THE STABILITY OF TETANUS TOXIN IN 50 % GLYCERINE AND OF TETANUS ANTITOXIN IN SATURATED SALT SOLUTION.

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The Standard Tetanus toxin sent out by the Hygienic Laboratory of the United States Public Health Service is in the form of a dry powder, and the required quantities have to be weighed out.

This weighing must be done as quickly as possible, for the powder, being hygroscopic, is apt, on exposure, to absorb moisture from the air, in consequence of which the weighing is affected and the stock of standard toxin is liable to deteriorate in potency. This is a great inconvenience in the case of laboratories which are situated, as we are, where the air is usually moist. With the idea of simplifying matters a trial was made of a liquid toxin and as the results have so far proved satisfactory they are published as a possible matter of interest to other users of tetanus toxin.

Some of the Standard Tetanus toxin of the Hygienic Laboratory was, on January 17, 1921, dissolved in a mixture of equal parts of pure neutral glycerine and distilled water, and labelled G.T.T. 1. (Glycerinated Tetanus Toxin, No. 1.)

On January 21, 1921, a test was made to ascertain the M.L.D. of this mixture. Doses of 1/800 c.c., 1/900 c.c. or 1/1000 c.c. were injected subcutaneously in guinea-pigs weighing 340–380 grms. All three animals died on the 4th day.

On February 8, 1921, a further test was carried out:

Guinea-pig	Toxin dose	Result
350 grms.	1/1000 c.c. subcut.	+4th day
355 „	1/1200 „	Had tetanus but lived

+4th day = death on 4th day.

About one year later a similar test was performed:

25. i. 22.	Guinea-pig	Toxin dose	Result
	350 grms.	1/900 c.c. subcut.	+5th day
	340 „	1/1000 „	+6th „
	345 „	1/1100 „	+6th „

It is obvious that the M.L.D. had undergone little change during the twelve months that had elapsed since the previous test.

Tests having been carried out to ascertain the L + dose of this toxin it was taken to be 1/12th of a cubic centimetre. Between 19. iii. 21 and 21. vii. 22 this toxin was tested 33 times, one animal each time, in a dose of 1/12 c.c. against 1/220 c.c. of an antitetanus serum (Wilhelmina, 19. ix. 16).

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The results of these tests were:

Day of death	3rd	4th	5th	
No. of animals	7	23	3	Total 33

The justifiable conclusion is that the glycerinated tetanus toxin remained stable during this period—the supply, unfortunately, did not last out longer—provided that the test serum also remained stable.

This test serum (Wilhelmina, 19. ix. 16) was an antitetanus serum which had been saturated with table salt, as it had been found that saturation with salt increases the stability of antitoxic sera.

Between 13. i. 21 and 11. i. 24, 1/220 c.c. of (Wilhelmina, 19. ix. 16) brined serum was tested 31 times, one animal each time, against 0·00075 grm. of the standard dry tetanus toxin issued by the Hygienic Laboratory, U.S. Public Health Service, with the following results:

Day of death	*2nd*	*3rd*	*4th*	*5th*	
No. of animals	1	10	17	3	Total 31

The question now arises, How does the brined serum compare with the standard tetanus antitoxin issued by the Hygienic Laboratory?

The answer to this question is given in the protocols of the comparative tests which follow.

1. xi. 20. L+ U.S.A. Standard Tetanus Toxin against U.S.A. Standard Tetanus Antitoxin.

Guinea-pig	340 grms.	0·00075 grm. std. toxin + 1/10 A.U. std. a.-t.	+3rd day
	340 „	0·0008 „ + „	+end 2nd day
	340 „	0·009 „ + „	+2nd day

L+ U.S.A. Standard Tetanus Toxin against "Wilhelmina" brined serum.

345 grms.	0·00075 grm. std. toxin + 1/220 c.c. "Wilhelmina"		+4th day
340 „	0·0008 „ + „		+3rd day
350 „	0·0009 „ + „		+end 2nd day

17. xii. 20. "Wilhelmina" brined serum against U.S.A. Standard Tetanus Toxin.

350 grms.	1/220 c.c. "Wilhelmina"	serum	+	0·00075 grm. U.S.A.	toxin	+3rd day
340 „	„	„	+	„	„	+3rd „
365 „	„	„	+	„	„	+3rd „

2. xi. 21. L+ U.S.A. Standard Tetanus Toxin against U.S.A. Standard Tetanus Antitoxin.

340 grms.	0·0007 grm. std. toxin + 1/10 A.U. std. a.-t.		+4th day
340 „	0·00075 „ + „		+4th „
340 „	0·0008 „ + „		+3rd „
360 „	0·00085 „ + „		+3rd „

L+ U.S.A. Standard Tetanus Toxin against "Wilhelmina" brined serum.

345 grms.	0·0007 grm. std. toxin + 1/220 serum		+4th day
340 „	0·00075 „ + „		+4th „
360 „	0·0008 „ + „		+3rd „
340 „	0·00085 „ + „		+3rd „

19. iv. 22. L+ U.S.A. Standard Tetanus Toxin against U.S.A. Standard Antitoxin.

