing the length of the hydrocarbon linkage, substituting bivalent atoms and groups, such as oxygen, sulphur or an imino-group, for one or more of the methylene groups in the linkage, the use of unsaturated linkages and studying the effect of substitution in the amidine group itself.

Altogether we have so far examined about one hundred compounds. For activity *in vivo* it has been found essential that two amidine groups be present and while the most active diamidines are those in which these groups are in the 4 : 4′-positions, the 3 : 3′-derivatives and also the unsymmetrical 3 : 4′-derivatives are but little inferior. Invariably monoamidines have failed to show any trypanocidal activity in the experimental animal.

Our original results appeared to indicate that alkyl or aryl substitution in the amidine group resulted in diminished activity, but more recent work suggests that this is not necessarily the case. Replacement of a methylene group in the linkage by oxygen led to increase; by an imino-group to some diminution; and by sulphur to a definite reduction of activity. Replacement of the methylene group by a carbinol or ketonic group brought about greatly diminished activity.

Some of the more active of these compounds have been examined in greater detail and over a wide range of protozoal and parasitic infections by Yorke and Fulton. Their findings are summarised by Yorke in the following statement:

"Certain of these aromatic diamidines exert a powerful curative action on *T. rhodesiense* infections, on *Babesia canis* infections and on *Leishmania donovani* infections, a less powerful action on *T. congolense* infection and on certain malarial infections of man and monkeys, but they have no effect on *T. cruzi* infections or on infections due to *Spirochæta recurrentis* or to *Spirella minus*."

From the results of this experimental work it was concluded that from the chemotherapeutic point of view the most interesting of these compounds were 4 : 4′-diamidinostilbene (stilbamidine), 4 : 4′-diamidinodiphenoxypentane (pentamidine), 4 : 4′-diamidinodiphenoxypropane (propamidine), and 4 : 4′-diamidinodiphenyl ether (phenamidine). The chemotherapeutic ratios of the first three products, as determined by Yorke in mice infected with *T. rhodesiense*, are from 15 to 5 times as great as that of tryparsamide. Their trypanocidal activity is very great; Yorke obtained permanent cures in infected mice, using doses of the order of 0·01 to 0·05 mg. per 20 g. mouse. Chemotherapeutic ratios of the same order were obtained in our own experiments.

Stilbamidine, pentamidine and propamidine have been the subject of clinical investigation in African trypanosomiasis and in kala-azar, a tropical fever due to infection with *L. donovani*. All three drugs were found to be effective in the early stages of trypanosomiasis, but their usefulness in the later stages is doubtful and much more work is needed in order to assess their real value. Like all powerfully active compounds, they are apt to give rise to toxic effects. Stilbamidine would appear to be too toxic for use in sleeping sickness, and pentamidine perhaps to be the most likely to prove of practical value. The products have been given both intravenously and intramuscularly. Intravenously they produce temporary, but sometimes alarming reactions, such as flushing of the face, nausea, faintness, and an almost imperceptible pulse rate. These are due to a sudden but transient fall in blood pressure. Fortunately these symptoms are of short duration, passing off within a few minutes, or at most within ½ hour. The experimental findings suggest that these symptoms might be minimised by intramuscular or subcutaneous administration.

In the treatment of kala-azar all three compounds have been shown to be curative and stilbamidine is, at present, the most widely used. The disease is widespread and is found in India, China, in the countries bordering on the Mediterranean and in the Sudan and elsewhere in North Africa. It is worthy of note that previous to the use of these amidines, kala-azar had been found to be amenable only to treatment with organic antimony compounds. In Mediterranean kala-azar, which is more resistant to treatment than that met with in India, cases resistant to antimony have been cured by the amidines, the first wholly organic compounds to be employed successfully for this purpose.

