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104

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EXPERIENCES WITH THE SCHICK TEST AND ACTIVE IMMUNISATION AGAINST DIPHTHERIA.

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AFTER a preliminary investigation into the value of the Schick test and active immunisation against diphtheria (Copeman, 1921), the Ministry of Health instituted a more extensive test at the Mitcham Poor-Law Schools with the consent of the Guardians. In the present paper the immunological results are described.

The Mitcham schools contain a resident population of rather over 300 children between the ages of three and sixteen years. All, until quite recently, not only lived, but received their education, in one or other of the three adjoining institutions. The weekly rate of admission and discharge is small, and the population is thus a very stable one. Prior to November, 1920, these schools had been practically free from infectious disease, but since then they have been affected with scarlet fever and diphtheria almost continuously. No case of diphtheria has, however, been notified since August 4th, 1921.

The general scheme of work included a routine swabbing of all children and testing the bacilli isolated for virulence. The Schick test was applied in every case and the positives immunised with toxin-antitoxin mixtures, the effect of this being judged by re-testing by Schick's method at a later date.

BACTERIOLOGICAL EXAMINATION.*

All the children in the school and annexed infirmary, numbering 329, were swabbed on two separate occasions. The first set of swabs, hereinafter referred to as Series I, was taken eight weeks before the second set, called Series II. Children found on either occasion to be harbouring bacilli morphologically resembling *B. diphtheriæ* were re-swabbed until three consecutive negatives were obtained.

* The whole of the technical work was planned and carried out by the staff of the Wellcome Physiological Research Laboratories, the bacteriology being done by one of us (A. J. E.), Dr. Okell and Miss Baxter, the examination of the blood samples for antitoxin and the control and testing of the Schick toxin and toxin-antitoxin mixtures by A. T. G. and Miss Allen, the Schick testing and the inoculations with toxin-antitoxin mixtures by R. A. O'B.

In all some 1400 swabs were examined by cultural methods. The following results were obtained:

No. of Cases Diagnosed as "M.D." in Series I.

"M.D." = bacilli morphologically resembling *B. diphtheriæ* in smear from "Loeffler" overnight culture.

"M.D." throat only	1
"M.D." nose only	8
"M.D." nose and throat	9
Total positive	18
Total swabbed	329
Percentage positive	5·47

Cultures of B. diphtheriæ *Isolated in Series I.*

	"M.D." diagnosis.	Cultures isolated.	Sugar reactions. Correct.	Not done.	Virulent.	Avirulent.
Throat	1	1	1	0	1	0
Nose	8	3	1	2	2	1
Throat and nose	9	4*	3	1	2	2

"Correct" sugar reactions were acid production in glucose, but not in saccharose. Virulence was tested by the intracutaneous method checked by the subcutaneous (Eagleton and Baxter, 1921).

* Four cultures from two patients who both harboured virulent and avirulent strains, but at different times.

No. of Cases Diagnosed as "M.D." in Series II.

"M.D." throat only	1
"M.D." nose only	3
"M.D." nose and throat	2
Total positive	6
Total swabbed	327
Percentage positive	1·8

Cultures of B. diphtheriæ *Isolated in Series II.*

	"M.D." diagnosis.	Cultures. isolated.	Sugar reactions. Correct.	Not done.	Virulent.	Avirulent.
Throat	1	0	0	0	0	0
Nose	3	2	2	0	0	2
Nose and throat	2	2	2	0	0	2

Results of Series I and II Compared.

	"M.D." diagnosis.	Cultures isolated.	Sugar reactions. Correct.	Not done.	Virulent.	Avirulent.
Series I	18	8	5	3	5	3
Series II	6	4	4	0	0	4
Common to both	3	2	2	0	0	2
Total	21	12	9	3	5	7

The swabs in Series I were taken at the end of a series of cases of clinical diphtheria, when opportunities for infection of throats were fairly plentiful; one would naturally expect that the later series, taken at a date two months further away from the existence of manifold opportunities for infection, would show a lower reading of infection, and this is what we found.

