THE DIPHTHERIA TOXOID-REACTION (MOLONEY) TEST: ITS APPLICATIONS AND SIGNIFICANCE

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(With 4 Figures in the Text)

THE diphtheria toxoid-reaction test was introduced in 1927 by P. J. Moloney and C. J. Fraser for the purpose of detecting those individuals who would be likely to give undesirable reactions on the injection of toxoids for diphtheria prophylaxis (Moloney and Fraser, 1927). The test was soon used as a routine measure in Toronto, and Burke (1930) collected the results; a larger series was published by McKinnon and Ross (1933). Although the Moloney test has now been very extensively used in Canada, very little work has been done on it in this country. O'Brien and Parish (1932) described the results of testing 906 individuals, and decided that the Moloney test was equally effective in picking out persons who were likely to react unfavourably to the much stronger toxoids which they had used as it was in indicating those who would react to the weaker toxoids of the Canadian workers: and Parish (1933) later published a smaller series. Underwood (1934) published the results of 905 tests and discussed their relation to the reactions which followed the injection of potent formol toxoids; he also described a delayed reaction to the Moloney test (+D reaction), which will be discussed in more detail later. The paper by McSweeney (1935) discussed certain features of 837 tests performed at Cardiff.

This communication is based on 2666 Moloney tests performed before the injection of a prophylactic, and 595 tests carried out after the injection of toxoids. The whole of the work, including both the injections and the subsequent readings of the reactions, was carried out by the writer, so that the fallacies which are sometimes associated with the different standards of reading adopted by individuals in groups of observers do not arise. It should be mentioned that all the tests were performed in Leeds, mostly in children of school or pre-school age.

Before proceeding to discuss the results a word should be said regarding nomenclature. In their original communication Dr P. J. Moloney and Miss C. J. Fraser used diluted toxoid not only to detect sensitive individuals, but also as a substitute for the Schick control test. They said: "Previous work has shown that diluted toxoid is as effective as the heated control for determining pseudo reactors; in addition diluted toxoid serves to indicate those individuals who may react to a subcutaneous injection of toxoid." In Canada the test

appears to have been known generally by the name of the "diphtheria toxoid-reaction test", and the name "Moloney test" seems to have been given first by O'Brien and Parish (1932). In a recent address Dr O'Brien (1934) referred to the test as the "Moloney-Fraser reaction". As the test is now fairly well known in this country by the name of the "Moloney test" this name will be adopted in this paper. It should also be noted that Zoeller (1925) introduced a modification of the Schick test which consisted of the injection of a 1/100 dilution of Ramon's anatoxin at the same time as the Schick test was performed. Zoeller thought, however, that a positive result at this site was a transition stage between susceptibility to diphtheria and complete immunity. He also thought that such a positive reaction could again become negative, and accordingly complete immunity was represented by a Schick-negative combined with an anatoxin-negative reaction. In any case Zoeller does not seem to have recognised the important use of the test for the purpose of detecting toxoid-reactors, and hence the name "Moloney test" is justifiably retained.

Description of the test

The test consists of the intradermal injection of diluted toxoid. The amount injected is usually 0·2 c.c. and the toxoid dilution 1 : 200. A positive reaction usually develops within 24 hours and is at its maximum in about 48 hours. The test can be read either after 24 or after 48 hours. In the series which will be described all the tests were read after 48 hours, though some were also read at 24 hours. The positive reaction is usually described as of three grades, +, + +, or + + +. Fitzgerald, Moloney, Fraser and their colleagues (1932) describe the different types as follows: "1+ signifies an area of redness no greater than 1 cm.; 2+ an area of redness greater than 1 cm., but with little or no induration; 3+ indicates definite induration at the site of the injection." In the paper by McKinnon and Ross (1933) the reactions are classified as follows: + = redness of 1 cm. or less, with no induration; + + = redness greater than 1 cm., with no induration; + + + = redness accompanied by induration. O'Brien and Parish (1932) gave the following description of the "definite" reactor (corresponding to + + +): "'Definite' indicates a central zone of redness with a palpable thickening of the skin 15 to 35 mm. wide; this is surrounded by a flushed zone varying from an easily delimited area 40–120 mm. to a faint flush 10–15 cm. wide, merging into the colour of the surrounding skin." A "mild" reaction (corresponding to + +) they describe as "a faint red flush 25–100 mm. wide". In a later paper Parish (1933) gave the following description: "M+ + + or 'definite' indicated a large zone of redness with palpable thickening of the skin; M+ + or 'mild' was an area of bright hyperaemia of more than 10 mm. in diameter, with very slight induration; where induration was absent, a very faint flush irrespective of its diameter, or a bright reaction of less than 10 mm. was classified as M+ or 'faint'". The classification which the writer adopted was more or less that of Parish, but was modified slightly as

a result of preliminary work. All the tests described in this paper were classified according to the definitions which were given in a previous paper (Underwood, 1934): "(1) 'Definite reaction' (M+++): a large area of erythema up to 40 mm. or more in diameter, with a definite palpable area of induration in the centre; (2) 'mild reaction' (M++): an area of erythema of more than 10 mm. in diameter, with an area of slight induration; (3) 'faint reaction' (M+): a definite area of erythema of less than 10 mm. in diameter, without induration, *or any area of a faint pink colour which showed no thickening, and which was not due to trauma.*" With the exception of the classification of McKinnon and Ross, these definitions are all more or less similar, but the one which is used in this paper probably allows of the most satisfactory classification of the reaction which consists of a definite area of erythema of about 15 mm. in diameter, but which shows no thickening. In actual practice all reactions fall naturally into one or other of these three groups, and reactions which are difficult to classify are not often met with. The British systems of classification seem to be finer and more useful than that of McKinnon and Ross. For example, the cases classified in this paper as "++" or "+++" would all be classified in the latter category by these writers. Yet the distinction is easily made, and there is often an appreciable difference in the reactions of the two classes on the injection of toxoids. The writer is of opinion that it is better to have the finer division in the region of the strong positive reactors.

DISTRIBUTION OF POSITIVE REACTORS IN THE POPULATION

The Moloney state of 2666 children and young adults in Leeds before the inoculation of a prophylactic is given in Table I, the individuals being grouped according to their Schick state and age. The essential features of this table, so far as age is concerned, are given in Table II, from which Fig. 1 was drawn.

Table I

Age group	Moloney result	Schick result: Negative	Slight (+)	Moderate (++)	Marked (+++)	Totals	
Under 1	0	8	8	30	2	48	
	+D	—	—	—	—	—	
	+	—	—	1	—	1	
	++	—	—	—	—	—	
	+++	—	—	—	—	—	49
1+	0	3	16	136	26	181	
	+D	—	—	—	—	—	
	+	—	—	4	—	4	
	++	—	—	—	—	—	
	+++	—	—	—	—	—	185
2+	0	—	13	124	30	167	
	+D	—	—	2	—	2	
	+	2	1	2	1	6	
	++	1	—	1	—	2	
	+++	—	—	—	—	—	177
3+	0	6	14	121	38	179	
	+D	—	—	1	—	1	
	+	1	—	6	1	8	
	++	1	1	4	—	6	
	+++	—	—	—	—	—	194

Table I (*continued*)

Age group	Moloney result	Schick result: Negative	Slight (+)	Moderate (++)	Marked (+++)	Totals	
4+	0	16	16	143	46	221	
	+D	—	—	—	—	—	
	+	4	1	5	—	10	
	++	1	—	—	1	2	
	+++	—	2	—	—	2	235
5+	0	29	29	175	65	298	
	+D	1	—	3	1	5	
	+	5	5	8	3	21	
	++	4	1	1	2	8	
	+++	—	—	1	—	1	333
6+	0	45	36	197	67	345	
	+D	5	—	1	—	6	
	+	9	—	5	4	18	
	++	6	4	4	1	15	
	+++	2	—	—	—	2	386
7+	0	21	14	77	36	148	
	+D	3	1	—	1	5	
	+	7	4	5	1	17	
	++	5	1	7	1	14	
	+++	2	1	1	—	4	188
8+	0	20	12	60	29	121	
	+D	3	—	—	1	4	
	+	9	—	4	2	15	
	++	5	1	6	1	13	
	+++	2	—	1	—	3	156
9+	0	18	10	65	18	111	
	+D	1	1	1	—	3	
	+	7	1	9	1	18	
	++	14	—	2	1	17	
	+++	4	2	2	1	9	158
10+	0	17	4	43	21	85	
	+D	2	—	1	—	3	
	+	13	2	3	—	18	
	++	7	—	2	3	12	
	+++	6	1	2	2	11	129
11+	0	17	5	32	7	61	
	+D	1	—	1	—	2	
	+	12	—	5	2	19	
	++	14	1	5	1	21	
	+++	7	—	2	—	9	112
12+	0	26	7	27	23	83	
	+D	2	1	1	—	4	
	+	17	6	4	3	30	
	++	9	3	3	—	15	
	+++	8	—	—	1	9	141
13+	0	13	9	13	14	49	
	+D	—	1	—	1	2	
	+	8	1	2	3	14	
	++	9	1	1	—	11	
	+++	7	2	—	1	10	86
14+	0	5	—	3	2	10	
	+D	1	1	—	1	3	
	+	2	2	—	—	4	
	++	8	1	1	—	10	
	+++	3	—	—	—	3	30
15–19	0	33	1	3	3	40	
	+D	5	—	—	—	5	
	+	19	2	2	1	24	
	++	19	—	5	3	27	
	+++	10	—	—	1	11	107
							2666

https://doi.org/10.1017/S0022172400032496 Published online by Cambridge University Press

