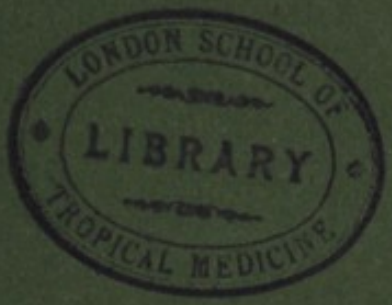
A modification of diphtheria antitoxin / by A.T. Glenny.

No. 66

A MODIFICATION OF DIPHTHERIA ANTITOXIN



BY

A. T. GLENNY, B.Sc.

(*"Journ. of Hygiene," Vol.* xiii., *No.* 1, *April* 30, 1913)

From

THE WELLCOME PHYSIOLOGICAL RESEARCH LABORATORIES
BROCKWELL HALL
HERNE HILL
LONDON, S.E.

[From THE JOURNAL OF HYGIENE, Vol. XIII, No. 1, April 30, 1913.]

A MODIFICATION OF DIPHTHERIA ANTITOXIN.

By A. T. GLENNY, B.Sc.

(Preliminary Communication.)

(From the Wellcome Physiological Research Laboratories, Brockwell Hall, Herne Hill, London.)

One of the fundamental features of Ehrlich's method of standardising diphtheria antitoxic serum is the recognition of the so-called Lo and L+ limits, the L+ dose being the quantity of a toxin which when mixed with one unit of antitoxin and injected subcutaneously into a guinea-pig leaves sufficient excess of toxin free to cause the death of the animal about the fifth day after injection, the Lo dose being the largest quantity which when similarly mixed with one unit of antitoxin fails to produce a local reaction. In the region between the two doses, the so-called "Differential-Region" of Ehrlich, one can obtain a series of mixtures which, while they kill the guinea-pig later than the fifth day or not at all, cause local reactions of varying severity increasing as the L+ limit is reached.

It should be noted that Ehrlich draws attention to the fact that the oedema produced by these intermediate mixtures is far less severe than that produced by doses of unmixed toxin, even when these are small fractions of the M.L.D., and that it practically never terminates in extensive skin necrosis as does the reaction to toxin alone.

In the standardisation of an ordinary specimen of diphtheria antitoxic serum, when graduated doses are mixed with one test dose of toxin, the local and general effects obtained are of the type shown in Table I.

For a given toxin the volume of any normal diphtheria antitoxic serum, necessary for complete neutralisation of one test dose, bears a fairly constant ratio to the volume of the same serum which, when

mixed with one test dose, leaves one fatal dose free; in other words, to the volume containing one unit of antitoxin. This ratio corresponds to that between the L+ and Lo doses of the toxin in question. In the example given above the ratio would be as 1·2 is to 1·0.

TABLE I.

	Units of antitoxin	Toxin	Local reaction	Death
	0·95	1 test dose	Very large swelling	Death on 3rd day
L+ mixture	1·00	,,	Large swelling	,, 5th day
	1·05	,,	,, ,,	,, 10th–20th day
	1·10	,,	Moderate swelling	Survival
	1·15	,,	Small swelling	,,
Lo mixture	1·20	,,	No swelling	,,

The purpose of this note is to call attention to the behaviour of certain sera, the effects of which upon the action of toxin differ from the above type in that they possess in a relatively high degree the property of neutralising the power of diphtheria toxin to produce local reaction, but have comparatively little effect upon its lethal power. The serum exhibiting this anomaly in the most marked degree was obtained under the following conditions.

In the course of a study of the rate of deterioration in unit value of diphtheria antitoxic serum at various temperatures over a long period, the result of which I hope shortly to publish, I have had occasion to test serum which has been kept for seven years at 37° C. Table II shows the results of tests of a certain serum after different periods at this temperature. The serum has been kept in hermetically sealed phials and is sterile. The initial unit value was 440 units.

These tests were made against a toxin (J. 967) with average minimal lethal dose 0·004 c.c., Lo 0·33 c.c., and L+ 0·40 c.c., and injections of mixtures of fresh serum and this toxin usually gave rise to local reactions if less than 1·2 units of antitoxin were present in the mixture with one test dose of toxin.

The history of other batches of serum which have been kept for a considerable period at high temperatures is very similar and one more example is given in Table III.

It will be seen that such sera have no true Lo value and consequently the "differential region" no longer exists.

Progressive stages of modification are now being traced in a batch of horse serum kept at 37° C. and tested every few months. Successive tests have shown a diminution in size of the local reactions caused by

L+ mixtures, and it is hoped that one may be able to trace the gradual reduction of the "differential region" until the mixture with just enough serum to produce no local reaction is identical with the L+, and further modification until such a mixture becomes acutely lethal.

TABLE II.

Diphtheria antitoxic serum (*Series E* 148). *Original value* 440 *units.*

Tests made at the end of 4½ years at 37° C.

