THE INTRADERMAL TEST IN WHOOPING COUGH

A REVIEW, WITH A STUDY OF 1300 CASES

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(With Graph I in the Text)

Introduction

Whooping cough remains one of the major problems confronting the paediatrician to-day, and an increasing realization of its importance as a cause of infantile mortality, and of morbidity in later life, has, during recent years, directed the combined efforts of both the clinician and the bacteriologist towards effective measures in its control and treatment. Apart from the virus hypothesis put forward by Rich (1932) and supported by McCordock & Muckenfuss (1932) and McCordock & Smith (1934), a considerable volume of evidence has now accumulated in support of the specific role played by *Haemophilus pertussis* in the aetiology of whooping cough; and among others, the experiments of the MacDonalds (1933) on human subjects in America, and of Culotta *et al.* (1935), Shibley (1934) and other workers on monkeys, have, in fulfilling Koch's postulates, helped to substantiate the claim that *H. pertussis* is the sole aetiological agent.

The work of Madsen (1925) and of Sauer (1935) suggests that prophylactic vaccination against whooping cough in infancy is at the present time the most important method at our disposal for combating the disease; and the researches of Gardner & Leslie (1931) in this country have, in elucidating the antigenic phases of *H. pertussis*, added knowledge of the greatest importance to our conception of the causative organism. Of recent years the wider application of the "cough plate" method in the early diagnosis of pertussis has introduced promising possibilities in early isolation and treatment, and has provided an important practical measure in its control.

Following the earlier experiments of Bordet & Gengou (1907, 1909), the successive attempts of a number of observers to elucidate the problem of immunity to whooping cough by means of animal experimentation have not yielded consistent results; and the difficulty of the whole subject has been increased by the well-recognized tolerance of the human and animal body to relatively enormous doses of *H. pertussis* (Teissier *et al.* 1929; Mishulow *et al.* 1930; Toomey *et al.* 1933–4; Miller *et al.* 1934; Koplick, 1934; Witebsky & Salm, 1936). This is reflected in the conflicting views of a number of observers concerning the interpretation of the intradermal response to injections of

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vaccines and other preparations of *H. pertussis*, and has not led to the general adoption of the skin test as an index of immunity to whooping cough.

BRIEF SUMMARY OF SOME EARLIER INVESTIGATIONS

In 1921 Modigliani & de Villa reported that they had obtained papular reactions in the skin of children suffering from whooping cough by the intracutaneous injection of a suspension of *H. pertussis*, which reaction was not present in those persons who had recovered from the disease. Subsequent observers, using stock pertussis vaccines in various strengths, claimed that the reaction was a specific allergic response present in clinical whooping cough at all stages as well as in those individuals with a past history of *pertussis*, while it was stated to be absent in persons with no history of the disease; a positive reaction in their view being of value both in indicating immunity to pertussis and in the early diagnosis of suspected cases (Lubrano, 1929; Orgel, 1922; Garzia, 1923; Craster & Smith, 1931).

Riesenfeld (1923) and Hull & Naus (1923), as a result of their investigations with vaccines, toxic filtrates and extracts of *H. pertussis*, testing large numbers of children subject to epidemics of whooping cough in residential institutions, concluded that the reactions observed were of no specific value, and similar findings were reported by Toomey & McClelland (1933), Truschina *et. al.* (1934) and more recently by Bonnet (1936). Siebler & Okrent (1934), however, in reporting skin tests on 372 children, placed an entirely different interpretation on the intradermal reaction. They discarded the use of stock vaccines in favour of a phenolized vaccine containing 10 billion organisms per c.c. prepared according to the method of Sauer from freshly isolated strains, and obtained positive reactions in 80% of children with no previous history of pertussis and negative reactions in 76·4% of those giving a history of having had it, the test according to them becoming negative about the fifth week of the disease and also following a course of prophylactic injections with Sauer's vaccine. In this country, on the other hand, Paterson *et al.* (1935), in recording tests with Sauer's vaccine on 100 children, obtained positive reactions which they considered allergic in nature in 80% of those with a past history of pertussis and in 19% of individuals with no such history. They noted that negative reactors became positive about 1 month after immunization with Sauer's vaccine but could produce no evidence in support of the diagnostic value of the test in the early stages of the disease. Recently, similar findings have been reported by O'Brien (1937) who tested 371 children with Sauer's vaccine and obtained negative reactions in 90% of those with no history of whooping cough; while of children showing positive reactions, only 71% had definite histories of pertussis, latent immunization possibly accounting for these results. Finally, Paton (1937), using various pertussis vaccines and antigens, concluded that the intradermal test failed to give specific reactions and could not be correlated with the complement-fixation test during the active phase of the disease (cf. Miller, 1934). The latter reaction, he found, became positive

before the third week of whooping cough, thus confirming the original work of Chievitz & Meyer (1916) and the later investigations of Sauer & Hambrecht (1930). These findings have received further confirmation in the more recent work of Donald & Cruickshank (1937), who consider the complement-fixation test to be a reliable diagnostic reaction.

