DIFFERENT AMOUNTS OF TRANSFORMED ATOXYL PRODUCED BY INCUBATING ONE PER CENT. AND TEN PER CENT. ATOXYL IN BLOOD.*

By B. T. TERRY, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

INTRODUCTION.

Atoxyl in 5 per cent. solution *in vitro* has at room temperature no influence on trypanosomes for several hours, but dissolved in blood and incubated at 37° C. for 2 or 3 hours it gives rise to a substance (transformed atoxyl, or trypanotoxyl) that is quite toxic for these parasites.

This toxic substance has probably not been isolated in purity from the blood atoxyl mixture, but the relative amounts of it present in different solutions may be estimated by seeing to what extent these solutions may be diluted before the toxic influence on the trypanosomes disappears.

While working in 1910 in the George Speyer House, Frankfurt a/M., on a problem suggested by Professor Ehrlich, it became desirable to discover whether the strength of the atoxyl which was incubated with blood had any influence on the amount of transformed atoxyl produced. A number of different strengths were tested, first in Frankfurt, and later at The Rockefeller Institute, but most of my experiments were with 1 per cent. and 10 per cent. atoxyl in blood, and it is the results with these two strengths that will be reported in this paper. Moreover, as the technique in the later experiments yielded results that were somewhat easier to tabulate than those obtained in the earlier experiments, the tables given in this paper will be taken exclusively from the experiments done at The Rockefeller Institute. The results reported are, however, in exact accord with those obtained by me in the George Speyer House.

* Received for publication, December 30, 1914.

TECHNIQUE.[1]

The atoxyl was dissolved in fresh rabbit blood to make 1 and 10 per cent. solutions, respectively. These solutions in blood were incubated at 37° C. for 1 to 3 hours, diluted with salt solution (0.9 per cent.), so as to give, after the addition of 0.5 c.c. of a standard trypanosome suspension to each 0.5 c.c. of dilution, end dilutions of 1:500, 1,000, 2,000, 4,000, 8,000, and 16,000.

It should be noted that these end dilutions contained some unaltered atoxyl and varying numbers of rabbit corpuscles, the number decreasing as the dilution increased. The corpuscles were usually not removed as the rest of the experiment was of short duration and was to be carried out at room temperature. Under these conditions it was thought that the small number of corpuscles present would at this temperature have a negligible influence on the unchanged atoxyl. Whether or not this assumption was justified will be seen in the results reported in this paper.

The adding of the trypanosomes to each of these dilutions of transformed atoxyl was so timed that the examination of each dilution could be made after exactly 30, 60, and 90 minutes' contact with these parasites. The influence which the dilution exerted on the trypanosomes was then recorded in terms of motility of the parasites, this being reckoned on a per cent. basis. 100 per cent. indicates that the trypanosomes were perfectly active, 0 per cent. that all of them had been immobilized. Intermediate influences were indicated by intermediate percentages.

The Tables.—In interpreting the results in the tables we should recall:

1. That the dilutions of atoxyl in the tables are comparable, for they represent in all cases the number of times one part of atoxyl has been diluted. To obtain these dilutions the 10 per cent. solution was in each instance diluted ten times as much as the 1 per cent. solution.

2. The motility per cent. of the trypanosomes is in inverse ratio to the amount of free toxin present in the dilution. Great motility of the trypanosomes indicates the absence of much toxicity, but little or no motility shows the presence of much toxin.

3. To make the comparison of the results more striking the figures which indicate a greater production of toxin by one solution than by the other are printed in heavier type.

Incubation for One Hour.—When 1 per cent. atoxyl in blood and 10 per cent. atoxyl in blood were incubated at 37° C. for 1 hour it was at once obvious that the 10 per cent. atoxyl solution produced distinctly more than 10 times as much free toxin (transformed atoxyl) as the 1 per cent. atoxyl solution. This result has been obtained many times and three typical experiments showing it are recorded in table I.

[1] The technique of my experiments with atoxyl is given in greater detail in a paper entitled: The Effect of Heat on the Transforming and Binding Power of Blood (Terry, B. T., *Jour. Exper. Med.*, 1915, xxi, 267).

TABLE I.

