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In order to standardize anti-dysentery serum, it is necessary to have an accepted unit; before this can be established, a test toxin and test antitoxin must be available to all workers at the subject. In this paper we describe—

(1) Preparation and testing of "test toxin" (dried bacillary bodies); (2) Testing of "test anti-dysentery (Shiga) serum," as they have been carried out in these laboratories for some years past (Sudmersen and Eagleton, *Lancet*, 1921, **2**, 1109).

The animals employed were mice and rabbits; intravenous inoculation was used throughout.

Questions relating to different toxins, use of other animals and other methods, and many other obvious points of interest are not touched upon in this present paper.

Toxin.—"Toxin" is prepared by growing *B. dysenteriæ* (Shiga) on nutrient agar for 48 hours, washing off the growth in distilled water, heating just to the lethal point, *i.e.* 58° to 60 C. for 10 minutes, centrifuging and drying rapidly in a desiccator. The dried scales are finally ground so as to make a homogeneous powder.

For some reason which we cannot explain, some of the toxins prepared for a period of many months were poor in potency and irregular in action. The obvious causes of failure might be the use of improper "peptone," or of cultures of *B. dysenteriæ* (Shiga) which had become "rough." To neither of these causes could we definitely assign our occasional failures; we may remark in passing that "rough" colonies have produced as good toxin as "smooth" ones. We have for some months past been able to make supplies of antigen which have a satisfactory potency. Our first standard toxin, of which a supply is available, is called AA.

Results of intravenous injection into mice.—Animals of 20 gm. were used for all confirmatory tests, though frequently animals of 15 to 25 gm. were the only ones available for preliminary work. The dose, made up to 1·0 c.c. in sterile 0·75 per cent. solution of NaCl in distilled water, was injected into

* The substance of the present paper is to be presented as the first of a series of reports to the League of Nations Standardization Committee, which has requested workers in a number of different laboratories to investigate the subject.

one of the tail veins. The mice were inspected daily until the seventh day; those alive at this time were called survivors. In Table I are given the results of the injections made to ascertain the *minimum lethal dose.* Two to four mice were injected with each dose in each experiment. It will be seen that the range is fairly wide, a dose of 0·04 mgm. being needed to produce a fatal result in all mice, whereas some mice died when given 0·005 mgm.

TABLE I.—*Minimum Lethal Dose; Shiga Toxin AA. (Intravenous Injection: Mice.)*

Amount of toxin in mgm.	Number dying.	Number living.	% deaths.
0·1	1	0	100
0·05	1	0	100
0·04	6	0	100
0·03	5	1	83
0·025	13	3	81
0·02	13	3	81
0·015	8	6	57
0·01	9	9	50
0·0075	5	7	42
0·005	4	12	25
0·0025	0	8	0

The test dose of toxin.—A weighed amount of toxin is put into a stoppered cylinder containing the requisite volume of saline solution and shaken violently for a few moments to produce a homogeneous suspension; this is agitated between the withdrawal of each of the doses used in the experiment. The varying doses are then made up to 0·5 c.c. with saline solution and mixed with 0·5 c.c. of a saline solution containing 0·025 c.c. of our standard antitoxin, C.950, a total volume of 1 c.c. being injected. The mixture of toxin and antitoxin must stand for at least 30 minutes before injection.

Antitoxin, standard.—We have decided to adopt 0·025 c.c. of this serum C.950 as the standard test dose. This antitoxin of low potency was chosen because it was apparently stable. It has been in use for several years, and the amount, 0·025 c.c., was, as nearly as we could ascertain in tests continued for several years, the amount necessary to protect more than half the mice or rabbits injected with approximately 10 lethal doses of the original toxin which had been in use by one of us (H. J. S.) for some years. It will be seen from Tables I and II that if we take as the minimum lethal dose of toxin AA, 0·005 mgm., the lowest amount that kills an appreciable number of mice, then 0·025 c.c. of serum C.950 will protect an occasional mouse when injected with 25 to 30 minimum lethal doses, *i.e.* 0·125 mgm. or 0·15 mgm., and will protect more than 50 per cent. of those receiving 0·1 mgm. It is not easy, however, to name a certain quantity as the minimum lethal dose, and it is not therefore possible to say with accuracy against how many "minimum lethal doses" of the toxin AA the test dose of antitoxin will protect. For testing sera we decided to adopt for the present 0·2 mgm. of toxin AA as the test dose, since at that point any survival is apparently "significant." We

have in some of our work used 0·05 c.c. or 0·1 c.c. of this serum; it may be advisable to reconsider this point when dealing later with the "unit" of antitoxin for therapeutic purposes. Our results lead us to anticipate that if we double or quadruple the test dose of both toxin and antitoxin we shall obtain the same ratios for any series of antitoxins under test.