Preliminary considerations and scope of the present investigation

The conflicting views set out in the foregoing summary prompted an investigation into the intradermal reaction in a comprehensive series of cases and suggested that, as far as *H. pertussis* was concerned, any cutaneous response would be allergic in nature, as distinct from the toxin-antitoxin reactions elaborated in the Schick and Dick tests. Comparative tests were therefore performed, using a freshly prepared pertussis vaccine containing organisms in phase I of Leslie & Gardner and controlled by simultaneous skin tests with an endotoxin extract of *H. pertussis* in order to try the effect of a reagent containing a minimum amount of protein in its purest form (Miller, 1934; Kreuger *et. al.* 1933). The preparation chosen was suggested by the work of Krueger (1933) who prepared endotoxin extracts of the *H. pertussis* and other bacteria by the mechanical disruption of living organisms without denaturation of the resulting product by heat, chemicals or passage through adsorbent filters. Working with an extract prepared in this manner from *H. pertussis*, Frawley *et al.* (1934) elicited occasional erythematous reactions in the skin of 300 children with no history of whooping cough, but they placed no interpretation on these findings and did not investigate the intradermal response to this preparation in clinical whooping cough.

Assuming the response to be allergic in nature, a positive reaction might—as in the case of the Mantoux test for tuberculosis—be interpreted as indicating no more than a previous acquaintance with *H. pertussis* and of no significance as far as immunity to the disease is concerned. Bearing in mind, however, the fairly solid type of immunity conferred by one attack of whooping cough, together with the fact that many children escape the disease in spite of almost certain exposure, it has been the purpose of this investigation to attempt to correlate the appearance of a positive reaction with a state of presumed immunity developed in the individual as a result of a previous attack of whooping cough, or in response to subinfecting doses of the causative organism. The value of the test in the early diagnosis of the disease has also been studied.

The present investigation deals with observations on a total of 1300 individuals, 1182 of whom were under 10 years of age and 300 of whom were suffering from pertussis. For purposes of record they have been divided into the following two groups:

Group I. Cases not suffering from pertussis and in whom a careful enquiry was made into a previous history of whooping cough or of a history of contact with this disease (e.g. at home or school).

Group II. Cases suffering from clinical whooping cough at all stages of the disease.

CLASSIFICATION AND INTERPRETATION OF THE REACTION

With the foregoing assumptions as a working hypothesis, the reactions observed were classified and interpreted as follows:

(*a*) *Positive* = a well-marked area of cutaneous erythema 1 cm. or more in diameter = immunity to pertussis.

(*b*) *Negative* = no reaction, or reactions of less than 1 cm. in diameter = susceptibility to pertussis.

(*c*) *Doubtful* = a faintly marked area of erythema 1 cm. or more in diameter = sensitization due to contact. Possible latent immunization or partial immunity.

(*e*) *Papular* = a papule in the skin, up to 5 mm. but less than 10 mm. in diameter = non-specific, probable susceptibility.

In an attempt to explain the presence of positive or doubtful reactions in children with no past history of pertussis, the tabular results include a column indicating the number of individuals giving a history of non-specific respiratory diseases (mostly termed "bronchitis") as the possible explanation of a sub-clinical attack of whooping cough.

PREPARATION OF PERTUSSIS ENDOTOXIN AND OTHER REAGENTS USED

These were prepared in collaboration with Dr H. A. Ash using the following technique. Fresh strains of *H. pertussis* were isolated from early cases of whooping cough, using cough plates containing Bordet-Gengou medium (Kristensen's formula) with 20% and later 50% fresh horse or rabbit blood. Nine to twenty strains were used, and all were confirmed, as in phase I of Leslie & Gardner, by agglutination tests with a high-titre rabbit serum prepared in the laboratory from organisms known to be in the correct antigenic phase. They were then grown in 2·5 c.c. of blood broth for 24 hr. and the resulting growths used to inoculate 30% human-blood Bordet medium in 20 oz. medical flats fitted with screw caps. A good growth was obtained from these after 24 hr. incubation and this was washed off with 5 c.c. of sterile saline, primary subcultures only being utilized. The suspension was centrifuged and washed three times with saline and once with distilled water, after which it was well shaken, counted by Brown's opacity tubes and made up to a strength of 50,000 million organisms per c.c. The volume was noted and the suspension again centrifuged, the supernatant fluid being discarded. The deposit was then put into an agate ball-mill and dried in a desiccator. When dry it was ground for four working days (about 36 hr.) and then resuspended in saline, the volume being adjusted to the same as it was before centrifuging and drying. The suspension was again centrifuged, and the water-clear supernatant fluid pipetted off constituted the endotoxin. The protein content of this preparation is very small, being present in sufficient concentration to give the standard tests for protein, while nitrogen estimations on quantities up to 5 c.c. of the

concentrated product yielded results less than the experimental error by Kjeldahl's method. Injected intraperitoneally into white mice, guinea-pigs and rabbits in doses up to 0·5 c.c., it is without effect (personal communications from Dr H. A. Ash and Dr H. J. Parish). Given therapeutically to patients suffering from pertussis, the endotoxin elicits fairly constantly a focal reaction with exacerbation of coughing paroxysms, and it is at present being subjected to therapeutic trial in an attempt to confirm the good results reported following its employment in America in the prophylaxis and treatment of whooping cough (Krueger *et al.* 1933; Frawley *et al.* 1934; Munns & Aldrich, 1934; Slesinger, 1936).

