

but to return a known carrier from hospital back to school, even to a segregation ward, is not without risk to others. In either case the loss of three or four months school time may be a serious matter educationally.

In view of the findings recorded above in which 15 cases produced 6 carriers, I am inclined to think that if found to be practicable, a more logical method of institutional diphtheria management would be to proceed *ab initio* on the assumption that every case of diphtheria will ordinarily become a carrier and, as soon as the clinical condition permits of it, to endeavour to exalt the bacteriolytic powers of the patient's blood by administering, as a routine, suitable doses of polyvalent bacterial vaccine, combined, perhaps, with a direct attack on the strongholds of the bacillus by cauterisation of the crypts of the tonsils, thus dealing with an assumed carrier condition during convalescence from the clinical disease.

Acknowledgments.

I am indebted to my assistant, Assistant Surgeon A. C. Victor, I.M.D., for his valuable work in connection with this investigation. The epidemic involved the examination of nearly 700 separate cultures from throat swabs, most of which were taken in the Health Department laboratory at a time when the ordinary routine work was unusually heavy.

THE SCHICK TEST AND IMMUNISATION BY TOXIN-ANTI-TOXIN IN ASSAM.

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SHILLONG is the administrative capital of the Province of Assam and is a hill station, in which there are three schools for European children. During the school season, these schools receive pupils as boarders, who come from all over India.

In 1917 a serious outbreak of diphtheria occurred in one of these schools, when 40 cases of diphtheria occurred with two deaths. Since then, in spite of scrupulous attention to school hygiene, isolation of cases and search for contact carriers, there has been an annual crop of diphtheria cases which has caused so much trouble to the school authorities, and so much anxiety to parents, that fear of these outbreaks was tending to empty the schools.

The actual number of cases occurring is shown below:—

TABLE I.

Year.	Number of cases of diphtheria.
1917	40
1918	16
1919	2
1920	3
1921	26

Before describing the work done by us in Shillong, it may be of interest to give brief details of the Schick test, and toxin-anti-toxin immunisation as carried out in America and England.

SCHICK TEST.

It is well known that this test determines by a local reaction, or its absence, whether an individual is, or is not, susceptible to diphtheria.

That the reaction is specific may be judged from the following:—

(a) Schick has found that those giving a negative reaction have at least 1/30th of a unit of natural diphtheria anti-toxin in their blood, sufficient to protect them from an attack of diphtheria, whilst those giving a positive reaction have less than this amount of diphtheria anti-toxin or none at all in their blood. This has been confirmed by other workers.

(b) The Schick reaction is found to be negative in cases which have recently been given diphtheria anti-toxin, and in cases where the anti-toxin has been given at the same time as the test.

(c) Cases giving a negative reaction do not develop diphtheria, even when exposed to infection. Thus Zingher(1) states, of 2,200 scarlet fever patients who gave negative reactions on admission to the Wellard Parker Hospital, none developed diphtheria though they were exposed to cases of diphtheria among the Schick positive patients, even though some of them became carriers of virulent diphtheria bacilli during their stay in hospital.

In 500 cases tested by Leete(2), eleven cases developed diphtheria. Of these, ten were among cases who gave a positive Schick reaction, and one was a case who gave a doubtful reaction.

Judging by the results of the Schick test, the susceptibility to diphtheria varies with age. The following figures of Park and Zingher(3) are based on many thousands of cases tested.

TABLE II.

Age.	Susceptible.
3 to 6 months	30 per cent.
6 months to 1 year	60 " "
1 to 2 years	70 " "
2 to 3 years	60 " "
3 to 5 years	40 " "
5 to 10 years	30 " "
10 to 20 years	20 " "
Over 20 years	12 " "

Four types of reactions are met with(4).

(1) Positive reaction.—Those having less than 1/30th unit of natural diphtheria anti-toxin per c. c. in their blood.

(2) Negative reaction.—Those having at least 1/30th unit of anti-toxin per c. c. in their blood.

(3) Pseudo-reaction.—Those reacting to the bacillary protein but not to diphtheria toxin.

(4) Pseudo and positive-combined reaction. Those reacting to both the diphtheria toxin and the bacillary protein.

