SKIN TESTS FOR SUSCEPTIBILITY TO MEASLES.

By J. SMITH, M.D., D.Sc., D.P.H. and A. M. FRASER, M.B., Ch.B.

(*From the City Hospital, Aberdeen.*)

In 1918 Tunnicliff claimed that she was able to isolate a Gram positive diplococcus from the blood of measles cases, in cultures placed under anaerobic conditions. Later (1925) she found that when an anaerobic dextrose broth culture of this green producing diplococcus (killed by the addition of 0·5 per cent. phenol) was used for skin tests, an inflammatory reaction was obtained in those who had not had measles and no reaction in 96 per cent. of persons who gave a history of having had this disease. Ferry and Fisher (1926) confirmed the work of Tunnicliff and found that the green producing coccus elaborated an extracellular toxin which, when injected in a suitable dilution into human beings, gave negative skin tests in those who gave a history of having had measles and positive reactions in those who did not. Results were later reported by Tunnicliff and Taylor (1926), by Hibbard and Duval (1926) and by Musser (1927) which supported the findings of Ferry. Park and his co-workers (1927), however, carried out an extensive series of skin tests with toxins obtained from the Tunnicliff strain and from the Ferry strain, but were unable to confirm that skin tests could differentiate between those who were immune and those who were susceptible.

Methods.

Three strains of these green producing diplococci were obtained from Dr Ruth Tunnicliff, Chicago, and from Prof. Duval, New Orleans, but unfortunately, one of the strains from Prof. Duval was subsequently lost. Before examining the toxins from these cultures a preliminary serological examination of the various strains was made. The results obtained will be reported in detail later, but the agglutination and absorption tests showed that Tunnicliff's No. 1 strain and both Duval's strains were identical, while Tunnicliff's No. 2 and No. 3 each belonged to different serological types, but agglutination tests showed a group relationship.

To obtain the toxins the cocci were grown for 2 days at 37° C. in a proteose peptone (Difco) broth containing 0·02 per cent. glucose. Phenol to a concentration of 0·4 per cent. was then added and the broth culture was then passed through an L 2 Chamberland filter. It was found that when 0·2 c.c. of these filtrates was inoculated in a dilution of 1 in 10 into certain individuals, reactions similar to those produced by the Dick test occurred, while control tests made with similar uninoculated culture medium produced no reactions.

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Later it was found that when the incubation period of the bouillon culture was extended to 6 days, toxins could be obtained which, when diluted 1 in 100, produced inflammatory reactions in human beings. As pseudo reactions occur infrequently even with a 1 in 10 dilution of inactivated toxin and as the strength of the toxin appeared to be suitable, it did not seem necessary to discard the results obtained with the weak toxin and to start afresh with the more potent materials.

The reactions recorded in the following pages were classified according to their size as seen 24 hours after making the test, thus:

No inflamed area = 0
Inflamed area 1 cm. in diameter = +
Inflamed area 1–2 cm. in diameter = + +
Inflamed area 2–3 cm. in diameter = + + +

Results.

The Effect of Heat on the Toxins.

In order that the various skin tests might be adequately controlled the effect of heating the toxins was first studied. The results of skin tests with two toxins which were heated at 100° C. for various periods are given in

Table I. *Skin Tests showing Effect of Heat on two Toxins.*

Toxin from strain	Case no.	Results of skin tests: Active Toxin diluted 1 in 10	Toxin heated for 1 hour	Toxin heated for 2 hours	Toxin heated for 3 hours	Toxin heated for 4 hours
Tunnicliff, No. 1	1	+ + +	+ + +	+ + +	+	0
	2	+	+	0	0	0
	3	+ + +	+ + +	+ + +	+	0
	4	+ +	+ +	+	0	0
Tunnicliff, No. 2	1	+ + +	+ + +	+ + +	+	0
	2	+ + +	+ + +	+ + +	+	0
	3	+ + +	+ + +	+ + +	+	0
	4	+ +	+ +	+ +	+	0

Table I. From these tests it is seen that the toxins are heat resisting and that heat must be applied for 4 hours at 100° C. before the toxic substance is completely destroyed.

