THE PSEUDO-SCHICK REACTION AND THE INTRADERMOL TOXOID TEST OF MOLONEY: THEIR RELATIONSHIP AND SIGNIFICANCE

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(With 2 Figures in the Text)

Historical review

The Schick test, as originally performed, consisted of a single injection of toxin filtrate. In 1913 Schick noticed that whilst a negative reaction was evidence of enough antitoxin for immunity, a positive reaction was not always proof of the absence of immunity. These false positive reactions differed from the true ones in appearance and duration and in their persistence despite the simultaneous administration of antitoxin. Park, Zingher and Serota (1914) described them as false or pseudo reactions which were dependent on local sensitisation phenomena of a general protein character, for they could be obtained with broth or a dialysate of diphtheria bacilli. Bessau and Schwenke (1915) found that suspended diphtheria bacilli and heated toxin filtrate produced a similar reaction. As a practical application of this knowledge, a second—control—injection was introduced in the Schick test. Three substances were tried: (1) Kolmer and Moshage (1916) used a suspension of diphtheria bacilli; (2) Zingher (1916*b*) used (*a*) heated toxin filtrate, (*b*) neutralised toxin filtrate. The information sought in employing these substances was whether they produced a reaction comparable with that obtained with the test fluid. If they did, the reaction was a false one. Thus the original function of the second injection was to serve as a control for the Schick test. It was soon observed, however, that pseudo reactions gave other information. Park, Zingher and Serota (1914), Zingher (1916*a*,*b*, 1922), Roubinovitch, Loiseau and Laffaille (1924), Zoeller (1924*a*,*c*), all noticed that pseudo reactors were particularly liable to unpleasant reactions after immunising injections.

Thus, a pseudo reaction served two purposes:

(1) it constituted a control for the Schick test,

(2) it acted as an indicator of possible reactors to immunising injections.

The former was the more important function and decided workers to employ heated toxin filtrate as the most suitable material.

With the increasing use of immunising injections the need for detecting possible reactors became more pressing. Zoeller (1924 *a*) therefore introduced his "anatoxi-réaction". This consisted of an intradermal injection of 0·2 c.c. of a 1 in 100 dilution of the toxoid used for immunisation. He maintained

that the anatoxi-réaction was as efficient as the pseudo reaction for indicating individuals who would react to immunisation, and it had the additional advantage that the diluted toxoid employed could be kept for 8 days, whereas the material used for the Schick test had, at that time, to be diluted immediately before use. This question of how long the diluted material could be kept is of the utmost importance in the history of the evolution of the test. It explains why heated toxin filtrate was superseded by toxoid. We now know that this objection to diluted, heated toxin filtrate is not valid. But before this was realised, the intradermal toxoid test had established a place for itself which it still holds. Thus the position to-day is:

(*a*) Heated toxin filtrate is used as a control for the Schick test.

(*b*) The intradermal toxoid test is employed to indicate possible reactors to immunising injections.

Moloney and Fraser (1927) employed a similar intradermal toxoid test, using 0·1 c.c. of a 1 in 20 dilution, in association with the Schick test. Actually they did not perform a complete Schick test, but replaced the control by diluted toxoid. When O'Brien and Parish (1932) introduced the test to this country they associated Moloney's name with it.

THE PRESENT INVESTIGATION

The scope of the investigation here recorded is as follows:

(i) To determine the relationship between:
  (*a*) the pseudo-Schick reaction produced by heated toxin filtrate,
  (*b*) the Moloney reaction obtained with diluted toxoid.

(ii) To assess the relative efficiency of these two reactions:
  (*a*) as a control of the Schick test,
  (*b*) as an indicator of reactors to immunising injections.

(iii) To consider the significance of these reactions.

The investigation was conducted at the North-Eastern (Fever) Hospital by permission of Dr E. H. R. Harries, the Medical Superintendent. The subjects were 212 new members of the nursing and domestic staffs who joined the hospital between June 1933 and March 1934, and who consented to be tested. The material was provided by Dr R. G. White, Director of the Belmont Laboratories of the London County Council. The formol toxoid had an *Lf* value of 28 antigenic units per c.c. For immunisation the doses were 0·2, 0·4 and 0·6 c.c. for children, and half these doses for adults. The intervals between injections were 3 weeks between the first and second and 2 weeks between the second and third. For the Moloney test 0·2 c.c. of a 1 in 40 dilution was employed.

Each case, on arrival at the hospital, was subjected to a complete Schick test (toxin and control) and a Moloney test. Readings and measurements were made in 24 and 48 hours, and, if necessary, at 1- or 2-day intervals thereafter. All Schick-positive reactors were immunised with the standard adult doses of toxoid, and any reactions recorded. Six weeks after the last

injection a post-Schick and Moloney were performed. Following the usual practice, Moloney and pseudo reactions were classified as follows:

\+ redness no greater than 1 cm.
\+ + redness greater than 1 cm. but with little or no induration.
\+ + + redness greater than 1 cm. with definite induration.

Findings

It was soon apparent that a striking resemblance existed between the pseudo-Schick reaction and the Moloney response. The two occurred in the same individuals and were roughly of the same size, type and duration. The only difference was that the Moloney was usually more intense. Whether the pseudo reaction was a papule, an area of mottled erythema, an indurated plaque with or without a halo of erythema, the Moloney reaction was almost always of the same type. The frequency of the two reactions is given in Table I. It shows that of 212 subjects, 106 (50 per cent.) gave a pseudo reaction and 109 (51 per cent.) a positive Moloney.

Table I. *Comparison of pseudo-Schick reactions and Moloney tests in* 212 *subjects*

Reaction	Pseudo response	Moloney test
Negative –	106 (50 %)	103 (49 %)
Positive +	44 (21 %)	34 (16 %)
+ +	30 (14 %)	8 (8 %)
+ + +	32 (15 %)	57 (27 %)
Total positive	106 (50 %)	109 (51 %)

If all the tests performed, both pre- and post-immunisation, are included, the numerical conformity is even closer. 271 Schick tests and Moloney tests gave 150 pseudo reactions and 151 positive Moloneys.