It should be emphasised here that the following discussion applies to the community as a whole and not to individuals. The assumption that the individual always behaves in the same manner as the herd is liable to lead to certain fallacies in the interpretation of the Moloney test. These fallacies, and the significance of individual tests, will be discussed in detail later.

Table II. *Influence of age on positive Moloney incidence*

		Moloney-positive		Primary Schick tests	
Age	Total tests	No. positive (all types)	% positive	No. negative	% negative
Under 1	49	1	2·04	8	16·33
1+	185	4	2·16	3	1·62
2+	177	10	5·65	3	1·69
3+	194	15	7·73	8	4·12
4+	235	14	5·96	21	8·93
5+	333	35	10·51	39	11·71
6+	386	41	10·62	67	17·36
7+	188	40	21·28	38	20·21
8+	156	35	22·44	39	25·01
9+	158	47	29·74	44	27·85
10+	129	44	34·11	45	34·88
11+	112	51	45·54	51	45·54
12+	141	58	41·14	62	43·97
13+	86	37	43·02	37	43·02
14+	30	20	66·66	19	63·34
15–19	107	67	62·61	86	80·37

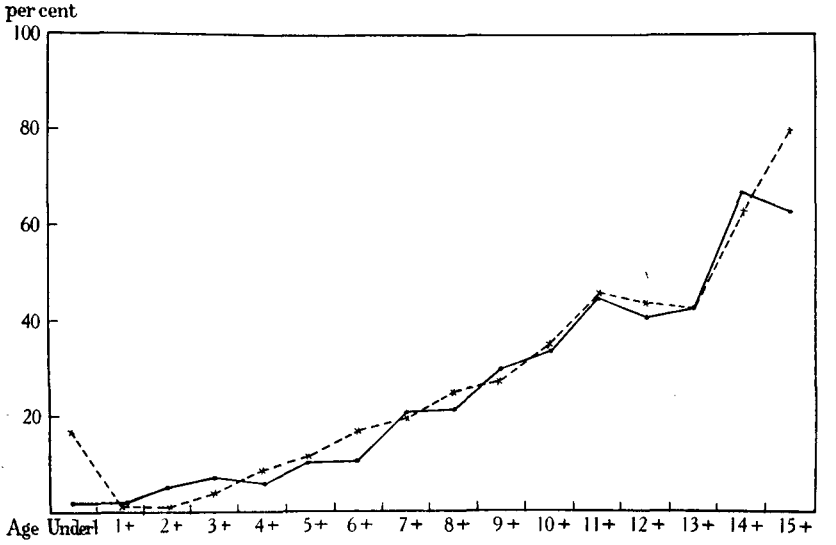

Fig. 1. 2666 tests. Black line = per cent. of total tested at each age who showed a positive Moloney test. Dotted line = per cent. of total tested at each age who showed a negative Schick test.

From the data presented in Table II it is seen that the percentage of children who show a positive Moloney reaction increases more or less continuously as age advances. The table also shows that, as a positive Schick

https://doi.org/10.1017/S0022172400032496 Published online by Cambridge University Press

is most common in the early years of childhood, a positive Moloney test is on the contrary most often met with in later childhood or in adult life. It should be emphasised here that the figures for the Schick test at the different ages are given solely for reference purposes. The remarkable parallelism between the graphs for Moloney-positive reactors and for Schick-negative reactors, which is apparent in Fig. 1, is to a large extent fictitious, and the graphs cannot therefore be used for any other purpose than that of stating the incidence of the two reactions at the different ages. *The figure does not in itself express any fundamental relationship between the incidence of the respective conditions.* This point is important, since it is often assumed that the reactions not only run a parallel course, but that in the later years of childhood a positive Moloney reaction is practically synonymous with a negative Schick reaction. Reference to Table I will show that at the age of 12 years, of 62 negative Schick reactors, 26 showed a negative Moloney reaction; and of 79 positive Schick reactors, 22 showed a positive Moloney reaction. This feature may be found in a corresponding degree in the other age groups, and the early recognition of this fact was one of the starting points of the present paper. This question will be investigated more fully by other methods in a later section, and it is introduced here merely to emphasise the point that no conclusions can be drawn from the apparent similarity, which is seen in the figure, between the frequency of Moloney-positive and Schick-negative reactors.

The incidence of positive Moloney reactors at different ages may be compared with the figures in the corresponding series of McKinnon and Ross. In the present series it may be said that, in children who had not attained their second year only about 2 per cent., and in those in the next three succeeding years not more than 7 per cent. showed any reaction at all to the Moloney test. Thereafter the percentages of positive Moloney reactors increased steadily as age advanced with the exception of a slight falling off between the ages of 11 and 13 years, and in the group of 15 years and over. McKinnon and Ross found that over 90 per cent. of children under 6 years of age gave negative reactions, and that only 46 per cent. of the 14-year-old group gave negative readings. In the present series the corresponding percentages are 93·3 and 36·5 respectively. From these figures it would be possible to say something regarding the tendency of persons at different ages to develop reactions after the injection of prophylactic doses of toxoid, but it is advisable to consider this question in relation to the different types of Moloney reaction.

The results for the Moloney tests at different ages were plotted on a logarithmic scale (Fig. 2). It is seen from the graph that the process of sensitisation starts very early in life, and, although the rate is slow during the first 18 months, it is definitely in progress during this period. During the first and second years of life, however, the rate increases very markedly. In fact, we may say that in these two years the rate of development of a positive Moloney reaction is higher than at any other period of the child's existence. Between the third and fourth years there is a temporary, though appreciable, retardation; but from

the fifth year onwards until the eleventh year there is a more or less continuous increase in the development of sensitivity to toxoid.

There is a considerable amount of evidence, much of which will be discussed in later sections, that the development of a positive Moloney test is due to the acquaintance of the individual with the proteins of the *C. diphtheriae*. McKinnon and Ross rightly emphasise the point that immunity may be obtained without sensitivity being developed at any time during the process. They also suggest that age *per se* may be a factor in the development of sensitivity. From an examination of Fig. 2 it would not appear that the results of the present investigation give much support to this suggestion. In certain

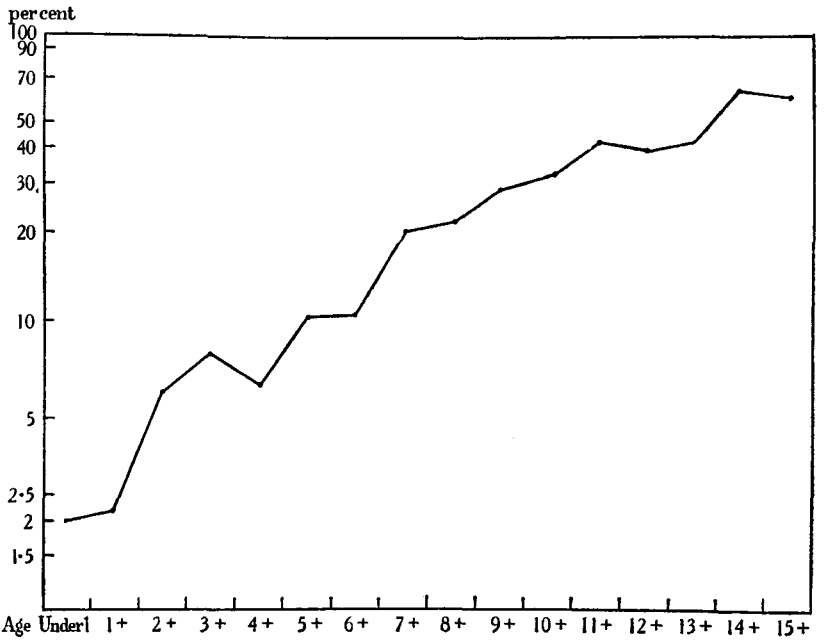

Fig. 2. Logarithmic scale. Per cent. of total tested at each age who showed a positive Moloney test.