With regard to the difficulties of isolation, one or two short notes may be made. The digestion of Loeffler's medium by organisms occurring in the throat and nose, but especially the latter, is one fruitful source of trouble. Another great difficulty consists in the fact that "M.D." may be present in very small numbers indeed. We do not think it is going too far to say that the ease with which *B. diphtheriæ* can be isolated from the nose or throat is a measure of the abundance of this organism in the nose or throat, and therefore, most probably, of the danger to the community arising from the patient under examination.

In our opinion it follows that the percentage of cases from which virulent *B. diphtheriæ* can be isolated is more important than a carrier rate based on morphological criteria, which disregard the pathogenic power of the organisms found and the number present.

In support of this view, and as a contrast to the results in these two series, the following brief account of some work that has been largely contemporaneous, and so has acted as a control, will be of interest. One hundred and fifty convalescents from clinical diphtheria were swabbed at different intervals after the disease. In 14 "M.D." was diagnosed, and although one swab only from each case was submitted to us, from 13 out of those 14 virulent *B. diphtheriæ* were isolated.

In Series I five virulent carriers were found, one of them being convalescent from clinical diphtheria at the time of swabbing. The most persistent gave an initial "virulent"; subsequent cultures were "avirulent." In Series II no "virulent" carriers were found.

There had been a series of cases of clinical diphtheria in this institution, and it was to be expected that one or more carriers of virulent bacilli, easily isolated and continuously excreted, would be found. No such carrier was, however, discovered. But our examination was not made at the height of the epidemic, and we know from general experience (*cf.* also Hartley and Martin, 1920) that most convalescents rapidly become clear of bacilli. It may be that one of the five "virulent" carriers (or, less probably, one of the seven "avirulent" carriers) was the cause of the epidemic, no evidence having been obtained that the infection was derived from a source outside the institution.

SCHICK TESTING.

Toxin.—The same toxin was used throughout. Fresh dilutions were made for each day's work, and the potency of the diluted toxin remaining over was tested on guinea-pigs (Glenny, Allen and O'Brien, 1921). We adopted the original Schick formula as used by Park and Zingher, 0·2 c.c. containing $\frac{1}{50}$ guinea-pig M.L.D. being injected. In one group of about thirty children in which the first Schick test had given rise to some slight uncertainty, it was repeated, the original Park formula being used simultaneously with Zingher's later modification, in which 25 per cent. more toxin is used on the left arm

and 50 per cent. extra toxin in the heated control on the right arm. In this small group of cases we could not find that the Zingher modification gave any clearer readings than the original formula.

Readings.—In a discussion of the reaction it is necessary to have concise descriptive signs or terms which should convey as much information as possible. We think this question is so important that we append a short table of explanation of the conventions that we would suggest.

Schick Test Nomenclature.

Type of reaction.	Written description of reaction.	Verbal description of reaction.	Description of patient.	Description previously in use.
1	−	Negative	Immune = I.M.	Negative.
2	− (ψ)	Negative and pseudo	Immune (pseudo reactor) = I.M.P.	Pseudo.
3	+	Positive	Non-immune = N.I.M.	Positive.
4	+ (ψ)	Positive and pseudo	Non-immune (pseudo reactor) = N.I.M.P.	Combined.

The readings were made daily up to five or seven and occasionally thirteen days. If one decides to make one reading only, the most satisfactory is that made from the fourth to the seventh day after the injection.

The results of our tests are set out in Table I. The percentage of positive reactions obtained for this group of children, between the ages of three and sixteen, correspond closely with that published by Park.

