ON THE AMERICAN METHOD OF STANDARDIZING TETANUS ANTITOXIN.

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In October 1907, the United States Government issued a circular defining the unit which should be used for measuring the strength of tetanus antitoxin.

Details of the method of standardization in which this unit is used were published by Rosenau and Anderson in Bulletin No. 43 of the Hygienic Laboratory of the Public Health and Marine Hospital Service.

It is claimed for this method that it is *simple, direct, and accurate*—attributes, which, if proved true, would compel its adoption universally. It seemed advisable therefore to study the method somewhat carefully and ascertain whether the claims made for it were well grounded.

The UNIT is defined as "ten times the least quantity of antitetanic serum necessary to save the life of a 350 gramme guinea-pig for 96 hours against the official test dose of a standard toxin furnished by the Hygienic Laboratory of the Public Health and Marine Hospital Service" and it is the *toxin*, in the form of a dry powder, and *not*, as in the case of the diphtheria unit, the antitoxin which is given out to manufacturers for the purpose of standardizing serum.

The L + or official test dose of the toxin at present issued contains just 100 M.L.D.'s for a 350 gramme guinea-pig.

The Bulletin carefully draws attention to certain points in technique which are considered of great importance for the success of the method. They are :—

(1) *Diluting fluid for both toxin and antitoxin.*
A 0·85 % solution of chemically pure sodium chloride sterilized by boiling.

(2) *Time and temperature.*
The mixtures of toxin and antitoxin are kept 1 hour at room temperature in diffused light before injection.

(3) *Amount injected.*
A total amount of 4 c.cm. of the toxin-antitoxin mixture is always injected into the guinea-pig.

(4) *Concentration of the toxin.*
1 c.cm. of the toxin dilution = the test (or L+) dose.

(5) *Concentration of the antitoxin.*
Dilution tables are given on p. 50 of the Bulletin.

(6) *Weight of guinea-pig.*
The test animals should weigh 340–370 grammes.

(7) *Site of injection.*
The injection is always given subcutaneously into the tissues of the abdomen about the level of the umbilicus.

(8) *Time of death.*
The number of immunity units contained in the serum is determined from the amount given the animals that are *living* 96 hours after the inoculation of the mixtures.

We must now proceed to analyse some of the results given in the Bulletin.

On p. 7, it is stated that "the L+ dose is the smallest quantity of tetanus toxin that will neutralize 1/10th of an immunity unit plus a quantity of toxin sufficient to kill the animal in just 4 days" and in Table II, pp. 9 and 10, are given the results of injecting the L+ dose of toxin + 1/10 unit of antitoxin.

Tests were made on 19 occasions, on all but 2 of which two or more animals were inoculated on the same day. On only one occasion did the two animals die at the same time. The intervals between the times of death of guinea-pigs inoculated with the same volume of the same mixture on the same day varied between 0 and 74 hours. There was a difference of four days between the earliest and the latest death. Of the 42 animals used only three died in just four days, *i.e.* only some 7–8 % died at the time specified in the definition of the L+ dose. Twenty-six died within four days (two under three days) and thirteen in over four days (one in over five, and one in over six days), *i.e.* about 57 % died during the fourth day, and about 25 % during the fifth day, or taking them together 83 % died during the fourth and fifth days.

Again in Table IV, p. 16, are given the results of inoculating the M.L.D. of the standard test toxin into 58 guinea-pigs. Of these animals

4	died	during	the	3rd	day
21	,,	,,	,,	4th	,,
6	,,	in 96 hours exactly			
17	,,	during	the	5th	day
7	,,	,,	,,	6th	,,
2	,,	,,	,,	7th	,,
1	,,	,,	,,	8th	,,

Thus only about 10 % of the animals died at the time specified in the definition of the L+ dose; while 75 % of them died during the fourth or fifth day. The longest interval between the death-times of animals inoculated on the same day was 3 days 23 hours.

We find then that 83 % of the deaths in Table II and 75 % of those in Table IV occurred during the fourth and fifth days and consequently it would seem that for practical purposes the death-time-limit may be taken as the fourth or fifth day.

It is necessary here to consider the meaning of the word "day" in this paper. From the American Bulletin one gathers that each period of 24 hours counting from the hour of inoculation is held to constitute a "day" and this definition is adhered to in all experiments detailed below, which refer to the *minimal lethal dose only*. When however it is a question of testing a serum, then the remaining hours of the day of inoculation are neglected and the first day commences on the morning after inoculation. This method of calculation is found to be most convenient from the point of view of routine work and does not lead to a serum being overestimated.

Now it is claimed (as stated above) for this method of standardizing tetanus antitoxin that it is simple, direct and accurate. As it is based on and is practically identical with Ehrlich's method of evaluating diphtheria antitoxin we may allow the first two claims and confine our attention to ascertaining whether the method is accurate.

The first point to determine is whether the test toxin is stable.

In the following Table I are given the results of the tests made to ascertain the minimal lethal dose of the standard dry powdered toxin.

This toxin has been sent on several occasions by Dr J. F. Anderson (I am pleased to have this opportunity of thanking him most sincerely for his kindness) and has been preserved in a vacuum desiccator in the ice chest. The powder was dissolved in 0·85 % salt solution so that 1 c.cm. of the solution contained 0·000006 grm. of toxin. Each dose was made up to about 4 c.cm. with the same saline solution and was injected beneath the skin of the abdomen between the ensiform cartilage and the umbilicus.

A consideration of this table shows that this standard dry test toxin has been tested on 17 occasions during a period of 23 months and that 0·000006 grm.—the dose stated on the label to be the M.L.D.—of this toxin has killed 14 out of 17 guinea-pigs weighing 340–380 grms. on the fourth or fifth day. In other words 82 % of the animals died within the specified time limits. This is quite in accordance with the results

TABLE I.

Examination of Sample No. 3 of the United States standard dry powdered tetanus toxin received March, 1911. The M.L.D. was stated to be = 0·000006 grm. One guinea-pig was used for each dose. Weight of guinea-pigs, 340–380 grms. unless otherwise stated. The figures in the columns beneath the doses give the number of the 24 hour period, counting from the time of inoculation, during which the animal died or, where noted, first showed signs of tetanus.

Toxin 3.

Date of test	Dose 0·00001	0·000009	0·000008	0·000007	0·000006	0·000005	0·000004	0·000003	Remarks
6. 4. 11	...	...	...	...	4	3	did not kill	...	
,,	...	...	5	5	7	6	8	...	Guinea-pigs, 445–470 grms.
8. 8. 11	...	...	4	4	4	5	...	...	
9. 10. 11	...	...	4	4	5	6	6	...	
2. 11. 11	...	...	4	4	4	6	6 slight tetanus	...	
29. 2. 12	...	...	6	4	4	3	4	...	Inoculated in back
,,	...	...	3	4	4	5	6	..	,, thigh
,,	...	..	3	3	4	6	6	...	,, abdomen
25. 6. 12	...	...	4	4	5	5	...	...	
24. 9. 12	...	...	4	5	5	6	7 marked tetanus	...	
21. 10. 12	...	...	4	4	7 distinct tetanus	5	no symptoms	...	
29. 10. 12	...	...	4	5	5	...	...	...	
8. 11. 12	...	...	4	4	4	5	6	...	Dilutions made with NaCl
,,	...	...	4	4	4	5	6	...	Dilutions made with dist. water
,,	...	...	4	4	5	5	5 slight tetanus	...	Dilutions made with tap-water
28. 1. 13	...	..	...	4	5	5 slight tetanus	...	...	
28. 2. 13	...	...	6	7 tetanus	6	7 tetanus	7 tetanus	...	
17. 3. 13	9 tetanus	5	5	7	...	...	...	...	
18. 4. 13	5	4	5	6	5 slight tetanus	...	..	...	
Toxin 4. Received July, 1912.									
28. 2. 13	...	...	...	...	4	4	4	4	
5. 5. 13	...	...	...	3	3	4	4	5	

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TABLE II.