No.	Method.	Dilution 4,000. Motility per cent.	Dilution 2,000. Motility per cent.	Dilution 1,000. Motility per cent.	After contact for min.
9.100	A (1%, 37°, 1 hr.)	100	100	100	30
		100	100	100	60
		100	100	50	90
9.101	A (10%, 37°, 1 hr.)	100	**40**	**20**	30
		30	**10**	**0**	60
		0	**0**	**0**	90
9.114	A (1%, 37°, 1 hr.)	100	100	100	30
		100	100	100	60
		100	100	60	90
9.115	A (10%, 37°, 1 hr.)	100	**50**	**10**	30
		50	**20**	**0**	60
		20	**0**	**0**	90
9.134	A (1%, 37°, 1 hr.)	100	100	100	30
		100	100	100	60
		100	30	5	90
9.135	A (10%, 37°, 1 hr.)	100	**30**	**0**	30
		30	**0**	**0**	60
		0	**0**	**0**	90

In table I we observe that the motility of the trypanosomes was but little affected in the dilutions from the 1 per cent. atoxyl. In these dilutions after contact for 30 and 60 minutes no effect was seen, but after contact for 90 minutes an effect was observed in four instances, three times in the dilution 1 to 1,000, and once in the dilution of 1 to 2,000.

In striking contrast was the effect produced by the dilutions made from the 10 per cent. atoxyl. In dilutions of 1 part in 1,000 and 1 in 2,000 the motility was decreased markedly after a contact of 30 minutes, and in two of the three experiments the trypanosomes were completely immobilized after 90 minutes' contact with dilutions of 1 part in 4,000. If we look at the figures printed in heavier type we see that in twenty-four instances out of a possible twenty-seven, the dilutions of the 10 per cent. atoxyl showed more toxicity than the corresponding dilutions of the 1 per cent. atoxyl. In the remaining three instances on account of the greatness of the dilution (1 to 4,000) and the shortness of the contact (30 minutes) no perceptible effect on the trypanosomes was seen in the dilutions either of the 1 per cent. or of the 10 per cent. solutions.

Incubation for Three Hours.—Where the incubation of the 1

and 10 per cent. atoxyl solutions in blood was for 3 hours instead of for 1, the result was quite different, as will be seen in table II, in which the results of three experiments are recorded. Some of the dilutions of the 10 per cent. solution continued to be more toxic than those of the 1 per cent. solution, but the differences in the toxicity of the dilutions was no longer great. In seven instances the dilutions of the 10 per cent. solution were more toxic than those of the 1 per cent. solution, but in one instance a dilution, 8,000, of the 1 per cent. solution was more toxic than the corresponding dilution of the 10 per cent. solution.

TABLE II.

No.	Method.	Dilution 8,000. Motility per cent.	Dilution 4,000. Motility per cent.	Dilution 2,000. Motility per cent.	After contact for min.
9.96	A (1%, 37°, 3 hrs.)	100	100	60	30
		50	10	0	60
		10	0	0	90
9.97	A (10%, 37°, 3 hrs.)	100	**40**	**20**	30
		50	10	0	60
		10	0	0	90
9.118	A (1%, 37°, 3 hrs.)	100	100	50	30
		50	20	**0**	60
		20	0	0	90
9.119	A (10%, 37°, 3 hrs.)	100	**80**	**20**	30
		50	20	0	60
		40	0	0	90
9.138	A (1%, 37°, 3 hrs.)	100	80	20	30
		30	10	0	60
		0	0	0	90
9.139	A (10%, 37°, 3 hrs.)	100	**20**	**0**	30
		30	**0**	0	60
		0	0	0	90

If we examine table II more closely we note that of the seven instances in which the dilutions of the 10 per cent. atoxyl were more toxic than those of the 1 per cent., six were seen where the contact with the trypanosomes was shortest; *i. e.*, 30 minutes; that the seventh instance was seen after a contact of 60 minutes; and that the one instance in which a dilution of the 1 per cent. was more toxic than the corresponding dilution of the 10 per cent. solution, was observed after a contact of 90 minutes.

These observations suggested that the 10 per cent. dilutions were probably more toxic immediately after incubation than the 1 per cent. dilutions, but that the 1 per cent. dilutions became correspondingly more and more toxic the longer they were allowed to stand. If this were really the case the increased toxicity was probably due to the red blood corpuscles continuing to act after the incuba-

tion was over. This explanation seemed the more probable because the number of red blood corpuscles in the dilutions of the 1 per cent. solution was ten times as great as those in the 10 per cent. solution.

It was easy to test the influence which the red blood corpuscles might exert after incubation, for the corpuscles could be removed at once by centrifugalization and the corpuscle-free solutions could be tested as before and the figures compared. This was done and the results after 3 hours' incubation are seen in table III.