Similarity of Toxins from Tunnicliff and Duval Strains.

Toxins obtained from the three Tunnicliff strains were tested on 10 individuals (Table II) and the results suggest that these toxins are similar in nature but that strain No. 1 produced a more potent filtrate than any of the others. Next, 20 individuals were tested simultaneously with toxin from a Tunnicliff strain and toxin from a Duval strain. These tests (Table III) indicate that the toxic principles obtained from these cocci were also identical, as was to be expected since they were identical serologically.

Table II. *Comparative Series of Skin Tests made with Toxins from three Tunnicliff Strains.*

Case no.	Toxin from Strain 1	Toxin from Strain 2	Toxin from Strain 3	Control fluid
	10	10	10	10
1	0	0	0	0
2	0	0	0	0
3	+ +	+	+	0
4	0	+	0	0
5	+ + +	+ +	+	0
6	+ + +	+ +	+	0
7	+ +	+	0	0
8	+ +	+ +	+	0
9	+	0	0	0
10	+	+	0	0

Table III. *Comparative Series of Skin Tests with Toxins from Tunnicliff's* (MT 2) *and Duval's* (MT 18) *Strains of Green Producing Cocci.*

Case no.	Age	History of Measles	MT 2	MT 18	Control fluid
			10	10	10
1	3	No	+ +	+ +	0
2	6	No	0	0	0
3	5	Yes	+ +	+ +	0
4	2	No	+ +	+ +	0
5	5	Yes	0	0	0
6	7	Yes	0	+ ?	0
7	2	Yes	0	0	0
8	1½	No	0	+ ?	0
9	5	No	+	+	0
10	4	No	+	+	0
11	4	Yes	+ ?	0	0
12	2	No	0	0	0
13	5	Yes	+ +	+ +	0
14	5	Yes	0	0	0
15	6	Yes	0	0	0
16	3	Yes	0	0	0
17	4	No	+ +	+ +	0
18	4	No	0	0	0
19	4	Yes	+	+	0
20	6	Yes	+	+	0

Skin Tests in Relation to Age Group.

Preliminary tests with the toxin from No. 1 Tunnicliff strain appeared to indicate that a 1 in 10 dilution would be suitable for testing a series of individuals in relation to their previous history of having, or not having had measles. A control fluid consisting of heat inactivated toxin was used. The results of the tests made on 254 individuals are summarised in Table IV, from which it will be seen that few positive reactors were obtained in the age group 0 to ½ year; the majority of individuals in the age group ½ to 5 years gave a positive reaction; in the 5 to 10 year period, 27 per cent. reacted positively, but from this age period onwards, the number of positive reactors rapidly increased again, until in those over 20 years of age 62 per cent. gave a positive reaction. In other words it was found that in individuals under the age of 10 years the majority of the tests agreed with the clinical histories of the cases, but from this time onwards there were more positive than negative tests in those who had had measles.

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Table IV. *Skin Tests in Relation to Age Groups and in Relation to Clinical History.*

Age group	Had measles		Not had measles		Totals			
	Number positive	Number negative	Number positive	Number negative	Number positive	Number negative	% positive	% negative
0– ½ year	0	0	1	10	1	10	9	91
½–1 „	0	0	3	1	3	1	75	25
1–5 years	9	21	39	10	48	31	60	40
5–10 „	15	44	3	5	18	49	27	73
10–20 „	12	25	3	1	15	26	36	64
20+ „	24	15	6	3	30	18	62	38

Skin Tests before and after the Occurrence of Measles.