The dry powdered toxin was dissolved in 0·85 % NaCl solution in such a proportion that 1 c.cm. contained 0·0006 grm. of toxin. Then 1 c.cm. of this dilution was added to 99 c.cm. of saline, distilled water or tap-water respectively, thus giving a solution of which 1 c.cm. = 0·000006 grm. of toxin or 1 M. L. D. The doses were measured out into test-glasses and made up to about 4 c.cm. with saline, distilled water or tap-water as the case might be.

Test of Toxin No. 3.

Diluting fluid	Guinea-pig Weight	Grm. of toxin: 0·000008	0·000007	0·000006	0·000005	0·000004
0·85 % saline	365	4	...	...	...	...
	350	...	4	...	...	...
	350	...	...	4	...	...
	345	...	...	...	5	...
	345	...	...	...	...	6
Distilled water	340	4	...	...	...	...
	370	...	4	...	...	...
	365	...	...	4	...	...
	340	...	...	...	5	...
	340	...	...	...	...	6
Tap-water	340	4	...	...	...	...
	350	...	4	...	...	...
	350	...	...	5	...	...
	350	...	...	...	5	...
	340	...	...	...	...	symptoms of tetanus on 5th day

Test of Toxin No. 4. Received July, 1912. Tested February, 1913.

0·85 % saline	365	3	...	...	...	...
	350	...	3	...	...	...
	370	...	...	3	...	...
	380	...	...	...	3	...
	345	...	...	...	...	4
Distilled water	350	3	...	...	...	...
	345	...	4	...	...	...
	370	...	...	4	...	...
	365	...	...	...	5	...
	345	...	...	...	...	5
Tap-water	380	3	...	...	...	...
	350	...	3	...	...	...
	340	...	...	3	...	...
	360	...	...	...	4	...
	340	...	...	...	...	5

The numbers in the columns beneath the doses denote the day on which the animal which received that dose died.

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given by Rosenau and Anderson and so we may accept this dry powdered toxin as a product which remains stable for 23 months when kept under proper conditions.

It is necessary to mention that the saline solution used in these experiments was made up with an ordinary "table salt[1]" and not with chemically pure sodium chloride such as is recommended by the authors of the Bulletin.

This raises the question as to whether one might use distilled water or tap-water as the diluting fluid should it by chance happen that on a very urgent occasion the amount of saline available was not sufficient for all purposes.

With the object of furnishing an answer to this question two experiments were made, one with Toxin No. 3 and one with Toxin No. 4.

The results are given in Table II.

From these results we conclude that once the dry powdered toxin has been dissolved in 0·85% NaCl solution we may use either distilled water or tap-water for the making of any further dilutions which may be necessary.

The next point to determine is whether this standard test toxin gives consistent values when used on several occasions to evaluate the same serum.

Now Rosenau and Anderson consider it most important that the total quantity injected each time should be exactly 4 c.cm. so that the pressure effects may be always the same. In Ehrlich's method of testing diphtheria antitoxin such accuracy is not considered to be absolutely necessary and the quantities to be inoculated are made up to *about* 4 c.cm. Experiments were therefore made to ascertain at the same time (1) whether the slight latitude allowable in testing diphtheria antitoxin would render the method valueless for the standardization of tetanus antitoxin, and (2) whether the standard dry powdered toxin gave consistent results with the same serum.

A serum, T. 26, was taken and a rough test made, using total quantities of *about* 4 c.cm.

The details of the test are given below.

[1] This analysis of the salt was kindly supplied by the manufacturers, Messrs D. Bumsted and Co., and was stated to be an average analysis:

Sodium chloride	99·28 %
Insoluble	0·02
Calcium sulphate	0·33
Magnesium chloride	0·05
Sodium sulphate	0·32

TABLE III. *Testing serum T.* 26.

Weight of guinea-pigs	Amount of toxin	Amount of serum (c.cm.)	Day: 1	2	3	4	5	6
370	0·0006 grm. U.S.A. 3	1/1500	—	S	—	—	—	—
340	,,	1/1600	—	,,	—	—	—	—
340	,,	1/1700	—	,,	—	t	ttt	ttt
340	,,	1/1800	—	,,	?	ttt †		
345	,,	1/1900	—	,,	ttt †			
360	,,	1/2000	—	,,	ttt †			

S = Sunday, no observations made. t = tetanus, the number of letters showing the intensity of the symptoms. † = death, the position of the cross indicates approximately the time of death. — = no symptoms.

Note. The animal's day begins at 6.30 a.m. and ends at 5.30 p.m. when the houses are closed for the night. Notes of the condition of the animals are made each morning between 9 a.m. and 10 a.m. A death taking place between 5.30 p.m. and 6.30 a.m. is indicated by a † between two days. Should death occur between 6.30 a.m. and 9.30 a.m. then a † is placed on the left-hand side of the space for the day. A note of the animal's condition shows that it was alive at say 9.30 a.m. After that the † is placed to indicate approximately the time of death up to 5.30 p.m. Full details of the experiments are given so that the reader may form his own opinion as to the value of the method.

These results give a value of between 180–190 units per c.cm.

The test was then repeated—using two series of animals. In the case of one series the dilutions were made as in the last test but in the case of series 2 according to the dilution tables given in the Bulletin.

In both series the total volume injected was not measured accurately but was *about* 4 c.cm.

TABLE IV. *Second test of T.* 26.

Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day: 1	2	3	4	5	6	Remarks
345	0·0006 U.S.A. 3	1/1650	—	—	—	t	S	ttt	
350	,,	1/1700	—	—	—	tt	,,	tt	Dilutions made
360	,,	1/1750	—	—	t	tt †			in way usual
360	,,	1/1800	—	—	—	ttt †			in testing diph-
380	,,	1/1850	tt † accident during inoculation						theria antitoxin
380	,,	1/1900	—	—	tt	ttt†t	S		
365	,,	1/1666	—	—	—	tt	,,	tttt	
355	,,	1/1739	—	—	t	tt	,,	tttt	Dilutions made
350	,,	1/1786	—	—	t	tt	,,	tttt	according to the
365	,,	1/1818	—	—	t	t	,, †		American tables
360	,,	1/1850	—	—	tt	ttt	,, †		
360	,,	1/1905	—	—	tt †				

S = Sunday, no observations. — = no symptoms. † = death, the position of the cross indicating approximately the time of death. t = tetanus, the number of letters showing the intensity of the symptoms.

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These results agree with those of the previous test, but to make quite certain the test was repeated. On this occasion the total quantity injected was made to vary considerably.

TABLE V. *Third test of T.* 26.

Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm)	Day: 1	2	3	4	5		Remarks
365	0·0006 U.S.A. 3	1/1650	—	S	—	—	tt	ttt	
355	,,	1/1700	—	,,	—	—	tt	tttt	Doses made up to *about* 4 c.cm.
350	,,	1/1750	—	,,	—	tt	†		
345	,,	1/1800	—	,,	—	ttt	†		
345	,,	1/1850	—	,,	—	ttt	†		
375	,,	1/1650	—	,,	—	—	t	tt	
370	,,	1/1700	—	,,	t	t	ttt	tttt	Doses made up to *exactly* 4 c.cm.
360	,,	1/1750	—	,,	—	t	ttt	†	
370	,,	1/1800	—	,,	t	tt	tttt †		
365	,,	1/1850	—	,,	t	tt	tttt †		
375	,,	1/1650	—	,,	—	tt	tt	tttt	
370	,,	1/1700	—	,,	?	tt	tt	tttt	Doses made up to exactly 3 c.cm.
380	,,	1/1750	—	,,	—	ttt	ttt†t		
370	,,	1/1800	—	,,	—	ttt	ttt†t		
365	,,	1/1850	—	,,	—	ttt	ttt†t		
									Doses
350	,,	1/1650	—	,,	—	t	tt	ttt	2·21 c.cm.
375	,,	1/1700	—	,,	?	tt	tt	tttt	2·18 ,,
345	,,	1/1750	—	,,	—	tt	tt	tttt	2·14 ,,
360	,,	1/1800	—	,,	—	tt	†		2·11 ,,
365	,,	1/1850	—	,,	—	ttt	tttt†		2·08 ,,
355	,,	1/1666	—	,,	—	ttt †			Doses made up to exactly 4 c.cm. American tables of dilution used
370	,,	1/1739	—	,,	tt †				
350	,,	1/1786	—	,,	ttt†t				
350	,,	1/1818	—	,,	ttt †				
375	,,	1/1850	—	,,	ttt†t				

S = Sunday, no observations made. † = death. — = no symptoms. t = tetanus.