Skin tests were also carried out with the following other preparations:

The centrifuged deposit of disrupted bacilli was again adjusted to its original volume (equivalent to 50 billion organisms per c.c.), stored in the cold for 1 month, and then incubated at 37° C. for 1 week, after which it was again centrifuged and the supernatant fluid, called preparation F, drawn off.

Preparation B.E. consisted of the emulsion of ground-up organisms after primary extraction of endotoxin and diluted to the equivalent of 10, 4 and 1 billion organisms per c.c. respectively.

A vaccine containing 10,000 million organisms per c.c. was prepared according to the method of Sauer from freshly isolated *H. pertussis* in phase I of Leslie & Gardner, suspended in 0·5% phenol in physiological saline and stored in the cold until sterile (about 14 days). In addition, a potent phase I commercial vaccine of a strength of 4 billion organisms per c.c. (Parke, Davis pure pertussis vaccine "A") was used in performing tests on 300 cases and was found to give reactions identical with the former vaccine in a similar dilution. Control tests were carried out with a vaccine prepared by Sauer's method from *Chromobacterium prodigiosum* in strengths varying from 4 billion to 10 million organisms per c.c. suspended in 0·5% phenol in saline. As concentrated endotoxin proved too strong for skin-testing purposes, it was diluted just prior to use with boric buffer solution, this preparation being found not to give rise to reactions on intracutaneous injection (borax crystals, 57 g.; boric acid, 84 g.; sodium chloride, 99 g.; made up in a 1·5% solution of the mixture of salts in distilled water and autoclaved).

The reactions were read at 12, 24 and 48 hr. intervals, the standard test dose in every case being 0·1 c.c. of one or other of the reagents used.

Group I

(*a*) *The intradermal response to pertussis vaccine*

The predominant response to the intradermal injection of 0·1 c.c. of Sauer's vaccine in a strength of 10 billion organisms per c.c. was seen to consist of either a papule or a pustule surrounded with a varying sized area of erythematous induration, appearing in about 4 hr., reaching its maximum intensity in 18–24 hr. and thereafter fading, the papule persisting for a few days. Reactions

of a similar intensity followed injections with vaccine diluted to a content of 4 billion organisms per c.c., and well-marked though less papular reactions were elicited with dilutions to 1000 million *H. pertussis* per c.c. Weaker suspensions than this failed to produce satisfactory reactions, even in well-developed clinical cases of whooping cough. In fact, the anomalous reactions that were early apparent with Sauer's vaccine rendered necessary some similar preparation to act as a control. For this purpose it was decided to exclude the group reactions that might occur with *H. influenzae* and other pathogenic organisms, and to employ a vaccine prepared from *Chromobacterium prodigiosum* ($1{\cdot}0 \times 0{\cdot}7\,\mu$) as this organism approximates in size to *Haemophilus pertussis* ($1{\cdot}5 \times 0{\cdot}3\,\mu$) and is non-pathogenic. In addition, fifty skin tests were performed with 0·1 c.c. of 0·5% phenol in saline in order to exclude non-specific reactions from this source. In a mixed series of sixty-eight children, thirty of whom were suffering from whooping cough, tested simultaneously with these two vaccines, it was seen that the reactions produced in response to *Chromobacterium prodigiosum* far outstripped those observed with *Haemophilus pertussis* in both the frequency and the severity of the local lesion present, a wide area of inflammatory induration 2–5 cm. in diameter resulting in fifty-six out of sixty tests performed with suspensions containing 100 million or more *Chromobacterium prodigiosum* per c.c. On this account, and because *Haemophilus pertussis* in a similar concentration (100 million per c.c.) failed almost constantly to elicit any cutaneous response, control tests with *Chromobacterium prodigiosum* were subsequently abandoned. These results are summarized in Tables I and IV.