Table II. *To illustrate the frequency of agreement between the pseudo reaction and the Moloney reaction*

Agreement or disagreement	Pseudo reaction	Moloney test	No. of cases: In immunes	No. of cases: In susceptibles	Totals
Agreement	–	–	51	46	199 = 94 % agreement
	+	+	81	21	
Disagreement	–	+	6	1	13 = 6 % disagreement
	+	–	5	1	

In Table II a comparison between the pseudo and Moloney responses in the 212 subjects has been made for the purpose of determining the frequency of individual agreement between the two. It illustrates the additional but important fact that 94 per cent. of the subjects reacted in the same way to the Moloney and pseudo tests.

The table does not, however, indicate the degree of agreement or disagreement. Where there was agreement it was considerable—a strongly positive pseudo reaction occurring with a strongly positive Moloney; where there was

disagreement it was slight—a negative pseudo being associated with a faintly positive Moloney, or *vice versa*. The 6 per cent. therefore gives an exaggerated idea of the disagreement, which, for all practical purposes, may be considered to fall within the limits of experimental error.

All this suggests that the two reactions are one and the same. No reference was found in the literature to simultaneous Schick and Moloney tests performed for the purpose of comparing the pseudo with the Moloney; but Zoeller, Moloney and his co-workers, and later others realised that the two were comparable. Below, drawn up side by side, are the most important observations made on the two reactions:

PSEUDO REACTION	MOLONEY REACTION
1. Pseudo reactions occur in both susceptibles and immunes (Park, Zingher and Serota, 1914).	1. Positive reactions occur in both susceptibles and immunes (Zoeller, 1924 *b*).
2. Pseudo reactions are not observed in babies; become increasingly common as age advances (Park, Zingher and Serota, 1914; Shaw and Youland, 1916).	2. (*a*) Positive reactions are not observed in babies; become increasingly common as age advances (Fitzgerald, Defries, Fraser, Moloney, McKinnon, 1932). (*b*) Positive reactions become increasingly severe with age (McKinnon and Ross, 1933).
3. Percentage of pseudo reactions increases with age in both susceptibles and immunes (Baranski and Brokman, 1926).	3. Percentage of positive reactions increases with age in both susceptibles and immunes (McKinnon and Ross, 1933).
4. (*a*) Increase of pseudo reactions with age runs parallel with the increase in immunity with age (von Groer and Kassowitz, 1919). (*b*) The percentage of pseudo reactions increases with each increase in antitoxic concentration of the blood (Young, Bunney, Crooks, Cummings and Forsbeck, 1934).	
5. (*a*) There is a much higher percentage of pseudo reactions in immunes than in susceptibles (Zingher, 1921). (*b*) Pseudo and negative reaction is three times as common as pseudo and positive (Dudley, 1929).	5. (*a*) Positive reactions in non-immunes are uncommon (Moloney, 1927). (*b*) Positive reactions are four times more frequent in Schick negative than in Schick positive group (Underwood, 1934).
6. Pseudo reactions occur in those recently in contact with diphtheria bacilli (overt or latent infection) (Dudley, 1923, 1929). The order of descending frequency of pseudo reactions is the following: diphtheria convalescents, recently recovered cases, diphtheria carriers, inhabitants of places where diphtheria is or has just been especially prevalent at time of testing. The increased frequency occurs in both susceptibles and immunes.	6. Positive reactions depend upon recent or remote exposure to the diphtheria bacillus. Recent attacks of diptheria appear to have a particularly marked action for the percentage in diphtheria convalescents is high (Zoeller, 1924 *b*).

7. Susceptibles with pseudo reactions become immune more quickly after exposure to infection than susceptibles without pseudo reactions (Dudley, 1923).	7. Susceptibles who are positive are more easily immunised artificially than negative reactors (Zoeller, 1924*c*; Defries, 1928). Positive reactors recover from diphtheria more easily than negative reactors (Zoeller, 1924*d*).
8. Children with pseudo and positive reactions almost always show more severe local and constitutional symptoms after immunising injections than plain positive reactors. (Using toxin-antitoxin: Park, Zingher and Serota, 1914; Zingher, 1916, 1922. Using toxoid: Roubinovitch, Loiseau and Laffaille, 1924; Zoeller, 1924 *a*, *c*.)	8. Subjects with positive reactions show severe local and constitutional symptoms after immunising injections (Zoeller, 1924 *b*, *c*; Moloney and Fraser, 1927).
9. Pseudo reactions can be lost and gained in both susceptibles and immunes (Dudley, 1923).	
10. (*a*) Pseudo reactions are not, in many cases, very lasting (Dudley, 1923). (*b*) Pseudo reactions, once they have appeared, become a stable property of the organism (Baranski and Brokman, 1926).	

This summary emphasises further the similarity of the two reactions. On all points on which comparison is possible there is agreement. The evidence is overwhelming that these two reactions are one and the same. Why, then, retain both? Moloney and Fraser (1927) dispensed with heated control because they believed that diluted toxoid was a better indicator of reactors to immunisation, and was as efficient as heated toxin for controlling the Schick test. This view is contrary to the findings in this investigation. The figures above show that for all practical purposes the pseudo reaction and the Moloney reaction occur with equal frequency and in the same individuals. It must, therefore, be equally efficacious in indicating possible reactors to toxoid. The fact that the pseudo reaction is less intense is, if anything, an advantage. Moreover, the Moloney is not an accurate control of the Schick test because the material cannot be standardised. The diagnosis of a pseudo and negative Schick reaction is made when the reaction in the control arm is of the same type, size, intensity and duration as that in the test arm; and of a pseudo and positive when there is a significant difference between the two, especially in the duration. Using heated toxin as control, any appreciable difference in size and intensity in the first few days is significant, because the factor responsible for pseudo reactions is present in equal quantities in the two arms, whereas there is not this equality of content when the Moloney is used as control. In consequence, the size and intensity of the reaction may be different. A few days makes the position clear in most, but not in all, cases. Moloney and Fraser admit the difficulty of drawing conclusions as to the immunity of certain

individuals who react to both toxin and toxoid. This difficulty depends upon the fact that the Moloney test is not an accurate control to the Schick test. Actually, Zingher (1916*b*) had discarded an autolysate of diphtheria bacilli as a control because it could not be standardised.

Thus it may be said that the pseudo reaction is as effective as the Moloney test for detecting possible reactors to toxoid, and, in addition, provides an accurate control for the Schick test. The intradermal toxoid test was introduced for one reason only. Its originator, Zoeller, believed that the material used for the Schick test and its control had to be diluted immediately before use. Since this immediate dilution is no longer the practice, the advantage of using diluted toxoid has disappeared; the Moloney test is no longer necessary.