individuals sensitisation evidently starts to develop at a very early period of life, though the numbers who develop a positive reaction are very small. It is at this period that the individual is most cut off from contact with other children. But during the second year, when the opportunities for contact with the *C. diphtheriae* are greatly increased, the process of sensitisation becomes very marked. The fall in the rate between the third and fourth years is possibly associated with the fact that the child has, during the preceding three years, more or less exhausted the possibilities of fresh contact in the neighbourhood of his home. When he goes to school he enters into a new environment with considerably increased possibilities of contact with children who may be

harbouring the *C. diphtheriae*. At any rate from the fifth year onwards there is a more or less continuous increase in the percentage of children who become Moloney positive. This evidence certainly suggests that sensitisation, like the development of acquired immunity to the *C. diphtheriae*, is due to the individual's acquaintance with the products of that organism. It is, of course, difficult to draw any definite conclusions with regard to the influence of age. If, however, age in itself is an important factor in the development of sensitisation, we would be inclined to expect on the logarithmic scale a curve which would not show the marked variations which are brought out at the earlier ages in the present series, and which can be quite well explained by the view that sensitisation is due to exposure to the *C. diphtheriae*.

Material information on this question could probably be obtained by a study of Moloney test results in virgin races, or, failing that in closed or semi-closed communities. It should be noted that McKinnon and Ross state that less sensitivity is encountered in rural communities, and it is well known that in these communities there is a higher ratio of Schick-positive individuals.

Degree of reaction

Although these considerations are interesting from a scientific point of view, they have little bearing on the practical application of the test because the different grades of positive Moloney reaction do not indicate equal liability to the development of appreciable reactions on the inoculation of strong toxoids. In a previous paper (Underwood, 1934) it was suggested from preliminary work that care was necessary when using strong toxoids, especially new and untried toxoids, in the case of an individual who showed any type of positive Moloney reaction. This point will be discussed more fully later. Meanwhile it may be said that with known toxoids individuals who show the +D or + reactions may be injected with the usual doses; great care must be exercised with + + reactors, and + + + reactors should not be given toxoid at all. The statement is frequently made that infants and pre-school children can be inoculated with toxoid without a Moloney test, since they seldom or never show a + + or + + + reaction (see McKinnon and Ross; Burke; also *Lancet*, 1935, Editorial, etc.).

This point is brought out in Fig. 3, which shows the percentage incidence of each type of Moloney reaction at each year of life. The figure also shows the combined incidence of + + and + + + reactors at each year. It will be seen that all three grades of reaction increase in frequency as age advances. The +D and + reactions (combined grouping) are most frequent, and the + + + reaction least so. The graph for the + + reaction is almost throughout in an intermediate position between the two extreme types. This statement does not apply to groups over the age of 13 years. After this age M + + reactions become much more frequent.

From the utilitarian point of view the reactions which are most important are the + + and the + + +. Any individual who gives one or the other of these reactions is definitely liable to develop some symptoms on the injection of toxoid in prophylactic doses. These reactions will not necessarily be inconvenient or troublesome; they may consist merely of marked erythema at the injection site. On the other hand, a local reaction *may* be accompanied by a general reaction, and it is obviously important to decide which individuals are likely to give this. In this paper persons who are liable to show a reaction of some type on the injection of toxoid in prophylactic doses are called for convenience "potential toxoid reactors", and the term is only used in this special sense. The incidence of such "potential toxoid reactors" is given by the barred

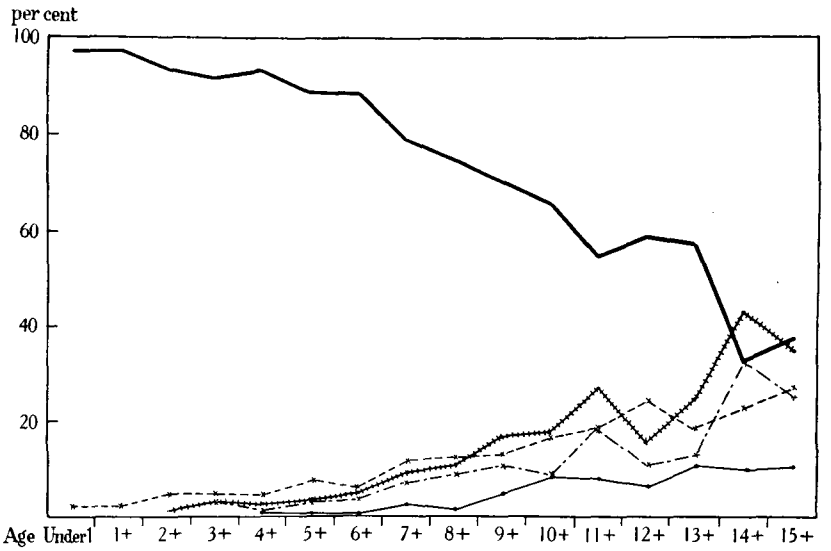

Fig. 3. 2666 tests. Percentages at each year of age.

——— = negative. - - - - - = + *D* and + – · – · – = + +
—— = + + + –|–|–|–|– = combined percentages (+ + and + + +)

line in Fig. 3: this line represents the combined percentages for + + and + + + reactors. It will be seen that up to the age of 5 years the combined percentages of children who show such reactions do not exceed 3 per cent. at any age. At 6 years the incidence is 4·4 per cent.; at 7 years 9·6 per cent.; and thereafter the percentages increase rapidly. In this series of cases the maximum incidence of such "potential toxoid reactors" was reached at the fourteenth year, when 43·3 per cent. of the individuals at this age showed a + + or a + + + reaction.

McKinnon and Ross state that in infancy and the pre-school group sensitivity to toxoid is practically a negligible quantity, and that children at these ages can, therefore, in general be given toxoid without a preliminary Moloney test. In comparing their figures with those of this series it should be remembered that the + + + reaction of the Toronto workers practically em-

braces both the + + and the + + + types of reaction here described. In the Canadian series no reaction which showed any induration was found in 184 children under the age of 5 years. In the Leeds series 12 such reactors were met with in 840 children (1·4 per cent.) at these ages. Hence, although such "potential toxoid reactors" are rare in the pre-school group, they undoubtedly exist. The course of action which is to be adopted depends upon the individual who is actually giving the inoculations; but if it is particularly desired to exclude or treat specially all "potential toxoid reactors" then the Moloney test must be employed for children over the age of 2 years.

Relation of test to symptoms on injection of prophylactics

These considerations are to a certain extent theoretical by reason of the fact that not all positive Moloney reactors do actually show undesirable reactions on the injection of toxoids in prophylactic doses. Most + + + reactors will do so, and many + + reactors also show such symptoms with full doses of prophylactic. A more practical approach to the problem is arrived at by the consideration of those children who actually showed undesirable symptoms on the injection of prophylactics. In this paper reactions will not be dealt with in detail, but the main results are set out in Table III. This table is based on 2041 persons who were injected. All of these had FT or alum toxoids, except 100 who had TAF. (In Table III "++" includes 2 persons and "+++" 4 persons who had only TAF.) In a later section the question of any reaction whatever will be considered. The persons detailed in Table III are only those who showed significant reactions, which are defined here as follows: (1) any local reaction which was more than a mere erythema—*e.g.* swelling, tenderness, fullness, etc.; (2) general reactions—including slight vomiting, marked nausea, headache, or definite distaste for at least one meal. Of the 42 persons included in Table III, 25 were seen by the writer on the day following the injection.

