

LITERATURE

1. MONTGOMERY AND KINGHORN. Ann. Trop. Med. and Paras., 1908, 11, p. 97.
2. DUTTON AND TODD. The Liverpool School Trop. Med., Memoir XXI, 1906.
3. LAVERAN. Ann. Path., 1907, XXI, p. 347.
4. BRODEN. Rapport sur la Lab. de Leopoldville, Bruxelles, 1906.
5. MARTIN, LEROUEUX AND ROUBAUD. Bull. Soc. Path. Exot., 1908, 1, 6, p. 351.
6. DUTTON AND TODD. Thompson Yates and Johnston Lab. Rep., 1903, Vol. 5.
7. CAZALBOU. Rev. Gen. Med. Vet., 1907, IX, p. 564.
8. LAVERAN. C. R. Soc. Biol., 1905, p. 292.
9. BALFOUR. Report of the Wellcome Research Labs., 11, 1906.
10. LAVERAN. C. R. Acad. Sc., July, 1906, p. 94.
11. LAVERAN AND MESNIL. Les Trypanosomes et Trypanosomiasis, Paris, 1904.
12. ZIEMANN. Quoted by 17.
13. LAVERAN. Quoted by 15.
14. SCHILLING. Quoted by 17.
15. LÜHE. In Meuse, Handb. der. Tropenkrank., Bd. III, Leipzig, 1906.
16. SANDER AND HENNIG. Idem.
17. NABARRO. Translation of Laveran and Mesnil, Trypanosomes and Trypanosomiasis, London, 1907.
18. Report of the Sleeping Sickness Commission, Nos. 4, 5 and 6.
19. Quoted by 17.

EXPERIMENTS ON THE COMBINED ATOXYL - MERCURY TREATMENT IN MONKEYS INFECTED WITH TRYPANOSOMA GAMBIENSE

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(Received for publication 24 March, 1909)

Prolonged experience of Sleeping Sickness treatment in man, and to a certain extent in experimental animals, has proved beyond doubt that Atoxyl by itself effects a really permanent cure only in comparatively few and exceptionally favourable cases of Sleeping Sickness in man, and if administered over a very prolonged period. Nearly all experiences with horses and cattle infected with the different strains of pathogenic trypanosomes lead necessarily to the conclusion that Atoxyl alone is insufficient for a successful issue of the treatment, but only prolongs life to a certain extent, and that finally the animal nearly always succumbs to the disease.

Laveran's¹ experiments showing that a combination of Arsenious Acid and Trypanred was superior on small animals to the use of either drug by itself, led the way for further advance in the treatment of Trypanosomiasis. Thomas and Breinl² after the introduction of Atoxyl recommended the combination of Atoxyl and Trypanred, the latter being then, after Arsenic, the best trypanocide known.

The idea of a combined treatment in Trypanosomiasis was taken up later by Moore, Nierenstein and Todd,³ and their extensive study of the experimental treatment of Trypanosomiasis was directed by the assumption that "perhaps the recurrence in cases of trypanosome infection after treatment with Atoxyl might be due to some resistant stage of the parasite which survived the first treatment of Atoxyl

and later gave rise to recurrences of trypanosomes more or less refractory to Atoxyl treatment. The Atoxyl was, therefore, followed by a second drug; the best results, experimentally, were obtained by using mercury salts, after the disappearance of the parasites from the peripheral circulation had been brought about by Atoxyl. In small animals, such as rats and rabbits, infected with *T. brucei*, the results were far superior to treatment by Atoxyl alone; in large animals, as donkeys, on the other hand, the combined treatment with Atoxyl and Mercury was not found to be efficacious enough to be of practical value.

Plimmer and Thomson¹ repeated the experiments on rats infected with Surra and Ngana, obtaining similar results to those of Moore Nierenstein and Todd, but using different mercury salts. In their hands the combination of Atoxyl and Succinamide of Mercury gave the best results. In a further communication,² however, they state that, in small animals at any rate, Mercury has not given altogether satisfactory results, which fact they attribute to the small doses of Mercury. On enlarging the doses of Mercury, chronic kidney, and to a lesser degree, liver lesions, were observed. They conclude, therefore, that more favourable results might be obtained in dealing with a more chronic trypanosome disease, such as Sleeping Sickness in man.