Table I

Past history of whooping cough					No past history of whooping cough					
+	−	±	Papular	Non-specific respiratory disease	+	−	±	Papular	Non-specific respiratory disease	Reagent
96	11	26	24	5	118	52	19	64	37	Sauer's vacc. 10 billion H.P. per c.c.
40	1	5	8	4	33	13	11	10	10	,, 4 ,,
51	7	13	15	4	37	21	12	30	6	,, 1 ,,
1	1	0	3	0	4	0	1	1	1	,, 500 million H.P. per c.c.
1	4	2	5	0	0	14	1	5	0	,, 100 ,,
0	10	0	0	1	0	10	0	0	2	,, 10 ,,
189	34	46	55	14	192	110	44	110	56	Total = 780
1	0	0	0	1	1	0	0	0	0	*Chr. prodigiosum* 4000 million per c.c.
2	0	0	0	0	2	0	0	0	0	,, 1000 ,,
6	0	0	1	0	16	0	0	1	0	,, 100 ,,
2	0	0	2	0	2	0	0	2	0	,, 10 ,,
11	0	0	3	1	21	0	0	3	0	Total = 38
										Infants under one year of age:
3	0	0	0	0	21	4	3	14	0	Sauer's vacc. 10 billion H.P. per c.c.
2	0	0	0	0	4	2	2	6	0	,, 4 ,,
1	0	0	1	0	2	5	0	4	0	,, 1 ,,
0	2	0	0	0	0	4	0	1	0	,, 100 million H.P. per c.c.
6	2	0	1	Total tested = 3	27	15	5	25	0	Total tested = 66
0	0	0	0	0	11	6	3	1	0	*Pertussis* endotoxin 1 : 1
1	0	0	0	0	4	20	1	1	0	,, 1 : 5
2	0	0	0	0	0	33	2	0	0	,, 1 : 10
3	0	0	0	Total tested = 3	15	59	6	2	0	Total tested = 66
					4	0	0	1	Total tested = 5	*Chr. prodigiosum* 100 million per c.c.

N.B. H.P. = *Haemophilus pertussis.*

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Comment. A study of this table suggests that the intradermal response to Sauer's vaccine, more particularly in the strength commonly employed, is of no value as a reaction of immunity to whooping cough. The bacterial content of this vaccine appears to be too high for skin testing purposes, giving rise to reactions in the skin of the majority of individuals tested, irrespective of a past history of whooping cough, while a suspension one-tenth the original concentration appeared to be quite adequate in eliciting a satisfactory cutaneous response. Moreover, the reaction is of such a character that it is not possible to exclude concomitant inflammatory reactions of a non-specific nature which would appear to have been responsible for the conflicting interpretations placed upon it by previous observers. These conclusions are supported if reference be made to that portion of Table I which deals with reactions in the skin of sixty-six infants of 1 year of age or less, in whom a past history of pertussis or of contact with this disease could be definitely excluded. Of a total of forty-two tested with full strength Sauer's vaccine, no less than twenty-one showed well-marked positive reactions, while four out of five of them reacted positively to the control tests with *Chr. prodigiosum.*

(*b*) *The intradermal response to pertussis endotoxin*

Used in suitable dilution, this preparation gives rise to a reaction consisting of a circumscribed and uniform area of erythematous induration, not essentially papular in character, and resembling that seen in the Schick and Dick tests. It appears in 4–6 hr., reaching its maximum intensity in 18–24 hr., and then fades during the next 48 hr., leaving an area of staining in well-marked cases. Control tests performed with 0·1 c.c. of boric buffer solution were found constantly to produce no reaction in the skin of fifty children tested and were subsequently abandoned, simultaneous tests with vaccine and endotoxin being carried out on the same patient for purposes of comparison. Concentrated endotoxin, the equivalent of 50 billion organisms per c.c. proved to be too strong a reagent for skin-testing purposes, producing reactions in the skin of most individuals tested, the size and frequency of which were comparable to those seen with full strength Sauer's vaccine (see Table II). Trial dilutions up to 1 : 5 or 1 : 10, however, proved on subsequent investigation to give more consistent readings, higher dilutions than this yielding equivocal reactions except in persons suffering from whooping cough where the heightened allergic state of the patient provoked reactions with dilutions up to 1 : 100. Like the Mantoux test and other cutaneous reactions, the pertussis endotoxin test is suppressed during the eruptive phase of measles, and to a less extent also in that of other exanthemata or in the presence of severe intercurrent disease such as bronchopneumonia. As noted by Toomey (1934), working with pertussis toxic filtrate, a proportion of negative reactors, when tested 5–10 days later, showed positive tests suggesting sensitization to a dose as small as 0·1 c.c. of a 1 : 10 dilution. Autoclaving to 100° C. for 30 min. did not inactivate the endotoxin for skin-

testing purposes, but it tended to lose its potency if stored in a diluted condition for more than 3 months. Attempts at skin neutralization tests with convalescent serum removed from a patient during the sixth week of pertussis yielded indefinite results when tried out on known positive reactors during the active phase of the disease. Tests performed with secondary endotoxin extracts (preparation "F") yielded similar reactions to those with primary endotoxin in the same dilution. Skin testing with suspensions of disrupted bacilli after primary extraction of the endotoxin (preparation "B.E.") gave rise to reactions almost identical with those seen with Sauer's vaccine of a similar strength, suggesting a non-specific factor in their production.

