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ATOXYL AND TRYPANOSOMIASIS

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Early observations upon the use of Arsenic in the treatment of Fly disease

The brilliant discovery by the late Dr. Dutton in 1901 of the presence of trypanosomes in the blood of a patient under the care of Dr. Forde of Bathurst, to which he gave the name of *Trypanosoma gambiense*, the finding very shortly afterwards, by an expedition sent out to Africa under the auspices of the Royal Society and Colonial Office, composed of CASTELLANI, BRUCE, NABARRO and LOW, that sleeping sickness was caused by the same parasite (*Trypanosoma gambiense*), stimulated investigation throughout the civilised world into the life history of this group of haematozoa, their mode of action in the blood and tissues of man and animals, and the effect of various drugs upon them.

During the year 1907 very material progress has been made in the treatment of sleeping sickness, and it appears to us that the time is a suitable one in which to review the history of how arsenic and its compounds came to be employed, and to state the results of the treatment with this and allied drugs, in the light of the great experience gained in 1907.

There is no doubt that for a very long time Arsenic has been looked upon as a remedy useful in trypanosomiasis in animals. Long before the nature of sleeping sickness was understood there existed much speculation with regard to the nature and treatment of *Tsetse Fly disease* in horses and cattle. First and foremost among those who suggested *Arsenic* as a means of treating this disease in animals stands the great observer and explorer Dr. Livingstone.

DAVID LIVINGSTONE, in a letter (dated March 22, 1858) to the *British Medical Journal* of May 1st, 1858 mentioned that it had occurred to him in about the year 1847-8 to use Arsenic in the disease which followed the bite of the tsetse fly. He mentions how he tried the drug on a mare.

In thanking Mr. BRAID for some remarks (published in the *British Medical Journal*) Livingstone states 'that though my hopes are not sanguine, I still mean to try the remedy, if opportunity offers. Our instructions require us to examine the whole subject carefully, and the results will be communicated to the Royal Society.'

In the *British Medical Journal* of February 13, 1858, there appears a letter from Mr. Braid, dated from Manchester, February 6, 1858, in which he says: 'On reading the interesting facts communicated by Livingstone, one of the most notable is his narrative of the remarkable and fatal phenomena manifested in oxen and sheep from the bite of the tsetse fly. It immediately occurred to me that it would be highly interesting to institute some experiments with the view of discovering a remedy for this curious and fatal malady, and my mind immediately reverted to the prophylactic powers of arsenic against the poison of the most venomous reptiles . . . .

Braid then quotes Dr. Honigberger's case of a fakir who was an Arsenic eater, and who ascribed to this reason his immunity to the bite of the serpent.

Such at any rate was the source from which the idea developed of *giving small doses of Arsenic to oxen bitten by the tsetse fly.*

Surgeon-Major RANKING had been struck with the similarity existing between the disease Surra in horses and mules and malaria, and recommended a treatment similar to that used in malaria, but although he freely used *Quinine*, we find no evidence that he employed Arsenic.

Arsenious and Sodium te

We then come to the period of definite organised experiments, and foremost amongst investigators stands Bruce, who added an immense number of new facts to our very scanty knowledge of trypanosomiasis. BRUCE, in his classical report entitled "Further Report on the Tsetse Fly disease or Nagana in Zululand" (May 1896), gives a very useful and detailed account of the effect of *Arsenic* as a curative and prophylactic agent.

The experiments he set on foot demonstrated that *Arsenic* had a material effect, and that it was capable of driving haematozoa out of the blood; as Col. Bruce himself states, the drug *'undoubtedly markedly modified the course of the disease'*. As a prophylactic agent he argued that if Arsenic modified the course of the disease,

it seemed probable therefore that the disease would be prevented altogether if the Arsenic were given for some time previous to the animals being exposed to the infection.

From experiments set on foot to determine whether Arsenic had a prophylactic action, he concludes that it would be quite useless as a prophylactic agent, but that it was useful in prolonging life, and especially in the 'Fly Country,' after the disease had begun. Following upon Bruce's observations come those of LINGARD (1893).

LINGARD (Report on Horse Surra, vol. I, p. 104, 1893) mentions in connection with his experimental enquiry into the treatment of Surra, that Pottinger had tried Arsenic without any appreciable effect. Lingard then conducted himself a series of experiments upon numerous animals with *Arsenic compounds*, *Cinchona alkaloids*, *Arsenic* alone, and also *Mercury perchloride*. The results showed in the case of *Mercury perchloride* that there was no diminution in the number or activity of the parasites in the blood, and that they continued to be present in the blood for three days following the injection of the drug.

Combined ment with and the C alkaloids

With regard to the treatment with *Cinchona alkaloids and Arsenic*, we quote Lingard's own words (l. c.): 'In the case of two animals, it was decided to attempt curative treatment, although it was recognised that the animal had already been suffering from the disease for a period of at least twenty-three days. It was thought advisable to commence the treatment when the number of haematozoa in the blood should be on the decline, and accordingly on the 14th June, the haematozoa being few, treatment with *Cinchona alkaloids and Arsenic* in large doses was commenced. On the 20th the haematozoa disappeared from the blood, but the temperature and respirations remained above normal. The general symptoms of Surra increased up to the 24th; oedema and swelling of the limbs, sheath and under the abdomen increased in extent. The animal steadily losing condition, the muscles wasting, and the gait staggering and uncertain. On the 25th the animal had decidedly improved, and it was noticed that the oedema of the sheath and abdomen was distinctly less in amount; the animal seemed brighter and moved freely about the loose box.

'This amelioration of the symptoms was gradually progressive, and on the 2nd July the oedema under the belly had completely

disappeared. On the 3rd July, however, the temperature began to rise steadily, and it continued high until the 9th, notwithstanding the fact that no haematozoa were present in the blood. During this time, the animal presented symptoms, which might be accounted for by the continued high temperature, although on the 6th symptoms of kidney disorder presented themselves, followed by epithelial casts and a small number of blood corpuscles in the urine. The large doses of Arsenic and Alkaloids have been persisted in, notwithstanding the intestinal and hepatic derangements, as it was feared that the high temperature indicated the probable return of the Surra haematozoon. On the 9th there were symptoms of gastro-intestinal catarrh, and taking these complications into consideration, as well as the fact that the haematozoa had been absent from the blood for a period of twenty days, it was decided to discontinue the Arsenic and Alkaloids entirely, and a laxative and simple febrifuge was substituted.

'From the 10th to the 14th all went well, and the animal seemed to improve, the temperature remained low, and the animal became much brighter and fed well. On the 15th, however, he was dull, and the respiration and pulse had increased in frequency, the visible mucous membranes becoming of a dirty-yellow colour and petechiated. On the 16th, although his appearance was much brighter, the respiration had risen very high, and the heart's action was irritable; the symptoms of gastro-intestinal catarrh and the kidney disorder had entirely disappeared. On the 17th the animal appeared much in the same condition, but the respirations were less although the temperature was a little higher. On this date one Surra haematozoon was discovered in two cover-glass specimens of blood, and this was the first organism observed during a period of twenty-eight days. The previous treatment of Arsenic and Cinchona alkaloids was at once resumed. On the 18th the animal had not altered much in appearance, the respirations were greatly improved in character and the pulse in frequency, but the temperature had risen. The blood contained a few haematozoa, but not so active in their movements as the one noticed the day before. On the 19th the condition was worse, the haematozoa very numerous in the blood, and the temperature much elevated, viz., 41° C. On the 20th the appetite was normal, but otherwise the animal did not manifest any improvement; only two haematozoa were observed in two cover-glass

specimens of the blood, and these were exceedingly torpid. On the 21st the haematozoa were entirely absent, and from this date the animal appeared unable to throw off the effect which the return of the haematozoon had given rise to, and he gradually became weaker, and died on the 29th of July, although the haematozoa were absent during the last nine days.

'It was decided in this case to try again the effect of the mixed Cinchona alkaloids and Arsenic on the haematozoon, as it had shown promise of efficacy in that of Horse No. LXV, in which case after a short exhibition of the drugs, the haematozoon was kept in subjection for a period of twenty-seven days. Consequently as a fresh trial was resolved upon, the animal was first put upon small doses, beginning with one drachm of the Alkaloids and four grains of Arsenic, given in the form of the *Liq. arsenicalis*. On the morning following the first dose, the temperature was found to have fallen over a degree, and the haematozoon was absent from the blood, the temperature remained low, and the organism absent for the next five days, during which period two doses of the medicine were administered daily.