Laveran's and Thiroux's³ observations on the combined treatment of Atoxyl and Bichloride of Mercury on Surra in guinea-pigs are, although not very good, superior to the treatment by Atoxyl alone.

According to Uhlenhuth, Hübner and Woithe,^{4,5} the combined treatment on rats infected with Dourine was of considerable value. Criticising Laveran's and Thiroux's experiments, they remark that in guinea-pigs, Atoxyl treatment in general does not give good results, this being due to the fact that these animals very often die after one injection of Atoxyl without any apparent reason.

The good results obtained by the combined treatment of Atoxyl and Mercury in experimental animals, justified its use in human Trypanosomiasis. The Segregation Camps for the medical treatment of Sleeping Sickness in Uganda, gave this method a thorough trial.

Mercury was administered in different forms and at different

intervals; in one series of cases concurrently with the Atoxyl, in another series some time after the last injection of Atoxyl. It is of interest to compare the different modes of administration and the results obtained either with Atoxyl alone or with Atoxyl followed by Mercury. The following methods have been used since the camps were started:—

I. ATOXYL ONLY

- (a) 0.4 gm. every twentieth and twenty-first day.
- (b) 0.4 gm. every tenth and eleventh day.
- (c) 0.4 gm. increasing gradually every tenth and eleventh day up to 0.7 gm.
- (d) Van Campenhout's method (very similar to method (c)).
- (e) 1 gm. every fifteenth and sixteenth day.
- (f) 0.6 gm. every fifteenth and sixteenth day.

II. ATOXYL AND MERCURY

Course of Atoxyl treatment lasting a month or six weeks, during which time at least 4 gm. of the drug were given, followed by—

- (g) Mercury perchloride, $\frac{1}{8}$ grain, twice daily (Dr. J. Collins).
- (h) Mercury perchloride, $\frac{1}{4}$ grain, hypodermically, for six doses spread over fourteen days (Dr. Collins).
- (i) Metallic Mercury (Lambkin's cream), 5 minims once a week (Dr. C. J. Baker).

- (j) Intramuscular injections of 1 c.c. of a 1 per cent solution of soluble mercury salts, repeated every five days (Drs Goodliffe and Bayon).

Combined simultaneous Atoxyl and Mercury treatment (Dr. van Someren)—

- (k) First day, Atoxyl, 1 gm.; Mercury perchloride, 0.01 gm.
- Second day, Atoxyl, 1 gm. On fourteenth day, Atoxyl, 0.5 gm.; Mercury perchloride, 0.01 gm. On fifteenth day, Atoxyl, 0.5 gm.; repeating every fourteenth and fifteenth day.

- (l) Same as above, except that one initial dose of 1 gm. Atoxyl is given, the remaining doses being 0.5 gm.

A comparison of the results obtained at the various camps with Atoxyl and Atoxyl and Mercury treatment, during the period December, 1906, to November, 1907, is seen in the following table (Table VII, p. 8, of the report):—

Present State on February 29, 1908.	BUSHO. (Dr. Collins.)		KVAGWE. (Dr. van Someren.)		USOGA. (Dr. C. J. Baker.)	
	Atoxyl only. Method (g) or (h)	Per cent.	Atoxyl and Mercury. Method (h) or (i).	Per cent.	Atoxyl only. Method (h), few (i).	Atoxyl and Mercury. Method (i)
Improved	...	8	19	40.5	35	30
Relapsed	...	6	15	11	7	5.5
Continue in same state	...	38	62	12.5	3	40
Absent at time of examination	...	3	—	21	20	—
Died	...	45	4	15	35	25.5
Number of cases	...	382	100	328	252	73

The following table shows the comparative results from November-February, 1908, wherein the state of the disease is classified. A, meaning very early cases without symptoms, except gland enlargement; B, early cases with symptoms, itchy skin, &c.; C, advanced cases; D, very advanced cases (Table XVIII, p. 19, of the report):—

Present State on February 29, 1908.	CLASS OF CASE ON ADMISSION.							
	A		B		C		D	
	Atoxyl only.	Per cent.	Atoxyl only.	Per cent.	Atoxyl only.	Per cent.	Atoxyl only.	Per cent.
Improved	42	60.5	35	63	24	41	7	12
Relapsed	1	3	4	4	3	7	—	—
Continue in same state	50	23	46	23	56	36	53	44
Absent at time of examination	3	10	5	8	5	6	8	—
Died	4	3.5	10	2	12	10	32	44

The method of treatment according to 'improved' cases is, therefore:—

- I. Method (k), 64 per cent.
 - II. Method (j), 58 per cent.
 - III. Method (g), 39 per cent.
 - IV. Method (i), 34.5 per cent.
 - V. Method (b), 34 per cent.
 - VI. Method (b), 32 per cent.
 - VII. Method (f), 10 per cent.
- Atoxyl and Mercury.
Atoxyl alone.