Table III. *Moloney state of persons who showed reactions after prophylactic doses of toxoid*

Negative		+D		+		+ +		+ + +	
Age (years)	Persons	Age (years)	Persons	Age (years)	Persons	Age (years)	Persons	Age (years)	Persons
5	1	3	1	5	1	7	5	4	1
6	1	5	1	7	1	8	1	9	2
—	—	—	—	9	1	10	3	10	3
—	—	—	—	13	1	11	4	11	1
—	—	—	—	14	1	12	3	13	1
—	—	—	—	—	—	13	1	15	1
—	—	—	—	—	—	14	1	—	—
—	—	—	—	—	—	16	3	—	—
—	—	—	—	—	—	17	2	—	—
—	—	—	—	—	—	18	1	—	—
—	2	—	2	—	5	—	24	—	9

Table III shows quite clearly the value of the Moloney test, especially in children over the age of 5 years. It will be seen that only two children failed

to give a positive response to the test. Neither of these was seen until some days after the inoculation, but from the description which was given of the condition of the children one probably had a definite toxoid reaction, and in the other case there was considerable doubt whether the injected toxoid was actually the cause of the condition. It is obvious that the most important reactions occurred in the + + and the + + + groups. The total for the + + + is small when compared with that for the + + group. But it should not be inferred from this that + + + persons were less likely to develop unpleasant symptoms than were + + persons. This is far from being the case. The writer's experience is that the majority of + + + reactors will give such symptoms if injected with full doses of toxoids, and that many of them will behave in this way even after the injection of much smaller doses. The total of 9 for these + + + reactors is small because nearly all such reactors were either dealt with specially (*e.g.* injection of TAF) or were naturally immune, and were consequently not inoculated. This statement applies in less degree to the + + reactors. The evidence from this section of the investigation points to the fact that children of school age should not be inoculated without a preliminary Moloney test.

ASSOCIATION BETWEEN THE SCHICK-NEGATIVE AND THE MOLONEY-POSITIVE STATES

A casual examination of Fig. 1 might suggest that the practically simultaneous transition from Schick-positiveness to Schick-negativeness and from Moloney-negativeness to Moloney-positiveness, which is so marked in the community might apply to the individual as well. This suggestion has in effect been made, since certain workers have stated that the majority of strong positive Moloney reactors are immune and do not require inoculation. If this statement is true, then the Moloney test may to a certain extent be used as a substitute for the Schick test.

It was thought desirable to investigate the association between these two conditions more closely, and for this purpose Pearson's methods for contingency were used. Table IV is a 4×4 table compiled from the main table (Table I) and showing the distribution of the 2666 persons according to their Schick state and their Moloney condition. For this table $\chi^2 = 504{\cdot}76$; for $n = 9$

Table IV

	Schick-positive				
	Marked (+ + +)	Moderate (+ +)	Slight (+)	Schick-negative	Totals
Moloney-negative	427	1249	194	277	2147
Moloney + (+D and +)	27	76	30	139	272
Moloney + +	14	42	14	103	173
Moloney + + +	6	9	8	51	74
Totals	474	1376	246	570	2666

the value of P corresponding to this value of χ^2 is $< 0{\cdot}01$, which shows that the distribution is not a chance one. The degree of association between the Schick-negative and Moloney-positive states was estimated by calculating Pearson's

coefficient of mean square contingency (C_2). The value of C_2 is $0{\cdot}399 \pm {\cdot}013$, which would seem to indicate a moderate degree of association. While there is therefore a considerable degree of association between the two conditions, this is by no means perfect. It should be noted that, of a total of 74 + + + Moloney reactors, 15 showed a marked or moderate reaction to the Schick test (20·3 per cent.). Again, of a total of 173 individuals who gave a + + Moloney reaction, 56 showed a marked or moderate Schick reaction (32·4 per cent.). The tendency in the individual, as in the community, is for increasing toxoid sensitivity to develop *pari passu* with Schick immunity. The degree of association between the two conditions is brought out more clearly by a fourfold table (Table V). In this table the +D and + reactions are included with the

Table V

	Schick-positive (all degrees)	Schick-negative	Totals
Moloney-negative Moloney +D Moloney +	2003 (*a*)	416 (*b*)	2419
Moloney + + Moloney + + +	93 (*c*)	154 (*d*)	247
Totals	2096	570	2666

Moloney-negative reactions, since we are really interested in Schick-negativeness and Moloney reactions which indicate definite susceptibility to toxoid. For this table $\chi^2 = 271{\cdot}81$. For this value of χ^2 with $n = 1$, P is less than 0·01—which indicates that the divergence in the table is not random. The amount of association was determined by calculating Q_5 (the coefficient of association), which is $0{\cdot}632 \pm {\cdot}024$. For the same table the value of r_p (Pearson's equiprobable coefficient) is 0·667, which is practically the same. These values confirm the previous findings.

Association between positive Moloney reactions and positive Schick-control reactions

It has already been stated that, in their original paper, Moloney and Fraser (1927) used the diluted toxoid which they employed in their test, not only to distinguish toxoid reactors, but also as a substitute for the heated control in the Schick test. Suggestions have been made on several occasions that the one test might be made to serve for both purposes. Burke (1930) admitted that in the Toronto campaign the number of Schick tests which were difficult or impossible to interpret by this procedure was increased; as a result it was finally decided to abandon this modified Schick test altogether and to employ only the Moloney test. This procedure was adopted on the assumption that most of the + + + Moloney reactors were already immune—a suggestion which has already been dealt with. Dudley (1934) stated that those who give strong Moloney tests will generally give a pseudo-Schick as well; and in a recent editorial (*Lancet*, 1935) it was pointed out that "the occurrence of a pseudo-reaction, if a preliminary Schick test has been done, is evidence of probable

sensitivity to bacterial protein", and the writer of this editorial recommends that if this sensitivity is shown to exist either by the presence of a pseudo-Schick *or* by a positive Moloney test, special methods should be adopted for immunisation. The question is of importance, since if these statements are correct, one of these tests can evidently be discarded. The present writer has never been convinced that these two reactions are more or less interchangeable; and the accumulation of these personal records of Moloney reactions, practically all of which have been read at least twice after the test injections, has rendered possible a more detailed examination of the problem than has hitherto been attempted.

This is no place to discuss the rather complex features of pseudo-Schick reactions, and in this paper a positive Schick-control test is taken to mean any reaction at all at the site of the injection of heated toxin—excluding, of course, reactions which are due to trauma. The whole of the individuals in the series were grouped according to the nature of the reactions to the Schick-control and Moloney tests, and the results are set out in Table VI. In the following discussion +D and + reactions to the Moloney test are grouped together. For this table $\chi^2 = 309{\cdot}31$; for $n = 3$ the P corresponding to this value is $< 0{\cdot}01$,

Table VI

All Schick tests: incidence of pseudo-reactors (all degrees)	Moloney results					
	Negative	+D	+	++	+++	Totals
Control test: no reaction	2119	45	216 (261)	153	48	2581
Control test: +reaction	28	—	11 (11)	20	26	85
Totals	2147	45	227 (272)	173	74	2666

which shows that the distribution is not a random one. The degree of association between the Moloney-positive state and the liability of the individual to show a reaction at the site of the Schick-control injection was estimated as before by calculating the value of the coefficient C_2. This value was found to be $0{\cdot}322 \pm {\cdot}033$. Table VI was then investigated by the fourfold method, making the dichotomy between Moloney-negative reactors on the one hand and Moloney-positive reactors (all types) on the other. For the table thus formed the value of χ^2 was 126·84; for this value of χ^2 with $n = 1$, P was found to be $< 0{\cdot}01$, indicating again that the distribution was not random. The value of the coefficient of association (Q_5) was $0{\cdot}672 \pm {\cdot}022$, and the equiprobable coefficient (r_p) was 0·679. After making the dichotomies at two other points in the main table, it was found that the resulting coefficients were not materially affected by the alteration. These coefficients are set out in Table VII.

Table VII

Type of grouping	χ^2	P	C_2	Q_5	r_p
4×2 grouping	309·31	<0·01	0·3224 ± ·0329	—	—
(i) Fourfold: (*a*) Moloney-negative (*b*) Moloney + (all types)	126·84	<0·01	—	0·672 ± ·022	0·679
(ii) Fourfold: (*a*) Moloney-negative, M + D, M + (*b*) Moloney + + and M + + +	210·12	<0·01	—	0·642 ± ·023	0·720
(iii) Fourfold: (*a*) Moloney-negative, M + D, M +, M + + (*b*) M + + +	251·67	<0·01	—	0·603 ± ·025	—

Though these coefficients indicate a considerable or even a fairly high degree of positive association between positive Moloney and positive Schick control reactions, this association is far from being perfect. When it is remembered that the object of this part of the investigation is to find whether either of these tests can be used as a substitute for the other, it will be obvious that the answer to this question must be in the negative. Substitution implies perfect or practically perfect association if the information gained from the tests used is to be of any value whatever, and this series of cases does not give much support to the contention which was expressed at the beginning of this section.