A total of 1000 individuals, 882 of whom were under 10 years of age, were tested with one or other of these reagents in standard test doses of 0·1 c.c. and these findings are set out in the subjacent Tables II and III.

Table II

Past history of whooping cough					No past history of whooping cough						
+	−	Doubtful	Papular	Non-specific respiratory disease	+	−	Doubtful	Papular	Non-specific respiratory disease	Reagent used	Remarks
49	3	7	0	4	55	41	15	1	33	Concentrated endotoxin	Too strong
80	9	15	0	2	25	64	7	3	20	Endotoxin 1 : 5	More accurate
89	30	28	11	6	74	204	37	13	35	Endotoxin 1 : 10	More delicate
4	0	1	0	0	2	6	0	1	0	Endotoxin 1 : 10 heated	Inconclusive
6	1	1	2	0	5	14	3	3	4	Preparation F 1 : 10	More delicate
19	0	1	0	0	6	10	3	0	2	Preparation F 1 : 5	More accurate
5	0	0	3	0	9	0	0	3	0	B.E. 10,000 million per c.c.	Too strong
1	0	0	0	0	0	1	1	2	0	B.E. 4000 million per c.c.	Inconclusive
53	43	53	16	12	176	340	66	26	94	Totals	

Comment. The following facts emerge from a study of the 1074 tests recorded in Table II. Neglecting reactions with concentrated endotoxin and dealing first with the response to a dilution of 1 : 5 in a series of 202 individuals tested, it appears that 91·3% of 104 children with a past history of whooping cough showed manifestations of cutaneous allergy to pertussis endotoxin, 77% of these being well-marked reactions; while of ninety-nine with no such history, 32·3% were allergic but of these 20% had a record of non-specific respiratory disease. Similarly, out of 586 children tested with a 1 : 10 dilution of endotoxin, 84·1% of those with a past history of whooping cough gave positive reactions of some sort, 73·2% of which were well marked, while of 328 with no history of pertussis, 10·7% of whom had suffered from non-specific respiratory diseases, 66·1% reacted negatively. These results compare favourably with those produced in response to Sauer's vaccine, and the results are summarized for the sake of clarity in Table III.

The majority of tests were carried out with a 1 : 10 dilution of endotoxin, this being the weakest concentration found most constantly to give definite readings in the skin of children suffering from whooping cough. A study of Tables I–III suggests, therefore, that the endotoxin test would appear to be of some value in determining the state of immunity to pertussis. Latent

Table III

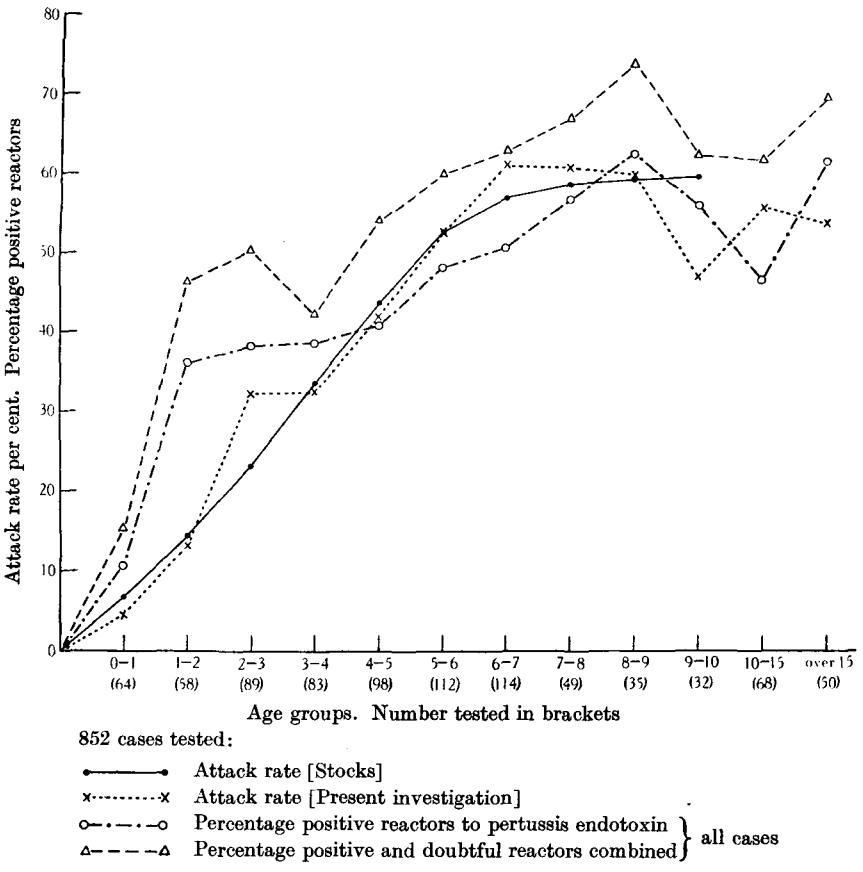
Reagent	Past history of whooping cough: Allergic	Not allergic	Total	Allergic %	Anergic %	No past history of whooping cough: Allergic	Not allergic	Total	Allergic %	Anergic %	Non-specific respiratory disease %
Endotoxin 1 : 5	95	9	104	91·3	8·7	32	67	99	32·3	67·7	20·0
Endotoxin 1 : 10	217	41	258	81·4	18·6	111	217	328	33·9	66·1	10·7
Sauer, 10,000 million per c.c.	112	35	147	77·7	22·3	137	116	253	54·1	45·9	14·6
Sauer, 1000 million per c.c.	64	22	86	74·4	25·6	49	51	100	51·0	49·0	6·0