Cross infection in several of the fever wards of the hospital offered an opportunity to examine more accurately the value of skin tests with toxin from Tunnicliff's strain. As soon as a case of measles occurred in a ward skin tests were applied to the remainder of the children, the results in the cases who subsequently developed measles being given in Table V. These tests

Table V. *Skin Tests with Toxin* (MT 2) *from Tunnicliff's Coccus on Children Before and After the Development of Measles.*

Outbreak	Case no.	Age	Results of skin tests before onset		Results of skin tests 10 days after disappearance of rash	
			Toxin 10	Control 10	Toxin 10	Control 10
I.	1	3	+	0	0	0
	2	3	+ + +	0	0	0
	3	5	+ +	0	0	0
	4	5	+	0	0	0
	5	1	+ +	0	+	0
	6	9/12	+ + + +	0	0	0
	7	5	+ +	0	0	0
	8	4	+ +	0	+	0
	9	7	+ + +	0	+ +	0
II.	1	5	0	0	0	0
	2	3	+	0	0	0
	3	5	0	0	0	0
	4	5	+ +	0	+	0
	5	3	0	0	0	0
	6	4	+ +	0	0	0
	7	4	0	0	0	0
	8	6	0	0	0	0
	9	4	0	0	0	0
	10	5	+ + +	0	0	0
	11	4	0	0	0	0
III.	1	3	0	0	0	0
	2	2	+	0	+	0

showed that the reactions appeared to have little relation to the occurrence of the disease since 8 out of 22 cases gave a negative test before the occurrence of the disease. On the other hand, when the cases were retested with the same toxin 10 days after the disappearance of the rash 9 out of 14 who were previously positive now gave no reaction.

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Skin Neutralisation Tests.

Blood was obtained from five patients suffering from measles at the time the rash appeared and another sample 10 days later. For the neutralisation tests each sample of serum was diluted 1 in 2, 1 in 5, 1 in 10, and 1 in 20 with normal saline, and to one volume of the various dilutions an equal volume of toxin (MT 2) diluted 1 in 5 was added. These mixtures were incubated in the water bath at 37° C. for 2 hours and inoculated intradermally in 0·2 c.c. amounts into known positive reactors who were also insensitive to protein. Thus the first sample of serum from each case was tested for its antitoxic properties on three or four individuals and the second sample was similarly tested on the same individuals some days later.

The results of these various tests are summarised in Table VI. The sera obtained at the onset of the disease are numbered 1, 2, 3, 4 and 5, while the

Table VI. *Skin Neutralisation Tests with Human Sera and a fixed amount of Toxin* (MT 2).

Serum	No. of reactors for test	Dilution of sera			
		1–2	1–5	1–10	1–20
1	4	0	0	0	+
1 A	4	0	0	0	+
2	3	0	+	+	+
2 A	3	0	+	+	+
3	3	0	+	+	+
3 A	3	0	+	+	+
4	4	+	+	+	+
4 A	4	+	+	+	+
5	3	+	+	+	+
5 A	3	+	+	+	+

0 = neutralisation.
+ = no neutralisation.

specimens obtained from the same patients during convalescence are numbered 1 A, 2 A, 3 A, 4 A and 5 A respectively. These skin neutralisation tests showed that no increase in the antitoxic content of the serum developed as a result of the infection.

DISCUSSION.

The filtrates obtained from broth cultures of the American strains and from strains of green producing cocci from measles cases in hospital have been found to contain only relatively weak toxins which appear to be of the same thermostable type as that obtained from the haemolytic streptococcus.

The skin reactions produced by the various filtrates were found to be much more difficult to interpret than those produced by scarlatinal toxins. The reactions are much less intense, the colour of the inflammatory response being frequently only of a pink or pale pink shade. As regards the skin tests on normal individuals under 10 years of age it might be suggested that if a more potent test dose of toxin had been used the test and the clinical history would have corresponded more closely. On the other hand, if the amount of toxin

in the test dose had been increased, a greater number of positive reactors would have been obtained amongst individuals who had already had the disease.

The result in the cases tested previous to and after the occurrence of measles suggested that some antitoxic immunity had been developed to the toxin from Tunnicliff's strain. The skin neutralisation tests with sera obtained from patients at the onset of the illness and during convalescence did not corroborate these findings and it seems probable therefore that an attack of measles produced in some individuals a condition of anergy so that no skin response was obtained to the toxin.

SUMMARY.

Skin tests made with the toxin obtained from Tunnicliff's strain of green producing streptococcus on normal individuals, and on cases prior to and after an attack of measles did not give any definite positive evidence of the etiological relationship of this organism to measles. Further, an attack of measles did not produce any apparent increase in the antitoxic content of a patient's serum for this toxin.

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