SOME EXPERIMENTS WITH HÆMOLYTIC STREPTOCOCCUS TOXIN OF VARYING ORIGIN IN CONNECTION WITH THE "DICK" TEST FOR SCARLET FEVER.

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In view of the somewhat varying estimates which have been attached to the value of the readings obtained, up to date, by means of the "Dick" test on children, the following controlled series carried out mostly at the Children's Hospital in Paris, and in the case of Series III at the West London Hospital, may be of interest.

When Drs. G. F. and Gladys Dick announced, in 1923, that in their opinion the short-chained hæmolytic streptococcus which, on many previous occasions, different observers had obtained from the throats of scarlatinal cases was in fact the causal agent of the disease, they apparently confirmed the findings of Klein, Tunncliffe, Mervyn, Gordon and others. Since, however, it would seem probable that several strains of this microörganism, differing in virulence, have been isolated, it would appear desirable when employing various samples of scarlet fever toxin for test and diagnostic purposes, to know something of their antecedents if we are to correlate the clinical results obtained by their use.

The Dicks' streptococcus exhibits no distinguishing characteristics by which it may be recognised with certainty in the test tube or under the microscope, their original test consisting in injecting small quantities of the toxin of the suspected organism mixed with antitoxic serum; and of the toxin alone, into susceptible individuals. Apart from this test the organism was described as being an hæmolytic streptococcus of which the colonies measured about 2 mm. in diameter, with an hæmolysed area of about 4 mm. It produced acid with dextrose, maltose, glucose, lactose and milk, and varied with mannite; it had no action on inulin⁽¹⁾.

Their toxin was prepared by passing the liquid medium, after four days, through a filter paper until clear, then through Berkefeld filters. The toxins used by the present writer were prepared by Dr. Gory at the Pasteur Institute, in similar fashion, and standardised; Cultures I and II being isolated from the tonsils of children with typical scarlet fever, before the fifth day of illness. Both these cultures presented similar characteristics.

Culture III was received from Dr. Anna Williams of the Public Health Department of New York, and appeared to have been isolated for some time, since it presented certain degenerative characteristics.

Culture IV (Dochez) was kindly supplied by Dr. O'Brien, from the Wellcome Physiological Laboratory, and had been carefully compared with a standard toxin.

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The following "pedigree" will serve to distinguish them:—

- TOXIN I.—(a) (No. R15 Inst. Pasteur) from culture grown on
Martin's broth for 3 days.
(b) " " " " " " " " 6 days.
- TOXIN II.—(a) No. S15 Inst. Pasteur) from culture grown on blood broth
for 7 days.
(b) " " " " " " " " from culture grown on Martin's
broth for 7 days.
- TOXIN III.—(a) (Williams) from culture grown on blood broth for 3 days.
(b) " " " " " " " " 6 "
- TOXIN IV.—(R.A.O'B.) made according to method described by Dr. Okell
and colleagues.

CULTURAL CHARACTERISTICS.

Cultures I. and II. and III.—In ordinary bouillon these cultures gave an abundant growth. The medium was clear with, later, finely granular masses. On gelatine slopes, small slightly translucent gray colonies were seen, with fairly regular contours, which were in parts finely dentate. On ascitic fluid medium and blood gelatine plates the colonies were typical, and gave, in 24 hours, an obvious zone of hæmolytic which was sharply defined for 2-3mm. Cultures I. and II. curdled milk in less than 24 hours, the clot being fissured from second day, and the fluid clear. In the case of III., clot only appearing after heating to 100°C. for three minutes.

Gelatine at varying pH incubated at 22°C., was liquefied by I. after 24 hours at pH 7.4, 6.5, and 6.0. II. was not tried, and III. failed to liquefy it.

All these cultures were without effect on Inulin.

Culture IV.—This culture was produced from an hæmolytic streptococcus of strain "Dochez." It gave acid with milk, and was without action on Inulin and Dulcitol.

ACTION ON SUGARS.

Culture	I.	II.	III.	IV.
Glucose ...	+	+	+	+
Maltose ...	+	+	+	+
Lævulose ...	+	+	+	+
Lactose ...	+	+	+	+
Saccharose ...	+	+	+	+
Mannite ...	0	0	0	+
Dextrin ...	0	0	0	+
Xylose ...	0	0	0	+
Salicin ...				+

The hæmolytic action of the filtered toxin was tested with a 24-hour culture; Culture I. yielding a fluid producing hæmolytic more rapidly, and in higher dilutions, than the other cultures.

METHOD EMPLOYED.

As will be seen by reference to the Tables below, each toxin was injected in varying dilutions, the dose, unless otherwise stated, being 0.2 cc.

A control consisting of the same dose of one of the toxins which had been heated for one hour at 100° C. in a water-bath was used in every case. The same group of children in a general ward of the Hospital (Enfants Malades) in Paris, was used; in each case the von Pirquet reaction was negative.

In the English series (III) the toxin supplied by Dr. O'Brien was used at the same time as the French Toxin II (a) and (b) and under the same conditions.