We see that the results of the first four series agree amongst themselves and confirm the results of the two previous tests. It is only the last series which is not concordant. It was therefore thought advisable to make a fourth test and to use total quantities of exactly 4 c.cm. in each case. (Table VI.)

From the result of this last experiment we must conclude that the discordant result which occurred in the third test of T. 26 should be

TABLE VI. *Fourth test of T.* 26.

Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day: 1	2	3	4	5	6	Remarks
340	0·0006 U.S.A. 3	1/1666	—	—	—	—	—	S tt	Dilutions according to the American tables.
340	,,	1/1739	—	—	—	t	t	,, ttt	
365	,,	1/1786	—	—	—	tt	tt	,, ttt	
340	,,	1/1818	—	—	—	—	t	,, ttt	
340	,,	1/1850	—	—	—	tt	tt †		
340	,,	1/1650	—	—	—	—	—	S t	
360	,,	1/1700	—	—	—	—	—	,, t	
375	,,	1/1750	—	—	—	—	—	,, ttt	
365	,,	1/1800	—	—	—	tt	ttt	,, †	
340	,,	1/1850	—	—	—	t	tt †		

neglected. All the others agree in measuring the antitoxin content of serum T. 26 at 185 units per cubic centimetre.

We have then found that the standard dry powdered test toxin, Sample No. 3, has given consistent results with serum T. 26 and that some latitude in the amount of the total quantity injected is permissible.

The question then arises whether another batch of toxin would give equally good results. A first answer to this question is given by the tests detailed in Table VII.

TABLE VII. *Evaluation of tetanus antitoxic serum T.* 8.

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day: 1	2	3	4	5	6	Remarks
28. 2. 13	350	0·0006 U.S.A. 3	1/1300	—	—	—	—	—	—	
	340	,,	1/1400	—	—	—	—	—	—	This test made with tetanus toxin U.S.A. 3 gave a titre of 150 units per c.cm.
	345	,,	1/1500	—	—	—	—	—	—	
25. 2. 13	350	,,	1/1500	—	—	—	t	S	ttt	
	340	,,	1/1750	—	t	ttt †				
	340	,,	1/2000	—	ttt †					
	340	,,	1/2225	—	†					
	370	,,	1/2500	—	†					
28. 2. 13	365	0·0006 U.S.A. 4	1/1100	—	—	—	—	—	tt	
	355	,,	1/1200	—	—	—	—	t	ttt	When tested against tetanus toxin U.S.A. 4 the titre was found to be 140 units per c.cm.
	345	,,	1/1300	—	—	—	tt	ttt	tttt	
	340	,,	1/1400	—	—	t	tttt †			
25. 2. 13	340	,,	1/1500	—	ttt †					
	340	,,	1/1750	—	ttt†t					
	340	,,	1/2000	— †						
	350	,,	1/2250	— †						
	370	,,	1/2500	t †						

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From these results we may conclude that (1) Toxin 3 has deteriorated somewhat, or (2) Toxin 4 is a little stronger that it was supposed to be, or (3) 10 units is too small a difference to make between the doses of serum.

A consideration of Tables I and II suggests that, at the time the experiments given in Table VII were made, Toxin 3 had begun to go off and that the L+ dose of Toxin 4, supposed to contain 100 M.L.D., might contain more.

To obtain further information upon these points these toxins were tested against a liquid standard tetanus antitoxin kindly supplied by Messrs Meister, Lucius and Bruning. (Table VIII.)

We see that the results given by Toxin 3 are not so uniform as those given by Toxin 4 and they value the serum higher. Thus, according to Toxin 3 the serum contains from 170–242 units per c.cm. and according to Toxin 4 from 170–187 units per c.cm. (one cannot lay much stress on the test of 24. 4. 13 as the bottle of serum by this time was almost empty (it contained 1·5 c.cm.) and the serum was six weeks older), a difference in the one case of 70 units and in the other of 17. One is inclined to ascribe this difference to deterioration in Toxin 3, but one cannot be certain.

Experiments were then made with a dry standard tetanus antitoxin for samples of which I am much indebted to the great kindness of Prof. Ehrlich. (Table IX.)

From the tests of 1. 5. 13 and of 3. 5. 13, 0·0006 grm., which was stated to be the L+ dose of Toxin 4, is neutralized to the requisite extent by 0·25 c.cm. of the serum dilution used. This amount of serum was therefore taken as the test dose for varying quantities both of Toxin 3 and of Toxin 4. The results are given in Table X.

We find that 0·0006 grm. of Toxin 4 gives the same result as before and is equal to 0·0008 grm. of Toxin 3. It is probable therefore that the want of agreement in the results of tests with the two toxins is due to deterioration of Toxin 3 but whether entirely so is impossible to tell from these experiments. A little light may however be thrown upon this point if we analyse the tests made to ascertain the keeping qualities of our serum given in Table XI.

We see that some of these sera were tested against Toxin 3 alone at a time when there was no doubt that deterioration had not begun. Seven sera thus tested give an average loss in unitage of some 18 % in 12 months whereas nine sera tested first against Toxin 3 (when at proper strength) and then against Toxin 4 show a loss of about 34 % in

TABLE VIII. *Evaluation of liquid standard tetanus antitoxin (Hoechst).*

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.c. of 1 in 425 dilution)	Day: 1	2	3	4	5	6	Origin and number of serum used. Remarks
12. 2. 13	340	0·0006 U.S.A. 3	1	—	—	—	S	—	—	
	355	,,	1/3	—	—	—	,,	—	—	
	340	,,	1/10	t †						
	360	,,	1/50	tt †						
19. 2. 13	340	,,	0·33	—	—	S	—	—	—	
	340	,,	0·3	—	—	,,	—	—	—	
	340	,,	0·25	—	—	,,	—	—	—	
	340	,,	0·2	—	—	,,	t	t	ttt	
	355	,,	0·15	—	tt	†				
28. 2. 13	340	,,	0·2	—	S	—	t	tt	tttt	
	355	,,	0·175	—	,,	—	tt †			
	355	,,	0·15	—	,,	ttt †				
	340	0·0006 U.S.A. 4	0·3	—	,,	—	tt	tttt †		
	355	,,	0·25	—	,,	—	tt	†		
	350	,,	0·2	—	,, †					
	355	,,	0·175	—	,,†					
	340	,,	0·15	—	,,†					
6. 3. 13	340	0·001 U.S.A. 3	0·25	—	tt†t					Hoechst standard serum 4·25 fach. Serum diluted 1 in 425.
	355	,,	0·2	—	†					
	350	,,	0·175	— †						
	340	0·0008 U.S.A. 3	0·25	—	ttt †					
	345	,,	0·2	—	ttt †					
	365	,,	0·175	—	tttt †					
	340	0·0006 U.S.A. 3	0·25	—	—	S	t	tt	tttt	
	340	,,	0·2	—	—	,,†				
	340	,,	0·175	—	tt †					
	345	0·0006 U.S.A. 4	0·25	—	—	S	ttt †			
	340	,,	0·2	—	ttt†					
	340	,,	0·175	—	ttt †					
	350	0·0004 U.S.A. 4	0·25	—	—	S	—	tt	tttt	
	365	,,	0·2	—	—	,,	t	tttt	†	
	340	,,	0·175	—	—	,,†				
	340	0·0002 U.S.A. 4	0·25	—	—	S	—	—	—	
	340	,,	0·2	—	—	,,	—	—	—	
	340	,,	0·175	—	—	,,	—	—	—	

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TABLE VIII (*continued*).