It may be objected that those are theoretical considerations and that possibly they have no marked connection with the practical question, whether a reaction at the site of the Schick-control test does actually indicate that the individual is liable to experience an appreciable reaction on the injection of toxoid, or alternatively, whether or not most individuals who react to toxoid react also to the heated Schick-control toxin. To answer this question Table VIII was prepared. It gives the Schick-control reactions and Moloney reactions in every case in which any reaction was noted after the injection of prophylactics—toxoid, alum toxoids, or floccules. It will be noted that the number of cases is larger than that given in Table III. The reason for the increase is that in Table VIII *all* cases of reaction were included, the object being to determine the relationship between these tests and *liability* to react after toxoid in prophylactic doses. Almost one-half of the cases in Table VIII therefore experienced reactions which were so mild as to be unnoticed by the individual inoculated, and many of them consisted merely of an area of erythema measuring 25 mm. in diameter or more. The standard adopted was rather a severe one, and this probably explains the number of cases in the last group of line (*b*). It should be noted that in Table VIII the phrases "cases not

Table VIII

No. of cases showing reactions (all types)	+ Moloney (any degree)	+ Schick-control	+ Moloney and + Schick-control	Both tests negative	Total
(*a*) Cases not seen	8	1	1	4	14
(*b*) Cases seen	55	—	6	16	77
Total	63	1	7	20	91

https://doi.org/10.1017/S0022172400032496 Published online by Cambridge University Press

seen" and "cases seen" do *not* apply to the actual reactors. In subgroup (*b*) every person who had toxoid in the particular batches of children was examined by the writer on the day following the injection; and in subgroup (*a*) every individual who was inoculated was not thus examined, whether they had reactions or not, though many of the actual reactors were seen.

Comment on this table is unnecessary. It is obvious that the test with heated Schick toxin is valueless for the purpose of determining "potential toxoid reactors", and the conclusions arrived at from more theoretical considerations are amply confirmed.

RELATION OF MOLONEY RESULT TO ANTITOXIN CONTENT OF BLOOD

The only investigation of any extent dealing with this question which has come to the writer's knowledge is that described in the original paper by Moloney and Fraser (1927). In the present investigation the blood sera of 108 persons upon whom the Moloney test was performed were titrated for diphtheria antitoxin. The complete results are given in Table IX. The serum from the blood samples was titrated to 100 per cent. differences. For example, $\frac{1}{50}$ unit in this table signifies $\frac{1}{50}$ unit or more of circulating antitoxin per c.c., but not $\frac{1}{25}$ unit. Titration to closer levels would not appear to be necessary. The majority of the samples were taken 6 days after the Schick and Moloney tests were performed; most of the samples were from persons who had not had any

Table IX

Antitoxin content of blood in units per c.c.

Moloney state	< $\frac{1}{100}$	$\frac{1}{1000}$	$\frac{1}{5000}$	$\frac{1}{250}$	$\frac{1}{100}$	$\frac{1}{50}$	$\frac{1}{25}$	$\frac{1}{10}$	$\frac{1}{5}$	$\frac{1}{2}$	1	2	5 or 5+	Total cases
Moloney-negative	8	2	—	1	1	1	1	1	2	3	1	—	—	21
M+D (7 days)	4	—	—	1	1	—	1	2	4	3	—	3	—	19
M+ (48 hours)	7	1	—	2	—	—	—	—	5	1	4	6	2	28
M++ (48 hours)	5	—	—	—	—	—	1	—	2	3	7	4	1	23
M+++ (48 hours)	2	—	—	1	—	—	1	—	2	2	3	3	3	17
Total cases	26	3	—	5	2	1	4	3	15	12	15	16	6	108

prophylactic treatment. From this table two 4×3 tables were prepared. In each case the divisions on the horizontal axis were $\frac{1}{500}$, $\frac{1}{250}$–$\frac{1}{25}$, $\frac{1}{10}$–$\frac{1}{2}$, 1 or 1+. On the vertical axis the divisions were: grouping (*a*)—(i) Moloney-negative, (ii) M+D and M+, (iii) M++ and M+++; grouping (*b*)—(i) Moloney-negative, (ii) M+D, (iii) M+, M++, M+++. The coefficients obtained were:

	χ^2	n	P	C_2
4×3 table: Grouping (*a*)	16·3647	6	0·0120	0·3628 ± ·0484
4×3 table: Grouping (*b*)	21·3765	6	<0·01	0·4065 ± ·0455

The question was then investigated by the fourfold method. In the following series the dichotomy on the vertical axis is in each case between Moloney-negative cases on the one hand and Moloney-positive cases (all types) on the other. The position of the dichotomy on the horizontal axis is given in Table X. For each grouping the values of χ^2 and Q_5 denote practically the same degree of association. It should be noted that the values increase progressively as the

https://doi.org/10.1017/S0022172400032496 Published online by Cambridge University Press

dichotomy is shifted towards the higher values of antitoxin content. From these figures it cannot be said that there was in Leeds more than a considerable intensity of association between antitoxic content of the serum and a positive Moloney reaction.

Table X

Type of grouping	χ^2	P	Q_5
(i) (*a*) Up to $\frac{1}{1000}$ unit; (*b*) $\frac{1}{50}$ unit and +	6·6503	<0·01	0·447 ± ·039
(ii) (*a*) Up to $\frac{1}{500}$ unit; (*b*) $\frac{1}{25}$ unit and +	8·8463	<0·01	0·506 ± ·050
(iii) (*a*) Up to $\frac{1}{250}$ unit; (*b*) $\frac{1}{10}$ unit and +	9·1199	<0·01	0·530 ± ·055
(iv) (*a*) Up to $\frac{1}{100}$ unit; (*b*) $\frac{1}{5}$ unit and +	10·1688	<0·01	0·568 ± ·063

The delayed (+D) Moloney reaction

It has already been stated that this reaction was described by the writer in 1934. In the earlier portion of this series of cases seven of these delayed reactions were found, and the number has now been increased to a total of 45 cases in the present series. The frequency is therefore 1·69 ± 0·17 per cent. In the delayed reaction there are no visible signs of erythema at the Moloney injection site when read at 24 or 48 hours; by the seventh day, however, a reaction which is usually faint has manifested itself. The exact day of appearance is unknown, and probably varies in different cases. It is possible, however, that the reaction really does develop late—possibly about the fifth or sixth day. The positive delayed reaction practically always consists merely of an area of erythema. In the 45 cases met with a slight degree of induration was felt only once. On the injection of 1 c.c. of toxoid the child had malaise and distaste for food which lasted for about a day, but there was no local reaction; almost identical symptoms were met with after a second prophylactic injection of toxoid. The reaction is sometimes of a bright red or a definite pink colour. More often it is faint red in appearance, and sometimes it consists merely of a blush, or even a dull stain. The edges are not infrequently difficult to delimit from the surrounding skin; but when this is possible it is seen that the erythematous area is nearly always circular. The diameter of the reaction varies. With such a small number of individuals an average value is not of much use; but it may be said that many of these delayed reactions measured from 8 to 14 mm. in diameter. The largest delayed reaction which the writer encountered was in a youth of 17 years; it measured 22 mm. in diameter. (The criticism may be raised that a reaction of 8 mm. in diameter is not of much consequence, and may be due to causes other than the material injected. It should be noted, however, that Moloney and Fraser in their original paper (1927) gave instances of ordinary positive reactions which measured 6 mm. in diameter.) The delayed reaction is therefore much less intense than an ordinary Moloney reaction. Further, this delayed reaction obviously develops spontaneously, and has no relation to the trauma of the needle prick. In this series there are few data on the duration of these reactions, but it would appear that the time of the disappearance, like the time of the appearance, is variable. Certainly in some instances the reaction persists for a few days and then fades, leaving a

faint stain. In those instances in which a stain alone was noted on the seventh day it may be that the actual erythema appeared a day or two previously and then faded quickly. In one instance the reaction consisted of a faint red area. The child, a boy of 9 years, was injected with toxoid and suffered no reaction. A month later a second Moloney test was performed: the resulting reaction was now definitely positive on the second day. On both occasions the Schick-control test was negative.