'On reference to the Chart, it will be seen that on the ninth day of the disease the haematozoon again appeared in the blood, consequently on the tenth the doses of Alkaloids and Arsenic were increased to one and a half drachms and five grains respectively; these were continued for a period of eight days, during which time the haematozoon varied in numbers between few and very numerous. On the eighteenth day of the disease a further increase in the doses was agreed upon, and during the next four days they were put up to Alkaloids two and a half drachms and Arsenic grains vi, and for the last or half of this period the organism was absent from the blood, but re appeared on the twenty-second day, and remained on the twenty-third at few. On the former date the Alkaloids were increased to three drachms, and the Arsenic to grains viii, and from the twenty-third to the thirty-fifth day of the disease, on which latter date the animal succumbed, the haematozoon was entirely absent from the blood. During the thirty-two days the animal was under treatment, three hundred and fifty-five grains of Arsenic in the form of *Liquor arsenicalis*, and one hundred and fifty-four and a half drachms of the mixed *Cinchona alkaloids* were administered.

'He further quotes the case of an animal belonging to the Bombay

Tramway Company. 'On the morning of the 7th October, 1892, the "Surra" haematozoon was discovered on microscopical examination of its blood. On admission to the Laboratory Hospital the following note was made: "Bay gelding, aged five years; appears to be in perfect health, and in splendid condition." On this date the haematozoon was absent from the blood, while for the next two days it was swarming: specific treatment was deferred, a simple febrifuge draught being given to try and reduce the temperature. On the evening of the 12th October, the organism was only present in small numbers in the blood, and the temperature had decreased considerably, as had also the pulse. It was decided to again attempt the destruction of the haematozoon by the exhibition of Cinchona Alkaloids and Arsenic, commencing in this instance with large doses (viz., Alkaloid drs. iii, and Arsenic grains iv, *bis die*). The reason why large doses of the former were exhibited was that few haematozoa remained in the blood, and in order to decide whether their destruction could be compassed by the drug, the dose was so regulated as to ensure its having a fair trial. No marked change took place in the animal's condition, nor in the number of the organisms present, until the 16th instant, when the haematozoa became numerous, the temperature rose considerably, respirations were increased in number, and it was noticed that the sheath was swollen; the treatment was continued, the dose of Arsenic having been gradually raised to six grains. On the 17th the organisms had again fallen to few, but the temperature and pulse were still above normal. On this date it was decided to try the effect of full doses of *Quinine*, as this drug has been said to be of much value when exhibited in this manner. Ranking has stated that "by the employment of full doses of *Quinine and Arsenic* he has been able to cure the disease." It appeared from our previous experiments on the use of similar drugs, that it would be futile to attempt the destruction of the haematozoa by giving only the full medicinal dose, and we considered it to be advisable therefore to commence with a dose of drs. iv of quinine, and arsenic grains xii, in the 24 hours, carefully watching its effects, and in case of any untoward symptom appearing, to discontinue its use.'

LAVERAN and MESNIL in 1902 published in the Annales de l'Institut Pasteur their researches 'Sur le Traitement et la prevention

du Nagana.' They obtained their best results by using *sodium arseniate* subcutaneously; with this drug they were able to bring about a quick disappearance of the parasites from the blood. The lives of the animals were prolonged, but a permanent cure was not effected. These observers also used *Sodium cacodylate*, *Sublimate of Mercury*, various *salts of Silver*, but without effect.

Serum

They were also led to try the effects of injecting *human serum* into ngana-infected rats and mice; they were led to do so as man was not susceptible to ngana. A marked improvement followed the injection of human serum; the duration of the disease was prolonged, but a real cure could not be effected.

E. J. MOORE, in a paper upon the beneficial effects of *Sodium arseniate* employed hypodermically in tsetse-fly disease, and published in the *Lancet*, Vol. II, 1904, records the most marked beneficial effects with large doses, and states that he would also recommend it for humans.

[illegible] CHICHESTER, who collaborated with him in a letter which he sent to Sir Patrick Manson dated May 5th, 1904, and published in the *Lancet* under the title of 'Arsenic in the treatment of Trypanosomiasis in [illegible] in Nigeria,' says that 'he used Arsenic hypodermically and produced most wonderful effects.' He adds that the experiment is not over, but says that 'I do not think it wise to wait longer. I tell you what I have found, and you may perhaps think it wise to ask others to try the same treatment, especially in those parts where it seems a scourge to human beings.'

[illegible] THOMAS, in 1903, whilst at the MacGill University, in Canada, repeated some of the experiments upon the action of Arsenic on human infected animals, and on joining the Liverpool School of Tropical Medicine, in August of the same year, immediately started extensive investigations upon the action of drugs upon trypanosomiasis. Amongst them he used *Sodium arseniate*, and with this drug his results were similar to those of Laveran. 'Many of the rats either died from the disease, were killed by the drug, or succumbed to an extensive ulceration around the site of injection.' Thomas' own words, taken from his paper entitled 'Some experiments in the treatment of trypanosomiasis' (*British Medical Journal*, May 27, 1905) were: 'Arsenic in the treatment of trypanosomiasis in animals seems rather to mitigate the disease, to cause the parasite to

apparently disappear from the animal's blood, and to prolong the life of the animal. Unfortunately the usual history is that if treatment be discontinued even after a prolonged course of the drug, the animal, after a varying length of time, once more exhibits the symptoms, and finally succumbs to the disease. Undoubtedly some of the more resistant animals do recover.' Thomas then quotes the view of Laveran and Mesnil (Trypanosomes et Trypanosomiases) that Arsenic kills the parasites which are free in the blood, but that when once the drug is eliminated or fixed in the tissues, the surviving parasites commence to multiply and the organisms once more reappear in the peripheral circulation unless another injection of Arsenic be given. On the administration of a second dose the parasites disappear only to reappear, and even though treatment be kept up, the majority of the animals succumb either from the disease or from the toxic effects of the drug. Some of the animals, such as rats and dogs, have been cured. Laveran also records the extensive necrosis and the pain which is apt to follow the administration of Arsenious acid.

THOMAS and BREINL, in a paper upon 'Trypanosomiasis and Sleeping Sickness,' published as Memoir XVI, 1905, of the Liverpool School of Tropical Medicine, report fully upon the treatment. *Sodium arseniate* was found the most useful form of Arsenic, but the usual disadvantages soon appeared and they stopped it. The sum total, therefore, of results obtained with Arsenic or its salts, dating from the early observation of Livingstone, through the elaborate experiments of Bruce and Lingard, to the more recent experimental observations of Laveran, Mesnil, Thomas, and Thomas and Breinl was such as to encourage hope of a successful treatment; there was no doubt that the disease was modified, the life of many of the animals was prolonged, and some of the animals were cured. All experiments showed equally well the disadvantages of the drug, such as the recrudescence of the disease on stopping the drug and the severe toxic symptoms caused by it.

ment of colours

EHRLICH and SHIGA at about this time (1904) published their '*Farbentherapeutische Versuche bei Trypanosomenerkrankung*,' in the Berliner klinische Wochenschrift, 1904, Nos. 13, 14. These observers were the first to introduce colouring matters belonging to the Benzopurpurin group in the treatment of trypanosomiasis; they

obtained better results with *Trypan-red* than with either Arsenic or human serum, upon mice infected with Mal de caderas. They were able to cure mice, and in rats the parasites disappeared, but reappeared after a short time. The result of these eminent observers drawing attention to the use of Anilin colours was to stimulate a great amount of investigation in this direction.

LAVERAN and MESNIL, '*Le trypanroth dans le traitement de quelques Trypanosomiases*' (Comptes rendus de l'acad. des sciences, Vol. 139, p. 19), confirm Ehrlich and Shiga.

HALBERSTADTER (Centralbl. f. Bakt., 1905, Vol. 38, p. 525) confirms Ehrlich as regards the action of Trypan-red in Caderas, similarly in Dourine, but had very little success in Ngana.