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.c. of 1 in 425 dilution)	Day: 1	2	3	4	5	6	Origin and number of serum used. Remarks
11. 3. 13	355	0·0007 U.S.A. 3	0·275	—	—	—	ttt	S	†	
	340	,,	0·25	—	—	tt	†			
	340	,,	0·225	—	t	†				Hoechst standard serum 4·25 fach. Serum diluted 1 in 425.
	380	,,	0·2	—	tttt †					
	365	,,	0·175	—	ttt†t					
	345	0·0006 U.S.A. 4	0·275	—	—	—	—	S	—	
	340	,,	0·25	—	—	—	t	,, †		
	340	,,	0·225	—	—	t	ttt†t			
	340	,,	0·2	—	tt	tttt †				
	345	,,	0·175	—	tt	ttt†t				
			(c.c. of serum)							Same serum. The amts. given in 3rd column represent the quantity of *undiluted serum* present in the mixture.
24. 4. 13	340	0·0006 U.S.A. 4	1/1500	—	t	S	ttt	tttt †		
	340	,,	1/1750	—	tt †					
	340	,,	1/2000	—	tttt†					
	340	,,	1/2250	—	tttt†					
	340	,,	1/2500	— †						

TABLE IX. *Evaluation of Frankfurt standard dry tetanus antitoxin.*

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of solution of serum (c.c.)	Day: 1	2	3	4	5	6	Remarks
1. 5. 13	350	0·0006 U.S.A. 4	0·4	—	—	—	—	—	—	
	340	,,	0·4	—	—	—	—	—	—	
	340	,,	0·2	—	ttt †					The contents of one tube of dry antitoxin were dissolved in exactly 26 c.cm. of 0·85 % NaCl solution.
	340	,,	0·2	—	ttt	†				
	340	,,	0·133	— †						
	340	,,	0·133	— †						
	350	,,	0·1	— †						
	345	,,	0·1	— †						
	365	,,	0·088	t †						
	350	,,	0·088	— †						
	340	,,	0·066	tt †						
	350	,,	0·066	tt †						
3. 5. 13	340	,,	0·4	—	—	—	—	—		
	345	,,	0·4	—	—	—	—	—		
	340	,,	0·35	—	—	—	—	—		
	360	,,	0·35	—	—	—	—	—		The contents of one tube were dissolved as before in exactly 26 c.cm. of 0·85 % NaCl solution.
	370	,,	0·3	—	—	—	—	—		
	370	,,	0·3	—	—	—	—	—		
	340	,,	0·25	—	—	tt	ttt	tttt		
	350	,,	0·25	—	—	tt	tt	ttt		
	355	,,	0·2	—	tt	tttt†				
	350	,,	0·2	—	ttt	tttt†				
	370	,,	0·15	tt	†					
	340	,,	0·15	—	ttt†t					
	340	,,	0·1	ttt †						
	340	,,	0·1	— †						

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TABLE X. *Testing toxins 3 and 4 against the Frankfurt standard serum.*

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum dilution (c.c.)	Day: 1	2	3	4	5	6	Remarks
20. 5. 13	340	0·0008 U.S.A. 4	0·25	—	tttt †					
	360	,,	,,	—	tt	ttt	tttt †			
	340	0·0007	,,	—	tt	tt	tttt †			
	355	,,	,,	—	tt	ttt	†			
	350	0·0006	,,	—	—	t	tt	S	ttt	
	350	,,	,,	—	—	t	ttt	,,	ttt	
	360	0·0005	,,	—	—	—	—	,,	—	
	345	,,	,,	—	—	—	—	,,	—	The contents of one tube of dry serum were dissolved in exactly 26 c.cm. of 0·85 % NaCl solution.
	340	0·0004	,,	—	—	—	—	,,	—	
	340	,,	,,	—	—	—	—	,,	—	
	340	0·0003	,,	—	—	—	—	,,	—	
	340	,,	,,	—	—	—	—	,,	—	
	345	0·0008 U.S.A. 3	,,	—	t	ttt	ttt	S	tttt	
	360	,,	,,	—	—	tt	ttt	,,	tttt	Serum and toxin in contact for 1 hour at room temperature before injection.
	340	0·0007	,,	—	—	—	—	,,	—	
	340	,,	,,	—	—	—	—	,,	—	
	340	0·0006	,,	—	—	—	—	,,	t	
	340	,,	,,	—	—	—	—	,,	—	
	355	0·0005	,,	—	—	—	—	,,	—	
	340	,,	,,	—	—	—	—	,,	—	
	340	0·0004	,,	—	—	—	—	,,	—	
	340	,,	,,	—	—	—	—	,,	—	
	340	0·0003	,,	—	—	—	—	,,	—	
	340	,,	,,	—	—	—	—	,,	—	

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TABLE XI. *Loss in unitage which occurs as serum ages.*

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day: 1	2	3	4	5	6	Remarks
2. 6. 11	355	0·0006 U.S.A. 5	1/800	—	S	—	—	—	—	
	365	,,	1/900	—	,,	—	—	t	t	Serum T. 2 110 units.
	340	,,	1/1000	—	,,	—	—	ttt†		
	370	,,	1/1100	—	,,	—	—	tt	†	
	370	,,	1/1200	—	,, †					
8. 11. 12	375	,,	1/700	—	,,	—	—	—	—	2nd test of same serum. Now 90 units. Loss of about 20 % in 17 months.
	380	,,	1/800	—	,,	—	t	t	tt	
	370	,,	1/900	—	,,	—	ttt	†		
	360	,,	1/1000	—	,,	ttt †				
	370	,,	1/1100	—	,,	ttt†				
9. 10. 11	385	,,	1/1400	—	—	—	—	—	S t	Serum T. 3 160 units.
	385	,,	1/1500	—	—	—	t	tt	,, †	
	380	,,	1/1600	—	—	—	t	tt †		
	355	,,	1/1700	—	—	tt †				
	370	,,	1/1800	—	— †					
8. 11. 12	365	,,	1/1200	—	S	—	t	tt	tttt	2nd test of same serum. Now 120 units. Loss of about 25 % in 13 months.
	350	,,	1/1300	—	,,	ttt †				
	355	,,	1/1400	—	,,	ttt †				
	355	,,	1/1500	—	,, †					
	365	,,	1/1600	—	,, †					
1. 7. 12	340	,,	1/1300	—	—	—	tt	tttt	tttt	Serum T. 4 150 units.
	355	,,	1/1400	—	—	—	tt	tttt	†	
	340	,,	1/1500	—	—	—	tttt †			
	350	,,	1/1600	—	—	t	†			
	345	,,	1/1700	—	ttt	†				
8. 11. 12	365	,,	1/1100	—	S	t	tt	tt	tttt	2nd test of same serum. T. 4 say 130 units. Loss of about 10 % in 4 months.
	360	,,	1/1200	—	,,	t	tt	ttt	tttt	
	340	,,	1/1300	—	,,	tt	tttt	ttt†t		
	345	,,	1/1400	—	,,	tt	ttt†			
	340	,,	1/1500	—	,,	ttt †				
30. 8. 12	370	,,	1/2000	—	S	—	—	—	—	Serum T. J 20. 8. 12. 300 units.
	360	,,	1/2500	—	,,	—	—	—	—	
	375	,,	1/3000	—	,,	—	tt	ttt	†	
	360	,,	1/3500	—	,,	ttt †				
	370	,,	1/4000	—	,, †					
19. 3. 13	340	0·0006 U.S.A. 4	1/2000	—			†			2nd test of same serum, T. J. Now only just 200 units. Loss of 33 % in 7 months.
	350	,,	1/2225	—	†					
	340	,,	1/2500	— †						
	340	,,	1/2750	— †						
	350	,,	1/3000	— †						

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TABLE XI (*continued*).