TABLE I.—*Schick Test Results at Various Ages.*

Age in years.	Immune and immune (pseudo reactor).	Non-immune.	Total.	Per cent. Immune and immune (pseudo reactor).	Per cent. Non-immune.
3–4	9	3	12	75·0	25·0
5–6	20	8	28	71·4	28·6
7–8	30	12	42	71·4	28·6
9–10	39	22	61	63·9	36·1
11–12	55	21	76	72·4	27·6
13–14	51	23	74	68·9	31·1
15–16	23	13	36	63·9	36·1
Total	227	102	329	69·0	31·0

Technique and reading of results.—So far as the technique is concerned, we have but little to add to the excellent description in the publications of Park and Zingher. The needle and syringe must work without any defect; the slightest bluntness of the needle or leak of the plunger at the junction of the needle results in unsatisfactory work. We used a Burroughs Wellcome No. 1 dental needle with a 1 c.c. all-glass syringe, and a 1 c.c. long

tuberculin syringe; in one series a "Record" 1 c.c. syringe was used. It is most convenient to use two syringes, one for the toxin, the other for the control; these should be of the same make, with the plunger working equally well, and the needles should be equally sharp, otherwise a slight difference in the depth at which the intradermic injection is made may occur, with resultant blurring of the readings, particularly those of the first day. The control syringe may be identified by a rubber band fixed around the barrel.

Readings were made daily in most of the cases up to five or seven days, each day's reading being made without reference to the previous readings. Wherever there was any discrepancy the Schick test was repeated. Fifty-five of the children were thus re-tested because some slight doubt or discrepancy had occurred in the course of the first test (Table II).

TABLE II.—*"Doubtful" Schick Results among 329 Children Tested.*

	First Schick test. Readings regarded as probably:			Second Schick test.	
	− & − (ψ)	+ & + (ψ)		− & − (ψ)	+ & + (ψ)
	44			44	
		11		4	7
Total	44	11 = 55		48	7 = 55

Consideration of these cases shows that when a reading is doubtful, it proves on further investigation in the great majority of cases to be negative or "negative (and pseudo.") When deciding that a given reaction is "negative (and pseudo)," one has always present in one's mind a slight fear that the reaction may be "positive (and pseudo)." Fortunately these latter reactions are rare. Of the 329 children only two showed a "positive (and pseudo)" reaction, *i. e.* on the right arm a reaction which, although smaller, resembled the left in depth of colour and degree of desquamation.

Table III gives details of the four cases in Table II in which differences were obtained on re-testing.

TABLE III.—*Apparent Discrepancies.*

	First test.	Second test.	Blood sample.
1.	Positive (and pseudo) .	Negative (and pseudo) .	More than $\frac{1}{30}$ unit of
2.	Positive . . .	Negative (and pseudo)	antitoxin per c.c. of
3.	Positive . . .	Negative (and pseudo)	blood found in every
4.	Positive (and pseudo) .	Negative (and pseudo)	case.

On referring to the readings we had recorded in the first test, we found in every case that the final entry, on which the child was classed, recorded a "very faint" or "very, very faint" stain on the left arm, greater in size than on the right, and that some of the readings had suggested a "negative (and pseudo)" reading. With the experience gained in these cases and the help supplied towards the interpretation of doubtful readings by the determination of antitoxin in the patient's blood, we should, with similar readings now, return the case as "immune (pseudo reactor)."

Blood samples.—Our confidence in the ultimate accuracy of our classification of the children as immune or non-immune is founded partly on the results of the repeated Schick tests in "doubtful" or difficult cases, but to a greater extent on the interpretation of the titration of antitoxin in the blood of individual children by the Römer method (Glenny and Allen, 1921). This we regard as a very important part of our investigation.

Many observers state that an antitoxin content per c.c. of blood of less than $\frac{1}{30}$ unit of antitoxin will fail to neutralise the toxin injected in the Schick test and so make the readings positive, while, with a greater antitoxin content, a negative Schick reaction will result. Our own experience in several hundred tests has hitherto afforded no ground for disagreement with this statement.

Sixty-six blood samples were taken and the antitoxin titrated. The findings are shown in the table.

TABLE IV.