All results were read 24 hours after injection, and the following symbols only are used. Positive (+)=red area 1-2 cms. in diameter. Slight positive ($\pm$)=distinct red colour up to 1 cm. in diameter. Pseudo and combined reactions are, for convenience, recorded as positive or negative. There were no strongly positive reactions (over 2 cms.).

SERIES I.

		I. (a)	I. (b)	II. (a)	II. (b)	III. (a)	III. (b)
1	a	$\pm$	N	+	+	+	N
	b	+	N	+	N	+	N
2	a	N	N	N	N	N	N
	b	N	N	N	N	N	N
3	a	N	N	N	N	N	N
	b	N	N	N	N	N	N
4	a	N	N	N	N	N	N
	b	N	+	+	+	+	N
5	a	N	N	N	N	N	N
	b	N	N	N	N	N	N
6	a	+	$\pm$	N	N	N	N
	b	+	N	N	N	$\pm$	N
7	a	+	$\pm$	+	N	N	N
	b	N	N	$\pm$	N	N	N
8	a	N	N	N	N	N	N
	b	N	N	N	N	N	N
9	a	+	+	+	+	+	+
	b	+	+	+	+	+	+
10	a	N	N	N	N	N	N
	b	N	N	N	N	N	N

(a) = 1 : 1,000.

(b) = 1 : 5,000.

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It will be noticed that the results with the six toxins used in this series are by no means in accord with each other. The chief point of interest would appear to be the varying results recorded in the case of different dilutions of the same toxin in these children, the positive not invariably being registered by the toxin in the lower dilution.

Toxin II from the culture grown in Martin's (peptone) broth gave less positive results than that grown in blood broth; while in the case of Toxins I and III, it appears to be the younger culture (three days' growth) whose toxin proved the more active.

Toxin No. II (a) was next injected in two weak dilutions, with a control, into a series of infants ranging in age from 21 days to 11 months; the dose in this case being 0.1 cc.

SERIES II.

No.	Age: Months	1:100	1:500
1	8	N	N
2	9½	+	+
3	3½	N	N
4	7½	N	±
5	10½	+	±
6	2½	N	? N
7	8	+	±
9	21 days	N	N
10	8	N	N
11	6	N	N
12	4½	N	+
13	6½	N	N
14	11	±	±
15	4½	N	N

According to most authorities, infants below the age of 5 and 6 months retain a transmitted placental immunity. In No. 12 of this series it will be observed, however, that a positive result was obtained at the age of 4½ months. The parents were, unfortunately, unavailable for testing purposes. Again it is seen not to be the stronger toxin which invariably recorded the positive reaction in a doubtful case at this age.

In Series III which was undertaken by kind permission of Dr. Arthur Saunders, toxin supplied by Dr. O'Brien (IV) was employed in conjunction with the French toxin, No. II (a) with the following results:—

SERIES III.

	IV.		II. (a)	
	1 : 5,000	1 : 1,000	1 : 5,000	1 : 1,000
1	±	±	±	+
2	N	N	N	N
3	±	N	N	N
4	+	+	+	+
5	N	N	N	N
6	N	N	N	N
7	N	N	N	N
8	+	+	+	+
9	N	N	N	N
10	+	+	+	+

Here it will be seen that in Case 1, whilst the lower dilution of the French toxin gave a definite +, the other three yielded ± results. In Case 3 the reactions are in the reverse direction.

The high percentage of negative reactions observed in these series is due to the fact that some of the children tested had doubtless suffered with scarlet fever. This was, however, ignored since positive results were not the object of testing—the comparison of results being the primary object of the series.

CONCLUSIONS.

It was found in the course of the small series of trials of the " Dick " test with toxins of various origins and strengths, that anomalous results were in some cases recorded. These results did not appear to be accounted for by lack of standardisation of the toxins, so much as by lack of standardisation of the strain of the original streptococci employed, and the methods and time employed in culturing them. Brown⁽²⁾ states in a recent paper that he found that the use of different dilutions of toxin may show divergent results. He found, however, that the positive result would always be given by the stronger toxin, a finding which was not entirely borne out in the present series. Experimental error must, of course, be allowed for, and also variations of the personal equation as regards interpretation of results. Possible error in this direction Park estimated at about 2%.

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It is of interest to note that a positive Dick reaction was found in a child of $4\frac{1}{2}$ months of age; this, however, has previously been recorded; amongst others, by Rosen and Korobincina of Moscow⁽³⁾.

If the foregoing results be accepted, it would accordingly seem desirable to arrange for (a) an internationally standardised strain of scarlet fever streptococcus to be procurable for purposes of obtaining "Dick test" toxin; (b) a standard method of culture of the organism (*i.e.*, time, medium, etc.); and (c) a standard method of preparation of the toxin, if experimental results from different sources are to be correlated accurately.

I must, in conclusion, thank Professor P. Nobécourt and Dr. Max Lévy for giving me all facilities in France, and Dr. Arthur Saunders for permission to publish Series III.

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