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day: 1	2	3	4	5	6	Remarks
28. 1. 13	365	0·0006 U.S.A. 3	1/1500	—	—	—	—	S	—	
	370	,,	1/2000	—	—	—	t	,, †		*Canterbury*, 14. 1. 13. 200 units.
	345	,,	1/2500	—	—	ttt †				
	360	,,	1/3000	—	ttt†					
	370	,,	1/3500	— †						
10. 4. 13	340	0·0006 U.S.A. 4	1/1250	—	—	S	tt	†		Same serum, C, 14. 1. 13. 125 units. Loss of about 37 % in 3 months.
	340	,,	1/1500	—	—	,,†				
	340	,,	1/1750	—	ttt †					
	340	,,	1/2000	—	†					
23. 1. 13	340	0·0006 U.S.A. 3	1/750	—	—	S	—	—	—	
	370	,,	1/1000	—	—	,,	—	—	—	*Juno*, 14. 1. 13. 250 units.
	350	,,	1/1500	—	—	,,	—	—	—	
	345	,,	1/2000	—	—	,,	—	—	—	
	345	,,	1/2500	—	—	,,	ttt †			
10 4. 13	340	0·0006 U.S.A. 4	1/1500	—	tttt †					Same serum, J, 14. 1. 13. 125 units. Loss of about 50 % in 3 months.
	340	,,	1/1750	—	†					
	340	,,	1/2000	— †						
	340	,,	1/2500	— †						
16. 4. 13	340	,,	1/1000	—	—	—	S	—	—	
	340	,,	1/1250	—	—	—	,,	—	—	
14. 5. 12	345	0·0006 U.S.A. 3	1/1500	—	—	—	—	S	—	
	345	,,	1/2000	—	—	—	—	,,	ttt	Serum T. C, 9. 11. 11. 200 units.
	350	,,	1/2500	—	—	ttt†				
	375	,,	1/3000	—	t †					
	375	,,	1/3500	—	ttt †					
29. 11. 12	340	,,	1/1000	—	—	—	—	—	—	2nd test of same serum. Still 200 units. No loss in 6 months.
	340	,,	1/1500	—	—	—	—	—	—	
	345	,,	1/2000	—	—	—	t	ttt	ttt	
31. 3. 13	360	0·0006 U.S.A. 4	1/1000	—	—	—	—	—	—	3rd test of same serum. Now 150 units. Loss of 25 % in 4 mos.
	340	,,	1/1250	—	—	—	—	—	—	
	340	,,	1/1500	—	—	t	ttt	†		
	350	,,	1/1750	—	—	ttt †				
	370	,,	1/2000	—	—	tt†t				
30. 10. 11	380	0·0006 U.S.A. 3	1/2000	—	—	—	—	—	—	
	380	,,	1/2500	—	—	—	—	—	—	Serum T. J, 30. 9. 11. 350 units.
	365	,,	1/3000	—	—	—	—	—	—	
	375	,,	1/3500	—	—	—	t †			
	360	,,	1/4000	—	t †					

TABLE XI (*continued*).

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day: 1	2	3	4	5	6	Remarks
29. 11. 12	340	0·0006 U.S.A. 3	1/2000	—	S	—	—	—	—	2nd test of same serum. 300units. Loss of about 15 % in 13 mos.
	355	,,	1/2500	—	,,	—	—	—	—	
	340	,,	1/3000	—	,,	—	—	t	t	
	345	,,	1/3500	—	,,	ttt†t				
11. 8. 11	355	,,	1/500	—	S	—	—	—	—	Serum T. B, 14. 7. 11. 100 units.
	365	,,	1/750	—	,,	—	—	—	—	
	350	,,	1/1000	—	,,	—	tt	ttt	ttt	
	345	,,	1/1250	—	,,	t †				
	345	,,	1/1500	—	,,	ttt†				2nd test of T. B, 14. 7. 11. Now 75 units. Loss of 25 % in 15 mos.
29. 11. 12	340	,,	1/500	—	S	—	—	—	—	
	350	,,	1/750	—	,,	—	—	—	t	
	355	,,	1/1000	—	,,	t †				
29. 7. 11	340	,,	1/500	S	—	—	—	—	—	Serum T. R, 27. 7. 11. 100 units.
	345	,,	1/750	,,	—	—	—	—	—	
	355	,,	1/1000	,,	—	—	—	—	—	
	355	,,	1/1500	,,	t †					
	355	,,	1/2000	,,	ttt†					
29. 11. 12	370	,,	1/500	—	S	—	—	—	—	2nd test of same serum, T.R. Now 75 units. Loss of 25 % in 16 mos.
	360	,,	1/750	—	,,	—	—	—	t	
	350	,,	1/1000	—	,,	t †				
30. 8. 12	375	,,	1/2000	—	S	—	—	—	—	Serum T. C, 20. 8. 12. 300 units.
	375	,,	1/2500	—	,,	—	—	—	—	
	375	,,	1/3000	—	,,	—	tt †			
	375	,,	1/3500	—	,,	t †				
	360	,,	1/4000	—	,,	ttt †				
9. 4. 13	340	0·0006 U.S.A. 4	1/1250	—	—	—	S	—	—	2nd test of same serum. Now 175 units. Loss of about 40 % in about 7 mos.
	340	,,	1/1500	—	—	—	,,	—	—	
	345	,,	1/1750	—	—	t	,,	ttt†		
	340	,,	1/2000	—	—	tt	†			
	340	,,	1/2250	—	t	tttt †				
1. 7. 12	345	0·0006 U.S.A. 3	1/750	—	—	—	—	—	—	Serum T. M, 19. 4. 12. 200 units.
	340	,,	1/1000	—	—	—	—	—	—	
	355	,,	1/1250	—	—	—	—	—	—	
	365	,,	1/1500	—	—	—	—	—	?	
	375	,,	1/2000	—	—	tt	ttt †			
7. 4 13	340	0·0006 U.S.A. 4	1/1250	—	—	—	tt	tt	ttt	2nd test of same serum. Now 125 units. Loss of 40 % in 9 mos.
	350	,,	1/1500	—	t	tttt †				
	340	,,	1/1750	—	t	tttt†				
	340	,,	1/2000	—	ttt †					

TABLE XI (*continued*).