Serum	Toxin	Local reaction	Death
1/200 c.c.	1 test dose	No record taken	2 days
1/100	,,	,,	3 ,,
1/75	,,	No swelling	7 ,,
1/50	,,	,,	20 ,,

Tests made at the end of 6¾ years at 37° C.

1/100 c.c.	1 test dose	No record taken	2 ,,
1/75	,,	,,	2 ,,
1/50	,,	No swelling	3 ,,
1/40	,,	,,	3 ,,
1/30	,,	,,	13 ,,

Tests made at the end of 7 years at 37° C.

1/60 c.c.	1 test dose	Very small swelling	2 ,,
1/50	,,	No swelling	2 ,,
1/45	,,	,,	2½ ,,
1/40	,,	,,	3 ,,
1/35	,,	,,	7 ,,
1/35	,,	,,	9 ,,
1/30	,,	,,	16 ,,
1/30	,,	,,	17 ,,
1/25	,,	,,	16 ,,
1/25	,,	,,	23 ,,
1/20	,,	,,	26 ,,
1/10	,,	,,	Survival

TABLE III.

Diphtheria antitoxic serum (*Series* 469). *Original value* 470 *units per c.c.*

Tests made at the end of 4½ years at 37° C.

Serum	Toxin	Local reaction	Death
1/75 c.c.	1 test dose	No swelling	3 days
1/50	,,	,,	8 ,,

Tests made at the end of 6¾ years at 37° C.

1/60 c.c.	1 test dose	No swelling	3 days
1/50	,,	,,	7 ,,
1/40	,,	,,	15 ,,

The cases quoted so far are those of very old sera kept under special conditions until the unit value (judged by the L+ dose) has fallen very low. This modification of antitoxin, although most frequently observed in such old sera, is not confined to them alone.

TABLE IV.

Tests upon samples of blood from guinea-pig T 3. 7. 12 actively immunised to diphtheria toxin.

	Date of removal of sample of blood	Volume of blood	Volume of toxin	Local reaction	Death	
	21 Aug.	1/7 c.c.	0·01 c.c.	Very large swelling	Death in 4 days	
	25 Sept.	,,	0·01	Large swelling	Survival	
	1 Oct.	,,	0·01	Small swelling	,,	
	4 ,,	,,	0·02	Very large swelling	Death in 4 days	
	21 ,,	1/70 c.c.	0·01	,, ,,	,, 5½ days	
	23 ,,	,,	0·02	No swelling	Survival	
	23 ,,	,,	0·05	Large swelling	Death in 8 days	
Modification of antitoxin	25 ,,	,,	0·05	No swelling	Survival	
	25 ,,	,,	0·1	,,	Death in 19 days	
	28 ,,	,,	0·1	,,	Survival	Probable period of maximum immunity
	28 ,,	,,	0·15	,,	Death in 16 days	
	28 ,,	,,	0·2	Small swelling	,, 3 ,,	
	30 ,,	,,	0·2	No swelling	,, 3 ,,	
	1 Nov.	,,	0·1	,,	Survival (paralysis)	
	1 ,,	,,	0·15	,,	Death in 13 days	
	5 ,,	,,	0·15	,,	,, 3 ,,	
	6 ,,	,,	0·1	,,	,, 20 ,,	
	11 ,,	,,	0·12	Small swelling	,, 4 ,,	
	21 ,,	,,	0·08	Large swelling	,, 4 ,,	
	2 ,,	,,	0·05	,,	,, 5 ,,	

Among routine tests upon the unit value of fresh antitoxic serum from horses, instances have been noted in which the local reactions caused by an L+ mixture were smaller than those usually observed. More frequently this phenomenon has occurred when testing the blood of guinea-pigs actively immunised to diphtheria toxin. These tests were made during an investigation still in progress by Dr H. J. Südmersen and myself. Table IV records the most marked instance that has occurred. At frequent intervals blood was withdrawn from the animal, mixed with diphtheria toxin, and injected subcutaneously into other guinea-pigs. For a time these tests followed the normal course, producing local reactions; then for a certain period marked modification of the antitoxin occurred; later the test resumed its normal course. It

should be noted that the modification of the antitoxin occurred round the period of maximum immunity.

Mention may be made of modified antitoxin appearing in human blood, but the case is still under investigation.

Conclusions.

The properties of the above mentioned sera seem to warrant two conclusions:

1. The constituent of diphtheria toxin which is acutely lethal in its action is not identical with that which causes the local reaction at the seat of injection.

2 The power of a serum to neutralise the acutely lethal constituent of a toxin may vary independently of its power to neutralise the constituent causing local reaction.

The possibility that antitoxin as well as toxin may be complex has already been suggested (*e.g.* by Pick and Schwoner, *Zeitschr. f. exp. Path. u. Ther.* I. p. 98, 1905), but, so far as I can trace, no evidence has yet been recorded of the existence of two forms of diphtheria antitoxin possessing different affinities for the lethal constituent of toxin and for that which causes local reaction. I hope that the continued study of the action of modified sera upon toxins of different ages will give further information upon the constitution of diphtheria toxin.