immunization following subclinical doses of *Haemophilus pertussis* is offered as a possible explanation for the allergic phenomena present in some 30% of individuals with no history of whooping cough. This view received some measure of support from observations on a series of thirty-eight children and adults, among them numbering several volunteers from the nursing staff of a fever hospital, in whom there was no history of pertussis but who had definitely been in contact with the clinical disease, twenty-nine of whom showed positive reactions with endotoxin and none of whom developed the disease subsequently. Finally, in a group of sixty-one infants under 1 year of age with no history of whooping cough or of contact, reference to Table I will show that, excluding tests with pure endotoxin, four only showed definite positive reactions with dilutions to 1 : 5 or 1 : 10.

An enquiry by Stocks (1932) revealed that 42·3% of London children under the age of 10 years gave a previous history of pertussis, but he computed that the presumed attack rate at this age was about 60%. The majority of the individuals who formed the subject of the present investigation were London children, and it is therefore of some interest to compare Stocks' findings with those at present under consideration. For this purpose only skin tests with endotoxin dilutions of 1 : 5 and 1 : 10 have been included, and the attack rates given by Stocks in the various age groups have been plotted for comparison in Graph I.

A study of this graph reveals in general a similarity in the attack-rate curves for the two investigations except in the age group 9–10, where an error in sampling due to the small number tested may account for the discrepancy. Furthermore, it was not found possible to obtain a satisfactory past history in every case, and after the age of 10, and in adults, too great a reliance could not be placed on the accuracy of the previous histories available, many adults for instance being unable to state whether or not they had had whooping cough. In Graph I, the curve indicating positive reactions to pertussis endotoxin includes all the subjects tested, irrespective of whether a past history of whooping cough was obtained or not, and it therefore merely indicates, in a broad manner, the degree of bacterial sensitivity towards *H. pertussis* that might be expected to be manifest among a mixed group of individuals living in an urban community. It will be noted that this

curve is at a higher level than the attack-rate curve, more particularly in children under the age of 4. This suggests either a recent contact with the causative organism or else the presence of a fairly widespread degree of latent immunization among the children at this age during the epidemic period at present under consideration (March 1936 to March 1937). Bennholdt-Thomsen (1934) and Gardner (1936) have drawn attention to the appearance



Graph I

of complement-fixing bodies for *H. pertussis* in the serum of contacts who do not themselves develop the disease, and these findings, coupled with those in the present investigation, suggest a parallel development of cutaneous sensitivity towards the causative organism in the same individuals. If the doubtfully positive reactions be considered as truly specific in origin, then a glance at the curve indicating combined positive and doubtful reactions renders this more obvious. These views are not incompatible with the following conclusions reached by Stocks when he states that "the bulk of the remaining 40% (who have not had whooping cough) must escape attack by virtue of some kind of

immunity rather than by avoidance of contact with infection through life, and a part of these no doubt escape by the repeated acquisition of transient latent immunity during epidemics and the remainder by virtue of an inherent immunity to the disease".

Group II

The intradermal test in clinical whooping cough

The skin test has been claimed to be of value in the early diagnosis of pertussis and, in the absence of other clinical and bacteriological criteria, it has been stated to be of use in confirming the diagnosis in atypical or late cases. These points have been investigated in 300 whooping-cough patients at all stages of the disease. Suspected cases in which the diagnosis was confirmed by clinical and bacteriological evidence later on, were tested and retested at all stages as the disease progressed and the reactions observed are set out in Table IV.