The writer was originally led to describe the reaction because two of the seven cases which had been met with up to that time had shown reactions of some degree after the injection of toxoid in prophylactic doses (Underwood, 1934). In one of these instances the reaction was quite a sharp one. Since little was known in this country 18 months ago regarding the reactions which an individual might experience on the injection of unconcentrated toxoids of high potency, this experience seemed to render it advisable to recommend greater care in the reading of the Moloney test. Although these two observations show that these delayed reactors may occasionally experience definite reactions, it would seem that the numbers who do so are not in excess of the Moloney-negative reactors who may likewise react—although actually, taking into account the fact that the incidence in negative reactors was 2 in 2147 cases (or 0·1 per cent.) whereas the incidence in +D cases was 2 in 45 cases (or 4·4 per cent.), the incidence in the latter was really very much greater. Even this latter percentage is too low, since only 24 of the 45 +D reactors were injected with toxoid—the remainder being either immune or having failed to attend at the time fixed. In these 24 cases, besides the two mentioned above who showed significant reactions, another seven manifested insignificant reactions such as redness at the injection site without other symptoms. It will be apparent from what has been written previously that the +D reaction may appear at any age over the first year of life, but generally the cases tend to be relatively more numerous in the later years of childhood.

THE MOLONEY TEST PERFORMED AFTER THE INJECTION OF TOXOID

In addition to the primary Moloney tests which have so far been dealt with, a further series of tests was performed on 562 individuals after the injection of toxoids in prophylactic doses; 33 of these were repeated for purposes of corroboration. In general these post-Moloney tests were performed 1 month after the injection of the prophylactic—or 5 weeks after the initial Moloney test. A Schick test was performed at the same time. Table XI gives the results of these tests on the 562 individuals.

Table XI

(*a*) Primary Moloney-negatives which remained Moloney-negative	514
(*b*) Positive cases which remained positive (approximately same degree)	12
(*c*) Cases originally faint positive which became negative	10
(*d*) Cases originally negative or faint positive which became definitely Moloney-positive ...	26

The most significant feature which emerges from Table XI is that primary negative Moloney reactors do not tend to develop a positive Moloney reaction on the injection of toxoid in prophylactic doses. Of the 26 cases classified under (*d*) 24 were originally Moloney-negative, and became Moloney-positive after inoculation. Some of these reactions were very faint, but a few were of moderate size though unaccompanied by induration. In addition to these 24 cases in this group, one case was originally Moloney-positive and after inoculation developed a more intense reaction: the other case was originally a +D reactor, and after inoculation showed a definite M+ reaction on the second day. Reference has already been made to this case. An interesting feature of the individuals in group (*d*) was the case of two brothers. They were originally tested on the same day and both gave negative reactions. When retested again after inoculation both manifested definite M+ reactions. Group (*b*) calls for little comment except to account for the small number of cases in this group. It should be remembered that most Moloney-positive reactors were dealt with by a special procedure and that they were not therefore due for testing along with the other members of the particular batches of children to which they belonged. Group (*c*) is more interesting. Two of the ten cases in this group were definite M+ reactors who became Moloney-negative. Another seven gave originally a definite but very faint reaction. The tenth case was originally a +D reactor on the seventh day. On retesting the patient was negative on the second day, but a reading on the seventh day was unfortunately not made. It should be especially noted in connection with this subgroup of cases that none of them showed any induration. It is possible that the features noted in this group may have been due to the element of error; for example, a slight variation in the amount or strength of the injected fluid may have been responsible. On the other hand, there is a possibility that there may be a transition field between the negative and positive Moloney states, in which a certain amount of fluctuation may take place. At all events, once a definite positive Moloney state—with induration—is established, it seems to be permanent within the limits of this experiment.

O'Brien and Parish (1932) did not note any "sensitisation" by the injection of toxoid, and they amplified this definition by saying that none of the children who showed Moloney-negative reactions before immunisation gave "mild" or "definite" reactions afterwards. On the whole the present series supports this view. It is obvious that "sensitisation", if it takes place, is comparatively rare. We must also bear in mind that transition from the Moloney-negative to the M+ + or M+ + + state is normally a gradual process. Hence we would expect that a certain number of children who had previously given a Moloney-negative reaction would on retesting, even without the injection of toxoid, give a faint positive reaction. From Table II it is seen that in 15 years the percentage of positive Moloney reactors changes from 2 to 66 per cent.—or roughly 64 per cent. Assuming that the rate of change over this period is constant—an assumption which is of course not strictly justified in the light of Fig. 3—this

gives an average rate of change of 4·3 per cent. per annum for all ages in the population under 15 years. On this basis the 562 individuals who were retested should have shown about 24 whose Moloney condition would have changed towards the positive end of the scale. It is impossible to decide whether or not the toxoid injections hastened the process in the 26 cases in which this actually happened. It may be that the toxoid caused a transition, which would naturally have taken about a year, to become effected in a few weeks.

SEX INCIDENCE OF POSITIVE MOLONEY REACTORS

The possibility of a difference in the sex incidence of positive Moloney reactors does not appear to have been investigated previously. In the present series, of the 2666 individuals, 1391 were males and 1275 were females. The incidence of the different types of reaction in the sexes was as follows:

	+D	+	+ +	+ + +	Totals
Males	33	136	97	40	306
Females	12	91	76	34	213

Even when account is taken of the total preponderance of males, these figures appear to indicate that a positive reaction is much more frequent in the male. The group 15–19 years in Table II, however, was composed entirely of males in a large residential school (and the total population investigated did not embrace any similar series of females at these ages). If the positive cases at these ages are deducted from the different groups, and the number of males in this school at these ages (105) is deducted from the total number of males, a more accurate comparison is obtained. The results, with the percentages for all males and females respectively for each type of reaction, are set out in Table XII. (The percentages are shown in brackets.)

Table XII

	+D	+	+ +	+ + +	Totals
Males (1286)	28 (2·18)	112 (8·71)	70 (5·44)	29 (2·25)	239 (18·58)
Females (1275)	12 (0·94)	91 (7·14)	76 (5·96)	34 (2·67)	213 (16·71)

It is obvious from these results that the incidence of a positive Moloney reaction is practically identical in males and females.

THE MOLONEY TEST IN DIFFERENT MEMBERS OF THE SAME FAMILY

The possible influence of fraternal relationships—and the close association which these imply—in hastening the development of positive Moloney reactions have not hitherto been investigated. Apart altogether from the congenital standpoint, we might expect that, if a number of brothers and sisters live together in a small house, and grow up there exposed to the unmeasured effects of subminimal infections, the result might be that sensitivity might develop earlier than it would do if the children were members of separate families. It is difficult to get definite evidence on these questions.

In the present series of 2666 cases, 1430 individuals belonged to as many separate families. The remaining 1236 individuals were members of 503 different families, and in each of these families at least two members were tested. Table XIII gives details of the number in each family and the number of these who gave a positive Moloney reaction. From this main table an abbreviated table was prepared, the divisions being: (*a*) on the horizontal scale, 2, 3, 4, 5 and over; and on the vertical scale, all negative, 1 positive, 2 or more positive. For this table $\chi^2 = 41{\cdot}68$; for $n = 6$, the corresponding value of P is $< 0{\cdot}01$, which shows that the distribution is not random. The value for C_2—viz. $0{\cdot}277 \pm {\cdot}030$—indicates that the association between the number of children in a family and the number of these who will give positive Moloney reactions is very slight.

Table XIII

	No. of children tested in family						
Moloney state	2	3	4	5	6	7	Totals
All negative	252	54	23	3	1	—	333
1 positive	66	32	12	1	—	—	111
2 positive	24	15	10	2	—	—	51
3 positive	—	3	3	1	—	—	7
4 positive	—	—	—	—	—	1	1
Totals	342	104	48	7	1	1	503

It has been pointed out that in a "family" investigation of this type it is impossible to exclude more than a few of the factors which may presumably influence the results. Nevertheless, these figures do suggest that, when we meet with several members of a family who happen to give positive Moloney readings, we can assume that this is due not to the fact that they are all living together under one roof, but to the fact that they must generally be of different ages, and that therefore the older members have been longer exposed to the *C. diphtheriae*. This conclusion is further borne out by the observation that in the present series there are 15 pairs of twins; in two of these pairs one twin gave a positive reaction and the other a negative reaction; in the other 13 pairs both members gave negative results. Certainly there are some grounds for considering that, in a large urban area in which diphtheria is prevalent, the chances of sensitization of children owing to the fact that they are members of large families, is not greater than it would be if they were members of families consisting of only one or two children, provided that they were still exposed to others of similar age outside their homes.