NISSLE (Arch. f. Hygiene, 1905, Vol. 53, p. 181) found *Trypan-red* better against Ngana than against Caderas.

EWALD FRANKE, 'Therapeutische Versuche bei Trypanosomen erkrankung' (Inaugural dissertation, 1905, Jena), as the result of an extensive study of *Trypan-red* on *T. equiperdum* and Mbori strains, recommends especially the *combined treatment of Trypan-red and Arsenic*.

WENDELSTADT (Deutsche med. Wochenschrift, 1904, No. 47) found that *Trypan-red* administered in small doses internally caused the trypanosomes to disappear, but that they soon reappeared again. He was able to cure rats infected with Ngana.

WENDELSTADT (Deutsche med. Wochenschrift, 1907) by the internal application of trypan-red only succeeded in obtaining prolongation of life in the case of ngana-infected animals.

THOMAS and BREINL in a paper entitled 'Trypanosomes, Trypanosomiasis and Sleeping Sickness' (Memoir XVI, 1905, of the Liverpool School of Tropical Medicine), published a full report of treatment experiments with various drugs, amongst them *Trypan-red*, but they only succeeded in demonstrating that it helped to prolong the life of infected animals.

WENDELSTADT and FELLMER, in a paper entitled 'Uber die Einwirkung von *Brillantgrün* auf Naganatrypanosomen' (Zeitschrift für Hygiene und Infekt. Krankheiten, Vol. LII, 1906, pp. 263-289), state that the parasites disappear from the peripheral circulation, and that after repeated doses the blood of the animals becomes negative as tested by subinoculations.

MESNIL and NICOLLE, 'Traitement des Trypanosomiases par les couleurs de benzidine' (Première partie, étude chimique. Seconde partie, étude experimentale. Annales de l'Institut Pasteur, Tome XX, June, July, 1906), tried to find out the connection between the chemical constitution and the trypanocidal action of the *benzidine colours*. *P. di-amido-diphenyl urea + Ac. H. (Ph.)* and *p. di-chloro-benzidine + Ac. H. (Cl.)* were the only colours which showed any marked action on the trypanosomes. Amongst the Arsenic preparations with which they experimented *Atoxyl* was found to be the most efficient.

MESNIL, NICOLLE and AUBERT (Annales de l'Institut Pasteur, January, 1907) give their experiments upon, amongst other drugs, the *benzidine colours*, and state that the blue colours are superior to the red colours, their best results being obtained with p. di-amido-diphenyl urea + Ac. H. (Ph.).

MESNIL and NICOLLE (Annales de l'Institut Pasteur, December, 1907) describe in a third paper the final results of their experiments in the case of twelve monkeys infected with *T. gambiense*, of which six were cured by Atoxyl alone, four by the combined treatment of Atoxyl and Ph., and two by Ph., first employed alone, followed up by only one injection of Atoxyl in the first case, and two in the second.

EHRLICH, in a paper entitled 'Chemotherapeutische Trypanosomenstudien' (Berl. klin. Wochenschrift, Nos. 9-12, 1907), says *Benzidine colours* (*Trypan-red*), *Triphenylmethane colours* (*Brilliant green* and *Malachite green*, Wendelstadt and Fellmer) have been found to be trypanocidal. Ehrlich experimented with *Parafuchsin* by feeding mice on parafuchsin cakes, which gave very good results.

WEBER and KRAUSE, in a paper entitled 'Farbstoffbehandlung der künstlichen Trypanosomeninfektion' (Berliner klinische Wochenschrift No. 7, 1907), tested systematically different colouring matters (*Crystal violet*, *Victoria blue*, *Fuchsin*), as regards their trypanocidal action in Ngana, with the view to find relations between chemical constitution and action. Fuchsin seemed to have the best effect, also because of its relative harmlessness for animals. They were unable to obtain *cures*, but the advantage of Fuchsin seems to reside in its power of prevention.

WENYON, in a paper entitled 'Action of the *colours of Benzidine* on mice infected with *Trypanosoma dimorphon*' (Journal of Hygiene,

Vol. VII, April, 1907), describes how he treated mice infected with *T. dimorphon* and 54 *Benzidine colours*. He finds, in contradiction to Mesnil, Nicolle and Aubert, that the *red* colours have a more powerful effect on *T. dimorphon* than the *blue* ones.

PLIMMER and THOMSON have used Mesnil's, Nicolle's and Aubert's *Cl. colour* (*p. di-chloro-benzidine + Ac. H.*) and obtained the same results as with Trypan-red.

KOCH, in a paper entitled 'Schlussbericht ü. d. Thatigkeit d. deutschen Expedition z. Erforschung der Schlafkrankheit' (Deut. med. Wochenschrift, No. 46, 1907), states that *Afridol blue* and *Afridol violet* had not the least effect upon trypanosomes, neither had *para-Fuchsin* nor *para-Rose aniline*.

C. BROWNING, 'Experimental Chemotherapy in Trypanosome infections' (Brit. Med. Jour., No. 2,446, November 16th, 1907, p. 1405), gives the results of colour treatment, especially with *para-Fuchsin*. He recommends treatment with *Atoxyl and Dye*.

YAKIMOFF, 'Zur Behandlung der Dourine' (Centralbl. f. Bakt. Orig., Vol. XLV, h. 5, Dec. '07) used *Trypan-red* for treatment of dourine in mice, rats and rabbits. Several injections of Trypan-red are able to prevent relapses in *mice* and to effect a *cure*. During the incubation period, if Trypan-red is given very early, the appearance of trypanosomes is prevented.

It does not act prophylactically, and there is no immunity after cure. The mechanism of Trypan-red action is trypanolytic, trypanosomes being killed by Trypan-red by immune substances and other products. The immunity, however, is of very short standing.

THOMAS in his paper referred to above, and published May, 1905, states that after the publication of Ehrlich's and Shiga's results with Trypan-red, he repeated the experiments with animals infected with different species of trypanosomes. 'The best results were obtained with mal de Caderas-infected animals; the results were not so good with animals infected with nagana and surra, and still worse in the case of animals infected with dourine, the Gambian horse-strain and *T. gambiense*. The parasites disappear for a few days to reappear, and the duration of the disease was not greatly prolonged, and on analysing the evidence given above of those who have carefully experimented with the aniline dyes, we are driven to conclude that the colours do not possess any advantage over the arsenic salts, that

they are not even as efficacious.' In other words, sodium arseniate had given better results and still held out to investigators a more promising field for ultimate success than the use of colours.

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However, following up the experiments of Laveran, Thomas determined to try a combination of the two drugs *Trypan-red* and *Arsenic* and the results were more encouraging, but he states, 'unfortunately trypan-red also caused a nephritis, and by its chemiotaxic properties very extensive necrosis sometimes resulted On monkeys, especially, the subcutaneous injection of the dye either alone or in combination with arsenious acid induced ulceration, which so undermined the health of the animals that they succumbed to any outbreak of disease, which occurred only too frequently amongst my animals. *It was these untoward accidents which induced me to seek a preparation of arsenic less toxic and the subcutaneous injection of which entailed less danger of necrosis.*' Before, however, proceeding to describe the compound of arsenic (*Atoxyl*), which he demonstrated had such a marked action upon the trypanosome, we wish to record the observations made upon the *combination of Arsenic with colours* and other bodies.

THOMAS and BREINL, in their publication referred to above, came to the conclusion:—

'That in trypan-red we possess an agent of some use in the treatment of trypanosomiasis. That certain trypanosomic diseases appear to be more amenable to its action than others. That in the substance at present available there is need for improvement in order to abolish its toxic effects.

'That a combination of arsenic and of an improved form of trypan-red would seem indicated in the further investigation of the cure of trypanosomiasis.'

LAVERAN wrote a paper entitled 'Traitement mixte des Trypanosomiases par l'acide arsenieux et le trypanroth des infections au *Trypanosoma gambiense*,' published 30th January, 1905, in the Comptes rendus de l'Academie des Sciences. He was the first to use a combination of trypan-red and arsenious acid. He made a large number of experiments upon this combination, and we owe to him most of our knowledge upon the effects of this combined treatment upon small animals. He demonstrated the curative action of the combination of trypan-red and arsenious acid in the case of rats

and mice infected with *Mbori* or *Surra*. In the case of animals infected with *T. gambiense* the results appeared to him less encouraging.