Table IV. *The intradermal reaction in whooping cough*—300 *cases tested*

Re-agent	P.E. 1 : 100	P.E. 1 : 50	P.E. 1 : 20	P.E. 1 : 10	P.E. 1 : 5	S. 100 million	S. 1000 million	S. 10,000 million	Week of pertussis	% + to P.E. 1 : 10	% + to P.E. 1 : 5
+	0	0	5	22	16	2	7	18	1st	38·6	48·4
−	1	9	27	35	17	6	2	7			
+	0	3	10	47	34	1	1	23	2nd	72·3	69·4
−	0	8	24	18	15	1	1	13			
+	2	1	8	49	29	0	0	27	3rd	75·3	74·3
−	1	5	13	16	10	3	3	12			
+	0	1	1	30	22	5	5	21	4th	70·0	75·8
−	0	4	11	13	7	1	1	7			
+	0	1	5	15	16	0	0	3	5th	93·7	94·1
−	4	1	2	1	1	0	0	3			
+	1	1	1	10	12	1	1	2	6th	77·0	92·3
−	0	2	2	3	1	0	0	1			
+	1	0	1	2	5	2	2	5	7th	100	83·3
−	1	1	1	0	1	1	1	1			
+	1	3	5	9	13	0	0	5	8th	70·0	81·2
−	5	5	3	4	3	0	0	3			
+	0	1	1	10	13	0	0	8	9th	62·5	81·2
−	3	4	6	6	3	0	0	1			
+	0	1	3	3	6	0	0	2	10th	75·0	85·7
−	2	1	1	1	1	1	1	1			
+	0	0	2	4	4	1	1	4	11th	66·6	80·0
−	1	2	4	2	1	0	0	2			
+	0	1	1	3	3	0	0	4	12th	75·0	75·0
−	1	2	2	1	1	1	1	2			
+	0	0	0	7	2	2	2	2	13th	100	100
−	1	1	1	0	0	0	0	0	and over		
Totals	25	58	140	311	236	28	29	177	All stages	Average % + after 2nd week	
% +	20·0	22·5	30·7	67·8	74·2	50·0	65·5	70·0		78·6	83·9

N.B. P.E. = pertussis endotoxin. S. = Sauer's vaccine.

In this table, no reaction was considered as positive unless a well-marked area of cutaneous erythema at least 1 cm. in diameter was present. This was considered advisable in order to exclude as far as possible any reaction that might be non-specific in origin, and for this reason all doubtful and papular

reactions were classified as negative. A marked feature in many of these reactions was an increase both in the size and in the intensity of the resulting cutaneous lesion which on occasion extended for several centimetres in diameter and could be elicited with both vaccine and endotoxin in higher dilutions than was found possible when testing the children in group I. A series of comparative tests, using endotoxin extracted from haemolytic streptococci, performed on cases of scarlet fever during convalescence in an attempt to correlate the onset of specific cutaneous allergy with the development of the Dick-negative state, yielded less striking reactions, and a further investigation of this matter is contemplated.

A study of Table IV suggests that there would appear to be a definite and early development of cutaneous hypersensitiveness towards pertussis endotoxin in patients suffering from whooping cough, the degree of sensitivity becoming heightened during the subsequent course of the disease. Thus, during the first week of illness, approximately 38% of individuals reacted positively; during the second week 70%; and thereafter between 75 and 95% in subsequent stages up to the 12th week. The intradermal injection of whole vaccine, on the other hand, produced reactions that appeared in an irregular fashion and which were less consistent with the development of a true specific sensitivity.

(*a*) *The diagnostic value of the test.*

An attempt was made to assess the value of the intradermal reaction in the early diagnosis of pertussis, a matter by no means easy to determine. The onset of the disease is characteristically insidious; moreover, the incubation period is not constant, varying according to different authorities between 4 and 21 days. The problem arises, therefore, of determining the exact day on which the disease could be said to have commenced. For this reason the grouping of the tests as set out in Table IV has been arranged at weekly intervals throughout the course of whooping cough and is thus approximate only in accuracy as far as the early stages of the disease are concerned. Nevertheless, it is now recognized that both the incubation period and the period of prodromal catarrh can, in some cases of pertussis, be much shorter than was previously believed (Stocks, 1930).

In a number of children, however, close inquiry combined with careful observation of definite early cases of pertussis and of contact cases numbering 14 who were subsequently found to be incubating the disease, led the observer to the following conclusions based upon the findings set out in Table V.

Comment. Sauer's vaccine in strengths of 10 and 4 million organisms per c.c. respectively can be excluded as giving too high a percentage of positive reactions in all individuals tested. Employed in a strength of 1000 million organisms per c.c. it appeared to be of little diagnostic value, doubtful or positive reactions appearing in twenty-seven out of forty children during the first 2 weeks of the disease. Weaker suspensions than this were found to be of no value.

Table V. *The intradermal reaction in the early diagnosis of pertussis—164 cases tested*

