Standardization of anti-dysentery (shiga) serum. 2

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THE STANDARDIZATION OF ANTI-DYSENTERY (SHIGA) SERUM.—II.

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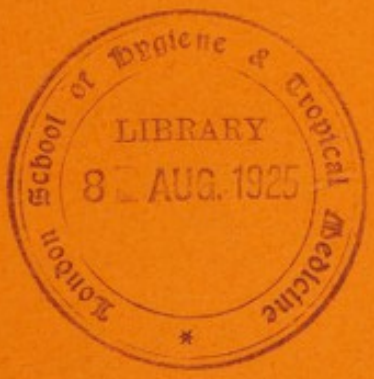

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THE STANDARDIZATION OF ANTI-DYSENTERY (SHIGA) SERUM.—II.*

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DETAILS of the method used for the standardization of anti-dysentery serum by intravenous injection into mice have already been published (Sudmersen, Runge and O'Brien, 1924), and the standard materials, "toxin" and antitoxin, described. We adopted a fixed dose, 0·025 c.c. of serum C. 950, as a test-dose and titrated the first standard toxin A.A. against this dose, using a large number of mice. The smallest amount of toxin, which, when mixed with the test-dose of serum, would kill approximately 100 per cent. of mice, was taken as the test-dose of toxin A.A. A second toxin, 3 A., has now been similarly titrated against the test-dose of C. 950, and a second serum, S. 9, standardized against the two toxins. The results of these tests are shown in Table I. Toxin 3 A. is almost exactly equal to toxin A.A. when

TABLE I.—*Interrelation of two "Standard" Anti-Dysentery (Shiga) Toxins and two "Standard" Antitoxins.*

Serum.	Dose c.c.	Toxin.	Dose mgms.			Per cent. deaths.			No. of mice used.
C.950	0·025	A.A.	0·2	0·15	0·1	100	91	51	90
C.950	0·025	3.A.	0·2	0·15	0·1	100	87	56	160
S.9	0·002	A.A.	0·2	—	—	95	—	—	40
S.9	0·002	3.A.	0·2	0·15	0·1	100	97	60	60

TABLE II.—*Tests of Various Anti-Dysentery (Shiga) Sera. Test Dose of Toxin* 0·2 *mgm.*

No. of serum.	Serum c.c.			Results. Lives/No. injected.			Value.
T	0·1	0·05	0·025	4/4	4/7	1/7	0·1
Frankfurt	0·05	0·025	0·01	8/9	3/9	0/6	0·05
L	0·02	0·01	0·005	6/6	2/8	0/5	0·02
W	0·01	0·005	0·0025	6/6	2/6	0/8	0·01
B	0·005	0·003	0·002	4/4	1/6	0/2	0·005
Varsovie	0·006	0·003	0·0015	5/6	7/9	1/12	0·003
A	0·002	0·001	0·0005	4/4	3/8	0/2	0·002

In the "results," the figure after the stroke gives the total number of mice injected, the figure before the stroke the number surviving.

* The work reported in this paper will form a report to the League of Nations Standardization Committee.

TABLE III.—*Antitoxic Value in Units per c.c. of 18 Commercial and Standard Anti-Dysentery (Shiga) Sera.*

Serum.	Units per c.c.	Serum.	Units per c.c.
A	500	G4	40
V and G3	300	H, D and E	30
B, G1 and G5	200	Fr	20
W, G2 and K	100	T	10
L, M and S	50		

tested against either of the two sera. Tables II and III give the results of tests on anti-dysentery sera from various laboratories. In Table II are given the details of a few of the tests in order to indicate the degree of accuracy of the method. Table III gives the general summary. The general scheme for the test was to estimate three particular points, small numbers of mice being used. At the first point 80 to 100 per cent. of the mice are protected against the test-dose 0·2 mgm. of toxin; at the second point between 20 and 50 per cent. are protected, and at the third point less than 20 per cent.

These points represent roughly the L 0* and the L + values of the serum with an intermediate point. Two, three or more mice were injected with the same mixture of toxin and serum, and the test was repeated on several occasions either at exactly the same points or at points very near to the previous one. Thus, where the total number injected is given as four, two mice were used on each of two occasions. Where only two mice are shown at the last point, other tests at lower levels had given no protection. The range from L + to L 0 varies a certain amount in the different samples, the L 0 dose being generally about three to four times the L + dose.

Individual mice vary a good deal in their susceptibility to Shiga toxin, and in addition to this they are delicate animals, and easily succumb to changes of temperature or feeding during a period of observation extending over seven days. In order to test within narrow limits a large number of animals would be necessary. In such an instance as No. 6 on the table there is some doubt as to the value to be assigned. The highest point, 0·006, gives one death in six mice, and the next point, 0·003, two deaths in nine. However, when the details of the tests, put up on three separate occasions, were taken into account, it appeared probable that two of the mice had died from other causes and the second point was taken as the true value. In all other cases there was no difficulty in distinguishing, with the small number of mice used, the points at which the serum was tested.

