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NOTICE TO BINDER.

This page should be inserted in *The Journal of Hygiene*, Vol. 29, so as to face page 28.

The omission of Table XI from this paper was notified to the Editors in a letter from Dr Stuart, dated 11 Jan. 1930, who overlooked the omission when he read the proof. We regret that it was left to a reviewer in *The Tropical Diseases Bulletin*, Vol. 26, p. 733 (September, 1929) to note the oversight.—ED.

Table XI. *Twenty rabbits were used for these experiments.*

Rabbit No.	Dose and period for immunisation: 2 c.c. daily for 14 days	4 c.c. daily for 14 days	6 c.c. daily for 10 days	15 c.c. daily for 3 days	One injection of 30 c.c.	Whether or not animal survived treatment	Subdural test 2 weeks after treatment tested with 0·2 c.c. of 1 % emulsion of fixed virus
1	1	.	.	.	.	Survived	Survived
2	1	.	.	.	.	,,	Died
3	1	.	.	.	.	,,	,,
4	1	.	.	.	.	,,	Survived
5	.	1	.	.	.	,,	,,
6	.	1	.	.	.	,,	Died
7	.	1	.	.	.	Died	.
8	.	1	.	.	.	Survived	Died
9	.	.	1	.	.	,,	Survived
10	.	.	1	.	.	,,	Died
11	.	.	1	.	.	,,	,,
12	.	.	1	.	.	,,	,,
13	.	.	.	1	.	,,	,,
14	.	.	.	1	.	,,	,,
15	.	.	.	1	.	,,	,,
16	.	.	.	1	.	,,	,,
17	.	.	.	.	1	,,	,,
18	.	.	.	.	1	,,	,,
19	.	.	.	.	1	Died	.
20	.	.	.	.	1	Survived	Died

Table I.

Clinician	Toxin	Description of patient	Dilution (in thousands) (6 = 1/6000)	No. of patients	% positive
A	*A*	Not scarlet fever	6	25	32
			1		64
B	*B*	Not scarlet fever	6	30	33
			3		63
			1		86
C	*A*	Convalescent after scarlet fever	6	25	0
			1		32
D	*B*	Convalescent after scarlet fever	3	11	55
			1		91
E	*B*	Admitted for scarlet fever	6	17	30
			3		35
			1		65
F	*A*	Acute scarlet fever and convalescents	3	58	12
			1		43
G	*B*	Not scarlet fever	6	35	77
			3		77
			1		80
H	*B*	Acute scarlet fever and convalescents	3	128	57
			1		71
H	*B*	Acute scarlet fever and convalescents	2	78	61
			1		61

In Table II are given the results of the injection of two or more different dilutions of the same toxin into each of some 422 patients convalescent from scarlet fever. The different strengths of toxin give differences in percentage.

Table II.

Toxin	Dilution (in thousands)	No. of patients*	% positive
B	2	70	31
	6	70	21
B	2	188	43
	4	188	32
A	1	164	28
B	1	164	52
B	2	154	42
B	4	150	26

* Many of the patients referred to in column 3 were used for several tests and consequently appear several times in the enumerations.

From the observations on the group of 164 patients it seemed reasonable to conclude that, as an indicator of immunity, the second toxin *B* in a dilution of 1/4000 was approximately equal to toxin *A* in 1/1000 dilution. In Table III

Table III. *Comparison of Toxin A* 1/1000 *with Toxin B* 1/1000, 1/2000 *and* 1/4000.

Toxin *A* 1/1000 was	>	*B* 1/1000 in	4	comparisons
,,	,,	=	,,	9	,,
,,	,,	<	,,	105	,,
Toxin *A* 1/1000 was	>	*B* 1/2000 in	17	,,
,,	,,	=	,,	12	,,
,,	,,	<	,,	61	,,
Toxin *A* 1/1000 was	>	*B* 1/4000 in	43	,,
,,	,,	=	,,	18	,,
,,	,,	<	,,	21	,,

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the measurements are somewhat further analysed and the suggestion arising therefrom is that toxin *A* 1/1000 is stronger than *B* 1/4000 but weaker than *B* 1/2000 and that therefore *B* 1/3000 would be nearly equal to *A* 1/1000.

In an attempt to see with what certainty one could distinguish dilutions of 1/1000 from 1/2000 and 1/4000 of the same toxin (*i.e. B*), the measurements made on patients receiving two or three of these dilutions were further analysed. The correct answer was obtained in 63 per cent. of the observations, *i.e.* reaction 1/1000 was consistently greater than 1/2000 and 1/4000; a nearly correct answer, *e.g.* 1/1000 stronger than 1/2000 which was equal to 1/4000, in 23 per cent., and an incorrect answer, *e.g.* 1/2000 stronger than 1/1000, in 14 per cent.

Table IV records another small series of comparisons of 1/2000 and 1/4000 of the same toxin. The correct answer, *i.e.* "reaction 1/2000 stronger than 1/4000," is given in 72 per cent. and an incorrect answer in 28 per cent. of

Table IV.