THE CONFORMITY OF THIS INVESTIGATION WITH PREVIOUS OBSERVATIONS

It will be of interest to see how this series of cases conforms with the previous findings tabulated above. To avoid repeating "Moloney and/or pseudo reaction" their unity will be accepted and the term "MP-reaction" used to indicate either or both, unless it is necessary in the context to distinguish between the two, when they will be referred to by their separate names.

Relationship of the MP-reaction to age

As all the subjects of this investigation were adults, they fall into the same age group. It is not possible, therefore, to study the variations of the MP-reaction with age. Nevertheless, the frequency and intensity in this series can profitably be compared with similar observations carried out on different age groups by other workers. Fig. 1 is constructed from the figures of McKinnon and Ross (1933). It consists of a diagrammatic representation of the percentage frequency and intensity of Moloney reaction obtained from 30,766 children of different ages up to 14 years of age. By the side two additional columns, constructed from Table I, indicate the percentages for the pseudo and Moloney reactions in the 212 subjects of this investigation.

The increase in the frequency and intensity of the reaction with age is well illustrated. A comparison of their last age group with the results of the Moloney tests performed in this series shows a sufficiently close resemblance to merit mention. The greater intensity of the Moloney reaction compared with the pseudo is also well shown.

Relationship of the MP-reaction with immunity

Table III is an analysis of the results of the Schick tests in this series. It will be seen that:

Of 69 susceptibles, 22, or 32 per cent., gave a pseudo reaction.
Of 143 immunes, 84, or 59 per cent.,

This is in agreement with the finding that there is a much higher percentage of MP-reactions in immunes than in susceptibles.

Of 106 pseudo reactors, 84, or 79 per cent., were immune. This conforms with the statement that a high percentage of MP-reactors are immune.

The relative ease with which MP-reactors and non-reactors can be immunised will next be considered. For the purposes of the following calculations, an MP-reactor will be considered as one who gives either a positive Moloney or a pseudo reaction or both. From Table II it will be seen that most gave both reactions, if they reacted at all; a few gave one only.

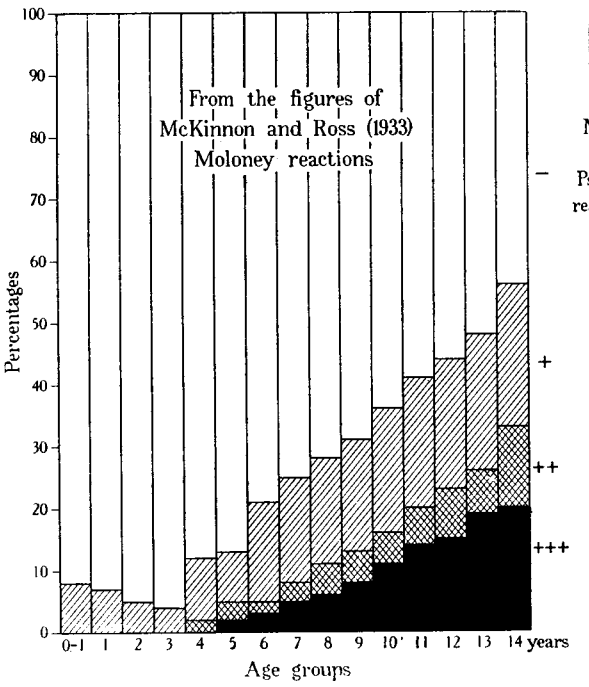

Fig. 1.

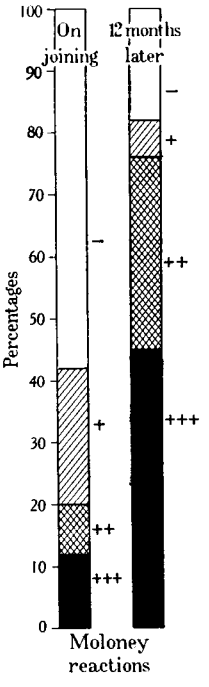

Fig. 2.

Table III. *Analysis of Schick tests in* 212 *subjects*

	Schick test: Susceptibles	Schick test: Immunes	Totals
Non-pseudo reactors	+47 (22 %)	–59 (28 %)	106 (50 %)
Pseudo reactors	Ps +22 (10 %)	Ps – 84 (40 %)	106 (50 %)
Totals	69 (33 %)	143 (67 %)	212 (100 %)

Ps = pseudo reaction. + = positive Schick. – = negative Schick.

(Owing to the correction of percentages to the first digit, the vertical additions do not agree with the calculated totals.)

Of 69 susceptibles, 11 failed to complete the course and are excluded from the calculations. The remaining 58 susceptibles consisted of 20 MP-reactors and 38 non-reactors. The ease with which they were immunised will be judged

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from the state of their immunity (their Schick test) after the usual three adult doses of toxoid.

Of 58 susceptibles, 50 were immune after three doses of toxoid, *i.e.* 86 per cent. If these susceptibles are divided into MP-reactors and non-reactors a definite difference is observed for:

Of 20 MP-reactors, all were immune after 3 doses, *i.e.* 100 per cent.

Of 38 non-reactors, 30 ,, ,, 79 per cent.

This difference is accentuated if the 38 non-reactors are further analysed. 22 of them developed an MP-reaction during, or as the result of, immunisation, and *all these were successfully immunised.* Thus, *all the failures were found among the* 16 *non-reactors who remained non-reactors throughout.* These results are summarised in Table IV.

Table IV

MP-reactors at the beginning	20.	Remained reactors	20.	Successes 20	42 MP-reactors at some stage
Non-reactors at the beginning	38	Became reactors	22.	Successes 22	
		Remained non-reactors	16.	Successes 8	16 Non-reactors
				Failures 8	

Total injected	58
Failed to complete course	11
Total susceptible	69

Thus:

(*a*) 100 per cent. success was obtained in 42 subjects who were either MP-reactors at the beginning or became MP-reactors.

(*b*) Only 50 per cent. success was obtained in 16 subjects who were constantly non-reactors.

This agrees with the observation that non-immunes with an MP-reaction are more easily immunised than non-immunes without a reaction.