In a second paper upon the mixed treatment, published April 17th, 1905, in the Comptes rendus de l'Academie des Sciences he discusses the combined treatment of monkeys infected with *T. gambiense*. His observations confirm the results previously obtained with other animals. We reproduce his own comments upon the combined treatment in the case of *T. gambiense*.

'Il n'y a pas de motif pour que le traitement qui a réussi dans les infections expérimentales du rat, du chien et des singes par *Trypanosoma gambiense* ne reussisse pas également dans les infections naturelles, chez l'homme, et je crois que, dès maintenant on serait autorisé à essayer de ce traitement chez les sujets atteints de trypanosomiase. La difficulté sera de detérminer les doses d'acides arsénieux et de trypanroth qui devront être préscrites; des tatonnements seront inévitables. Les chances de succès seront d'autant plus grandes que la maladie sera à une période moins avancée de son évolution. Il est douteux que le traitement puisse donner encore de bons résultats quand les accidents du côté du système cérébrospinal ont acquis une certaine intensité: on se rappellera d'autre part que le trypanroth est irritant pour les reins (1), on surveillera les urines et l'on ne préscrira pas ce médicament aux malades atteints de néphrite.'

FRANKE (loc. cit.) also strongly recommended, as the result of his extensive trials, the use of the Trypan-red-arsenic treatment. WENDELSTADT and FELLMER also advocate the treatment with a combination of *Arsenic and Brilliant green*. They also combine Brilliant green and Nucleinic acid.

MAGALHAES, in a paper entitled 'De l'Action des Composés arsenicaux et du vert brilliant sur le *Tryp. gambiense* et le *Tryp. brucei*' (Arch. de. R. Inst. bact. Camera pestana, T. I, Jan. 2nd, 1907) treated rats infected with *T. gambiense* with Sodium arseniate and Brilliant green. The parasites disappeared for a time but reappeared again.

As seen from the above notes, the combined Arsenic-colour treatment led Laveran to believe that as it had succeeded in his hands

so well in the case of animals artificially inoculated with *T. gambiense*, so it might succeed in man in the case of sleeping sickness.

THOMAS, however, as we have seen, had given it up on account of the nephritis and local necrosis which was induced, and set himself to find a preparation of Arsenic less toxic than Arsenious acid or Sodium arseniate, and the subcutaneous injection of which produced less danger of necrosis. An aniline compound, the anilide of met-arsenious acid (*Atoxyl*), having the supposed formula $C_6H_5NH\,AsO_2$, attracted his attention. This preparation had been before the medical profession since 1900, and various workers had recorded its use in the treatment of various skin affections and in anaemia.

F. BLUMENTHAL, in a paper 'Uber Metaarsensäure anilid' (Die medizinische Woche., 14th April, 1902, No. 15), describes how he had used Atoxyl on rabbits in order to test the toxicity of the drug. He concluded from his experiments that Atoxyl was 40 times less toxic than *Solutio Fowleri*.

SCHILD (Berl. klin. Wochenschrift, 1902, No. 23, and Dermatologische Zeitschrift, Band X, Heft 1, 1903) employed Atoxyl against different skin diseases as psoriasis, lichen ruber, etc., with very good results.

F. BIRINGER published in the Therapeutische Monatshefte, August, 1903, a paper on 'Klinische Erfahrungen mit Atoxyl.' He used Atoxyl in the treatment of different skin diseases, and as a result regarded it as a valuable and welcome substitute for Arsenious acid.

MÖLLER, in a paper in the Berliner klin. therap. Wochenschrift, entitled 'Uber die Heilung der Tuberkulose mit Atoxyl,' employed Atoxyl administered intravenously in 50 cases of tuberculosis with very encouraging results.

F. MENDEL (Therapeutische Monatshefte, April, 1903), 'Zur endovenösen Application der Medikamente,' states that Atoxyl is especially suitable for either subcutaneous or intravenous administration. It produces no necrosis, no fever, and very much larger doses of arsenic can be given without producing toxic results. '*I have myself*,' wrote Thomas, '*tried the drug in high doses intravenously without ill effects.*'

To Thomas, and to Thomas and Breinl, belongs the credit of having, after a very careful experimental investigation, introduced

to the notice of the medical profession the superior claims of Atoxyl as a curative agent in cases of trypanosomiasis. Before, however, recommending it to the profession, they put it to a most searching test in animals. We quote Thomas's own words:—'Tentative experiments on rats and rabbits appeared so favourable that I decided to institute a series of experiments. These observations have been in progress for the last ten months, and have on the whole been most promising. In all my therapeutic experiments certain conditions were laid down to be followed out.

1. That the animal should be well infected and the presence of parasites determinable in its blood.

2. That the disease should have been established some time (one cannot expect to treat either man or beast suffering from the disease in the very early stages when no definite symptoms are manifested).

3. That some symptoms besides the presence of parasites should be in evidence:

(*a*) Anemia;
(*b*) Loss of weight.

'If the treatment of such infected laboratory animals be successful, then such a line of medication ought to be of service in the practical treatment of the disease.

TRYPANOSOMES

The trypanosomes experimented with are the following:—

Trypanosoma gambiense—

(*a*) Gambian fever strain (Gunjur).
(*b*) Congo Free State fever strain.
(*c*) Uganda sleeping sickness.
(*d*) Congo Free State sleeping sickness.
(*e*) A highly virulent strain derived from one of my cases of sleeping sickness which had only been passed through a monkey, baboon, and a rabbit.

Trypanosoma brucei (nagana).
Trypanosoma evansi (surra).
Trypanosoma equinum (mal de Caderas).
Trypanosoma equiperdum (dourine).
Trypanosoma dimorphum (Gambian horse-disease).

ANIMALS USED

T. gambiense.—Monkeys, dogs, pups, kittens, rabbits, guinea-pigs, rats and mice.

T. evansi.—Rabbits, guinea-pigs, dogs, rats, mice.

T. brucei.—Rabbits, guinea-pigs, rats, mice.

T. equinum. Dogs, rabbits, guinea-pigs, rats and mice.

T. equiperdum. Pups.

T. dimorphum. Dogs, pups, rabbits, guinea-pigs, rats and mice.'

We reproduce also his conclusions upon the advantages of Atoxyl in trypanosomiasis:

'With five exceptions every animal has had one or more controls which were infected at the same time; the controls of *T. gambiense*, strain '*e*,' surra, nagana, caderas, and Gambian horse parasites have all died in the usual time. It is, therefore, evident, from the great majority of the experiments, that the treatment of animals infected with trypanosomes with this preparation or in combination with the dye either arrests the disease, thus prolonging the life of the animals, or apparently cures them. This is especially the case in animals infected with the ordinary strain of *T. gambiense*, and even, though to a less extent, with the abnormally virulent strain called '*e*.'

'A comparison of animals infected with the same strains, but treated according to Laveran's method with sodium arseniate has made me conclude that treatment with this aniline compound is in many ways superior to the ordinary arsenical treatment, on account of the quicker action of the drug on the parasite, the fact that the action seems to be prolonged, that large doses can be given without toxic symptoms and the entire absence from any tendency to cause sloughing.

'In my opinion treatment is indicated, of cases of trypanosomiasis in man, with this drug in high doses administered intravenously and for a long period, pushing it to the maximal amount that the case can stand without headache and nausea, at the same time building up the patient in every way possible that will conduce to a lessening of the anaemia. In the European the combination of trypan-red would probably be objectionable on account of the intense colouring of the tissues and secretions, but the native exhibits only a reddening of the conjunctivae and staining of the secretions. One of my cases of

trypanosome fever had been under treatment with trypan-red and arsenic for a short time before he returned to the Congo with favourable results the parasites were longer absent from the peripheral circulation; his general blood condition was better; his temperature was almost normal.

'I present the results of my experiments tentatively. I have used the term "apparently cured," as any one with an intimate knowledge of *T. gambiense* and other forms of trypanosomes in animals knows how difficult it is to say that an animal is not infected. This is especially the case with the human parasite. In a previous paper I have recorded cases in which the blood has been negative for nearly a year in rats known to have been infected, and which at the expiration of that time showed parasites once more in their blood.