1/2000 Dick reaction	Relation of 1/2000 reaction to 1/4000	1/4000 Dick reaction	No. of patients	%
+	Greater	+	25	42
+	Greater	−	18	30
+	Equal	+	14	23
+	Less	+	3	5

instances. In another series of 13 volunteers, dilutions of 1/500 and 1/1000 of a certain toxin were used. Here 85 per cent. gave a correct answer; in a further series of 12 where dilutions of 1/1000 and 1/2000 were used, the correct answer was given in 75 per cent. and an incorrect one in 25 per cent.

In 10 instances Dr Okell and Dr Parish made three injections of the same toxin, one of 1/1000, and two injections of 1/2000 into each patient. The correct reading later should have been "1/1000 greater than 1/2000 but both 1/2000 reactions equal." The reactions were carefully measured, both observers being in ignorance of the identity of the reaction; the correct answer was got in only 4 of 10 observations.

Table V records this series.

Table V. *Comparison of Toxin B* 1/1000 *with two dilutions of* 1/2000.

$1T > 2T = 2T$	4
$1T > 2T > 2T$	3
$2T > 1T = 2T$	1
$1T = 2T = 2T$	2

$1T = 1/1000$
$2T = 1/2000$

DISCUSSION.

We have been unable to find any large groups of published figures dealing with the particular points we aimed at investigating, and we cannot from our own experience give any satisfactory answer to the question—is it possible to distinguish with certainty toxin of value x from those of values $1{\cdot}5x$, $2x$, $3x$, or $4x$? The test for the purpose of answering this question we visualise

somewhat as follows: Observer A makes three bottles of strength x and three of the nearest strength which Observer B thinks he can differentiate, e.g. $2x$. The six bottles are lettered indiscriminately so that Observer B has no clue to their identity. B then tests the six samples on the number of patients he thinks necessary, and writes down his conclusions before seeing the key. If B identifies all six bottles correctly, such a result is beyond the operation of chance and would amount to proof that B can distinguish x from $2x$.

The toxins made in large batches for the immunisation of horses are tested intracutaneously on groups of volunteers and before a toxin may be passed for horse injection it must give a clear reaction in a dilution of 5000 in all of a group of three or four volunteers. We often inject a series of dilutions, *e.g.* 1/1000, 1/5000, 1/2000. It has occasionally happened that one of us, seeing the patient but once and reading the reactions without knowing anything of the dilutions used or of the identity of the various injections—and this we regard as an indispensable condition in any work of this kind—has concluded that the 20,000 reaction on one or more of the individuals used in the test was equal to or greater than the 5000 or 1000.

We are convinced that an increase of accuracy in comparing two toxins can be got by careful choice of the volunteer for test. We have found, for instance, that though it was difficult to distinguish a strength of 1/1000 from 1/2000 on a "strong positive reactor," yet in repeat tests in another subject negative to the ordinary Dick strength we could distinguish 1/100 from 1/200 of the same toxin. It is obvious that in titrating toxins in subjects negative to the ordinary Dick test we are testing the given strength of the toxin against the amount of antitoxin present in the skin of the subject and really doing a toxin-antitoxin titration.

Dr Okell and Dr Parish are at present investigating various aspects of the subject, particularly whether, by careful preliminary tests on the subjects available for titration, it is possible to distinguish with regularity a toxin of strength x from one of $2x$.

With regard to the choice of a suitable dilution for clinical use on the large scale, the toxin we originally chose, and the dilution for the indication of immunity, have proved satisfactory in practice, for by their use nursing staffs considered to be immune on the basis of the test have continued free of scarlet fever; and in groups of children, whereas those negative have almost consistently remained free of the disease even when exposed to continued risk of infection, the cases of scarlet fever have occurred amongst the positive reactors. In patients suffering from scarlet fever, in the first two days of rash, the ideal toxin should give approximately 100 per cent. of positive reactions. (Joe records 95 per cent. in a series of 700 patients; the toxin was made from a local strain of streptococcus.) Sutherland records 86 per cent. positive of 251 patients with toxin *A*, and when those with vivid rash were deducted (in whom presumably the toxin injected in the Dick test could produce no further dilatation) 97 per cent. of 223 patients gave a positive reaction. But on large

numbers of figures obtained from various hospitals where this bulk of toxin was used, the average percentage of positive reactions in patients believed to be, on the history available, in the first three days of the disease has been about 80.

In passing we may say that all toxins for the immunisation of horses must be able to kill two or three of three rabbits injected intravenously with 10 c.c. We find that with care we can usually ensure that our toxins reach this level.

SUMMARY.

The final choice of a suitable dilution of a given toxin will be made, partly from the difficult comparison with other known toxins, but mainly from the results of clinical observations in patients in the early stage of scarlet fever and in convalescents.

It is difficult to titrate scarlet fever toxin with accuracy. It will probably be found that by careful choice of subject and by several readings of the reactions at different intervals after the injection, one can differentiate between toxins of values x and $2x$.

(*MS. received for publication* 24. VIII. 1929.—Ed.)