In Table III are summarized some series of experiments by two investigators working independently to ascertain the ratio between the dose of toxin

TABLE II.—*Determination of "Test Dose" of Toxin AA. (Intravenous Injection: Mice. Antitoxin C.*950, 0·025 *c.c. constant.)*

Amount of toxin in mgm.								
0·25.	0·2.	0·15.	0·125.	0·1.	0·075.	0·05.	0·025.	0·015.
3	3	3	3	3	3	2	4	s
3	3	3	3	3	5	5	4	s
2	5	4	3	3	5	7	s	s
3	5	4	4	4	6	s	s	s
4	3	5	4	4	6	s	s	s
6	3	6	4	5	7	s	s	s
4	3	3	4	7	s	s	s	s
6	3	4	3	5	s	s	s	s
4	3	5	4	s	s	s	s	
4	2	4	3	s	s	s	s	
4	4	4	7	s	s	s	s	
3	4	4	5	s	s	s	s	
4	3	4	7	s	s	s	s	
s	3	4	3	s	s			
		4	2	s				
		2	1	s				
		2	s	s				
		6	s	s				
		4		s				
		4						
		1						
		s						

Figures indicate day of death. s = survived 7 days.

TABLE III.—*Determination of "Test Dose" of Toxin AA. (Intravenous Injection: Mice. Antitoxin C.*950, 0·025 *c.c. constant.)*

Amount of toxin in mgm.	Number dying.		Number living.		% deaths 7th day.		% deaths 4th day.
	R.	S.	R.	S.	R.	S.	R.
0·25	22	—	0	—	100	—	100
0·2	35	14	0	0	100	100	88
0·15	32	21	3	1	93	95	68
0·125	15	16	5	2	75	84	55
0·1	11	8	9	11	54	44	20
0·05	3	3	5	10	37	23	37

R and S indicate two observers working independently.

which, when mixed with 0·025 c.c., our test dose of antitoxin, would cause 100 per cent. of deaths, and those causing approximately 20 per cent. of deaths.

The intervals between the doses are much wider than in standardization tests of diphtheria and anti-tetanic sera; the "error of the test" is of a different order. But with this reservation there is a satisfactory degree of agreement between the results obtained by two investigators working independently. In the final column are shown the percentages that would have resulted if the period of observation of the mice had been limited to four days, and all mice living after that time had been considered as survivors. We consider it preferable to adopt seven days as the limit of observation.

Results of intravenous injection into rabbits (Tables IV and V).—It was unfortunately impracticable during these experiments to obtain rabbits of a standard weight. It proved in practice to be more convenient to use whatever

TABLE IV.—*Minimum Lethal Dose Shiga Antigen AA. (Intravenous Injection: Rabbits.)*

Amount of toxin in mgm.	Number dying.	Number living.
0·05	2	0
0·025	2	1
0·015	0	2
0·01	0	2
0·005	0	1

TABLE V.—*Toxin (Shiga) AA against Antitoxin C.*950. *(Intravenous Injection: Rabbits. Antitoxin C.*950, 0·025 *c.c. constant.)*

Amount of toxin in mgm.	Number dying.		Number living.	
	R.	S.	R.	S.
0·2	2	—	0	—
0·175	1	—	1	—
0·15	5	8	4	1
0·1	3	9	6	2
0·075	0	1	3	5
0·05	—	0	—	4
0·025	—	0	—	1

R and S indicate two observers working independently.

rabbits were available and to give the desired dose of toxin without making any allowance for weight. We should have preferred to use rabbits of 1500 gm. for all experiments, but those that were available varied in weight from 1400 to 2500 gm. It is probable that our practice of making no allowance for weight is at least partly responsible for the small difference between the results of the two workers shown in Table V. It will be noted that one worker found that 0·15 mgm. of toxin AA when mixed with 0·025 c.c. of standard antitoxin and injected would kill 8 rabbits out of 9, whereas the other found only 5 out of 9.

When using a dose of 0·1 mgm. of toxin, one worker found that 9 out of 11 rabbits died, whereas the other found only 3 out of 9.

The dose chosen as a test dose of toxin is that which when mixed with 0·025 c.c. of C.950 would kill all rabbits injected; this dose is 0·2 mgm.

Summary.—A dysentery (Shiga) "toxin" consisting of dried bacillary bodies is described as a standard toxin for the titration of anti-dysentery sera.

The test dose of the toxin has been found by titration against a test dose of standard anti-dysentery (Shiga) serum.

We are at present undecided whether further experience will lead us to prefer mice or rabbits for standardization.

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