Antitoxin per c.c. of blood.	No. of cases.	
5–20 units	7	8 of these children had had toxin-antitoxin mixtures injected.
2–5 ,,	8	
1–2 ,,	15	
$\frac{1}{2}$–1 unit	9	
$\frac{1}{5}$–$\frac{1}{2}$,,	9	
$\frac{1}{100}$–$\frac{1}{10}$,,	5	
? $\frac{1}{1000}$,,	5*	
Less than $\frac{1}{2000}$ unit	8	

Note.—In many instances the sample of blood for titration of antitoxin was not taken until approximately a week after the Schick test was done.

Recent experiments on animals (yet to be published) show that the minute amount of toxin used in the Schick test is sufficient, under certain circumstances, to cause the development of such a degree of immunity that an animal which has just given a non-immune Schick positive result, will, a week or two later, when the test is repeated, give an immune negative response. This may possibly be the explanation of some of the discrepancies recorded.

It is probable that these results will be confirmed on the human subject, but the point will be dealt with in a subsequent paper.

Error on first day's reading.—It is obviously of great importance in the presence of an epidemic of diphtheria to be able, if possible, to make a decision that the given patient is immune or not within the first twenty-four hours of the Schick test. We did in all approximately 400 Schick tests. In four instances the first day's reading was entered as "negative," whereas later readings showed that the reaction was "positive." First day's readings of the reaction in eight children were "positive," but the later readings showed clearly that the reaction was "negative" and the children therefore immune. In nine instances the first day's reading entered as "doubtfully" positive or negative differed from the final reading. Thus an actual error was made in

* All of these five sera, when injected intradermically without toxin into guinea-pigs, gave some reaction; it was, therefore, difficult to estimate the antitoxin content with the small amounts of serum available.

twelve cases and a dubious but erroneous reading in nine, *i. e.* 21 in all. In 400 tests, therefore, a decision based on the first day's reading proved erroneous in 5 per cent. of the cases.

"*Carriers.*"—The following table gives the results of the Schick test and examinations of the blood of children who harboured "morphological diphtheria bacilli." It is to be noted that the carriers of virulent bacilli possessed a fairly high degree of immunity.

TABLE V.

	Virulent cultures isolated.	Avirulent cultures isolated.	"Hofmann" isolated.	"M.D." not isolated.
	5	7	4	6
Schick test positive	0	1	2	1
Schick test negative	5	6	2	5
Blood samples examined	5	1	—	3
Antitoxin content per c.c.	4, 1 unit 1, $\frac{1}{50}$ unit*	$< \frac{1}{2000}$		1. $< \frac{1}{2000}$ 2. $\frac{1}{2}$–1 3. 2–5

Note.—In some of these instances the sample of blood for titration of antitoxin was not taken until approximately a week after the Schick test was done.

ACTIVE IMMUNISATION.

All children giving a positive Schick reaction, 102 in all, were given three weekly doses of 1 c.c. of toxin-antitoxin mixture (Park-Zingher formula and American official standard). As a precautionary measure, in most cases a preliminary injection of 0·05 c.c. had been given.

Reactions.—The results were reassuring. Of the 102 children 17 were reported to the nurse (one child twice). Eight had a temperature of 102°, four of 101°, and two of 99°. Though this number (17) seems large, the reaction was so slight in all but three of the children that they did not wish to stay in bed for a whole day. One of the remaining three wished to stop in bed for two days.

W. G—, one of two that were ill, had a temperature of 102°, and was distinctly ill for three days after her first dose of 0·05 c.c. A week later she received 0·01 c.c., and at intervals of five days 0·05 c.c., 1 c.c., 1 c.c., 1 c.c. She remained quite well throughout.

It is therefore probable that the initial illness was due to some other cause than the injection of the mixture.

The other child, A. B—, vomited immediately after the first injection, and was ill for two days. Inquiry showed that she had had a pork dinner a few hours before the injection, and had suffered from abdominal pain before the injection. Unfortunately this was not known to us.