Discussion of the significance and applications of the test

A consideration of the mechanism of production of a positive Moloney test is an interesting speculation on the borderland of the study of allergy. It was recognised by the originators of the test that a positive result did not necessarily mean that the individual was immune, and the point was further amplified by McKinnon and Ross (1933). Further evidence on this question is presented in this paper in the section dealing with the relationship between a

positive Moloney reaction and a positive reaction to the heated toxin of the Schick control. There is a possibility that a positive reaction may be an expression of the development of allergy due to exposure of the individual to proteins of different types. The writer has never been satisfied with this view, and in this paper a certain amount of indirect evidence is adduced which suggests that a positive Moloney test probably indicates previous contact with the *C. diphtheriae*. So far as our present knowledge extends, the analogy with protein allergic reactions is fairly close. Very suggestive are the facts that the skin responses are similar in the two conditions; that the degree of reaction, for the herd at least if not for the individual, increases in proportion to the length of contact with the "allergen"—that is, the *C. diphtheriae*; and that repeated tests are usually identical in type, with the exception of the borderland reactions already discussed. If we accept this theory, however, a peculiar feature emerges. True protein skin allergy is found only in a very small percentage of normal individuals. For example, Bray (1931) quotes Baker's figures of ten positive and eleven doubtful reactions to various test proteins in 937 healthy children. On the other hand, a large percentage of allergics—for example, asthmatics—will give a positive skin reaction to test proteins, and in the particular case of the Moloney test it would seem that we must regard every individual in an urban community as a real or potential allergic for this particular protein which is contained in the Moloney test fluid.

Many other suggestive features emerge from a consideration of the test from this point of view. For example, Gibson and McGibbon (1932), working with soluble intracellular products of haemolytic streptococci, showed that in a considerable proportion of scarlet fever patients an allergic skin reaction could be produced by the intradermal injection of this material in the second week of the disease or later. It is noteworthy that all their patients who were over 7 years of age developed marked allergy. These reactions were at their height in 36 hours. Coburn (1931) had previously observed that cases of acute rheumatism were hypersensitive to the nucleo-proteins of haemolytic streptococci, and similar results were obtained by Collis (1931). If we assume that the Moloney reaction is in effect the result of hypersensitiveness to some protein derived from the diphtheria bacillus, other facts may be enlightened by the analogy. For example, Harley (1933), working with the sera of various animals, described delayed reactions which were perhaps similar in type to the delayed Moloney reaction.

Much work still remains to be done on the interval between the injection of test proteins and the time of appearance of resulting reactions. It is therefore impossible to give any rational explanation of the delayed Moloney reaction. There seems to be some evidence that it represents an intermediate stage between the Moloney-negative state and the development of a frank positive reaction. The infrequency of +D reactors and the evidence obtained from contingency coefficients discussed previously lend some support to this view. Obviously, it is not merely a time factor which is involved. Dickinson (1922)

saw six instances in 1091 positive Schick tests where the reaction did not appear till after the fourth day, and some work of the present writer confirms these observations. But, once this delayed Schick reaction has developed, it runs a normal course. The delayed Moloney reaction, on the contrary, is in effect a reaction which is so mild that it fails to appear at the usual time.

Some further features arise from a discussion of the view that a positive Moloney reaction is due largely to previous contact with the products of the *C. diphtheriae*, and is essentially an allergic phenomenon. It has already been stated in an earlier section that McKinnon and Ross thought that age might in itself be a factor in the development of the reaction; and it was suggested that the results of the present investigation did not give much support to their view. The data on which they base their opinion are worth considering more fully.

These authors divided their cases according to age and Moloney state respectively. They also gave a separate table which is entitled "Reaction test readings in 'Schick-negatives'". It seems probable from their paper that this table includes all the Schick-negative individuals in their main series, but it should be emphasised that this fact is not definitely stated. A portion of this table is reproduced here (Table XIV) with slight rearrangement.

Table XIV (*abridged from McKinnon and Ross*)

Age	No. Moloney-negative	% negative	No. Moloney-positive (all types)	% positive	Total Schick-negative
0–1	5	100	—	—	5
1	6	100	—	—	6
2	14	100	—	—	14
3	13	100	—	—	13
4	11	78·8	3	21·4	14
5	58	93·6	4	6·5	62
	* * * * * * * * * * * *				
14	231	52·7	207	47·3	438

McKinnon and Ross make the following comments on this table: "The fact that 33 Schick-negative children, 1–3 years of age, show no sensitivity is strong evidence that immunity may be obtained without sensitivity developing at any time during the process. (The fact, too, that when using the diluted toxoid as a control in the Schick test one finds in the pre-school group approximately one sensitivity reaction for every five or six immune reactions, while from 6 years on the ratio changes till at 14 it is 1 : 1, practically confirms this and suggests that age *per se* is a factor in the development of sensitivity.)" The argument of the authors is evidently along the following lines: In the early years of life certain individuals develop immunity as a result of contact with the *C. diphtheriae*, and yet no sensitisation occurs; as age advances sensitisation becomes increasingly frequent, and the process proceeds at a relatively greater rate than the process of natural immunisation. Hence it is possible that age is in itself a factor in the development. On further examination of the tables of McKinnon and Ross it was found that the total number of children tested at 0–1, 1, 2, and 3 years was 12, 30, 39, 55 respectively. In the present series the respective totals at these ages were 49, 185, 177, 194, and amongst the negative

Schick reactors in these children five positive Moloney reactors were found (see Table I). It might be suggested that the failure to find positive Moloney reactors in the Toronto series was due to the smallness of the samples at these ages. Further examination showed, however, that, whereas in the Toronto series no positive Moloney reactors were found in 38 Schick-negative children under 4 years, the five positive Moloney reactors in the Leeds series were found among only 22 Schick-negative children. Right through the different age groups marked differences were found in the percentages of children who were Schick-negative in the Leeds and Toronto series respectively; but, since it was mentioned above that it is not certain that the Canadian Schick-negative figures included all such cases in the main series, this question will not be considered further here. The percentages of Moloney-positive reactors in Schick-negatives were in a different category, since the figures were tabulated in the original paper. These figures for the two cities are given along with the probable errors in Table XV. Since delayed reactions are not included in the Toronto series, the delayed reactors in Leeds are here considered as Moloney-negative.

Table XV. *Frequency of positive Moloney reactors (all degrees) in Schick-negatives, expressed as percentage of total Schick-negatives at each age. (Delayed reactors are included as Moloney-negative in the Leeds series.)*

	Leeds			Toronto			
Age	Total Schick-negative	Moloney-positive	% positive	Total Schick-negative	Moloney-positive	% positive	Difference
0–1	8	0	0	5	0	0	—
1	3	0	0	6	0	0	—
2	3	3	100	14	0	0	—
3	8	2	25·0 ± 10·3	13	0	0	—
4	21	5	23·8 ± 6·3	14	3	21·4 ± 7·4	2·4 ± 9·7
5	39	9	23·1 ± 4·6	62	4	6·5 ± 2·1	16·6 ± 5·1
6	67	17	25·4 ± 3·6	439	70	15·9 ± 1·2	9·5 ± 3·8
7	38	14	36·8 ± 5·3	536	84	15·7 ± 1·1	21·1 ± 5·4
8	39	16	41·0 ± 5·3	762	173	22·7 ± 1·0	18·3 ± 5·4
9	44	25	56·8 ± 5·0	795	206	25·9 ± 1·1	30·9 ± 5·1
10	45	26	57·8 ± 5·0	824	237	28·8 ± 1·1	29·0 ± 5·1
11	51	33	64·7 ± 4·5	860	305	35·5 ± 1·1	29·2 ± 4·6
12	62	34	54·8 ± 4·3	862	337	39·1 ± 1·1	15·7 ± 4·4
13	37	24	64·9 ± 5·3	681	269	39·5 ± 1·3	25·4 ± 5·5
14	19	13	68·4 ± 7·2	438	207	47·3 ± 1·6	21·1 ± 7·4
15–19	86	48	55·8 ± 3·6	—	—	—	—

It is seen from the table that at nearly every age over 4 years—when the percentages become significant—the percentage of Leeds Schick-negative reactors who showed positive Moloney reactions was almost double the percentage for Toronto Schick-negatives at corresponding ages. These differences might be due to at least three separate factors. Firstly, a different standard of reading may have been adopted. In considering this point it should be remembered that, although the classifications adopted were not strictly identical in the two series, the broad term "positive Moloney reactor" includes for both series all persons who showed any reaction at all at the injection site. The second possibility is that the Moloney test fluid used in Toronto may have

differed in strength or quality or both from that used in this country. The writer has some evidence that the strength of the toxoid used does have an effect on the numbers of persons who give positive results, but the difficulty of excluding age and other factors prevents its presentation. Thirdly, the differences may be due to environmental or biological factors which cannot easily be assessed. It should be remembered, however, that during the time when these investigations were progressing in Leeds, the predominant type of *C. diphtheriae* in the city was the *gravis* strain, which in addition to causing a severe form of the disease also exerts its effects in the direction of the breaking down of established immunity (Underwood, 1935 *a*).