'I do not believe that sodium arseniate alone will be found of great practical value, nor do I think atoxyl is a perfect preparation, from its toxic effects on canines and felines, but it is an advance on arsenious acid, and, if further efforts be made to produce a substance like trypan-red, but less irritating in action, the combination ought to be of service in the treatment of trypanosomiasis in man.'

THOMAS at about the same time made a communication to the Royal Society (received April 8th, 1905, and read May 11th, 1905) upon the experimental treatment of Trypanosomiasis in animals with Atoxyl, Atoxyl and Trypan-red, and Trypan-red alone. The communication was the result of exhaustive experiments upon animals infected with five strains of *T. gambiense*, one a very virulent one taken from a case of sleeping sickness, and the rest the common animal strains. The Atoxyl was given in two ways.

(1) High doses at intervals of a week.

(2) High initial dose and then reduced amounts administered three times a week.

He concluded, '*In my hands the arsenic-anilin compound (Atoxyl) has given far better results than treatment with sodium arseniate. The advantages of its administration intravenously or subcutaneously in high doses over a length of time—namely, its less toxic properties, the absence of all tendency to cause sloughing, and the apparently longer action of the drug, make me believe that the employment of this compound is indicated in the treatment of human trypanosomiasis.*'

A third paper by THOMAS in collaboration with BREINE upon

'Trypanosomes, Trypanosomiasis and Sleeping Sickness,' was published as Memoir XVI, 1905, of the Liverpool School of Tropical Medicine.

This paper contains the full report of all the treatment experiments. They wrote:—

'We have, therefore, to realise that the ordinary arsenic compounds when administered, only produce a temporary favourable effect, that, if long continued, the animals will die either from the parasite or from the arsenic, or from both. Hence, some other compound is indicated. It is for this reason that the newer compounds of arsenic have been experimented with in order to find a preparation capable of being used over a long period and in high doses without producing toxic symptoms. Of the various preparations tried, a meta-arsenic anilin compound, atoxyl, has proved the most satisfactory, but it is not ideal. It is not non-toxic, as dogs, kittens, guinea-pigs, and rabbits have shewn toxic symptoms and succumbed, but it is not so toxic as sodium arseniate. It does not produce the sloughing which so often follows the subcutaneous or intravenous inoculation of sodium arseniate, it causes no pain, and its administration can be continued over a period of many months even when used in extremely high doses.

'It is the only remedy at present giving any prospects of a cure. In the treatment of cases a rational method of treatment must be adopted. It is useless, for instance, to only administer arsenic for a short period or until the parasites have apparently disappeared from the peripheral circulation. The drug must be administered in as high doses as possible, and it must be continued even until after all the favourable signs are present, viz., disappearance of parasites from the blood, increase of weight, improvement in the blood count and percentage of haemoglobin, loss of the auto-agglutination phenomenon of the blood corpuscles, decrease in and more regular temperature. From time to time susceptible animals ought to be inoculated with large quantities of the patient's blood, at least in amounts of 5·0 to 15·0 c.c. At the same time all aids in building up the physical condition of the individual should be used. If such a régime be carried out, and treatment commenced at an early period, the prognosis (based on the experience of treated animals) will be good.

'The drug was used only upon animals showing the effects of the parasites, such as loss of weight, anaemia, fever, and auto-agglutination of the corpuscles, and no animal was used until its blood contained numerous parasites. The numbers of the parasites present differed according to the species of animal and the disease. In the majority of the experiments, control animals which were not treated, and had been inoculated at the same time as the treated animals, were used. In all cases the control animal died.

'Intravenous inoculation was used only on rabbits, all other animals were injected subcutaneously. Treatment was continued for one to three months or until increase of weight, diminution of the anaemia, and entire absence of parasites from the blood, as far as microscopical examination could determine, was noted. At various periods susceptible animals were inoculated with the blood from a treated animal. When treatment had been discontinued for one to three months, or longer, the animal was bled or killed and all the blood available was used to inject susceptible animals. Inoculated animals whose blood has given negative results after three to six months, or after longer periods, have been inoculated with virulent blood, and have taken the disease, thereby showing that no immunity was conferred by the previous inoculation.

'*T. gambiense*.—Rabbit, male, weight, 2,010 grammes. Parasites appeared on the twelfth day. On the forty-sixth day numerous trypanosomes were present; it had lost weight (1,890 grammes). A blood count gave reds, 4,980,000; whites, 8,860; haemoglobin, sixty-seven per cent. For three-and-a-quarter months it received 1·0 c.c. of five per cent. solution atoxyl three times a week, gradually increasing the amount to 1·0 c.c. of ten per cent. solution. It then weighed 2,000 grammes. The blood count was reds, 6,640,000; whites, 6,200; haemoglobin, eighty-eight per cent. The blood in quantities of 10 c.c. was non-effective. The auto-agglutination of the corpuscles was lost. Thirty-two days later it was very ill; it was therefore bled to death and the whole of its blood injected into a monkey. This monkey has never become infected. The post-mortem showed severe haemorrhagic cystitis, the bladder in parts being almost gangrenous and acute septic peritonitis, especially around the bladder. The spleen showed no congestion, but the connective tissue was slightly increased. The kidneys and liver were normal.

'Rabbit, 889, inoculated October 26: weight, 1,760 grammes. Blood count: reds, 6,620,000; whites, 6,700; haemoglobin, eighty-nine per cent. Parasites were seen from November 8 up to January 10; the trypanosomes were always present but in small numbers; they then increased to eighteen to twenty to a field. The anaemia was pronounced, and loss of weight was noted. It then weighed 1,540 grammes. Blood count: red, 3,880,000; white, 11,800; haemoglobin, sixty-three per cent. It could hardly sit up, and remained most of the time lying down. This animal was given 0·8 c.c. of five per cent. solution atoxyl. At the end of eighteen hours the parasites were absent from the blood. Doses were given twice a week, beginning with 0·5 c.c., and increasing to 2·0 c.c. of a five per cent. solution. The blood in large doses is non-infective. The animal is vivacious; the coat is smooth and thick. Average weight, 1,600 grammes. Several guinea-pigs infected for about two months, and showing twenty to forty parasites to a field, have been treated. They were injected subcutaneously with 0·3 c.c. of ten per cent. solution. At the end of the fifteenth hour no parasites were seen. Treatment was 0·1 to 0·3 c.c. of five per cent. solution three times a week for two months. The animals all increased in weight. One died sixty-two days after treatment was discontinued. Three rats inoculated with its blood never became infected. The second and third pigs were killed at the end of eighty and one hundred days after stopping treatment, and the blood used to inoculate controls. No control has shown the parasites.

'Virulent strain.—Rhesus. Weight, 2,815 grammes. Inoculated November 16. Blood count: red, 5,220,000; white, 12,800; haemoglobin seventy-eight per cent. On November 31, parasites were seen. Two days later, twelve to seventeen to a field were noted. The weight was 2,420 grammes. Blood count: red, 4,600,000; white, 7,200; haemoglobin, seventy-three per cent. The parasites counted per mm.^{3} gave 100,000. Oedema of the eyelids and bridge of nose was present. The animal was given 0·8 c.c. of ten per cent. solution atoxyl subcutaneously. Four hours later: red, 4,450,000; white, 24,800; parasites 40,000 per mm.^{3} At the eighth hour: red, 4,740,000; white, 25,200; parasites one to eighty-nine fields. Between the fourth and eighth hours the parasites were seen to become remarkably degenerated and deformed; many phagocytes

were present. At the twenty-fourth hour after injection a count gave red, 5,050,000; white, 46,000. The blood was negative. The leucocytes remained high for a couple of days and then fell; in none of the phagocytes could any remains of trypanosomes be found. Treatment: 1·0 c.c. of ten per cent. solution was given twice a week. The animal increased in weight. The auto-agglutination of the corpuscles began to be less accentuated, and the number of erythrocytes and the haemoglobin rose. The local oedema disappeared. On the thirty-ninth day dysentery appeared, and the animal succumbed on the forty-eighth day after injection. The autopsy showed a very severe haemorrhagic and necrotic enteritis, with slightly enlarged spleen. Kidneys normal. Glands, small; inguinal group haemorrhagic. The blood was non-infective in amounts of 1·0 c.c., but infective if 15 c.c. of pure blood was used. Unfortunately, the arsenic was discontinued on the appearance of dysentery. A second monkey, inoculated from the first Rhesus just before treatment was begun, was treated with the same doses of arsenic; the parasites disappeared in the same way, but the animal quickly succumbed to dysentery.