Unpleasant reactions after toxoid injections

Although it is usual to separate unpleasant reactions after toxoid injections into local and general, it should be emphasised that the distinction is artificial, and that most severe local reactions are associated with some general symptoms. These may be headache, malaise, lassitude, nausea, vomiting, shivering, and pyrexia. Most of them are subjective. In consequence they are not so reliable statistically as objective responses such as local reactions.

For the purpose of comparing the severity of reactions, the general ones are excluded because they are too few in number and too subjective for treatment along statistical lines. Consideration was limited to the local ones. To obtain some numerical basis for comparison, the local reactions were graded as $\pm$, +, ++ and +++.

In Table V the average number of plus signs recorded after 100 injections of first, second and third doses of toxoid has been computed.

Since the Moloney test was introduced to indicate those subjects likely to react to immunising doses of toxoid, it would be expected on *a priori* grounds,

that reactions after immunisation would bear a close relationship with MP-reactions. This was the case. From Table V it will be seen that:

(1) The most severe reactions occurred in those who were MP-reactors at the beginning (column *a*).

(2) The next in severity were those who gave no MP-reaction at the beginning, but subsequently developed one (column *b*).

(3) The least severe reactions occurred in those who never showed an MP-reaction at any time. In Table V there are two columns of these: those who were successfully immunised (*c*), and those who were difficult to immunise (*d*). The subjects who showed the least reactions of all were those who were difficult to immunise.

Table V. Severity of local reactions to doses of toxoid in the various groups, *expressed as the number of* + *reactions for each* 100 *injections. The average for each injection can be obtained by dividing each figure by* 100

Response on joining	Reactor	Non-reactor	Non-reactor	Non-reactor	
Response after immunisation...	Reactor	Reactor	Non-reactor (successes)	Non-reactor (failures)	Averages
	a	*b*	*c*	*d*	*e*
First injections	153	43	13	0	69
Second injections	221	95	31	6	116
Third injections	189	121	131	50	135
Averages	187	86	58	19	106

Thus we are again brought back to the relationship of *immunity* with these reactions. The association is inescapable and the inference inevitable; there is some parallelism between MP-reactions and reactions to toxoid on the one hand, and immunity on the other. The greater the tendency to an MP-reaction, the more likely are reactions after toxoid to be severe, and the more easily will the subject be immunised. This, of course, is in agreement with the observations that reactions after immunising doses are more severe in MP-reactors, and that MP-reactors are easily immunised. The position in susceptibles may be summed up as follows:

(1) MP-reactors react vigorously to toxoid and are easily immunised.

(2) Reactions after toxoid—even severe ones—are not confined to MP-reactors. Just as subjects develop immunity, so may they develop an MP-reaction and a tendency to react severely to immunisation. In fact, there is some association in time between the appearance of these features. From this, the following inferences may be drawn:

(*a*) The absence of an MP-reaction (Moloney or pseudo) at the beginning is no guarantee that a subject will not develop severe reactions to doses of toxoid during immunisation.

(*b*) Just as an MP-reaction indicates that a subject will react sharply to toxoid, so is the converse true. The appearance of a severe reaction to toxoid during immunisation indicates that the subject is developing an MP-reaction.

This suggests that unpleasant reactions depend upon the MP factor and not upon the antigenic factor.

(*c*) The appearance of a reaction to toxoid, or the development of an MP-reaction may be taken to indicate that the individual is becoming immune. The more severe the reactions, the more likely is this to be true. The converse generally holds also. The absence of reactions to toxoid, or the failure to develop an MP-reaction, usually means that the subject is proving difficult to immunise. Nevertheless, it must be emphasised that these reactions do not appear to be *essential* for the development of immunity, for some become immune without showing any reaction at all.

Two cases illustrate the practical application of these views:

No. 179. A laundry woman aged 28 years.

5. iii. 34. Schick-positive; pseudo reaction + + +; Moloney test + + +.

12. iii. 34. 0·1 c.c. of toxoid caused a marked local reaction and slight general symptoms.

26. iii. 34. 0·2 c.c. of toxoid caused a marked local reaction and more severe general symptoms.

At this stage it was decided to stop the immunisation, as the presence of an MP-reaction and severe reactions to toxoid were taken to indicate that she was on the high road to immunity. To verify this she was retested and found to be Schick negative, and both her pseudo reaction and Moloney test were still + + +.

No. 181. A staff nurse aged 24 years, differs from the previous case in being a non-MP-reactor.

9. iii. 34. Schick test + + +; no Moloney or pseudo reaction.

12. iii. 34. 0·1 c.c. of toxoid produced on ill effects, local or general.

26. iii. 34. 0·2 c.c. of toxoid, injected into the deep subcutaneous tissues of the left deltoid region, caused severe local and general symptoms. Almost immediately after the injection, she experienced local pain which increased in severity. 8 hours after the injection she complained of general symptoms and was put to bed.

27. iii. 34. 36 hours after the injection the symptoms were at their maximum and consisted of the following:

Local signs: Severe redness and swelling involving the whole arm from the shoulder to below the elbow. The length of this area was 30 cm.; the circumference of the arm was 27 cm., compared with 23 cm. on the opposite side. In the centre of this area was a bulla 2 cm. in diameter. Pain and tenderness were marked. The axillary glands were palpable and slightly tender.

General symptoms: Malaise, headache, shivering and nausea; the temperature was 103°.

It was decided to retest her immediately, with the following result: Schick negative; pseudo reaction + + +; Moloney test + + +.

29. iii. 34. Three days after the injection the temperature was normal and all symptoms had disappeared; *the redness and swelling had almost gone, and the bulla* had collapsed. She was back at work next day.

This is a most important case; it illustrates most of the points mentioned. Although she was a non-MP-reactor, yet her reactions to the second dose of toxoid were the most severe in this series. This severe reaction indicates:

(*a*) That a negative MP-reaction is no guarantee that a subject will not react sharply to one or other of the immunising doses of toxoid.

(*b*) That during immunisation such a non-reactor may develop an MP-reaction.

(*c*) That the time of the appearance of an MP-reaction coincides roughly with the development of immunity. There can be no doubt that *in the fortnight between the first and second injections she developed both an MP-reaction and antitoxic immunity.*

(*d*) That severe reactions do not depend upon the antigenic factor, because she was immune at the time; they do depend upon the MP factor, because her pseudo and Moloney reactions were strongly positive.