In the last column of Table II are shown "values." We have for some time past used these values as "units," and propose to continue doing so until the international standard unit is fixed by the League of Nations Committee. Our provisional unit is the amount of serum which will neutralize the test-dose 0·2 mgm. of our standard toxin A.A. (this is approximately ten lethal doses). In Table III we have collected the results of titrations of sixteen sera issued by various laboratories, as well as Frankfort standard serum and

* It must be realized that the use of the symbols L + and L 0 here is not in direct accordance with Ehrlich's use of the terms. Despite the inaccuracy, we would retain previsionally these convenient terms instead of the cumbrous phrases "approximately 80 per cent. protection dose" and "approximately 20 per cent. protection dose."

Varsovie standard kindly sent us by Prof. Kolle and Prof. Hirszfeld respectively. "A" was a concentrated serum, the others unconcentrated. It will be seen that the range of values is undesirably wide. At present it is possible for a clinician to purchase and use two samples of commercial anti-dysentery serum which bear no indication on the label, and therefore would be presumed to be of equal "value," and yet when using the weaker serum he would be giving $\frac{1}{20}$th or $\frac{1}{50}$th of the antitoxin contained in the stronger serum.

It appeared to be desirable to find out how accurately one could titrate the antitoxic value of a standard serum if a moderate number of mice were used. From a review of our general results we had formed the opinion that we might be able to distinguish sera differing by 20 per cent. in value.

Two dilutions of a serum of known strength were made by mixing with normal horse-serum so that they contained 80 per cent. and 64 per cent. of the original serum, and the undiluted serum and the two dilutions were numbered and handed over for test. The strength of the undiluted serum was known, but the identity of the three samples was throughout concealed from the tester. Each serum was tested at three points at 20 per cent. intervals from one another, and the test was repeated three times, the samples being re-lettered between each test. Table IV shows the results obtained. A figure in the table represents the day of death of a mouse, L indicates that the mouse lived.

TABLE IV.—*Tests on three Dilutions of Serum. Test-dose* 0·2 *mgm.* 3 *A.*

	Serum c.c. 0·008	0·0064	0·005	Identification by tester.
1st test.				
Sample labelled A .	LLLLL	LLLLL	3444L .	80 per cent.
,, ,, B .	45LLL	33444	34446 .	64 ,,
,, ,, C .	6LLLL	LLLLL	457LL .	100 ,,
2nd test.				
Sample labelled A .	6LLLL	3445L	1133L .	64 per cent.
,, ,, B .	LLLLL	45LLL	244LL .	80 ,,
,, ,, C .	LLLLL	LLLLL	3LLLL .	100 ,,
3rd test.				
Sample labelled K .	36LLL	5LLLL	3345L .	80 per cent.
,, ,, L .	6LLLL	4LLLL	33444 .	64 ,,
,, ,, M .	LLLLL	14LLL	3LLLL .	100 ,,

Summary.

Serum.	Dose c.c. 0·008	0·0064	0·005
	15 mice at each dose. Mice protected.		
100 per cent. .	14 .	13 .	10
80 ,, .	13 .	12 .	4
64 ,, .	11 .	5 .	1

In the first test sample C appeared to be somewhat stronger than A, one death on the sixth day at the highest dose being considered accidental, and B

decidedly the weakest. In the second, C is again the strongest and the other two are in the order B and A. The third test gave the order M, K, L. The order in which the three sera were placed by the tester was correct in each case. In only one test was there any real difficulty in distinguishing between two dilutions. K and L in the third test are separated only by the life of a single mouse, and the decision rested more on the judgment of the tester as to the condition of the mouse than on the figures available. On the other occasions the 20 per cent. decrease in value of the three samples is easily recognizable.

CONCLUSIONS.

1. The antitoxic values of anti-dysentery (Shiga) serum can be determined with a reasonable degree of accuracy by the well-known mouse intravenous method.

2. It is possible with a limited number of mice to distinguish between sera differing in strength by about 20 per cent.

3. There is a wide variation in antitoxic strength between the strongest and weakest antitoxic sera used in clinical work in different parts of the world.

REFERENCE.

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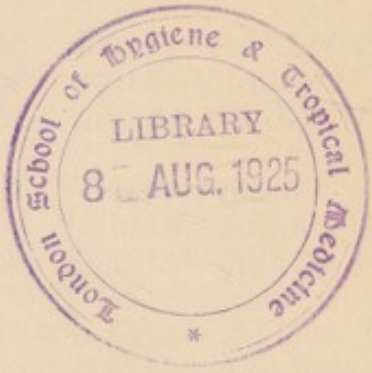

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