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day: 1	2	3	4	5	6	Remarks
9. 12. 12	365	0·0006 U.S.A. 3	1/1500	—	—	—	—	—	—	
	355	,,	1/2000	—	—	—	—	—	—	Serum T. J 1. 11. 12. 200 units.
	350	,,	1/2500	—	t	†				
	380	,,	1/3000	—	tttt †					
	340	,,	1/3500	— †						
10. 4. 13	340	0·0006 U.S.A. 4	1/1250	—	—	S	tt	ttt	ttt	2nd test of same serum. Now 125 units. Loss of about 35 % in 4 mos.
	340	,,	1/1500	—	t	†				
	340	,,	1/1750	—	†					
	340	,,	1/2000	—	†					
9. 12. 12	345	0·0006 U.S.A. 3	1/1500	—	—	—	—	—	—	
	340	,,	1/2000	—	—	—	—	?t	—	Serum T. C, 1. 11. 12. 200 units.
	360	,,	1/2500	—	—	t	ttt†t			
	370	,,	1/3000	—	t	ttt†t				
	365	,,	1/3500	—	ttt †					
10. 4. 13	340	0·0006 U.S.A. 4	1/1250	—	—	—	tt	tt		2nd test of same serum. Now 125 units. Loss of about 35 % in 4 mos.
	340	,,	1/1500	—	—	ttt†t				
	340	,,	1/1750	—	— †					
	345	,,	1/2000	—	ttt †					
20. 5. 12	370	0·0006 U.S.A. 3	1/2500	—	—	—	—	—	—	
	370	,,	1/3000	—	—	—	—	—	—	T. J, 19. 4. 12. 300 units.
	340	,,	1/3500	—	t	ttt†t				
	350	,,	1/4000	—	tt †					
	380	,,	1/4500	—	ttt†t					
7. 4. 13	340	0·0006 U.S.A. 4	1/2250	—	—	—	—	—	—	2nd test of the same serum. Now 250 units. Loss of about 16 % in 10 mos.
	340	,,	1/2500	—	—	t	ttt	tttt	†	
	340	,,	1/2750	—	tt	tttt †				
	340	,,	1/3000	—	tt †					

six months—a loss which one would not expect from general experience. It is therefore justifiable to presume that while Toxin 3 has deteriorated somewhat it is also probable that Toxin 4 is a little stronger than it was supposed to be. This presumption is borne out by the results of tests on samples of sera most generously given me by Dr Th. Madsen and by Prof. Paltauf.

TABLE XII.

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day: 1	2	3	4	5	6	Remarks
20. 2. 13	340	0·0006 U.S.A. 3	1/200	—	—	S	—	—	—	Copenhagen serum. On 10. 10. 11 the titre was 1·6 fach.
	365	,,	1/300	—	—	,,	ttt†			
	340	,,	1/400	—	ttt†					
	340	,,	1/600	— †						
	365	,,	1/800	— †						
20. 2. 13	340	,,	1/200	—	—	S	—	—	—	Copenhagen serum. On 26. 5. 11 the titre was 2·2 fach.
	340	,,	1/300	—	—	,,	—	—	—	
	340	,,	1/400	—	—	,,	—	—	—	
	340	,,	1/600	—	—	,,	—	—	—	
	340	,,	1/800	—	—	,,	—	—	—	
25. 4. 13	340	0·0006 U.S.A. 4	1/100	—	S	—	—	—	—	Copenhagen serum. On 18. 4. 12 the titre was 1·6 fach.
	340	,,	1/200	—	,,	tt	tt	tt	tt	
	340	,,	1/300	—	,,	ttt†t				
	340	,,	1/400	— †						
	340	,,	1/500	— †						
25. 4. 13	350	,,	1/100	—	S	—	—	—	—	Copenhagen serum. On 23. 10. 12 the titre was 1·6 fach.
	345	,,	1/200	—	,,	—	—	—	—	
	345	,,	1/300	—	,,	tt	tt	tt	tt	
	340	,,	1/400	—	,, †					
	340	,,	1/500	—	†					
21. 4. 13	345	,,	1/100	—	—	—	—	—	—	Copenhagen serum. On 1. 7. 12 the titre was 1·6 fach.
	350	,,	1/200	—	—	—	—	—	—	
	355	,,	1/300	—	—	—	t	t	t	
	350	,,	1/400	—	tttt †					
	340	,,	1/500	—	†					
21. 4. 13	340	,,	1/100	—	—	—	—	—	—	Copenhagen serum. On 4. 9. 12 the titre was 1·6 fach.
	350	,,	1/200	—	—	—	—	—	—	
	340	,,	1/300	—	—	tt	tt	ttt	ttt	
	355	,,	1/400	—	ttt †					
24. 4. 13 ●	340	,,	1/100	—	—	S	—	—	—	Copenhagen serum. On 2. 2. 12 the titre was 1·6 fach.
	340	,,	1/200	—	—	,,	tt	tt	tt	
	340	,,	1/300	—	ttt †					
	340	,,	1/400	—	†					
	340	,,	1/500	tt	†					
25. 4. 13	360	,,	1/100	—	S	—	—	—	—	Copenhagen serum. On 9. 3. 12 the titre was 1·6 fach.
	345	,,	1/200	—	,,	—	—	tt	tt	
	350	,,	1/300	—	,,	ttt†t				
	340	,,	1/400	—	†					
	350	,,	1/500	— †						

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TABLE XII (*continued*).

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day: 1	2	3	4	5	6	Remarks
31. 3. 13	370	0·0006 U.S.A. 4	1/500	—	—	—	—	—	S	
	365	,,	1/750	—	—	—	—	—	,, t	Vienna serum. On 27. 11. 12 was 15–20 fach.
	360	,,	1/1000	—	—	—	—	t	,, †	
	350	,,	1/1250	—	—	t	†			
	365	,,	1/1500	—	tt †					
31. 3. 13	340	,,	1/500	—	—	—	—	—	—	Vienna serum. On 1. 3. 11 was 25 fach.
	340	,,	1/750	—	—	tt †				
	370	,,	1/1000	—	tt †					
	340	,,	1/1500	— †						
	370	,,	1/2000	t †						
31. 3. 13	340	,,	1/1000	—	—	—	—	—	—	Vienna serum. On 4. 9. 12 was 40–50 fach.
	340	,,	1/1500	—	—	—	—	t	†	
	355	,,	1/2000	—	—	ttt†				
	340	,,	1/2500	—	ttt†					
	340	,,	1/3000	— †						
31. 3. 13	360	,,	1/1000	— †						Rotterdam serum No. 150. Titre unknown.
	350	,,	1/1500	— †						
	345	,,	1/2000	tt †						
	350	,,	1/2500	tt †						
	370	,,	1/3000	tt †						
2. 4. 13	345	,,	1/250	—	—	—	—	—	—	
	380	,,	1/500	—	—	—	—	—	—	
	380	,,	1/750	—	—	—	—	—	—	

If we suppose for the purposes of comparison that the German unit is equal to say 40 U.S.A. units, then in the case of the Copenhagen sera we see that of the two sera tested against Toxin 3 one has practically lost nothing in 21 months and the other about 46 % in 16 months, whereas the six sera tested against Toxin 4 show an average loss of some 57 % in 10 months. The loss of unitage in the Vienna sera appears to be even greater. These rates of deterioration are excessive and one is forced to the conclusion that the test dose of Toxin 4 is somewhat too large under our conditions of experiment.

Now before being sent out this toxin was tested against a standard antitoxin and the L+ dose fixed only after careful experiment by the U.S.A. Public Health Service. My results have disagreed with theirs too consistently and regularly for the want of agreement to be ascribed to errors in technique on my part and the only explanation I can offer is that the resistance of guinea-pigs bred in England may be less than

that of American animals. Such a racial variation in susceptibility with regard to diphtheria is referred to by Ehrlich and by Theobald Smith. (Cf. G. Dean, p. 471, The Types of Immunity in *The Bacteriology of Diphtheria* edited by G. H. F. Nuttall and G. S. Graham Smith. Cambridge University Press 1907.)

The deterioration of Toxin 3 is easily accounted for as it was not kept under absolutely the best conditions.

It is therefore advisable that in each laboratory where this method is used a standard dry antitoxin should be kept to control the toxin from time to time and to standardize each new batch of toxin.

Provided this precaution is taken this method of standardizing tetanus antitoxin may be accepted as being just as simple, accurate and reliable as Ehrlich's method of standardizing diphtheria antitoxin.

The following experiments may be considered unnecessary. They are given just to "complete the picture."

Rosenau and Anderson recommend that the toxin and serum should be allowed to remain in contact for one hour at room temperature before injection.

From Table XIII we gather that in the case of ordinary serum this duration of contact is ample.