340 grms.	0·00095 grm. std. toxin + 1/10 A.U. std. a.-t.		+2nd day
345 „	0·00085 „ + „		+3rd „
340 „	0·00075 „ + „		+3rd „
350 „	0·00065 „ + „		+5th „
340 „	0·00055 „ + „		Tetanus—lived over 9 days

L+ U.S.A. Standard Tetanus Toxin against "Wilhelmina" brined serum.

370 grms.	0·00095 grm. std. toxin + 1/220 c.c. serum		+2nd day	
350 „	0·00085 „ + „		+3rd „	
370 „	0·00075 „ + „		+3rd „	Killed because of tetanus
360 „	0·00065 „ + „		+5th „	
375 „	0·00055 „ + „		+7th „	

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L + G.T.T. 1 against U.S.A. Standard Tetanus Antitoxin.

375 grms.	1/10 c.c. G.T.T. 1	+	1/10 A.U. std. a.-t.	+3rd day
340 „	1/11 „	+	„	+4th „
350 „	1/12 „	+	„	+4th „
375 „	1/13 „	+	„	+5th „
375 „	1/14 „	+	„	Tetanus. Lived over 9 days

L + G.T.T. 1 against "Wilhelmina" brined serum.

365 grms.	1/10 c.c. G.T.T. 1	+	1/220 c.c. serum	+4th day
365 „	1/11 „	+	„	+4th „
360 „	1/12 „	+	„	+5th „
370 „	1/13 „	+	„	+7th „
370 „	1/14 „	+	„	Tetanus. Lived over 9 days

19. vi. 22. L + U.S.A. Standard Tetanus Toxin against U.S.A. Standard Antitoxin.

340 grms.	0·0008 grm. std. toxin	+	1/10 A.U. std. a.-t.	+3rd day
345 „	0·00075 „	+	„	+3rd „
340 „	0·0007 „	+	„	+3–4 days

L + U.S.A. Standard Tetanus Toxin against "Wilhelmina" brined serum

365 grms.	0·0008 grm. std. toxin	+	1/220 c.c. serum	+3rd day
340 „	0·00075 „	+	„	+3–4 days
345 „	0·0007 „	+	„	+3–4 „

25. i. 23. L + U.S.A. Standard Tetanus Toxin against U.S.A. Standard Antitoxin.

360 grms.	0·00085 grm. std. toxin	+	1/10 A.U. std. a.-t.	+3rd day
350 „	0·0008 „	+	„	+3rd „
340 „	0·00075 „	+	„	+3rd „
380 „	0·0007 „	+	„	+4th „
350 „	0·00065 „	+	„	+6th „

25. i. 23. L + U.S.A. Standard Tetanus Toxin against "Wilhelmina" brined Serum.

355 grms.	0·00085 grm. std. toxin	+	1/220 c.c. serum	+3rd day
345 „	0·0008 „	+	„	+3rd „
360 „	0·00075 „	+	„	+5th „
340 „	0·0007 „	+	„	+4th „
350 „	0·00065 „	+	„	+5th „

2 vii. 23. L + U.S.A. Standard Tetanus Toxin against U.S.A Standard Antitoxin

375 grms.	0·00065 grm. std. toxin	+	1/10 A.U. std. a.-t.	+5th day
380 „	0·0007 „	+	„	+4th „
380 „	0·00075 „	+	„	+4th „
365 „	0·0008 „	+	„	+3rd „
355 „	0·00085 „	+	„	+3rd „

L + U.S.A. Standard Tetanus Toxin against "Wilhelmina" brined serum.

340 grms.	0·00065 grm. std. toxin	+	1/220 c.c. serum	+4th day
340 „	0·0007 „	+	„	+3–4 days
350 „	0·00075 „	+	„	+3rd day
380 „	0·0008 „	+	„	+3rd „
373 „	0·00085 „	+	„	+3rd „

For comparison with the previous results those with 0·00075 grm. of Standard Toxin against Standard Antitoxin and against brined Antitoxin may be grouped together.

	1/10 A.U. std. a.-t.	1/220 c.c. "Wilhelmina" brined serum
1. xi. 20	3rd day	4th day
2. xi. 21	4th „	4th „
19. iv. 22	3rd „	3rd „
19. vi. 22	3rd „	3½ days
25. i. 23	3rd „	5th day
2. viii. 23	4th „	3rd „

From these results we may conclude that:

(1) The antitoxic serum dissolved in a saturated solution of common salt remained sufficiently stable to justify its use as a test serum.

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(2) That the glycerinated liquid tetanus toxin remained stable during the 18 months that the supply lasted; and

(3) That such a liquid tetanus toxin may be used for all preliminary testing.

An ordinary freshly prepared liquid tetanus toxin was mixed with an equal quantity of pure neutral glycerine and tested from time to time to ascertain its stability, 1/16th c.c. of the mixture being used with 1/220th c.c. of "Wilhelmina" brined serum. Between 27. ix. 22 and 18. vii. 23, 18 tests were carried out, one animal each time, with the result:

Day of death	3rd	4th	5th	6th	7th	
No. of animals	1	9	5	2	1	Total 18

Then the dose of toxin had to be increased to 1/14th c.c., and so it would seem that it would be safer to use a glycerinated solution of a dry powdered toxin precipitated by ammonium sulphate.

During the observations recorded above both toxin and serum were stored in the cold room.

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