

THE STABILITY OF SCHICK TOXIN.

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THE stability of the biological reagents used in routine immunological procedures is a matter of practical interest to the public health worker, and an attempt has been made in the following research to estimate the period during which the diluted toxin used in the Schick test for the estimation of susceptibility to diphtheria remains potent. In the early days of Schick testing, although Leete (1920) reported that a diluted toxin retained its potency for as long as 5 weeks, it has hitherto been considered inadvisable to use a toxin at a longer interval than 48 hours after it had been diluted to its proper strength. Glenny, Waddington and Pope (1928) and O'Brien, Okell and Parish (1928) have introduced a new diluent consisting of a solution of a combination of crystal borax 57, boric acid 84 and sodium chloride 99 parts. A 1.5 per cent. solution of this mixture is now used as a diluent and has the effect of sustaining the potency of the diluted toxin over a greater period of time. In our work over 400 persons have been tested with toxins made up according to this formula, a comparison being made in each individual between the reactions elicited by an aged toxin, stored for varying periods at room temperature or in the cold room, with those produced by a fresh toxin. Enough toxin was made up at the beginning of the experiment to last over a considerable period of time, samples being sent to us by post periodically together with batches of freshly diluted toxin. The testing of reagents was carried out immediately on receipt, the lower age groups being selected in any series of individuals as being likely to give the largest number of reactors. The actual testing was performed practically throughout by one of us (J. C. J. McEntee) so as to maintain uniformity of technique. Each pair of toxins was controlled by a single injection of inactivated toxin and the results were scrutinised at the 4th and 10th days, a record of the comparative intensities of the positives being made at the same time. The results are recorded in Table I.

COMMENTARY.

Regarding the simple issue, as to whether the reactions were positive or negative, it will be noted that the correspondence is complete during the whole time the toxin kept at room temperature was under observation, and up till the 194th day when the toxin was stored in the cold room. All the results given as positives were readable reactions which would have been classified as positive by anyone with a reasonable experience of the test, and as a matter of fact very few indeterminate reactions were met with in the course of the experiment. We expected to obtain some indication of waning potency of the stored toxin from our observations on the intensity of the

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reaction, but a study of Table I will show that our records of this feature are somewhat difficult of interpretation. It should be stated at once that no single standard of comparison was applied to all reactions, but each pair was considered separately. Apart from minor variations in technique, which even after a very large experience of intracutaneous work we can never be certain of fully eliminating, further difficulties seem to be introduced by the fact that

Table I. *Showing the Results of Comparative Tests performed with Aged and Fresh Schick Toxin at Various Intervals of Time.*

Length of storage of aged toxin	Total no. of persons tested	No. of cases reacting to both toxins	No. of cases reacting only to fresh toxin	Comparison of intensity of reactions*		
				$A = F$	$A < F$	$A > F$
I. At room temp.						
(days)						
13	18	14	nil	10	1	3
19	19	16	nil	14	1	1
26	21	14	nil	12	1	1
33	19	16	nil	7	9	0
40	21	13	nil	11	2	0
47	17	12	nil	10	1	1
53	24	20	nil	14	4	2
67	20	13	nil	2	10	1
81	20	14	nil	8	5	1
II. In cold room						
95	24	16	nil	7	7	2
109	20	16	nil	4	1	11
123	20	14	nil	11	0	3
139	22	15	nil	6	9	0
151	21	16	nil	5	11	0
165	22	13	nil	0	13	0
182	19	16	nil	9	7	0
194	22	13	nil	3	10	0
207	23	16	2	3	15	0
235	24	13	2	3	12	0

* A = Aged Toxin, F = Fresh Toxin.

the fresh toxin apparently varied within slight limits from time to time. In support of this we would draw attention to the records of the intensities of the reactions on the 33rd and the 109th days. In the former instance the fresh toxin seems to have been slightly more potent than usual, whereas in the latter, the opposite seems the case. Furthermore during the period from the 165th to the 235th day, reactions were encountered with the fresh toxin that exceeded in intensity any previously seen in an experience of the test now extending to nearly 7 years. The relatively small numbers of comparative observations therefore gives us no definite information on this point, and we can only make the general statement that weak positives with the aged toxin were usually so with the fresh, the differences in intensity of pairs of reactions due to storage not apparently being significant. Towards the end of the work with the cold room samples, when the toxin was showing signs of deterioration, observations were made to discover in what way the typical evolution of the reactions was modified. Nothing very characteristic emerged, although it may be said the chief effect was on the erythema and pigmentation, desquamation being almost unaffected.

A final experiment, not recorded in Table I, may be mentioned as showing the stability of a toxin which had undergone a rather severe test. The sample was diluted on 11. III. 27 and kept in the cold room until 2. v. 27. It was then kept at room temperature, unprotected from ordinary light, until 6. XII. 27, the 271st day, when a series of 23 comparative tests was made, a freshly diluted sample of toxin being used as control. Of these, 16 gave a positive reaction with both toxins. The intensities of the reactions were equal in 1 case, greater with the fresh toxin in 13 cases, and greater with the aged toxin in 2 cases.

CONCLUSION.

A sample of diphtheria toxin made up to its final state of dilution, as required for the Schick test with a diluent prepared according to the formula devised by Glenny, Waddington and Pope, has been shown by experiment to give accurate readings even after storage for 4 weeks at room temperature, and after storage for 4 months in the cold room.

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