Although severe reactions are due to the MP factor, they do not necessarily depend upon its amount. It would be expected that, as the dose of toxoid increases, reactions would increase correspondingly. Table V shows that in all columns except *a* there is an increased tendency to reactions with the increase in dose, but column *a* demonstrates that, in MP-reactors, who respond to all injections, reactions are most vigorous after the second injection, and columns *c* and *d* that the severity of third injections is out of all proportion to the other two. Thus the dose alone is not the only factor responsible. The other, and more important, factor is the appearance of a state of hypersensitiveness, which can be demonstrated by the MP-reaction. It has already been shown that such a state tends to develop, *pari passu*, with immunity. Just as immunity takes time to develop, so does this state of hypersensitiveness. In consequence, reactions are more liable after second injections than after first, and more likely after third than second. The appearance of a sharp reaction after the first dose suggests that the sensitising mechanism and the immunity mechanism, which runs parallel with it, are particularly active, and that success in immunising the subject can be predicted. *None of the failures showed the slightest sign of reaction after the first dose.* Just as it was difficult for them to develop immunity, so it appeared difficult for them to develop hypersensitiveness and a positive MP-reaction. Thus the degree of sensitiveness, and not the size of the dose, determines whether a subject will react severely to doses of toxoid. Since the tendency for this state to develop increases with each injection, the practical implication is obvious: the first dose of toxoid should be at least as big as the second and third, and not the smallest, as has been the practice.

The persistence of MP-reactions

Dudley (1933) suggested that pseudo reactions can be lost and gained in both susceptibles and immunes. Baranski and Brokman (1926) on the other hand, were of the opinion that, once a pseudo reaction had appeared, it became a stable property of the organism.

Sixty-four members of this series were subjected to a Moloney retest at intervals which varied from 6 to 15 months after their first examination. The

average period of observation was 11·5 months. Thus, after a year in a fever hospital, during which time the susceptibles were immunised and both naturally and artificially immunised were in contact with diphtheria, they were retested for hypersensitiveness. The results are interesting.

On joining, 27 of these 64, *i.e.* 42 per cent., gave a positive reaction. After a year the number had risen to 53, or 83 per cent. Not only was there a numerical increase, but there was also an increase in intensity. Both these points are illustrated in Fig. 2. This figure of 83 per cent. compares with Zoeller's (1924 *b*) 78 per cent. in diphtheria convalescents. It suggests that recent immunisation and/or recent contact with the diphtheria bacillus is responsible for this remarkable increase in severity and frequency of the reaction.

Table VI. *Results of Moloney retesting* 64 *subjects* 12 *months after arrival in hospital*

Group	State of immunity on joining	No. of cases retested	Moloney test			
			On joining	After immunisation	After 12 months	
					Positive	Negative
1	Immune	18	Negative	—	16	2
2	,,	24	Positive	—	23	1
3	Susceptible	3	Positive	Positive	3	0
4	,,	13	Negative	,,	10	3
5	,,	1	,,	Negative	0	1
6	,,	5	,,	,,	1	4
Totals	—	64	—	—	53	11

The behaviour of the various groups on retesting is given in Table VI. It will be seen that:

(1) Of 27 subjects who were *naturally* Moloney positive (groups 2 and 3), 26 were still positive after a year.

(2) Of 13 subjects who became Moloney positive *as the result of immunisation* (group 4), 10 were still positive on retesting.

Thus 40 subjects were either naturally positive or acquired a positive reaction as the result of immunisation, and 36 retained their reactivity after a year. This suggests that there is a considerable tendency for hypersensitiveness to persist, and bears out the finding of Baranski and Brokman (1926).

(3) Of 18 subjects who were naturally immune and who showed no Moloney reaction (group 1), 16 became positive. This is a most striking figure. It indicates that:

(*a*) The hypersensitive state may develop *after* antitoxic immunity is established.

(*b*) The tendency for this state to develop in those who are naturally immune is considerable.

It would appear that the members of this group found it easy to develop antitoxic immunity and hypersensitiveness, *but not at the same time.*

(4) Group 6 consists of five susceptibles who were difficult to immunise and who, as has already been emphasised, never produced a positive Moloney

during immunisation. Four of these remained negative and one developed a weak positive. Thus, the members of this group found it equally difficult to develop antitoxic immunity and hypersensitiveness.

The inferences to be drawn from these findings are:

(1) The general tendency is for antitoxic immunity and hypersensitiveness to develop *pari passu*; nevertheless, the one may appear before or after the other is established.

(2) The two are induced, both naturally and artificially, with roughly the same ease or difficulty.

(3) Once established they tend to persist, although both immunity and hypersensitiveness may be lost.

(4) The two processes may be considered as parallel but not dependent on each other.

The causative agent of MP-reactions

Diphtheria toxin filtrate obtained from broth cultures of diphtheria bacilli and used for the Schick and Moloney tests and for immunisation is a highly complex mixture. The constituents fall into three main groups:

(1) Exotoxin and its derivatives, such as toxoid.

(2) Altered and unaltered constituents of the original broth.

(3) "Bacterial proteins" from the disintegration of dead bacilli.

In the past, the pseudo reaction has been attributed to each of these constituents. Trauma and antiseptics have also been held responsible. Present knowledge may be summarised as follows:

(1) Uninoculated broth used for cultivating diphtheria bacilli produces either no reaction or a fleeting one that is nothing like the pseudo reaction (Zingher, 1916*a*; Zoeller, 1924 *b*).

(2) Heating the filtrate does not prevent the reaction, *i.e.* the causative agent is heat stable (Bessau and Schwenke, 1915; Zingher, 1916*a*). Since toxin is heat labile it is eliminated as the responsible agent. Also, on heating Schick "toxin" to prepare control, not only the toxin portion, but the toxoid also undergoes destruction to a very large extent (Glenny, A. T., personal communication).

(3) Neutralisation of toxin and toxoid by antitoxin does not prevent the appearance of the reaction (Zingher, 1916*a*).

(4) Products of the bodies of diphtheria bacilli are capable of producing similar reactions. To demonstrate this, Park, Zingher and Serota (1914) and Zingher (1916, *a*, *b*) used a filtered autolysate of washed diphtheria bacilli; Bessau and Schwenke (1915) and Kolmer and Moshage (1916) used a suspension of diphtheria bacilli washed free of toxin; and von Groer and Kassowitz (1920) used both a washed and ground suspension of bacilli and the nucleoproteins obtained from them, which they called diphtherin.