Reagent	+	±	−	Papular	Stage of pertussis day	Total	Positive %
Sauer 10,000 million per c.c.	1	1	0	0	1st– 3rd	2	50·0
	6	0	0	2	4th– 6th	8	75·0
	10	0	0	1	7th– 9th	11	90·9
	7	2	0	1	10th–12th	10	70·0
	11	2	0	3	13th–15th	16	68·7
Sauer 4000 million per c.c.	2	0	0	0	1st– 3rd	2	100·0
	2	1	1	2	4th– 6th	6	33·3
	3	0	0	2	7th– 9th	5	60·0
	2	0	0	1	10th–12th	3	66·6
	4	1	1	2	13th–15th	8	50·0
Sauer 1000 million per c.c.	4	0	0	0	1st– 3rd	4	100·0
	4	0	1	3	4th– 6th	8	50·0
	9	3	0	3	7th– 9th	15	56·6
	1	1	2	1	10th–12th	5	20·0
	4	1	1	2	13th–15th	8	50·0
Chr. prodigiosum vaccine controls	28	0	1	1	All stages	30	—
Pertussis endotoxin 1 : 5	1	0	1	1	1st– 3rd	3	33·3
	14	4	9	0	4th– 6th	27	51·8
	15	3	6	0	7th– 9th	24	62·5
	17	2	5	0	10th–12th	24	70·4
	15	1	5	0	13th–15th	21	71·4
Pertussis endotoxin 1 : 10	6	0	3	1	1st– 3rd	10	60·0
	13	0	20	1	4th– 6th	34	40·0
	25	4	11	0	7th– 9th	40	62·5
	13	2	7	0	10th–12th	22	60.0
	23	6	9	0	13th–15th	38	60·5

Pertussis endotoxin, however, employed in a dilution of 1 : 5, would appear to have some definite diagnostic value, sixty-two out of ninety-nine children showing positive evidence of cutaneous allergy during the first 15 days of the disease. The reaction was observed to become more definitely positive about the 10th day of pertussis, at which stage it may be expected to be present in approximately 70% of patients. The results of tests performed before the 4th day of whooping cough must, for the reasons given above, be accepted with reservation, and it is unlikely that, in the absence of an experienced observer, many cases at this stage of the disease would be suspected of suffering from pertussis. No reliance could be placed on the endotoxin test where solutions of a weaker potency than a 1 : 10 dilution were used; but a study of Table V will show that, employed in this strength, the earliest manifestations of cutaneous allergy can be traced by an increase in the number of doubtfully positive reactions that occurred during the second week of the disease.

(*b*) *The test in the later stages of pertussis.*

A perusal of the contents of Table IV suggests a characteristic development of cutaneous hypersensitiveness towards *H. pertussis* or extracts of this organism in patients suffering from whooping cough. This does not, however, appear to be a constant phenomenon. Excluding tests performed during the first 2 weeks of the disease, and making allowance for possible errors in sampling

due to the small numbers available in some of the later series of cases tested, positive evidence of cutaneous allergy may be expected in approximately 84% of all patients with developed evidence of the disease. Cutaneous sensitivity as judged by the intensity of the reaction, appears to become increased at this time, though the percentage of positive reactors is comparable to that found among the individuals of group I who gave past histories of pertussis.

A gradual diminution in titre of demonstrable antibodies (e.g. complement-fixing bodies) in the serum of patients after the acute phase of the disease is over, has long been recognized (Chievitz & Meyer, 1916), and it is analogous to assume that with recovery from whooping cough a similar decrease in cutaneous sensitivity follows. The findings in the present investigation suggest, however, that with the passage of time this regression in sensitivity is not always complete.

SUMMARY

The literature dealing with the intradermal test in whooping cough has been reviewed, and the claims as to the specific value of the test have been investigated in 1300 cases.

A method for the extraction of endotoxin from *Haemophilus pertussis* has been described and the intradermal response to this preparation compared with that following whole vaccine.

The findings in this investigation do not support the claim that the intradermal response to Sauer's vaccine in the strength commonly employed (10,000 million organisms per c.c.) is of value either in demonstrating immunity to whooping cough or in the early diagnosis of this disease. The bacterial content of this vaccine appears to be too high for skin-testing purposes, giving rise to inflammatory lesions of a non-specific character rather than to allergic reactions of specific value. These reactions are less apparent when the vaccine is employed in a diluted form.

The intradermal response to pertussis endotoxin, on the other hand, though not invariably consisting of a clear-cut reaction (and in this respect falling short of the ideal as a reagent) is more consistent with the development of the allergic state towards *H. pertussis*, reactions presumably of this nature being present in the skin of approximately 85% of children with a past history of whooping cough. Similar reactions, however, can be elicited in the skin of about 30% of individuals with no history of the disease; but in view of the fact that, unlike the more invasive virus diseases, many individuals never develop whooping cough in spite of almost certain exposure when young, it is suggested that latent immunization or recent contact with the causative organism might account for this phenomenon. Whether these reactions indicate merely sensitization or a definite immunity to pertussis must remain, for the time being, *sub judice*.

The onset of bacterial hypersensitiveness, as judged by the pertussis endotoxin test, appears about the 10th day of whooping cough and becomes

heightened during the subsequent course of the disease. Ultimately this cutaneous allergy undergoes a regression, which, however, is not always complete. For this reason the pertussis endotoxin test would appear to be of some value in assessing the immune state of the individual, and also as a diagnostic reaction in early, atypical or late cases of whooping cough where bacteriological findings by the cough plate method have proved disappointing.

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