Toxin-Anti-toxin Immunisation.—This consists of three injections at weekly intervals of 1 c. c. of diphtheria toxin, suitably neutralised with anti-toxin. The actual strength of the mixture employed is 85 per cent. L. dose of toxin per unit of anti-toxin.

As to the immunity conferred, the following figures are of interest:—

Bauer(5) immunised 1,100 children with the toxin-anti-toxin mixture who were found to be susceptible to diphtheria by the Schick test. Two hundred of these were re-tested after five months and all gave a negative test.

Park, quoted by M. Garcia Bonus(6), found 95 per cent. immunised after three injections of toxin-anti-toxin. These were among many thousands of cases done.

Schroedar(6) immunised 50,000 school children at Brooklyn, New York. After two years, 570 children were re-tested, of whom 92.4 per cent. were found to be immunised.

The length of time immunity lasts is not quite certain, but it is at least 5½ years and probably for life. Schroedar(6) has re-tested certain children yearly for over 5 years and found no variation in their immunity. Park(6) has tested cases 5 years after they were immunised and found them still immune.

TESTS DONE IN SHILLONG.

Having decided to test all the school children by the Schick test and to immunise those found positive with toxin-anti-toxin, it was discovered that the materials were not obtainable in India. Owing to rapid deterioration of diluted diphtheria toxin when exposed to heat or light it is not stored in the tropics. Undiluted toxin is more stable, but will not stand a temperature of 37°C. for many days.

Negotiations were opened however with an American firm by the Director of Public Health for the supply. It was received in April 1922, and put into cold storage immediately, and the following tests carried out (by E. C. R. F.).

The toxin for the Schick test was received in capillary tubes, each capillary tube containing sufficient for ten tests. As each test requires 1/50 M.L.D., each capillary tube contained 10/50 of one M.L.D.

1st Experiment.

Guinea pig weighing 250 grammes was inoculated subcutaneously with the contents of five capillary tubes representing one M.L.D.

Result. No local reaction, no induration, no signs of paralysis.

2nd Experiment.

Guinea pig of 250 grammes inoculated as in No. 1 with the contents of 10 capillary tubes or 2 M.L.Ds. Result.—Same as No. 1.

3rd Experiment.

Guinea pig of 250 grammes inoculated with the contents of 15 capillary tubes or 3 M.L.Ds. Result.—Same as No. 1.

This particular diphtheria toxin was thus found to be inert and was not used.

Through the kindness of Dr. O'Brien of the Wellcome Research Laboratory, a fresh supply of toxin for the Schick test was received and put to the following tests:—

The toxin was undiluted and received in capillary tubes. Each tube contained 1 M.L.D.

1st Experiment.

(A) Guinea pig (250 grammes) inoculated subcutaneously with 1 M.L.D.S. (= contents of one capillary tube).

Result: 1st day. Some local induration.

2nd day. " " "

4th day. Necrosis and sloughing.

9th day. Paralysis.

11th day. Death.

(B) Guinea pig (250 grammes) inoculated with 1 M.L.D. toxin—500 c.c. anti-toxin given 24 hours previously.

Result.—No local or general reaction.

2nd Experiment.

(C) Guinea pig (250 grammes) inoculated subcutaneously with 2 M.L.D. of toxin (2 capillary tubes).

Result: 24 hours. Marked induration.

48 hours. Necrosis and sloughing.

72 hours. Death.

3rd Experiment.

Intradermal inoculations were done with 0.2 c. c. of each of the following dilutions of toxin:—

(A) 1/50 of 1 M.L.D. (One capillary tube).

(B) 1/250 of 1 M.L.D.

(C) 1/500 of 1 M.L.D.

(D) 1/1000 of 1 M.L.D.

A. Gave a well-marked positive reaction.

B. Was positive but less definite.

C. Was doubtfully positive.

D. Was negative.

This Schick toxin, received from Dr. O'Brien, was used for all the tests carried out.

The toxin-anti-toxin received from America was found potent, *i.e.*, 5 c.c. injected subcutaneously into a guinea pig weighing 250 grammes, caused local induration and paralysis on the 7th day. The test laid down in America is that 5 c.c. should cause delayed paralysis but not acute death.