The conclusion is thus reached that the bacterial proteins of autolysed diphtheria bacilli are responsible. The term, "bacterial proteins" is loosely

employed to describe any material other than specific exotoxin and its derivatives, and may include endotoxin, if these exist, true bacterial proteins, and any other possible products of metabolism. Further differentiation beyond this point is not yet possible.

The question now arises as to whether this reaction is specific or non-specific. Von Groer and Kassowitz (1920) thought it was not, but their experimental evidence is not convincing. On the other hand, there is considerable clinical support for specificity. The close relationship with diphtheritic immunity and the high percentage in recent contacts and convalescents is very convincing. Nevertheless, although the reaction is specific, the type of response bears a striking resemblance to many other specific bacterial-protein reactions: these will now be considered in more detail.

The relationship of pseudo-reactions to other types of hypersensitiveness

The body may respond to an infection in one or more of three ways: firstly by developing the disease, secondly by developing a specific hypersensitiveness to the organism or its products, thirdly by acquiring a specific immunity.

The common method of detecting states of hypersensitiveness is by means of skin tests. Following the lines of Coca and Cooke (1923) and of Rich (1933), hypersensitiveness may be classified as follows:

(1) *Anaphylactic hypersensitiveness.* This is essentially a hypersensitiveness of the smooth muscle of such structures as the bronchi and blood vessels. The reaction, when activated, is a musculo-spasmodic one associated with shock. It is typically an animal (experimental) reaction and differs characteristically in different animals. A similar reaction may occur in man, but is not so typical. Detection of this state of hypersensitiveness by skin tests is often unsatisfactory because sensitisation of the skin and of smooth muscle do not necessarily go hand in hand.

(2) *Bacterial hypersensitiveness or the hypersensitiveness of infection.* The tissues, including the skin, are sensitised by a previous contact, and respond to the antigen with a *necrotising-inflammatory reaction.* This manifests itself by:

(*a*) A local reaction, consisting of tissue damage and inflammation.

(*b*) A general reaction, consisting of fever, malaise and prostration, and depending upon a spread *via* the blood stream. This general reaction is quite distinct from the shock in anaphylaxis.

This type of hypersensitiveness is readily detected by skin tests since cutaneous sensitiveness is an important feature. The material used for the tests is prepared from the specific "bacterial proteins". A positive reaction consists of an area of erythema, more or less indurated, which spreads and increases in intensity. It reaches its maximum in 36 hours and fades within a few days. Although the reaction is specific for each organism, the type is the same in all. This suggests some common factor, released or produced as the result of the specific reaction. The best known example is the detection of hypersensitive-

ness to the tubercle bacillus by the cutaneous tuberculin tests. But bacterial hypersensitiveness is very widespread and similar tests have been employed for a large number of organisms. Hypersensitiveness to the bacillus of glanders may be detected by mallein, to the typhoid bacillus by typhoidin (or typhin), to brucella abortus by abortin, to the spirochaete of syphilis by luetin, to the diphtheria bacillus by diphtherin, to the haemolytic streptococcus by streptococcal "endotoxins", to the leprosy bacillus by leprolin, and to certain ringworm infections by trichophytin. Nor does this exhaust the list. Hypersensitiveness to the pneumococcus, the staphylococcus, the bacillus of pertussis, and the fungus of favus can be similarly demonstrated. The early reaction to vaccination in those who have had small-pox or previous vaccination is an example of hypersensitiveness to a virus.

It has already been shown that pseudo reactions and diphtherin reactions are *of the same origin and type; hence the pseudo reaction is an example of a positive test of bacterial hypersensitiveness.*

(3) *Atopic hypersensitiveness.* To this category belong asthma, hay fever and eczema. The reactions obtained by skin tests are described as immediate. They are urticarial in type, appear rapidly in about 10 minutes, may reach their maximum in half an hour and fade in a few hours.

The relationship between infection, disease, immunity and hypersensitiveness

Disease is essentially a clinical condition and may be diagnosed on clinical evidence. Sometimes it is necessary or desirable to obtain other evidence of a non-clinical nature. These aids to diagnosis usually consist of tests for the presence or absence of infection, immunity or hypersensitiveness. In order to make clear the significance of such tests, some reference to the relationship between the three states is necessary.

Infection is not, of course, synonymous with disease for it may be overt or latent, but tests for the presence of infection are employed in the diagnosis of disease. These consist in examining for the causative organism. Whilst the presence of tubercle bacilli may be accepted as proving the existence of tuberculosis, a positive finding of diphtheria bacilli is not, by itself, proof that the subject is suffering from diphtheria. The fallacy of the test may be expressed thus: *If with any organism the carrier state be at all common, a positive test of infection is not, by itself, evidence of disease.*

Immunity may be defined as the capacity of the body to withstand invasion by bacteria, to prevent their growth and neutralise their toxins. It is an obviously protective state, depending upon the presence of an active antibody mechanism. The type of protective mechanism in any individual depends to a considerable extent upon genetic factors. There is no direct method at present available for classifying individuals along these lines, although certain racial characteristics permit of crude generalisations. When, however, such an individual is exposed to a specific infection, overt or latent, some indication of

the efficiency of his antibody mechanism may be obtained from the quantity of specific antibodies which appear in the blood. Such estimations of the amount present at any particular time may be called *immunity tests.* In some diseases it is necessary or more convenient to examine for one type of antibody, in others for another. The antibodies sought may be agglutinins, precipitins, lysins, antitoxins, etc. In diphtheria, the appropriate test is the estimation of the antitoxic content of the blood. As there are certain practical objections to the employment of this test, it has been replaced by a skin test which depends directly upon it. This is the Schick test. It cannot be too strongly emphasised that, strictly speaking, a positive immunity test means two things only: infection, past or present, and the presence of antibodies. It is evidence of previous activity of the antibody mechanism. If, therefore, it is taken to mean immunity to disease in the future, it becomes an inference—a prediction that the antibody mechanism will respond as effectively in the future as it has in the past. Such an inference is usually right, but may occasionally be wrong. On the other hand, an individual who gives a negative immunity test will not, of necessity, suffer from the disease when exposed to the ordinary mass of infection. His antibody mechanism may never have had the opportunity of demonstrating its efficiency, because he has not been previously exposed. Nevertheless, such immunity tests are often very accurate. This requires some explanation. When a particular disease is at all prevalent, or where the carrier state is common, and if latent infection is possible, many individuals will have become infected without their knowledge and will have had the opportunity of producing antibodies without realising that they were infected. These factors—prevalence of disease, the carrier state and latent infection—explain the various uses to which such tests are put. They are employed in three ways:

(1) *In healthy individuals* immunity tests are used for determining susceptibility or non-susceptibility to the disease. If latent infection is at all common, because of the nature of the disease, its prevalence and the existence of carriers, a high percentage of the population will have had the opportunity of producing antibodies. In such circumstances, a positive immunity test may be taken to indicate an active antibody mechanism and the existence of immunity. A negative test, although indicating susceptibility, cannot be taken to mean that disease must follow infection. The Schick test is employed in this manner. A negative result (corresponding to a positive immunity test) indicates the presence of antibodies in sufficient amount to confer immunity. A positive result means susceptibility.

(2) *In sick individuals* such tests are employed in two ways:

(*a*) At the *beginning* of the illness to decide that the patient is *not* suffering from the disease.

(*b*) *Later* in the illness to demonstrate that he *is* suffering from the disease.

These opposite inferences are explainable by consideration of the aforementioned factors. As shown above, *where latent infection is common*, a positive immunity test indicates immunity. If, therefore, a patient is examined *early*

in an illness, before specific antibodies could have accumulated in sufficient amount to be demonstrable, a positive test indicates that he is not susceptible and cannot be suffering from the disease. The Schick test is employed in this manner. It could not be employed later in the disease because it could not be decided if the antibodies present were due to the present illness or a previous latent infection.

On the other hand, *if latent infection is rare*, the presence of specific antibodies *later* in the disease may be taken as evidence that the patient is suffering from that specific disease. This is the principle of the use of the Widal reaction. There are, of course, pitfalls in arguing thus, but, used in conjunction with other signs, this evidence may be of considerable practical value.

The apparent paradox may be stated thus: Immunity reactions may be employed *early* in the illness to indicate *past* infection, therefore *immunity*; and *late* in the illness to indicate *present* infection, therefore *disease*.

Hypersensitiveness. Infection may be followed, after a latent period, by sensitisation, but not always. The tendency increases with age: in young children hypersensitiveness is rare; in adults it is not uncommon. Although it is more frequent in those recently exposed, it may persist long after contact with the organism has ceased. Dudley (1929, 1933) is very emphatic on the importance of *recent* contact in the production of hypersensitiveness to diphtheria bacilli. He maintains that when the individual is removed from contact, the hypersensitive state passes off fairly rapidly. Baranski and Brokman (1926) on the other hand, hold that it tends to persist. I have already shown that in this series hypersensitiveness persisted along with immunity. It must not be forgotten, however, that all the subjects of this investigation work in a fever hospital; and probably all were constantly exposed in varying degrees—even those Schick-positive reactors who were excluded from diphtheria wards. Thus, the findings in this series do not necessarily contradict the view expressed by Dudley. It is nevertheless a fact that almost all those who have worked in fever hospitals for any appreciable time are hypersensitive. Ten older nurses and sisters who were tested, but not included in this series, all showed a marked reaction. This agrees with similar findings with the Mantoux test in older nurses working in sanatoria (see Topley, 1933). Dudley (1933) has said that tuberculin sensitiveness is likewise not lasting unless exposure is continued. This also has been denied by Lloyd (1933).

Zoeller (1924 *c*) and Dudley (1933) contend that hypersensitiveness to diphtheria bacilli is produced only by an actual infection or contact with living or intact organisms. From this investigation alone this view cannot be refuted because, as has already been mentioned, such exposure cannot be excluded. Nevertheless from experience with other communities, there does appear to be some evidence that hypersensitiveness develops as the result of artificial immunisation. Roubinovitch, Loiseau and Laffaille (1924) stated that reactions after doses of toxoid may be taken to indicate the presence of a

positive Moloney reaction. With some of their subjects reactions occurred after second and third injections but not after the first. They concluded that sensitisation had occurred as the result of the injections. Their subjects were injected with the products of dead and disintegrated diphtheria bacilli and there was no evidence of exposure to living and intact organisms. Since, however, carriers and latent infection are common, chance contacts cannot be excluded. The question appears to remain open. There can be little doubt that Zoeller and Dudley found some support—and possibly inspiration—for their view from analogy with tuberculin sensitiveness. Guinea pigs can be sensitised to products of the bacillus of tuberculosis in two distinct ways. Anaphylactic hypersensitiveness can be readily produced by parenteral injections of tuberculo-proteins; subsequent intravenous or post-orbital injections cause anaphylaxis. On the other hand, to produce cutaneous hypersensitiveness it is necessary to proceed along quite different lines; and certain requirements must be fulfilled. Of these the most important, and the most pertinent, is that an actual focus of infection must be produced by the organism itself (Krause, A. R.—see Kolmer, 1917, and D'Arcy Hart, 1932). Strictly, then, the only inference to be drawn from a positive test of hypersensitiveness is the existence of a state of hypersensitive depending upon an infection—past or present, overt or latent.

I have considered certain aspects of disease, of immunity and of hypersensitiveness, and have mentioned that tests for these states have been evolved. I have tried to indicate the true interpretation of each test, and have shown that inferences beyond this are frequently made. These usually consist of arguing the presence of one state from a test designed for, and strictly dependent upon, another. Such arguments are possible because there is one common factor in the production of these three states: that factor is *infection.* From a positive test—whether it be of disease, of immunity, or of hypersensitiveness—it can be correctly deduced that infection, past or present, overt or latent, has occurred. Because infection results in one or more of these states, it is possible to argue from one to another. But such argument is beset with pitfalls. The relative efficiency of these tests when used for purposes other than their correct ones, depends upon the nature and prevalence of the disease, the age of the patient, the existence of carriers and the frequency of latent infection. Confusion arises when the limitations of each test are not appreciated. An example of the difficulty is provided by tuberculin tests. A positive result has, at different times and by different observers, been taken to mean past infection, latent infection, active disease and relative immunity to reinfection. O'Brien (1933) has said that he does not know whether he would rather be tuberculin positive or negative. Despite their drawbacks, these tests can be of great value if their limitations are realised. The following is a summary of their mode of employment:

(1) The presence or absence of *active disease* may be inferred from

(*a*) *Tests of infection:* carriers are the greatest cause of error in this test.