When, as in the case of tetanus, large doses of serum are necessary the question of the presence or absence of an antiseptic becomes of importance and it may be found to be preferable to make use of a serum which has been sterilized by filtration and heating and which contains no preservative.

The effect of heating tetanus antitoxin at 57° C. is shown in Table XIV, which also provides further evidence of the reliability of this method of testing.

We see that serum which is free from antiseptic can be heated daily for one hour for three days without loss of antitoxin.

If an antiseptic such as carbolic acid be present there is a loss of some 12 % which all occurs during the first hour. We find also that tetanus antitoxin differs somewhat from diphtheria antitoxin in being affected by heat in the presence of an antiseptic even though diluted with one third of its bulk with water and with NaCl present to the extent of 1·5 % (as in first stage of Gibson and Bauzhof's concentration process).

TABLE XIII. *Effect of temperature and duration of contact upon the union of toxin and antitoxin.*

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day: 1	2	3	4	5	6	Remarks
16. 4. 13	340	0·0006 U.S.A. 4	1/1750	—	—	—	S	—	—	
	340	,,	1/2000	—	—	—	,,	—	tt	
	340	,,	1/2250	—	—	tt	,, †			*T. 31 serum.*
	340	,,	1/2500	—	—	ttt†				
	350	,,	1/2750	—	tt †					
	340	,,	1/3000	—	ttt †					
21. 4. 13	340	,,	1/1500	—	—	—	—	—	—	*T.31serum.* Serum and toxin in contact ½ hr. at room temperature before injection.
	345	,,	1/1750	—	—	—	—	—	—	
	350	,,	1/2000	—	—	—	—	—	—	
	340	,,	1/2250	—	—	tt	tttt	†		
	340	,,	1/2500	—	—	tttt †				
	340	,,	1/1500	—	—	—	—	—	—	Serum and toxin in contact ½ hr. at 36° C. before injection.
	345	,,	1/1750	—	—	—	—	—	—	
	350	,,	1/2000	—	—	t	t	t	tt	
	340	,,	1/2250	—	—	ttt	ttt	tttt †		
	340	,,	1/2500	—	tt †					
	340	,,	1/1500	—	—	—	—	—	—	Serum and toxin in contact 1 hr. at room temperature before injection.
	340	,,	1/1750	—	—	—	—	—	—	
	340	,,	1/2000	—	—	—	tt	tt	tt	
	340	,,	1/2250	—	—	—	tt	ttt	†	
	350	,,	1/2500	—	—	ttt †				
	355	,,	1/1500	—	—	—	—	—	—	*T.31serum.* Serum and toxin in contact 1 hr. at 36° C. before injection.
	340	,,	1/1750	—	—	—	—	—	—	
	340	,,	1/2000	—	—	—	—	—	—	
	340	,,	1/2250	—	—	t	ttt	tttt †		
	345	,,	1/2500	—	—	t	ttt	tttt	†	
	340	,,	1/1500	—	—	—	—	—	—	Serum and toxin in contact 1½ hrs. at room temperature before injection.
	340	,,	1/1750	—	—	—	—	—	—	
	340	,,	1/2000	—	—	—	—	—	—	
	340	,,	1/2250	—	—	—	—	—	t	
	340	,,	1/2500	—	—	ttt †				
	340	,,	1/1500	—	—	—	—	—	—	Serum and toxin in contact 1½ hrs. at 36° C. before injection.
	355	,,	1/1750	—	—	—	—	—	—	
	340	,,	1/2000	—	—	—	—	—	—	
	340	,,	1/2250	—	—	t	tt	tt		
	340	,,	1/2500	—	—	tt †				
	345	,,	1/1500	—	—	—	—	—	—	Serum and toxin in contact 2 hrs. at room temperature before injection.
	345	,,	1/1750	—	—	—	—	—	—	
	340	,,	1/2000	—	—	—	—	—	t	
	345	,,	1/2250	—	—	—	tt	tttt	†	
	340	,,	1/2500	—	—	ttt †				
	350	,,	1/1500	—	—	—	—	—	—	*T.31serum.* Serum and toxin in contact 2 hrs. at 36° C. before injection.
	340	,,	1/1750	—	—	—	—	—	—	
	345	,,	1/2000	—	—	—	—	—	—	
	340	,,	1/2250	—	—	—	t	tt	tt	
	340	,,	1/2500	—	—	tt	tt†			

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TABLE XIV. *Showing the effect of heating tetanus antitoxin at* 57° C.

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day: 1	2	3	4	5	6	Remarks
3. 1. 13	360	0·0006 U.S.A. 3	1/1500	—	S	—	—	—	—	Serum T. C without antiseptic, Unitage *before* heating 1 hr. × 57° C. 200 units.
	340	,,	1/2000	—	,,	—	tt	tt†		
	365	,,	1/2500	—	,,	t †				
	375	,,	1/3000	—	,, †					
	375	,,	1/3500	—	,,†					
	350	,,	1/1500	—	,,	—	—	—	—	Serum T. C. Unitage *after* heating 200 units.
	340	,,	1/2000	—	,,	—	—	tt †		
	340	,,	1/2500	—	,,	t †				
	365	,,	1/3000	—	,, †					
	380	,,	1/3500	—	,, †					
6. 1. 13	340	,,	1/1500	—	—	—	—	—	S	Serum T. J without antiseptic *before* heating 1 hr. × 57° C. Unitage = 150 units.
	375	,,	1/2000	—	t	ttt†t				
	370	,,	1/2500	—	ttt†					
	365	,,	1/3000	— †						
	380	,,	1/1500	—	—	—	—	—	S	Serum T. J *after* heating. Unitage = 150 units.
	350	,,	1/2000	—	t	ttt†t				
	360	,,	1/2500	—	ttt†					
	370	,,	1/3000	— †						
15. 5. 13	340	0·0006 U.S.A. 4	1/1000	—	—	S	—	—		T. C serum 10. 5. 13, before heating. No antiseptic present.
	340	,,	1/1250	—	—	,,	—	—		
	345	,,	1/1500	—	—	,,	—	t		
	340	,,	1/1750	—	—	,,	tt	ttt		
	340	,,	1/2000	—	t	,,†				
17. 5. 13	340	,,	1/1250	S	—	—	—	—	—	Same serum after heating for 1 hr. at 57° C.
	340	,,	1/1500	,,	—	—	—	t	tt	
	340	,,	1/1750	,,	tt	ttt	tttt †			
	350	,,	1/2000	,,	tt	ttt†t				
	345	,,	1/2250	,,	ttt †					
	350	,,	1/1250	,,	—	—	—	—	—	Same serum after heating for 1 hr. at 57° C. on two succeeding days.
	340	,,	1/1500	,,	—	—	—	t	tt	
	340	,,	1/1750	,,	—	t	ttt †			
	370	,,	1/2000	,,	tt	tt	†			
	350	,,	1/2500	,,	tt	†				
	340	,,	1/1250	,,	—	—	—	—	—	Same serum after heating for 1 hr. at 57° C. on three succeeding days.
	360	,,	1/1500	,,	—	—	—	t	tt	
	365	,,	1/1750	,,	t	tt	ttt	ttt †		
	340	,,	1/2000	,,	— †					
	340	,,	1/2500	,,	ttt †					
7. 5. 13	370	,,	1/1500	—	—	—	—	—	—	Serum T. 31 containing a little antiseptic. On 16. 4. 13 the unitage was 225, see Table XIII.
	345	,,	1/1750	—	—	—	—	—	—	
	350	,,	1/2000	—	—	—			†	
	340	,,	1/2250	—	tt	ttt †				
	370	,,	1/2500	—	ttt †					

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TABLE XIV (*continued*).