Of 121 children immunised 74 were done with the American toxin-anti-toxin and the remainder with a supply received from Burroughs Wellcome and Co.

The toxin used was diluted immediately before use in the diluent sent out with the toxin; the strength of the dilution being 1/50 of 1 M.L.D. in 0.2 c.c.

One c.c. Roux syringes were used with very fine needles. Both forearms were exposed and sterilised with ether, and 0.2 c.c. of the toxin injected intradermally into the anterior aspect of the right forearm. Assistant Surgeon R. T. Sen, L.M.S., assisted the writers in the administration of the injection.

The same quantity of heated toxin, as a control, was similarly injected into the left forearm.

Great care was taken that the injections were really given intradermally and not subcutaneously. Forty to forty-five cases were dealt with at a time, and any diluted toxin left over was thrown away. Fresh toxin was used for each batch.

The arms were examined after 2, 3 and 10 days.

The appearance of a typical positive reaction is quite distinct, and no difficulty was found in judging the reaction even in those with a very dark skin.

Unfortunately there were some cases in which the reaction was doubtful; and it was not found possible, for want of sufficient toxin, to retest these.

TABLE III.

Showing the results of the tests in the three schools.

Name of school.	Number tested.	Number positive.	Number of pseudo-reactions		Number doubtful	Percentage positive.
			Positive	Negative.		
St. Edmund's College.	143	57	2	3	6	39.9
Pinemount School.	49	21	0	0	2	42.8
Loretto Convent.	79	47	2	5	6	59.5
TOTAL ..	271	125	4	8	14	46.1

TABLE IV.

Showing the number of positive Schick reactions and pseudo reactions according to age.

Age.	No. tested	No. positive.	Percentage positive.	No. of pseudo reactions.
1 to 5 years ..	11	6	54.5	0
5 to 10 years ..	97	50	51.5	3
10 to 15 years ..	137	57	41.6	5
Over 20 years ..	6	4	66.6	2
TOTAL ..	271	125	46.1	12

Owing to the small numbers tested these percentages are not of much value.

The pseudo-reactions were more common

among the older children, 15 per cent. giving a pseudo-reaction above the age of 15 as compared with 3 per cent. below that age. This is in accordance with general experience.

EFFECT OF CONSANGUINITY ON THE SCHICK TEST.

Of 4 families of 4 children each :—

In 2 families 3 were positive and 1 negative.

In 2 families 2 were positive and 2 negative.

Of 4 families of 3 children each :—

In 2 families all 3 were negative.

In 1 family all 3 were positive.

In 1 family 2 were positive and 1 negative.

Of 23 families of 2 children each :—

In 9 families both were negative.

In 7 families both were positive.

In 7 families 1 was positive and 1 negative.

Zingher(1), after an analysis of 93 groups, came to the conclusion that members of the same family are likely to give the same positive or negative reaction.

TOXIN-ANTI-TOXIN IMMUNISATION.

Of 125 children found to be positive, 121 were inoculated with toxin-anti-toxin, and 3 owing to want of material, and one owing to the refusal of the parents, were not immunised.

Each child received three injections of 1 c.c. at weekly intervals.

After the first injection, 18 of the older children above 12 years of age had a severe reaction, shown by a rise of temperature to between 101° and 103°F and a painful local reaction.

14 others had a moderate reaction, with a temperature up to 100°F. The others, which included all the younger children, suffered no inconvenience. The second and third injections caused no local or general reaction, except in those who reacted severely to the first injection in whom the subsequent injections caused a lesser degree of inconvenience.

Two months after the last injection, thirteen of the children who had shown a typical positive Schick reaction were retested, and all gave a definitely negative reaction.

RESULTS.

In the largest of the three schools, containing 144 boys, all were tested with the exception of one, and out of 57 who were positive, 56 were immunised. The 57th boy was not immunised owing to the refusal of his parents. This boy developed diphtheria, but made a satisfactory recovery.

At a later date, a boy who had given a negative Schick reaction developed a mild attack of sore throat and virulent diphtheria bacilli were isolated from the swab. We consider that this case may either have been one of septic sore throat in a healthy contact carrier or of modified diphtheria in an immune subject. This case was very mild, the throat clearing up in a day or two after a very small dose of anti-toxin.