Bohne¹⁰ describes one case of trypanosome fever which improved markedly under the combined Atoxyl-Mercury treatment, but the time of observation is too short to conclude that this patient has been definitely cured.

Broden and Rodhain,¹¹ on the other hand, come to the conclusion that the combined treatment did not prove superior at all to a treatment with Atoxyl alone.

As Moore's, Nierenstein's and Todd's work was mostly carried out on animals infected with *T. brucei*, it seemed advisable to repeat the work on monkeys infected with *T. gambiense*. Six animals (*Cercopithecus callithrix*) were inoculated with our laboratory strain of *T. gambiense*, and after varying time subjected to treatment. Acetylated Atoxyl, which, in former experiments, had proved itself less toxic than Atoxyl, was used.

EXPERIMENT I.—*Cercopithecus callithrix*, ♀, weight 2 k. 700 gm., was inoculated on April 28th with *T. gambiense*. At the time of the third relapse, on June 1st, treatment was begun after the animal had shown typical signs of the infection; the animal was markedly anæmic, and had lost over 300 gm. in weight. On June 1st and 2nd, an injection of 0.1 gm. of acetylated Atoxyl was given. The parasites disappeared after the first injection. On June 10th and 18th, the Atoxyl was followed by an intra-muscular injection of 0.05 gm. of Sublimat. On July 2nd and 3rd, the Atoxyl injection, and on July 15th and 16th the injections of Sublimat were repeated. Up to November, daily examinations of the blood were made, and since then the peripheral blood has been examined twice weekly. No trypanosomes have been seen since June 1st, the day of the injection. The blood count is normal (March 7th). The animal has increased in weight to 2 k. 845 gm., which has been maintained with slight variations since the middle of November.

EXPERIMENT II.—*Cercopithecus callithrix*, ♀, weight 2 k. 700 gm., was inoculated on April 28th with *T. gambiense*. The animal never showed parasites in great number. On June 16th, treatment was begun, at the symptoms of the disease became more and more marked. On June 6th and 10th, 0.1 gm. of acetylated Atoxyl was administered; these injections were followed on June 18th and 19th by 0.05 gm. of Sublimat, intra-muscularly in the gluteal region. The second injection of Sublimat was followed by complete paralysis of the hind legs.

which was most probably due to an injury of the sciatic nerve through the injection. This paralysis, however, passed off very gradually, and after two and a half months the mobility of the leg was normal. On July 2nd and 3rd, the acetylated Atoxyl injections were repeated, and on July 15th and 16th the intra-muscular injections of Sublimate. The animal is still alive, and parasites have never been seen since the first inoculation. The blood is normal, and the weight is at present 2 k. 895 gm.

EXPERIMENT III.—*Cercopithecus callithrix*, ♀, weight 3 k. 600 gm., was inoculated on April 28th, 1908, with *T. gambiense*. Treatment was started on June 6th, the time of the third relapse. 0.15 gm. of acetylated Atoxyl was administered. This injection was repeated on June 10th, and followed on June 18th and 19th by intra-muscular injections of 0.075 gm. of Sublimate. The Atoxyl injections were repeated on July 2nd and 3rd, and the Sublimate injections on July 15th and 16th. This animal is still alive; parasites have never been seen since the treatment was started. The blood count is at present normal, and the weight, which had increased by July 21st to 3 k. 850 gm., has remained constant since November at 3 k. 600 gm.