(*b*) *Tests of immunity:* latent infection and previous immunisation may interfere with the efficiency of this test.

(*c*) *Tests of hypersensitiveness:* this is probably the least accurate method. By itself it means practically nothing. Each disease requires separate consideration.

(2) The state of *immunity* may be inferred from:

(*a*) *Tests of immunity:* only a rough indication of the activity of the antibody mechanism is obtained. The stage of the disease at which the test is performed is important.

(*b*) *Tests of hypersensitiveness:* each disease requires separate consideration. The difference between the hypersensitive and immune states is held to be quantitative rather than qualitative, depending upon the balance between fixed and circulating antibodies. Hence this use of the hypersensitive test is logical.

(3) Hypersensitiveness can be detected only from tests designed to demonstrate its presence.

It is now possible to consider the more specific question of the significance of MP-reactions. One of the theories most attractive superficially is that of Zoeller (1924 *b*). He postulated that hypersensitiveness was a half-way stage between susceptibility and immunity. From analogy with the view then current on tuberculin sensitiveness he drew up the following scheme:

Stages of immunity to diphtheria

	Schick reaction	Anatoxi-reaction
First: subject never exposed: susceptible	+ +	−
Second: first contact; still susceptible; onset of hypersensitiveness	+	+ +
Third: immune, but still hypersensitive	−	+
Fourth: completely immune; hypersensitiveness gone	−	−

Against this view certain observations of other workers and some findings in this series may be quoted. Firstly: hypersensitiveness may develop *after* "complete" immunity is established. In Table VI it will be seen that group 1 consists of 18 subjects who were naturally immune on joining and had a negative MP-reaction. 16 of these subsequently developed a positive Moloney.

Secondly: whilst in this series it was possible, by artificial immunisation, to convert all the 58 susceptibles from the Schick positive to the Schick negative state, it was impossible to convert a single one of the 20 MP-reactors into non-reactors. If Zoeller's view is correct, not one of these 20 was completely immunised. This, however, is against the weight of evidence. From Table IV it will be seen that 16 subjects were non-MP-reactors at the end of immunisation. These included all the cases who were most difficult to immunise: moreover, at no stage did these show an MP-reaction. Thus, in this series, not a single artificially immunised subject passed through Zoeller's four stages. In some there was no stage 2 or 3; these were difficult to immunise; some had no stage 4: these were easily immunised. All this suggests that the

absence of an MP-reaction is associated, not with ease and completeness of immunisation, but with the very opposite. Also, the presence of MP-reactions in older nurses, and in the two cases detailed above, confirms the suggestion that the hypersensitive state is associated with complete immunity.

Thirdly: McKinnon and Ross (1933) calculated the ratio of Moloney reactors to Schick negatives in children of various ages. They found that in pre-school children the ratio was 1 to 5 or 6; whereas from 6 years onwards the ratio changed to 1 to 1. They inferred that age *per se* was a factor in the development of sensitivity, and that immunity, particularly in the lower age groups, appeared in a high percentage without evidence of sensitivity.

Lastly: consideration of the significance of tests of immunity and hypersensitiveness in general render it possible to say, on *a priori* grounds, that Zoeller's theory is improbable. The Schick test is an index of the response of the immunity mechanism to *exotin*, whereas the MP-reaction is evidence of hypersensitiveness, not to exotin but to *bacterial proteins*. If the MP-reaction is concerned with immunity at all, it must be with bacterial immunity.

Nevertheless, evidence of the relationship of the MP-reaction with immunity has been demonstrated both in this and in other work. This raises the whole question of bacterial, as distinct from antitoxic, immunity in diphtheria. It would be beyond the scope of this paper to enter into this question in any detail, but one or two points are worthy of mention. Side by side with diffusible toxin of the diphtheria bacillus—the variable factor in the toxicity of the organism, is the other and more constant factor, the fundamental substance of the body of the bacillus—sometimes called "endotoxin". Exotoxin and the antitoxic response it provokes have been extensively investigated and the results generally accepted. By comparison the endobacterial substance has received little attention. Its nature has been investigated; the existence of hypersensitiveness to it is known; and the presence of bacterial antibodies sought, but the results are equivocal and the significance not yet apparent. Nevertheless, this paper confirms that bacterial hypersensitiveness bears a close relationship with antitoxic immunity. It appears to conform with the view expressed from time to time, that immunity to diphtheria is something more than antitoxic immunity. If bacterial immunity exists, what is its relationship to bacterial hypersensitiveness? This is a more general question. Whilst most immunologists agree that the hypersensitive state bears a close relationship with the state of immunity, there is no agreement as to whether hypersensitiveness is necessary or desirable for the development of immunity. Rich (1933) states that it is not, and that in fact, it is definitely harmful. The impression obtained from this work is that it appears to have some place in immunity. In view of the detailed knowledge which exists about diphtheria, a further study of the problem in this disease would repay investigation.

SUMMARY

A total of 212 new members of the staff of the North-Eastern Fever Hospital were Schick and Moloney tested. The Schick-positive reactors were immunised with formol toxoid and post-Schick and Moloney tests were performed. The following conclusions were reached:

(1) The intradermal toxoid test of Moloney or Zoeller corresponds exactly with the pseudo response in the Schick test.

(2) The pseudo response is as efficient as the Moloney for detecting possible reactors to immunising doses of toxoid, and is a more accurate control of the Schick test. The Moloney therefore appears redundant.

(3) A positive MP (Moloney or pseudo) reaction accurately indicates those who will react to immunisation; but a negative MP is no guarantee that the subject will not react.

(4) The MP-reaction is evidence of bacterial hypersensitiveness to specific products of the body of the diphtheria bacillus.

(5) Zoeller's theory that hypersensitiveness is a half-way stage between susceptibility and immunity, is incorrect.

(6) MP-reactions usually, but not invariably, develop *pari passu* with immunity. Because of this parallelism tests of hypersensitiveness give information as to the state of immunity.

The significance of tests of infection, hypersensitiveness and immunity are considered; and the possible relationship of MP-reactions with bacterial immunity suggested.

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