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day: 1	2	3	4	5	6	Remarks
5. 5. 13	340	0·0006 U.S.A. 4	1/1500	—	—	—	—	t	t	
	340	,,	1/1750	—	—	ttt	tttt †			T. 31 heated for 1 hr. at 57° C.
	350	,,	1/2000	—	tt	†				
	370	,,	1/2250	—	ttt†					
	370	,,	1/2500	—	ttt†t					
7. 5. 13	340	,,	1/1000	—	—	—	—	—	—	T. 31 heated for 1 hr. at 57° C. on two successive days.
	340	,,	1/1500	—	—	—	—	—	—	
	340	,,	1/1750	—	—	—			tt	
	340	,,	1/2000	—	--	ttt †				
	340	,,	1/2250	—	tt	†				
	355	,,	1/1000	—	—	—	—	—	—	T. 31 heated for 1 hr. at 57° C. on three successive days.
	340	,,	1/1500	—	—	t			tttt	
	345	,,	1/1750	—	—	tt		†		
	360	,,	1/2000	—	ttt	†				
	370	,,	1/2250	—	tttt †					
5. 5. 13	350	,,	1/500	—	—	—	—	—	—	Tetanus antitoxin containing carbolic acid 0·4 %.
	350	,,	1/750	—	—	—	—	—	—	
	365	,,	1/1000	—	—	—	—	—	—	
	360	,,	1/1250	—	—	ttt	ttt†			
	340	,,	1/1500	—	tttt†					
10. 5. 13	340	,,	1/500	S		—	—	—	—	The same serum diluted with ⅓rd its vol. of distilled water, NaCl added to 1½ %, and heated to 57° C. for 7 hrs.
	340	,,	1/600	,,		—	—	—	—	
	340	,,	1/700	,,		—	t	tt	ttt	
	340	,,	1/800	,,		t	ttt†t			
	340	,,	1/900	,,	†					

Experiments on mice.

Some experiments were performed to ascertain the minimal lethal dose of these toxins for mice.

On 18. 4. 13 the M.L.D. of Toxin 3 for mice of 30 grms. was found to be 0·000002 grm. and on 17. 4. 13 Toxin 4 had an M.L.D. for mice of the same weight of 0·0000007 grm.

On 24. 4. 13, 0·0001 grm. of Toxin 4 was mixed with varying amounts of Copenhagen serum (sample of 2. 2. 12) and injected into mice weighing 35 grm.

Two mice each of which received 0·0001 gr. tox. + 1/750 c.c. serum—both lived.
,, ,, ,, ,, ,, + 1/1000 ,, —both died in 5 days, one not of tetanus.
,, ,, ,, ,, ,, + 1/1250 ,, —both lived.
,, ,, ,, ,, ,, + 1/1500 ,, —both died on 4th day.
,, ,, ,, ,, ,, + 1/2000 ,, —both died on 2nd day.

The mice with larger doses of serum lived.

SUMMARY.

The American method of standardizing tetanus antitoxin has been studied and two samples (Toxins 3 and 4 of this paper) have been examined.

It has been found that:

1. Toxin 3 remained stable for two years under conditions which were not absolutely ideal, as the tube containing the supply was repeatedly opened during that time and the air in this neighbourhood, especially in winter, is very moist.

Toxin 4 has so far remained stable for 12 months. This toxin has been distributed in small quantities in tubes which have been exhausted and sealed in the flame.

2. By itself each of these toxins has given results consistent within the error of experiment when used to establish the M.L.D. and also to test a serum. But when the same serum was evaluated by both toxins they gave differing values. This dffference in value appears to be due to

(*a*) Deterioration in Toxin 3 which raised the L+ dose from 0·0006—0·00075.

(*b*) Excess of toxicity in Toxin 4.

Whether this is due to increased susceptibility of the race of animals used for the tests can at present be only a matter of surmise.

3. The American method shows clearly that tetanus antitoxin can be heated at 57° C. for one hour on each of three successive days without loss of antitoxin provided no antiseptic (*e.g.* phenol) be present. Should such a preservative have been added then the loss on heating amounts to about 12 % and occurs during the first hour.

4. Tetanus antitoxin containing phenol appears to be more susceptible to heat than diphtheria antitoxin under the same conditions.

The general conclusion arrived at is that, provided control experiments are carried out from time to time (as in the case of standard diphtheria toxin), this method of standardizing tetanus antitoxin is—as claimed for it—simple, accurate and reliable.

ADDENDUM.

While this paper was in the printer's hands I had the opportunity of examining another sample of the dry powdered standard toxin for which I am greatly indebted to Dr J. F. Anderson. The results of these tests and of the evaluation of another market sample of antitetanic serum are given below.

It will be seen that the two toxins give practically identical results with both a strong and a weak serum and that the M. L. D. is much the same in each case.

On one occasion mentioned in the table and in several other experiments not given here the toxin was injected subcutaneously in the side over the lower ribs. It was noticed that in these cases the tetanic symptoms made their appearance earlier, but that death took place at about the same time as when the toxin was injected in the middle line of the abdomen.

Minimal lethal dose of toxins U.S.A. 4 and U.S.A. 5.

Date of test	Dose in grammes: 0·000007	0·000006	0·000005	0·000004	0·000003	0·000002	Remarks
U.S.A. 4.							
7. 7. 13	4	4	6	5	5	—	——
24. 10. 13	—	4	4	4	5	—	——
29. 10. 13	—	3	5	4	5	—	Injections made at side over lower ribs.
U.S.A. 5 received 6. 10. 13.							
10. 10. 13	—	4	5	5	Slight tetanus	Slight tetanus	

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Testing toxins U.S.A. 4 and U.S.A. 5 against the same sera.

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day: 1	2	3	4	5	6	Remarks
9. 12. 13	365	0·0006 U.S.A. 4	1/50	—	S	—	—	—	t	Serum Vulcan
	345	,,	1/60	—	,,	—	—	—	tt killed	
	340	,,	1/70	—	,,	—	—	t	tt killed	
	345	,,	1/80	—	,,	t	tttt	tttt†	—	
	355	,,	1/90	—	,,	tttt†	—	—	—	
	365	0·0006 U.S.A. 5	1/50	—	S	—	—	—	tt killed	Serum Vulcan
	375	,,	1/60	—	,,	—	tt	tttt †	—	
	340	,,	1/70	—	,,	tttt†	—	—	—	
	350	,,	1/80	—	,,	tt	tttt†	—	—	
	350	,,	1/90	—	,, †	—	—	—	—	
12. 11. 13	370	0·0006 U.S.A. 4	1/6000	—	—	—	S	—	—	Serum T. R. 4
	355	,,	1/7000	—	—	tt	,, †	—	—	
	340	,,	1/8000	—	ttt †	—	—	—	—	
	350	,,	1/9000	—	tttt †	—	—	—	—	
	340	0·0006 U.S.A. 5	1/6000	—	—	tt	S	tt	ttt killed	Serum T. R. 4
	340	,,	1/7000	—	—	ttt †	—	—	—	
	340	,,	1/8000	—	tttt †	—	—	—	—	
	370	,,	1/9000	—	tttt†	—	—	—	—	

Testing the value of Tizzoni's antitetanic serum.

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day: 1	2	3	4	5	6	Remarks
8. 11. 13	350	0·0006 U.S.A. 4	1/500	tt †	—	—	—	—	—	Market sample of serum Tizzoni 200,000 units in 5 c.cm.
	340	,,	1/1000	tttt†	—	—	—	—	—	
	340	,,	1/1500	tttt†	—	—	—	—	—	
	370	,,	1/2000	tttt†	—	—	—	—	—	
	340	,,	1/2500	tttt†	—	—	—	—	—	
	340	,,	1/3000	tttt†	—	—	—	—	—	
12. 11. 13	340	,,	1/50	—	—	—	S	—	—	
	340	,,	1/100	—	—	—	,,	—	—	
	340	,,	1/250	—	—	tt	,,	tttt	tttt killed	

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