THE DEVELOPMENT OF HOMOLOGOUS NEUTRALIZING ANTIBODY DURING A TYPE 1 POLIOMYELITIS EPIDEMIC*

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INTRODUCTION

The opportunity to reinvestigate the immunity status, after an epidemic, of a group of people whose sera had been tested for antibodies to polio-viruses shortly before that epidemic is not common, but such a situation has arisen in Dunedin, New Zealand, and we now report the results of our investigations on these people.

In April 1955 serum specimens were taken from 168 persons in Dunedin, among them a group of students at a boy's school and a group of University students. Because the virus laboratory at the University of Otago Medical School was not able to examine this material at that time, Dr J. H. S. Gear of the South African Institute for Medical Research, Johannesburg, kindly consented to test the sera and the results of this investigation have been published (Caughey, Douglas & Spears, 1956).

In October 1955 cases of poliomyelitis began to occur in Otago and rapidly increased to a peak in December when forty-seven cases were notified. In January and February 1956 there were thirty and twenty-four notifications, respectively, and in March and April three and one. The total number of notifications was 152 in a population in the province of approximately 150,000. Dunedin, with a population at that time just below 100,000, had just over 100 notified cases.

Material from cases in this epidemic was sent to Mr Murphy in Auckland who isolated two strains of Type 1 virus (all thirty-eight strains except three isolated during this epidemic in New Zealand were of Type 1 (Murphy & Brown, 1957)). More recently we have re-examined stored material from two fatal cases in the epidemic and have isolated Type 1 virus from each of them. It is, therefore, reasonable to think that this was a Type 1 epidemic.

In September 1956 we were able to obtain further serum specimens from fifty-five of the school-boys and twenty-four of the students tested previously and to examine them for alterations in the antibody pattern.

The individual results of Dr Gear's examination of the serum collected in 1955 were available to us and we now report a comparison of these two sets of results.

MATERIALS AND METHODS

Virus

The strains of virus used were the Mahoney strain of Type 1, M.E.F. 1 of Type 2 and Saukett of Type 3. These strains were obtained from Mr A. M. Murphy of Auckland.

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Tissue culture

The HeLa strain of epidermoid carcinoma cells was used in this investigation. This strain of cells was obtained from Mr Murphy and adapted in this laboratory to multiply readily in the following medium:

Hanks' balanced salt solution	77.5 %
1.4 % sodium bicarbonate	6.5
Heated rabbit serum	10
5 % lactalbumin hydrolysate	5
Antibiotic mixture	1
(10,000 units penicillin + 10,000 μ g. streptomycin in each ml.)	

The maintenance medium used in the neutralization tests contained only 3 % rabbit serum.

Neutralization tests

In the qualitative tests sera were diluted 1:5 in Hanks' balanced salt solution, heated at 56° C. for 30 min. and then mixed with an equal volume of virus diluted to contain 1000 TC₅₀ doses in each ml. Neutralization was allowed to proceed for 60 min. at room temperature after which 0.2 ml. of the virus serum mixture was added to each of three tubes which had been seeded 2 days previously with 100,000 HeLa cells. The fluid volume in each tube was made up to 1 ml. with maintenance medium. Controls were set up, including a titration of the virus used in the test, a known positive serum (prepared in a rabbit) and maintenance medium without virus.

In quantitative tests the serum was tested diluted 1:5, 1:25, 1:125 and 1:625.

The cultures were examined microscopically for tissue degeneration after 3 days at 37° C.

RESULTS

Antibodies to Type 1 virus

The results of tests with Type 1 virus, comparing the post-epidemic findings with those obtained by Gear on the pre-epidemic sera, are shown in Tables 1 and 2. Twelve boys in the two lowest classes in 1955 had only one doubtful positive among them. Eleven showed definite positive in 1956 with serum titres varying between 1:25 and 1:625. This group included all the boys between the ages of 5-7 years tested. In all the higher classes the proportion of changes from sero-negative to sero-positive was much lower, but showed a progressive decrease with increasing age.

The school was in session from the beginning of the epidemic until 8 December, when the summer holiday commenced which lasted until 1 February.

There were two clinical cases of poliomyelitis among the boys, both in School Form 2, but one of these had his onset at such a time during the holiday that it indicated he was infected away from school. The other boy was first absent on 17 February, towards the end of the epidemic and thus was at school throughout

the usual incubation period. Of four susceptibles tested in this Form (other than the poliomyelitis cases) only one had developed antibody to the virus. The ages of the boys in this Form were from 10–13 years.