TABLE III.

No.	Method.		Dilution 8,000. Motility per cent.	Dilution 4,000. Motility per cent.	Dilution 2,000. Motility per cent.	After contact for min.
9.98	A (1%, 37°, 3 hrs.)	CC	100	100	100	30
			100	100	60	60
			100	70	10	90
9.99	A (10%, 37°, 3 hrs.)	CC	100	**40**	**20**	30
			60	**10**	**5**	60
			10	**0**	**0**	90
9.120	A (1%, 37°, 3 hrs.)	CC	100	100	100	30
			100	100	100	60
			100	100	70	90
9.121	A (10%, 37°, 3 hrs.)	CC	100	**80**	**10**	30
			80	**10**	**0**	60
			20	**0**	**0**	90
9.140	A (1%, 37°, 3 hrs.)	CC	100	100	100	30
			100	100	30	60
			100	80	0	90
9.141	A (10%, 37°, 3 hrs.)	CC	100	**30**	**5**	30
			30	**0**	**0**	60
			30	**0**	**0**	90

CC means clear centrifugalized fluid containing transformed atoxyl but no corpuscles.

In table III we note that after 3 hours' incubation the 10 per cent. atoxyl dilutions are as much more toxic than the 1 per cent. dilutions, as they were found to be in table I when the incubation period was only 1 hour. In both table I and table III the 10 per cent. solutions were in twenty-four instances out of a possible twenty-seven decidedly more toxic than the corresponding dilutions of the 1 per cent. solution, and in both tables in three instances the effect on the trypanosomes was too slight to be detected.

As the results in table II differ markedly from those in table III, and as the removal of the corpuscles by centrifugalization is the only difference in the technique, we conclude that it was the corpuscles in table II which led to the relatively increased toxicity seen in the dilution of the 1 per cent. atoxyl.

If the presence of corpuscles could give misleading results when the incubation period was 3 hours, it was possible that they might do the same when the incubation was for 1 hour. It became desirable therefore to repeat the experiments recorded in table I, but this time removing the corpuscles by centrifugalization immediately after incubation. This was done and the results obtained in this way are recorded in table IV.

TABLE IV.

No.	Method.		Dilution 2,000. Motility per cent.	Dilution 1,000. Motility per cent.	Dilution 500. Motility per cent.	After contact for min.
9.102	A (1%, 37°, 1 hr.)	CC	100	100	100	30
			100	100	100	60
			100	100	100	90
9.103	A (10%, 37°, 1 hr.)	CC	100	80	20	30
			60	0	0	60
			30	0	0	90
9.116	A (1%, 37°, 1 hr.)	CC	100	100	100	30
			100	100	100	60
			100	100	100	90
9.117	A (10%, 37°, 1 hr.)	CC	100	70	5	30
			70	10	0	60
			30	0	0	90
9.136	A (1%, 37°, 1 hr.)	CC	100	100	100	30
			100	100	100	60
			100	100	100	90
9.137	A (10%, 37°, 1 hr.)	CC	100	30	0	30
			30	0	0	60
			10	0	0	90

Table IV shows plainly that after incubation for one hour and in the absence of all corpuscles the dilutions of the 10 per cent. atoxyl were much more than 10 times as toxic as the corresponding dilutions of the 1 per cent. atoxyl. We note that even after 90 minutes' contact with trypanosomes none of the dilutions of the 1 per cent. atoxyl showed the slightest evidence of toxicity in the lowest dilution examined, 1:500, whereas in all three experiments with 10 per cent. atoxyl the parasites were completely immobilized in 90 minutes in dilutions of 1:1,000.

It is evident from the experiments here reported that the strength of the atoxyl solution in blood may have a marked effect on the result. If it is desired to produce a large quantity of transformed atoxyl by incubating with blood for only 1 to 3 hours at 37° C., a strong solution of atoxyl (10 per cent.) should be chosen in preference to a weaker one (1 per cent.).

Suggested Explanation.—It may not be out of place to suggest at this point the explanation which seems to me to account most satisfactorily for the results here reported even though this explanation cannot be said to have been proved in every particular and is based in part on experiments not recorded in this paper. It is as follows: Red blood cells transform atoxyl and have a strong affinity for the transformed atoxyl. They unite, therefore, with transformed atoxyl, the quantity with which they can unite being considerable but having definite limits. Not until these limits have been passed does the fluid in which the corpuscles are suspended become toxic for trypanosomes, for the union of the red blood corpuscles and the transformed atoxyl seems to be firm. Blood that has bound as much transformed atoxyl as it can, continues for a time at least to transform atoxyl, and this transformation takes place even at room temperature, although it is faster at 37° C.