'Many rabbits inoculated with this strain have been treated. It was found that unless treatment was started early that the majority of animals died as it was so exceedingly virulent. With these animals treatment was begun earlier and higher doses given than with the standard "Gunjur" strain. Despite treating the animals early, some died. With this strain treatment had to be kept up longer. Some rabbits have survived eight months after injection, while all the controls have died in fourteen to thirty-six days. Guinea pigs infected with this strain do not react so well to the treatment. Rats must be treated early and with high doses if treatment is to be successful. Mice infected with this strain react if treatment is commenced early enough. The action of atoxyl on the various trypanosomes has been studied, and after numerous observations, continued for the whole period during which the drug was administered, the effect appears to be as follows:—

'On administration of arsenic compounds into an animal showing numerous parasites in the blood the following action on the trypanosomes will be noticed. For the first three-and-a-half to four hours, depending on the dose used, very little change in the parasites can

be noticed. Between the fourth and fifth hour the effect on the trypanosomes is evident. Some parasites appear to be swollen and their movement is less rapid. If now a series of blood specimens be examined at intervals of twenty to thirty minutes, the following changes will be seen. The number of slowly moving trypanosomes increases, many parasites will be seen to be almost motionless. The protoplasm takes on a peculiar ground-glass appearance, and dark granules appear in the protoplasm; very often a small series of granules one behind the other, sometimes in pairs or all clumped together, are seen lying between the macronucleus and the anterior end, or distributed through the whole body of the parasite. At the same time vacuoles are observed, oftentimes very large. The trypanosomes become deformed, assuming various shapes, the most common being a kite-shaped form with fairly long flagellum, and a tadpole-like one with hardly any free flagellum. These forms especially exhibit greatly impaired movements. At the same time a noticeable increase of the leucocytes is discernible; phagocytes begin to appear, very often groups of five to seven will be seen. Up to this time (sixth to seventh hours) the trypanosomes, though decreased in numbers are still present in considerable quantities. Suddenly, in the course of an hour, the numbers may drop from forty to two to three to a field or less; coincident with this is a very marked increase in the number of leucocytes, especially phagocytes. From the ninth to the fourteenth and sixteenth hours the changes are less pronounced and rapid, the trypanosomes gradually disappear. At the eighteenth hour, provided the animal has been injected with the correct amount the parasites are absent from the peripheral circulation and, even though the blood is centrifuged, none can be found.

'From a series of these observations, we have determined that in hardly any of the forty-six continuously observed animals were parasites to be found after the eighteenth. Should, however, the drug be given in smaller amounts the process takes longer, lasting from thirty-six to forty-eight hours.

'From the experimental work with various therapeutic agents the following conclusions can be made:—

'(1) That animals suffering from trypanosome infection react favourably to only a few agents, of which arsenic is the only drug which seems to exert a more than transient action.

'(2) That the greater the amount of arsenic introduced into the system of the animal the greater and more permanent the effect on the parasite.

'(3) That arsenic medication is indicated in the treatment of individuals suffering from trypanosomiasis. That the treatment ought to be long continued and regularly administered in as high doses as the case can stand. That all aids to building up the system should be employed.'

In a fourth paper upon 'Atoxyl in the treatment of Trypanosomiasis,' published in the British Medical Journal, Jan. 19th, 1907, by Drs. Breinl and Todd, these observers summarised our knowledge concerning the use of Atoxyl up to January, 1907. They quote Van Campenhout's private communication, in which he refers to the combination of Atoxyl and Strychnine and a cold bath, the latter for a tonic and stimulating effect. Three Europeans treated by Van Campenhout have gained weight and are apparently well. He has obtained good results by the treatment of Europeans by Atoxyl in the first stages of trypanosomiasis. He prefers a solution of 5 per cent. rather than 10 per cent.

Todd and Breinl recommend the use of a 20 per cent. solution administered in increasing doses, up to 0·2 gramme.

The following letter was published by Professor Ross to make it clear that atoxyl was first suggested and used by Thomas and Breinl in trypanosomiasis and sleeping sickness in man, as fully established in the preceding pages.

THE TREATMENT OF TRYPANOSOMIASIS.

Sir,—Many statements having been made recently in the lay press to the effect that trypanosomiasis has been cured by various persons by the means of atoxyl, I should like to point out that this drug was first suggested and used by Drs. Thomas and Breinl, of this School, for the purpose referred to. A full account of their experiments was given in the Proceedings of the Royal Society, November 9th, 1905, vol. 76, and also in our publications, Memoir XVI. At the instance of this School large quantities of the drug have been sent to the

Congo, and several patients are now under treatment with it. The result has been an apparent success so far, but it is of course too early to speak definitely as to the final result, because it is well known that patients may survive for several years, even without treatment, and yet ultimately succumb. For example, we have had cases under observation for four years without a fatal result.—I am, etc.,

RONALD ROSS,
Professor of Tropical Medicine,
Incorporated School of Tropical Medicine.

Sept. 21st., 1906.

Editor 'British Medical Journal.'

At the end of 1907 overwhelming clinical proof derived from the human subject is forthcoming, as we shall see. The statement that '*We have in Atoxyl the specific drug for trypanosomiasis, as we have in Quinine that for Malaria*,' fairly represents the view of Professor Koch.

Sir Patrick Manson, who has had the largest experience of this drug in human cases of trypanosomiasis in this country, concludes in the following words:—'The prospects of atoxyl treatment I consider most hopeful. As regards efficiency and mode of action, it seems to me that it is almost on a par with mercury in syphilis and quinine in malaria; and I think in using atoxyl we should conform our practice to what experience has taught us to be the best methods of using these other efficient and long-tried remedies. I don't believe we can kill the trypanosomes outright by one or two large doses of atoxyl, any more than we can kill the treponema of syphilis or the parasites of malaria by large doses of their respective specifics. Mercury does not immediately cure syphilis nor does quinine immediately cure malaria; but they deprive their respective parasites of their pathogenic properties and keep the patient alive and in good health till, in process of time, the parasites either die out or become permanently inert.'

By far the largest clinical experience of the drug has, however, been obtained by Professor Koch and his assistants, and their conviction is overwhelming on the advantages of this drug over all previous ones.

R. KOCH, 'Bericht über die Tathigkeit der deutschen Expedition zur Erforschung der Schlafkrankheit bis zum 25 November, 1906' (Deutsche med. Wochenschift, Jahr. XXXIII, No. 2, 1907).

Koch treated *986 cases of sleeping sickness with Atoxyl.* Out of 356 cases positive results were obtained in 347.* He points out the importance of the drug in early cases when it is much more efficient than in advanced cases. Koch divides his cases into two classes—'early' and 'advanced' cases. He gives a short clinical account of the symptoms. Debility, nervous excitement, trembling of the extremities, general disturbance of the psychical functions, and nervousness very often in the form of mania. In the latest stages of this disease, apathy and sleep. Atoxyl was usually administered in the forms of two double injections of 0·5 gramme. Usually, after the second injection the parasites had disappeared from the blood and from the lymph glands, but a real improvement is only to be seen three or four weeks after the injection. In advanced cases, it was impossible to drive out the parasites from the circulation, even after prolonged administration. Under the influence of Atoxyl, the trypanosomes sometimes disappeared for 30 to 40 days; when they reappeared they were only observed in very small numbers. Attempts to shorten the treatment by the administration of one large dose of Atoxyl only have given uncertain results as yet. In 12 cases, in which only one full dose of Atoxyl was given, the trypanosomes disappeared for 30 to 40 days.