EXPERIMENT IV.—*Cercopithecus callithrix*, ♂, weight 1 k. 800 gm., was inoculated on April 28th. Treatment was started on June 3rd with injections of 0.1 gm. of acetylated Atoxyl, followed by an administration on June 8th of 0.01 gm. of Sublimate in pill form, by the mouth. A very severe attack of diarrhoea, which lasted from June 9th–12th, followed this medication; the stools were slimy and haemorrhagic. On June 18th and 19th, 0.1 gm. of acetylated Atoxyl was given, followed on June 23rd by one injection of 0.05 gm. of Sublimate. On July 2nd and 3rd, 0.1 gm. of acetylated Atoxyl was given, followed on July 15th and 16th by 0.01 of Sublimate, intra-muscularly. The Sublimate injections were not followed by any untoward effects. The animal is still alive; parasites disappeared after the first injection of Atoxyl, and have never been seen since. The blood is normal, and the weight 2 k. 100 gm.

EXPERIMENT V.—*Cercopithecus callithrix*, ♀, weight 1 k. 900 gm., was inoculated on April 28th. At the time of the second relapse on June 3rd, treatment with acetylated Atoxyl was begun. Two injections of 0.1 gm. on June 3rd and 4th were followed by an administration of 0.1 gm. of Sublimate in pill form by the mouth. In this case, as in Experiment IV, a very severe diarrhoea with slimy and haemorrhagic stools occurred, which, however, passed off in a few days. The Atoxyl injections were repeated on June 18th and 19th, followed on June 26th and 27th by an intra-muscular injection of 1 c.c. of Donovan's solution. On July 2nd and 3rd the Atoxyl injections were repeated, followed on July 15th and 16th by an injection of 1 c.c. of Donovan's solution. The parasites disappeared from the peripheral circulation of the animal after the first injection, and were never seen again. The animal was found dead on July 17th. The post-mortem showed fatty degeneration of the heart; the spleen was slightly enlarged, of firm consistence, with well-marked hypertrophic malpighian follicles. The kidneys were enlarged and markedly congested, the cortical substance was not defined from the medullary substance, and haemorrhagic stripes intersected the yellow cortical substance. Microscopically the liver showed typical fatty degeneration of the liver cells; the kidneys showed on section a well-marked parenchymatous nephritis. The death of the animal was due in all probability to Mercury poisoning.

EXPERIMENT VI.—*Cercopithecus callithrix*, ♀, weight 2 k. 750 gm., was inoculated on June 6th with *T. gambiense*. Treatment was started on June 18th with a view to ascertaining whether a short treatment at an early stage can effect a permanent cure. 0.1 gm. of acetylated Atoxyl was given, and the same dose repeated on June 18th. The parasites, which were fairly numerous before treatment, disappeared promptly. In this case, however, the acetylated Atoxyl

produced a harmful effect. The doses which proved perfectly harmless in the foregoing experiments caused the whole complex of symptoms of a typical Atoxyl poisoning. Tremors occurred all over the body, and, at the same time, a severe slimy diarrhoea started. These symptoms, however, passed off in the course of a week. On June 26th and 27th, injections of 1 c.c. of Donovan's solution was given. This animal is still alive, weighing at present 3 k. 100 gm. The blood is normal; parasites have never been seen since treatment was begun.

The foregoing experiments show that in five cases out of six, the administration of Acetylated Atoxyl and Sublimate, and Donovan's solution, in monkeys (*Cercopithecus callithrix*), has effected a complete cure.

If we consider that in our experiments *Cercopithecus callithrix* usually succumb to an infection of *T. gambiense* in the course of 40–60 days, the results of Atoxyl-Mercury treatment in monkeys infected with *T. gambiense* must be looked upon as very encouraging indeed.

The length of time which has elapsed since treatment was discontinued is probably sufficient to permit of the animals being considered as definitely cured of the disease.

Concerning the action of the combined treatment by Atoxyl and Mercury, a conclusive opinion can hardly yet be expressed. Mercury has proved beyond doubt to have not the least effect on trypanosomes in the peripheral circulation. It is, therefore, very tempting to accept Moore's, Nierenstein's and Todd's suggestion of its action on a secondary resistant form of the parasite which it destroys; but whether we accept this hypothesis or suppose that the Mercury acts merely as an internal disinfectant after the destruction of the parasites by Atoxyl, the success of the combined treatment seems to depend upon the administration of the two drugs either concurrently or in rapid succession.

Volume II

May, 1909

No. 5

ANNALS
OF
TROPICAL MEDICINE AND
PARASITOLOGY

ISSUED BY

THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

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