Phenamidine has proved to be very effective in the treatment of *B. canis*, an infection to which dogs in the tropics are very liable. Warrington Yorke first drew attention to the curative action of the diamidines

in this infection. He found that the most active compounds were stilbamidine, propamidine and phenamidine and the curative effect of each of these has since been confirmed. Again stilbamidine is too toxic to be of real value. Phenamidine, however, has been reported by Carmichael, of the Veterinary Laboratory at Entebbe, to have a wide margin of safety and to be excellent in every way in the treatment of tick fever (canine babesiasis). Its main advantages are its specificity and therapeutic efficacy in a single dose, combined with low toxicity and absence of undesirable reactions. Relapses are few and, if they do occur, they usually respond to a second dose. Trials of this product in babesia infections of cattle and other animals are being carried out, but it is too early yet to assess its value in these infections. Prior to the discovery of the potency of the diamidines treatment had consisted in injecting trypan-blue or the German product acaprin (*NN'*-bis-methylquinolinium-methyl sulphate-6-urea). The latter is apt, however, to produce toxic effects in the dose employed.

I have already made brief mention of the use of antimony compounds for the treatment of kala-azar. In view of the chemotherapeutic value of arsenic compounds and of the traditional value of antimony in medicine, it was only natural that compounds of antimony should have been examined for similar properties. In 1908 tartar emetic had been shown by Plimmer and Thomas to possess some trypanocidal action. In 1913 Vianna, in South America, was led to try its effect on oriental sore, a disease caused by infection with *L. tropica*. The results were favourable. They led Caronia and di Cristina to try the substance in Italy, in 1915, for the treatment of infantile kala-azar (*L. donovani*), again with promising results which in turn were fully confirmed in that same year by Rogers, in the treatment of Indian kala-azar, and later by other workers.

Tartar emetic and the corresponding sodium salt are somewhat unsatisfactory drugs, since they can only be given intravenously and, on injection, are liable to give rise to inflammatory processes leading not infrequently to pneumonia. Nevertheless their introduction was a triumph for chemotherapy. The death rate from kala-azar in India was of the order of 80—90% : on occasion it was even higher. The use of these products converted this high death rate into a recovery rate of a similar order. In view, however, of the toxic effects of these salts, research has been directed to the preparation of other organic compounds of antimony which should be more free from these disadvantages and a number of preparations of antimony, mainly derivatives closely allied to *p*-aminophenylstibonic acid, in which compound antimony is quinquevalent, have been prepared and tested and have proved much more satisfactory. Of these neostibosan, the diethylamine salt of *p*-aminophenylstibonic acid, and neostam, the *N*-glucoside of sodium *p*-aminophenylstibonate, would seem to be in more general use at the present time. In addition mention should be made of urea stibamine and solustibosan.

Urea stibamine, introduced in India by Brahmachari in 1922, was thought originally to be a simple combination of *p*-aminophenylstibonic acid with urea, but later work by Ghosh showed it to be a mixture of substances. Gray and his collaborators were able to demonstrate that it consisted of the disubstituted urea, symmetrical diphenylcarbamido-4 : 4'-distibonic acid, together with antimonic acid and some *p*-acetamidophenylstibonic acid. It is quite widely employed and has been reported to be effective, but it has two drawbacks, its variable constitution and its instability.

Solustibosan has recently been shown to be sodium antimony gluconate and there are favourable reports on its use in the treatment of kala-azar.

Bilharzia is another tropical disease. It is caused by a parasitic worm, *Schistosoma hæmatobium*, infecting the bladder and urinary system. Another species, *S. mansoni*, produces intestinal lesions, visceral complications, enlargement of the spleen and cirrhosis of the liver. For the treatment of these infections tartar emetic is the usual remedy and is effective. Nevertheless its toxicity and the necessity for giving the drug intravenously are great drawbacks to its use. At the same time the compounds of quinquevalent antimony are of little or no value in the treatment of these infections. Attention has been directed, therefore, to the possibility of preparing tervalent antimony compounds which might be more satisfactory. For this purpose fouadin, the sodium salt of an antimony compound of catecholdisulphonic acid, prepared by Schmidt and first employed in 1929, has since been widely used, cures being obtained in a considerably shorter time than with other drugs and attaining the order of 80%. Still more recently lithium antimony thiomalate has given promise of being effective in the treatment of these same infections, but more extensive clinical data are required before its true value can be assessed. The fact that some 50% of the population of Egypt is said to be infected with these parasites emphasises the great importance of these chemotherapeutic agents and the urgency of the problem they are called upon to solve.