R. KOCH, 'Schlussbericht über die Tatigkeit der deutschen Expedition zur Erforschung der Schlafkrankheit' (Deutsch. med. Wochenschrift, 1907, No. 46), gives his further experiences. He believes that some of the appearances, after Atoxyl treatment, can only be explained by the resorbtion of the dead trypanosomes. He still uses as the mode of administration the double injection of 0·5 gramme Atoxyl on two successive days, then 10 days interval, and two more injections. Koch was not able to observe, as Erlich did, that the trypanosomes become resistant to the Atoxyl. Attempts to administer Atoxyl in doses of one gramme were unsuccessful in so far as after such large doses of atoxyl blindness very frequently occurred, due to atrophy of the optic nerve. Very interesting from an epidemiological point of view is the fact, which he has pointed out in his previous reports, that in Kisiba fifteen married women were found suffering from sleeping sickness. As

* Up to date he has treated some 2,000 cases.

these women had never left the place, and it was found that their husbands were all either suffering from sleeping sickness or had died of that disease, Koch brings forth his own explanation: that trypanosomes may be transmitted by coitus.

Koch quotes a further case, where three women were all infected with trypanosomes from one man.

Koch states that early cases of trypanosomiasis can be definitely cured by Atoxyl by a six months' treatment; in far advanced cases it is very difficult to drive out the parasites.

BRODEN, in a paper entitled 'Les Trypanosomes dans l'Etat du Congo' (Rapport sur les traveaux du laboratoire medicale de Léopoldville de 1900 à 1905, II, Bruxelles, Hayem, 1906, p. 71-143), used Atoxyl in the treatment of one case of trypanosomiasis with very encouraging results.

BRODEN ET RODHAIN 'Le traitement de la trypanosomiasé humaine (Maladie du Sommeil)' (Archiv für Schiffs und Tropen-hygiene, Band X, 1906, p. 693), treated cases of trypanosomiasis (three white men) with Atoxyl. Fever disappeared in all three and their general heath improved.

VAN CAMPENHOUT starts with 0·2 gramme of Atoxyl, and increases his doses 0·05 gramme every second day till he reaches 0·8 gramme. This does is given every second day for a fortnight or three weeks, and then he decreases his doses gradually 0·05 gramme. In the cases of the second period he uses strychnine and cold baths.

MESNIL, NICOLLE ET AUBERT, 'Recherches sur le traitement des infections experimentales à *Trypanosoma gambiense*' (Annales de l'institut Pasteur, Tome XXI, Jan., 1907, pp. 1-19), continue the researches on a number of Benzidine colours and Sodium arseniate and Atoxyl on *Trypanosoma gambiense*. Amongst the Benzidine colours in general, the blue colours show themselves superior to red colours. The best results were obtained with p. di-amido-di-phenyl-urea+AcH (Ph.). Atoxyl is considered superior to Sodium arseniate. Further, they combined the colour Ph. with Atoxyl with very good results.

KOPKE publishes his experiences in the following papers:—

'Trypanosomiases humaines,' (XV Congrés international de médecine de Lisbonne). 'Traitement de la maladie du Sommeil,' (Travaux de l'École de Médecine Tropicale de Lisbonne). 'Traitement de la maladie du Sommeil,' (Rapport presenté au XIV Congrés Intern. d'Hygiene et Demographie. Lisbonne, 1907).

His results with atoxyl are not very good. Out of 29 cases, 22 died from trypanosomes. Seven are still alive and of these only two are in good health.

Kopke used very large doses of Atoxyl (one gramme in one injection). Out of 29 cases, six had eye lesions which were diagnosed as atrophy of the optic nerve, and were certainly due to the over-doses of atoxyl given.

THIROUX ET D'ANFREVILLE, 'La maladie du Sommeil au Senegal,' record, 'trois cas traités, guérison dans un cas.' Rapport de Laveran, (Bull. Acad. Méd. Séance de 26 fev. 1907).

HOLLEBEKE, 'Traitement de la trypanosomiase par l'atoxyl. Notes cliniques et therapeutiques,' (Bull. acad. roy. de méd. de Belgique, Vol. XXI, 1907), treated eight Europeans for trypano-somiasis. Gave daily injections of 0·2 gramme of Atoxyl. Never observed any eye-symptoms. After six months, he stopped the treatment and the same improvement continues. He considers his cases as cured.

JAKIMOFF, 'Zur Atoxylbehandlung der experimentellen Dourine,' (Deutsche. med. Wochenschrift. 1907. No. 16), cured white rats infected with Dourine by Atoxyl.

MARTIN, in a paper entitled 'Maladie du Sommeil. Cinq nouveaux cas de trypanosomiase chez les blancs. Essais de traite-ment,' (Ann. de Inst. Past. Tome XXI, Mars, 1907), states that Atoxyl has a very noticeable action on the trypanosomes, which disappear from the blood, as in malaria the parasites disappear under quinine treatment. Concerning whether or not a permanent cure has been effected, he states that it is impossible to say anything as the time was too short.

COOK, at a meeting of the Society of Tropical Medicine and Hygiene, Oct. 26, 1907, discussed the question of sleeping sickness in Uganda, and stated that Atoxyl was by far the best drug for the cure of the disease that had yet been tried, and that it was greatly to the credit of English investigators that an Englishman, Thomas, of Liverpool, first applied it to the treatment of sleeping sickness.

UHLENHUTH, 'Demonstration von mit Atoxyl behandelten Dourinekaninchen' (Deut. med. Wochenschrift, 1907, No. 30).

UHLENHUTH, HUBENER and WOITHE, 'Experimentelle Untersuchungen über Dourine mit besonderer Berucksichtigung der Atoxyl behandlung' (Arb. a. d. Kaiserl. Gesundheitsamte, Bd. XXVII, h. 2, 1907);

UHLENHUTH, GROSS and BICKEL, 'Untersuchungen über die Wirkung des Atoxyls auf Trypanosomen und Spirochäten' (Deutsche med. Wochenschrift, 1907, No. 4), give the results of treatment of experimental animals infected with *T. equiperdum* (Dourine) with Atoxyl (*a*) preventive, (*b*) curative. Preventive results were not very encouraging. They were able to drive the Dourine parasites out of rabbits, rats and mice, and to keep the animals alive. The authors do not state whether it is a definite cure, as the time of observation was too short.

Ehrlich's experiences with Atoxyl in experimental treatment are very favourable. He was able to prove that the different trypanosome strains become after a time resistant to the drugs, and he got the 'festen' strains; an Atoxyl resistant, a para-Fuchsin resistant strain, which did not even in sub-inoculation react to the drug.

The Atoxyl resistance may partly explain the unfavourable results in some cases of sleeping sickness, as described by Kopke and Broden.

To FOURNEAU (Journal Phar. et Chim., 6ième série, T. XXV, 1 April, 1907) is due the credit of showing that, chemically, atoxyl is not a new preparation, having been synthetized as long ago as 1863 by Bechamp in the early days of the synthesis of aniline colours, fuchsin being produced in abundance at the same time. Bechamp supposed that he had in hand an anilide of ortho-arsenic acid. Fourneau supports this view, and the body is described as an anilide, but this from the evidence given by Moore, Nierenstein and Todd, and by Ehrlich and Betheim, is probably an error.

EHRLICH, in a lecture delivered before the Berliner medizinischen Gesellschaft, February 13th, 1907 (Berl. klin. Woch. No. 9-12, 1907), stated that Atoxyl was the sodium salt of p-amido-phenyl-arsenic acid, with four molecules of water of crystallization. The analyses of MOORE, NIERENSTEIN and TODD (Bio-Chemical Journal, Vol. II, Nos. 5-6, 1907, pp. 309-374) yield the formula $(NH_2)(C_6H_4)AsO_3ONaOH,3H_2O$, and they had independently come to the conclusion that the arsenic radicle was united directly to the ring. Since then

Ehrlich and Bertheim have published the details of their chemical work, showing that the arsenic radicle is in the para position to the NH_2 group.

Moore, Nierenstein and Todd show that Atoxyl is not, as it was originally described, an anilide of metarsenious acid, but is an exceedingly stable chemical substance with the arsenical radicle directly attached to the benzene ring. It was shown that the aqueous solution is strongly dissociated electrolytically, giving in consequence an apparently low molecular weight by the freezing point method and possessing a high electrical conductivity. Except on standing in aqueous solution, it is a most stable compound, and neither aniline nor arsenic are easily detachable from its molecule by chemical means.