The percentages which are given in Table XV were plotted on a logarithmic scale (Fig. 4). It is seen from the graphs that, although there are such marked

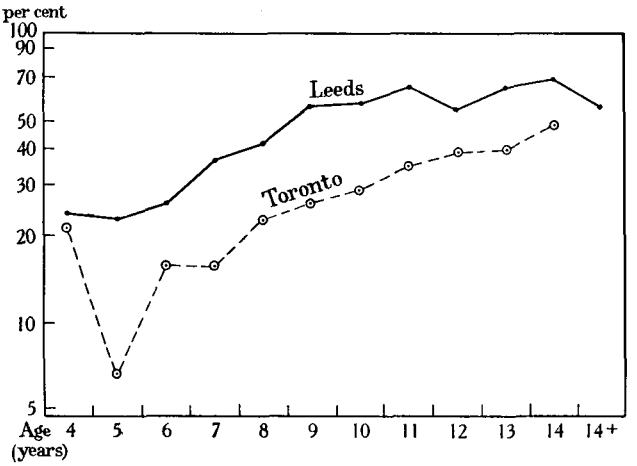

Fig. 4. Frequency of positive Moloney reactors (all degrees) in Schick-negatives, expressed as percentage of total Schick-negative individuals at each age. (Logarithmic scale.) Leeds (+*D* reactors excluded). Toronto (from data by McKinnon and Ross).

differences in the actual values, the rates at which Schick-negatives develop sensitivity to toxoid as age advances is practically the same in the two cities. It has already been mentioned that in the different age groups there were marked differences in the percentages of children who were Schick-negative in the Leeds and Toronto series respectively. Hence, this similarity in the rates of development of Moloney-positiveness *in Schick-negatives* is additional evidence that development of the two conditions, Schick-negativeness and Moloney-positiveness, is closely associated—possibly more so than would be the case if age in itself were an important factor in the development of sensitivity.

Practical considerations

The practical worker who is about to use undiluted toxoids of high potency will be faced with the question of whether it is possible or expedient to dispense with the Moloney test in view of the fact that by its inclusion three injections

https://doi.org/10.1017/S0022172400032496 Published online by Cambridge University Press

have to be given at the time when the primary Schick test is performed. The evidence of this paper is that the test is extremely useful from the practical point of view, and the writings of Burke and of McKinnon and Ross show to what extent it has been employed in Canada. The difficulty which faces workers in this country was evidently met with in Toronto also, for it will be remembered *that the Schick-control injection was abandoned in that city. Hence, when* toxoid is used as a prophylactic in this country there would seem to be a case for the retention of the test. In the 2666 cases which are described here, 519 positive Moloney reactions of all types were met with; but in the same cases the number of pseudo-reactions to the Schick test was only 85. In a paper which is at present in preparation it will be shown that it is impossible to decide, apart from antitoxin titration of the blood serum, whether many of these pseudo-reactors are, or are not, immune. The writer is consequently of opinion that, if one test has to be omitted, that test should certainly be the Schick-control and not the Moloney injection. It has been shown that the one test cannot be used as a substitute for the other, and of the two the information given by the Moloney test is much the more important.

A word should be said regarding the importance of the Moloney test where alum preparations are used. Many of the persons dealt with in this paper were injected with such preparations, and a previous communication (Underwood, 1935 *b*) dealt with the significance of the test when used prior to the injection in prophylactic doses of the latest development of the alum group—viz. alum-precipitated toxoid (A.P.T.). It is only necessary to say here that with this material Moloney-negative persons gave no trouble, whereas reactions of various types were met with in Moloney-positives. The writer is of opinion that this test is essential whenever such preparations are to be used in anything more than minimum prophylactic doses.

SUMMARY AND CONCLUSIONS

1. The investigation is based on 2666 Moloney tests performed before the injection of prophylactics and 595 tests carried out after the injection of toxoids in prophylactic doses. Methods of reading the test reactions are described.

2. Certain individuals develop sensitivity to toxoid at a very early stage of their existence—even during the first 18 months of life. In the herd the periods of most rapid development of sensitivity to toxoid are between the first and second years, between the fourth and seventh years, and just before the age of fourteen years.

3. For practical purposes the important reactions are those which are designated + + and + + +. Up to the age of 5 years the combined percentages of children who showed these + + and + + + Moloney reactions did not exceed 3 per cent. at any age. At 7 years the incidence was 9·6 per cent. and thereafter there was a rapid increase until the maximum (43·3 per cent.) was reached at 14 years. Not every individual who shows such + + or + + +

results will develop unpleasant reactions on the injection of toxoids in prophylactic doses. But an investigation into the age and Moloney state of 2041 persons who received prophylactics demonstrated quite definitely that children who showed general and local reactions after inoculation were nearly always over 5 years of age, and most of them (79 per cent.) showed + + or + + + results with the Moloney test. Hence the test should be used whenever it is intended to inoculate children of school age with prophylactic doses of toxoid or its alum preparations.

4. The assumption that strong positive Moloney reactors are usually immune is unjustified. Of 74 individuals who showed a + + + Moloney reaction 20·3 per cent., and of 173 individuals who showed a + + Moloney reaction 32·4 per cent. were definitely non-immune as judged by the Schick test. The coefficient of association (Q_5) between the two conditions was 0·632.

5. The relationship between positive pseudo-Schick reactions and positive Moloney reactions is examined statistically, and it is shown that the association is not sufficiently high to warrant the substitution of one test for the other. Confirmation of this conclusion was obtained from an examination of the pseudo-Schick and Moloney states of all individuals who showed any degree of local or general reaction after the injection of prophylactic doses of toxoids or other prophylactics.

6. The degree of association between the antitoxic content of the blood serum and the positive Moloney state was investigated in 108 cases. The coefficient (Q_5) was found to be in the region of 0·5.

7. The delayed Moloney reaction (+D) is further described and its significance is discussed. The frequency of +D reactions is $1{\cdot}69 \pm 0{\cdot}17$ per cent.

8. In general, a definite Moloney-positive state appears to be permanent. The injection of toxoid in prophylactic doses does not tend to render the injected individual sensitive to toxoid. Exceptions to this rule may be due to the acceleration of a normal process.

9. The incidence of positive Moloney reactions is practically identical in males and in females.

10. The association between the number of children in a family and the number of those who will give positive Moloney reactions is very slight. The effect of an urban environment appears to be more important than the actual family environment in determining the development of sensitivity to toxoid.

11. The relationship between protein skin tests and the Moloney reaction is discussed. Toxoid sensitivity is probably an allergic condition which is due to previous contact with the products of the *C. diphtheriae*. There is little evidence that age is in itself a factor in the development of sensitivity.

It is a pleasure to express my indebtedness to Dr R. A. O'Brien of the Wellcome Physiological Research Laboratories for his kindness in supplying

the materials which were used in this investigation, and to Mr A. T. Glenny for carrying out the numerous titrations of blood samples. To these gentlemen, and to Dr H. J. Parish I am grateful for advice regarding certain aspects of the work.

NOTE ON STATISTICAL METHODS

Although Pearson's coefficient of mean square contingency (C_2) is well known, his coefficient of association (Q_5) in a fourfold table is not so widely used. If individuals are classified by the characters into A and not-A, B and not-B, then a tetrachoric table may be formed in which the cell frequencies are represented by (a), (b), (c), (d) in the position indicated in Table V (Pearson, 1901). If the total frequency be N, then for the fourfold table

$$\chi^2 = \frac{N(ad-bc)^2}{(a+b)(c+d)(b+d)(a+c)}, \quad \text{and} \quad Q_5 = \sin\frac{\pi}{2}.\frac{1}{\sqrt{1+\kappa^2}},$$

where

$$\kappa^2 = \frac{4abcdN^2}{(ad-bc)^2(a+d)(b+c)}.$$

In the paper the probable errors of the values of C_2 are derived from $\phi_t{}^2$ and not from $\phi_a{}^2$, the approximate value of the mean square contingency, which is nearly always used for the calculation of C_2. The use of $\phi_t{}^2$, the true value of mean square contingency, for the calculation of the probable error has been shown to give satisfactory results (Pearson, 1915).

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(*MS. received for publication* 2. VII. 1935.—Ed.)