Application to Tables I and IV.—The small toxicity shown by the dilutions of 1 per cent. atoxyl is due to the fact that in 1 hour's incubation at 37° not quite enough transformed atoxyl is produced to satisfy the red blood cells. If, however, these cells are not removed from the dilutions (table I) they continue to transform the atoxyl during the time the dilutions are acting on the trypanosomes, so that after 90 minutes a little free toxin is present. In the case of the 10 per cent. solutions the transformation goes on more rapidly, and the point at which the corpuscles cease to take up transformed atoxyl is reached sooner. Thereafter the transformed atoxyl remains in solution and acts upon the trypanosomes. By the end of the first hour there is considerable free toxin in solution. As the 10 per cent. solution produces more toxin than the 1 per cent. solution, and as all, or nearly all, the toxin produced by the 1 per cent. solution is taken up by the corpuscles, the 10 per cent. solution appears to have produced many times as much transformed atoxyl as the 1 per cent., although in reality it may not have produced quite ten times as much.

This explanation should also be borne in mind when atoxyl is used in treatment of trypanosome infections. If a small dose is given it is quite possible that all the atoxyl transformed will be taken up by the cells of the host and that none will be available for

the trypanosomes. On the other hand, a large single dose may prove very much more toxic for the host than two half doses, for if a single large dose is given, the cells of the body probably bind what they can of the toxin they produce and the rest is free to poison the trypanosomes and cells of the host that may have an affinity for the transformed atoxyl. If the dose is divided and given on different days, the host may on the first day transform half as much as if the full dose were given; but since the body cells must be satisfied, the amount of free toxin present will not be half of the free toxin produced by the full dose. In 24 hours we assume that much or all of the transformed atoxyl will be bound or excreted. When, therefore, the second half dose is given, free toxin is again produced, but much of this will probably be bound by the body cells before any will be free in fluids which have already lost most or all of the toxin produced when the first half dose was given.

In this connection Koch's[2] experience in Africa may be referred to. At one time Koch injected into his sleeping sickness patients doses varying from 0.5 of a gram to 1 gram. In some of the patients that received the larger doses Koch observed a symptom he had never seen in patients that had received only 0.5 of a gram. Some of those receiving the larger doses became permanently blind in both eyes. As soon as Koch was convinced that the blindness was due to the large doses, he reduced these to 0.5 of a gram given on two succeeding days, and he states that no further cases of blindness resulted.

Before concluding the paper one other point should be mentioned. The four tables show that leaving the corpuscles in with the atoxyl and transformed atoxyl made a great difference in the result when the dilutions were from the 1 per cent. atoxyl in blood. On the other hand, the presence of corpuscles in the dilutions made from the 10 per cent. atoxyl in blood did not alter the result materially. This, of course, is due to the fact that the corpuscles were 10 times as numerous in the dilutions from the 1 per cent. atoxyl as they were in the dilutions from the 10 per cent. atoxyl.

[2] Koch, R., *Deutsch. med. Wchnschr.*, 1907, xxxiii, 1889.

SUMMARY.

1. 10 per cent. atoxyl in blood incubated at 37° C. for 1 hour gives rise to a solution that is much more than ten times as toxic as a 1 per cent. solution of atoxyl similarly incubated.

2. When the comparison is made after incubation for 3 hours instead of for 1 hour, the toxicity of the 10 per cent. solution is but slightly greater than ten times that of the 1 per cent., provided the red blood corpuscles are not removed from the dilutions.

3. If the corpuscles are removed from both the 10 per cent. and the 1 per cent. atoxyl solutions immediately after incubation at 37° for 1 to 3 hours, the dilutions of the 10 per cent. atoxyl are much more than ten times as toxic as the corresponding dilutions of the 1 per cent. atoxyl.

4. After incubation with atoxyl at 37° for 1 to 3 hours, red blood corpuscles left at room temperature in dilutions made from the 10 per cent. and 1 per cent. solutions in blood increase markedly the toxicity of the dilutions made from the 1 per cent. atoxyl, but increase very slightly the toxicity of the dilutions made from the 10 per cent. atoxyl.

5. If one desires to produce a large amount of transformed atoxyl by incubating atoxyl in blood at 37° for 1 to 3 hours, strong solutions of atoxyl should be chosen in preference to weaker solutions.