Its toxic properties are neither those of arsenic nor of aniline even when pushed to excess, and its therapeutic action is rapid; from this and its high conductivity, showing high dissociation, the conclusion was drawn that its activity must be ascribed, not to free inorganic ions or to free aniline, but to a complex organic ion containing both the arsenical and aniline radicles.

Since the introduction of Atoxyl for the treatment of trypanosomiasis a large number of observers have tested it, and all are now united in giving it the premier position as a trypanocide.

The results of most observers upon the treatment of sleeping sickness by Atoxyl alone completely confirm the results stated above as having been obtained by Thomas and Breinl in the experimental trypanosomiasis of animals. Thus, cases have been described which were apparently permanently benefited and might be described as cured, but in a great many of them recurrences were observed, and finally the infection became persistent, the trypanosomes becoming 'Atoxyl-fast' and being apparently no longer affected by the drug.

Quite recently a second distinct advance has been made in the experimental therapeutics of trypanosomiases by workers of the Liverpool School. This consists in treating infected animals, from which the trypanosomes have primarily been driven out of the blood by the use of Atoxyl, by a second drug, so as to prevent the recurrences which so often follow Atoxyl treatment alone.

The general principle underlying the combined method of treatment by two successive and quite different drugs is that when an

infective organism, such as a protozoon, shows two distinct phases in its life-history, then these two phases ought to be attacked by separate drugs, and it is not only possible but probable that a drug which affects the first will not affect the second, and *vice versâ*.

The application of this bio-chemical principle, which has led to success in the prevention of recurrences in trypanosomiases, may be very wide in experimental therapeutics, especially in protozoon diseases where different phases in the life-history occur in nearly all cases. The two drugs necessary to attack two successive stages of the parasite will certainly not always be found to be an arsenical compound and a mercurial compound; but it is established as a principle that given a parasite has two successive phases, A and B, then the problem of experimental therapeutics is to find two remedial agents, *a* and *b*, of which *a* kills phase A and *b* kills phase B, and then to apply the remedies *a* and *b* in succession, killing off phase A as completely as possible with remedy *a*, and then attacking phase B with remedy *b*, and continuing this rotation until the animal is free of infection.

The research on the combined treatment was commenced in the Bio-chemical Laboratory of the University of Liverpool by B. Moore, Nierenstein and Todd in October, 1906, and a preliminary report published in March, 1907, followed by a fuller account in May, 1907; the work is now being continued on large animals in the Runcorn Laboratory, and, as far as cases are available, upon human trypanosomiasis.

At the time of the commencement of this research, a second phase in the life cycle of the trypanosome was not known with certainty to exist, but since then the life-history has been more completely investigated by J. E. Salvin-Moore and Breinl, and these observers have clearly shown the existence of such a phase.

The existence of this other phase was suspected by Thomas and Breinl, Moore, Nierenstein and Todd, from the regular way in which, after the trypanosomes had been completely driven out of the peripheral blood by Atoxyl, recurrence again took place, although the investigation of organs and tissues other than the blood had failed to demonstrate anywhere a storage of the ordinary phase of trypanosome.

On these grounds, these observers determined to first drive out

the parasite from the peripheral blood by Atoxyl, and then when the blood was free to treat by other drugs, paying no attention to whether these drugs had any effect upon the usual phase of the trypanosome or not.

Accordingly a series of experiments was commenced, in which rats infected experimentally with *Trypanosoma brucei* were used on account of the rapidity of action of this type of trypanosome. After driving out the trypanosome with Atoxyl, the salts of the different heavy metals were given; salts of Silver, Lead, Copper and Antimony respectively were employed without any marked results, but when Mercury was used in the form of the bichloride a distinct beneficial result was at once obtained. While the entire series of controlled rats treated with Atoxyl alone succumbed, nearly 70 per cent. of the rats given the double treatment survived, never showing any recurrence of trypanosomes, and of the remaining 30 per cent. only 8 per cent. showed recurrences of trypanosomes.

It may be emphasized that the Mercury salt alone has not the slightest effect upon the ordinary phase of the trypanosome as seen in the peripheral circulation. This appears to demonstrate clearly that the two drugs act upon two quite distinct phases.

Similar results are at present being obtained with other classes of animals, and indications of like results with the more slowly acting *Trypanosoma gambiense* of sleeping sickness.

The two drugs are likewise being employed in the treatment of sleeping sickness in man, and the results so far obtained are distinctly encouraging.

PLIMMER and THOMSON, 'A Preliminary Summary of the Results of the Experimental Treatment of Trypanosomiasis in Rats' (Proc. Roy. Soc., July 20th, 1907), got the best results by combining Atoxyl and the different Mercury preparations (Sozoiodol, Donovan's solution), and certainly some of the rats were cured. They also used Iodipine. Plimmer and Thomson were able to confirm Ehrlich's experiments on atoxyl-resistance.

PLIMMER and THOMSON, 'Further Results of the Experimental Treatment of Trypanosomiasis in Rats' (Proc. of the Royal Soc., read Nov. 7th, 1907), recommend for the treatment of sleeping sickness Potassium antimonyl tartrate. Trypanosomes disappear very rapidly, but there is a bad effect on the rats treated. They

Antimon

suggest the use of Sodium antimonyl tartrate. Out of 25 rats which have been treated with this substance, 23 had no relapses from 25 to 26 days. No local disturbances are caused by the injection of the drug into rats, in doses of up to 0·5 centigramme.

In the hands of one of us (A. B.) Sodium antimonyl tartrate has not given the good results Plimmer and Thomson described. A fairly virulent strain of *T. equiperdum* was used. Out of twelve rats treated with two to four injections of 0·25 c.c. of a 1 per cent. solution (0·35 of the same solution was the fatal dose) only one rat which was treated during the incubation period of the disease is still alive, all the others having died ten to fifteen days after the last injection of the drug, their blood swarming with parasites. One horse infected with a strain of cattle trypanosomes, brought back from the Congo, has been under treatment since December 9th, having received five intramuscular injections, 10 c.c. of a 1 per cent. solution of Sodium antimonyl tartrate. It is still showing parasites in a very small number from time to time; they were seen even three days after the last injection. The local effects of the drug in rats are very severe: necroses and sloughing.

The parasites, however, disappear very rapidly indeed, generally speaking more so than after a corresponding injection of Atoxyl.

But notwithstanding our results, we consider that the introduction of another metal (Antimony) belonging to the same group as Arsenic is a further progressive step in the treatment of sleeping sickness, and is moreover very suggestive.

MY EXPERIENCE OF TRYPANOSOMIASIS IN EUROPEANS AND ITS TREATMENT BY ATOXYL AND OTHER DRUGS

BY

SIR PATRICK MANSON, K.C.M.G., F.R.S., &c.

(*Received January 23rd, 1908*)

In view of the recently recorded experiences of Campenhout, Broden, Kopke, Koch and others in the treatment of human trypanosomiasis, especially in negroes, by atoxyl, it may not be inopportune if I gave some account of my experience of this disease in Europeans, and of atoxyl and other drugs in its treatment.

My experience of trypanosomiasis in man extends to seventeen cases—seven negroes, ten whites. The negroes, who had been brought to Europe for purposes of clinical study, and because they had already entered on the terminal phase—sleeping sickness—of the infection, all died. They did not have the benefit of Thomas's important discovery of the therapeutic value of atoxyl. I shall not allude to them further.

Of the ten whites, three of the cases have been recorded already by myself and others. For the sake of completeness I shall briefly mention here these three cases, along with the seven unrecorded cases, giving them along with the latter in the order in which they came under my observation, but referring the reader to the medical journals for details.

I.—Mrs. H. M. was first seen by me on July 17th, 1901. She was then 40 years of age, and had resided on the Congo for two periods of two years and one year respectively. During the latter period she had suffered much from fever. Being pregnant at the time she came home, arriving in England in April, 1901. She had fever all the way home. A week after her arrival her child was born, and from that time till the date of her visit to me she had attacks of fever lasting for three days at a time and occurring at intervals of seven days with considerable regularity. She also suffered with pains in her

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