The constitutional reactions were slight. Local reactions, on the other hand, were at first rather alarming. In approximately half of the cases

* Patient G. M— referred to under "Results of Immunisation" (p. 49).

a large flushed area varying from 2 in. by 1 in. to even 6 in. by 3 in. appeared, but rapidly cleared up after the first twenty-four to forty-eight hours. We were very disturbed until we learned by experience that a large, angry-looking swelling would not prevent a boy from playing football two days later or a girl from skipping on the first day after the injection, and that it did not interfere to any great extent with sleep.

Results of Immunisation.—Approximately eleven weeks after the conclusion of the course of immunisation the whole of the children in the school were again subjected to the Schick test.

Of the 227 children who had been classified by the first Schick test as immune, 203 remained in the school; of these 201 again gave negative or negative (and pseudo) reactions, while two children showed positive reactions. Samples of blood were obtained from these two, and neither contained any antitoxin. Both children had had diphtheria, and had been removed to hospital, where they were given antitoxin shortly before the first Schick test was done. It appears probable that the first Schick test, which in one child was undoubtedly negative and in the other (G. M—) had been read as "pseudo (with a faint possibility of positive)," had been influenced by some remnants of the antitoxic serum which had been injected in hospital. Before the second Schick test was performed, some three months later, the last remnants of the horse antitoxic serum had been excreted, and the Schick results were therefore positive.

Of the 102 children who had given a positive reaction and had been inoculated with a toxin-antitoxin mixture, 99 remained and were re-tested. Two still gave a clear positive reaction; samples of their blood were tested; no antitoxin was present in either. These children will be re-inoculated.

CONCLUSIONS.

(1) In a residential school of 329 children, amongst whom a recent epidemic of diphtheria had occurred, eighteen (6 per cent.) carried morphological *B. diphtheriæ* in throat or nose. At the first swabbing, at the end of the epidemic, 5 per cent. of carriers were found; at the second, two months later, 2 per cent. From these eighteen children twelve cultures were isolated, of which five were virulent.

(2) The later swabbings from these five carriers showed either avirulent organisms or no "morphological diphtheria" bacilli.

(3) On the grounds of a bacteriological examination of a large number of cases of diphtheria, carriers and convalescents, the following suggestions are tentatively put forward:

(i) That the "avirulent carrier" is of no importance epidemiologically.

(ii) That the danger of a carrier of virulent bacilli is, at the time of examination, proportionate to the number of virulent bacilli present in throat and nose, and, therefore, to the ease with which the virulent bacilli can be isolated.

(4) Of 329 children, aged from three to sixteen, 102, *i. e.* 31 per cent., gave a positive Schick reaction; 95 per cent. of the readings made on the first day proved to be accurate.

Of the 227 children who had given a negative response when first

Schick tested, 203 remained in the school eleven weeks later. Of these, 201 on being re-tested again gave a negative or a negative (and pseudo) response, thus confirming the decision made two months previously. Two showed a positive response at the second test; these two children had had antitoxin injected shortly before the first test was made.

(5) These 102 children were inoculated with toxin-antitoxin mixtures (Park and Zingher formula). Local reactions occurred in about one-third of the children; though in some of these the area of inflammation was large, the activities of the children were but little interfered with. Constitutional reactions were slight in all but two of the children.

(6) Of the 102 children, 99 were still present in the school eleven weeks later when the Schick test was repeated. Two gave an undoubtedly positive response, the remainder a negative or negative (and pseudo) reaction; 98 per cent. were therefore immune.

We have much pleasure in thanking Dr. Manby for interesting the Guardians in the test, and Dr. Morrish, the Medical Officer; Mr. Drury, the Superintendent of the Schools; Mrs. Drury, the Matron, and the Infirmary Sister, for the valuable assistance afforded by them, especially in arranging practical details in connection with the work.

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CONTENTS.

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