Such cases have been met with in America and do quite well without treatment with anti-toxin. In this case virulent diphtheria bacilli persisted in his throat after recovery and resisted all attempts at sterilisation for some months. He was therefore present throughout the season as a carrier, despite which only one other case of diphtheria occurred, which by a curious coincidence, was in the person of the one boarder, who by oversight, had not been tested by the Schick test.

No cases of diphtheria occurred in the other two schools.

Thus out of 265 children and 6 adults who were running a risk of diphtheria during the term, there were three cases :—

(1) A case of diphtheria in a Schick positive case.

(2) A case of septic throat with virulent diphtheria bacilli present in a Schick negative case.

(3) A case of diphtheria in a non-immunised boy whose Schick reaction was unknown.

CONCLUSIONS.

In so far as is known, this is the first attempt to make use of the Schick reaction and toxin-anti-toxin immunisation in India, and our experience seems to indicate that the toxin for the Schick test and the toxin-anti-toxin mixture can, although not always, retain its potency after a journey to India during the hot weather months and give similarly useful results in India to those reported in Europe and America.

Thanks are due to the Irish Christian Brothers who run the School (St. Edmund's) in which these measures were initiated, for their enlightened confidence and cordial co-operation in new—and in India untried—measures of prevention and prophylaxis, and also to the Head Mistress of Pinemount School and the Mother Superior of Loretto Convent School for following the lead thus given by St. Edmunds. It is satisfactory to record that, as a result of the confidence inspired by these measures, on the opening of the session, all new pupils are now being tested and immunised.

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- (4) Bauer—*Therapeutic Gazette*, 1920, page 457.
- (5) Glenny, Allen & O'Brien—*Lancet*, 1921, Vol. 1, page 1236.
- (6) Garcia Bonus—*Int. Journal of Public Health*, Vol. II, No. 4.

THE DISSEMINATION OF HUMAN HELMINTHIC INFECTIONS BY ANIMALS.

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 COLONEL ANDREW BALFOUR, when delivering an address at the Royal Sanitary Institute on

April 26th, 1922, stated that recent observation by American workers had shown the pig to be a disseminator of hookworm larvæ.

In the annual report of the work done in the hookworm laboratory of the Calcutta School of Tropical Medicine during 1921, issued by the Indian Tea Association on May 10th, 1922, Dr. R. Borland McVail writes: "In view of the fact that pigs are sometimes alleged to spread hookworm disease, we tried to infect some young pigs by means of larvæ of human hookworm. The results were negative and as the Indian village pig is a natural scavenger and devourer of refuse, including fæcal matter, it is probable that the presence of pigs in a village or in garden lines does more good than harm."

In the tea garden practice which I supervise in Cachar it is a very noticeable fact that the most unhealthy gardens are those where coolies are allowed to keep pigs.

My most unhealthy garden, which has usually the unenviable distinction of being on Government's "Black List," has at present a human population of 465, composed chiefly of Bhuiya and Chamar castes, and a pig population of over 200. Practically every coolie on this garden over five years of age shows signs and symptoms of anæmia.

The vital statistics of this garden for the past seven years have been:—

Years.	Death-rate per 1000.	Birth-rate per 1000.
1916	45·32	31·09
1917	54·92	29·07
1918	91·63	25·72
1919	108·51	31·71
1920	59·92	20·59
1921	70·04	16·26
1922	46·21	29·4

On the garden where I reside, which has a population of over 2,000 coolies, and where they are prohibited from keeping pigs, the vital statistics during the same period have been:—

Years.	Death-rate per 1000.	Birth-rate per 1000.
1916	13·21	29·89
1917	12·45	24·31
1918	63·29	22·39
1919	39·6	21·02
1920	16·92	28·52
1921	18·21	28·84
1922	14·07	25·06

To confirm or disprove the theory of the dissemination of hookworm by pigs, I recently carried out the following investigations:—

Six pigs purchased from tea garden coolies were slaughtered, their gastro-intestinal tracts were examined for helminths, the gastro-intestinal contents examined microscopically