To deal adequately with the chemotherapy of malaria would require a whole series of lectures. This afternoon I shall concern myself more particularly with the two synthetic products, atebrin and plasmoquin, which have been found to be of practical value as chemotherapeutic remedies for the disease.

Quinine has long been known to have a specific effect upon malaria and in spite of the large number of products which have been examined for antimalarial properties, this alkaloid is still generally considered to be the most satisfactory remedy. So nearly do the principal cinchona alkaloids resemble one another in their mode of action that totaquina, a mixture of cinchona bark alkaloids, is now widely employed. It can, of course, be produced much more cheaply than quinine—a factor of the greatest importance in dealing with a disease of such widespread prevalence.

In 1880, Laveran discovered the plasmodia responsible for malaria and when, later, certain similar parasites were found in many animals and birds, it became possible to devise laboratory tests for the deter-

mination of antimalarial activity. The compounds examined in this way have, for the most part, been based on considerations of the structure of quinine and of methylene-blue.

In 1891 Ehrlich and Guttmann observed that methylene-blue had a weak, though quite definite antimalarial effect. It is to this observation that we owe the ultimate discovery of plasmoquin and atebrin. Schulemann, Schonhöfer, and Wingler, working in the laboratories of the I.G. Farbenindustrie, started from methylene-blue and systematically modified the length and character of the side chain, until Roehl was able to report that, by replacing one methyl group of a dimethylamino side chain in methylene-blue by a diethylaminoethyl substituent, activity was definitely increased.

Attention was next directed to heterocyclic systems other than the phenothiazine of methylene-blue, particularly to derivatives of quinoline closely allied to that in quinine, while retaining the type of side chain which had been found to be effective in increasing the activity of methylene-blue. As a result of these further experiments it was found that such derivatives of 6-methoxy-8-aminoquinoline in many cases showed marked antimalarial properties. The most promising of these was 6-methoxy-8-(ω-diethylamino-α-methylbutylamino)quinoline which Schulemann and his collaborators introduced into chemotherapy in 1924 under the name plasmoquin. This product (now officially recognised in this country as " pamaquin ") acts mainly on the gametocytes, the sexual forms of the malarial parasite, in contrast to quinine, which acts mainly on the schizonts or asexual forms.

In practice plasmoquin was found to be somewhat too toxic for very general employment and is now used mainly in conjunction with quinine or atebrin. The latter compound, now officially termed " mepacrine," was the result of further research in which the acridine nucleus was substituted for that of quinoline : it is 2-chloro-7-methoxy-5-(ω-diethylamino-α-methylbutylamino)acridine and was first synthesised by Mietsch and Mauss in 1930. Like quinine, it acts mainly on the schizonts of the parasite, but acts rather more rapidly than does quinine. It has proved to be a very effective antimalarial, although its use requires rather more strict medical supervision than that of quinine. Its main disadvantages are the production of gastric disturbances in a proportion of cases and a temporary yellowing of the skin following administration.

The loss of the main source of supply of cinchona—to the Japanese, soon after their entry into the war—brought the United Nations face to face with an almost complete cessation of supplies of quinine—supplies which, in view of the vast numbers of troops that would be exposed to infection with malaria, were of the utmost importance to the conduct of the war. All the available quinine both in America and in this country was immediately reserved for this essential service. The amount thus made available was, however, estimated to be sufficient only for a limited period of time and in both countries the manufacture of mepacrine on a scale never before envisaged was put into operation.

The full story of this great achievement will, no doubt, be told after the war, but the effectiveness of this antimalarial is now fully manifest and the use of this chemotherapeutic agent has rendered inestimable service in safeguarding our fighting forces from the worst effects of this widespread infection. Truly another great triumph for chemotherapy.

In this short review of some aspects of chemotherapy I have touched only on some of the more outstanding results achieved in the field of tropical medicine by chemotherapeutic research. For many years this field of investigation was one of the major concerns of the great German firms dealing with synthetic drugs. In this country interest in the subject has for long been far from that which our responsibilities to our Empire should demand. Fortunately there is every sign that the potentialities of research in this field are now being realised and great developments may confidently be expected.