Table 1. *Antibodies to Type 1 polio-virus (grouped in classes)*

Class	Age distribution	No. tested	No. positive	
			1955	1956
Primers	5–6	7	$\frac{1}{2}$ *	7
Standard 1	6–7	5	0	4
2	8	6	2	3
3	8–9	14	3	$6\frac{1}{2}$ *
4	9–11	4	0	2
Form 1	10–11	6	0	2
2	10–13	5	1	2
3	12–14	5	1	$\frac{1}{2}$ *
4–6	16	3	$\frac{1}{2}$ *	0
University students	20–26	24	8	10
Totals	5–26	79	16	37

* A doubtful positive is recorded as $\frac{1}{2}$.

Table 2. *Antibodies to Type 1 polio-virus (grouped by age)*

Age group	No. tested	No. positive	
		1955	1956
5–7	12	$\frac{1}{2}$ *	11
8–9	22	5	$10\frac{1}{2}$ *
10–14	18	2	$5\frac{1}{2}$ *
15+	27	$8\frac{1}{2}$	10
Totals	79	16	37

* A doubtful positive is recorded as $\frac{1}{2}$.

Table 3. *Proportion of susceptibles giving serological evidence of infection in different age groups compared with the proportion of notified cases in those age groups*

Age group	No. of susceptibles tested	No. gaining immunity	% gaining immunity	% of notified cases in Otago
5–9	27*	17	63	22
10–14	16	4	25	17
15+	15	1	7	43

* In this analysis doubtful positive reactors in 1955 were regarded as immune.

Among the older boys and the University students tested there was only one conversion from negative to positive. That is to say, there was one boy who had no antibody to Type 1 when examined in 1955, but who was found to have developed measurable amounts after the epidemic.

Table 3 shows the proportion of susceptibles in different age groups who developed antibody during the epidemic. The usual 5-year groups have been used here

so that the results could be compared with the figures for notifications obtained from the Department of Health. The contrast is striking and will be discussed later.

Altogether twenty-two who had no antibody to Type 1 before the epidemic gave evidence of infection (28 %). There was a small number of doubtful changes in each direction, but no clear-cut change from positive to negative.

Antibody titres

Table 4 shows the titres of neutralizing antibody in the sera of those positive, divided into two groups—those whose sera had been positive or doubtfully positive in 1955 and those who were negative at the first testing and became positive after

Table 4. *Titre of antibodies to Type 1 in the positive sera*

Group	No. in group	Titre				
		1:5	1:25	1:125	1:625	> 1:625
+ve or ±ve 1955, 1956	16	2	9	5	0	0
-ve 1955, +ve 1956	22	0	11	9	1	1

the epidemic. The mean titre of those who had antibodies before the epidemic was 1:54, and for those who had none 1:120, but these differences are not significant in view of the wide variation in titre. Reserve supplies of three of the pre-epidemic positive sera had been kept in this laboratory and in each case the post-epidemic titre was the same as the pre-epidemic. The titres were 1:5, 1:25 and 1:125.

Minor illnesses

The available school records for the years 1954–57 were examined to see whether the amount of minor illness during the epidemic period differed from that in the years before and after. The results are shown in Table 5. Minor illness was taken to mean an illness causing absence for at least one whole day, but not more than

Table 5. *Rate of absence from school for minor illnesses*

Class	1955	1956	3-year mean	3-year mean
	November–December	February–March	November–December	February–March
Primers	Not available	1.5 boys/days	0.80*	1.31
Standards 1, 2	0.70 boys/days	0.56	0.78	0.97
3	0.20	1.62	0.22	1.39
4	0.50	1.10	0.31	1.18
Form 1	0.33	0.72	0.49	1.01
2	0.31	1.14†	0.48	1.11
3	0.30	0.37	0.32	0.29
4–6	0.05	0.20	0.08	0.24

These figures include only those absent for one whole day or longer, but for less than 10 days. The absence rate is expressed as the average number of days absence per boy over the 2-month period.

* Two years only.

† Two boys in this class suffered paralytic poliomyelitis.

9 days. The more prolonged illnesses during the epidemic period had been given a definite diagnosis. The amount of absence has been expressed as the average number of days absence per boy in the class for the period under investigation.

Unfortunately, the figures for the youngest boys in November–December 1955 were not available, but apart from this it can be seen that the epidemic period was not associated with an amount of minor illness differing significantly from the 3-year average. The period November–December 1957 was associated with the terminal phase of an influenza epidemic and the amount of absence due to brief illnesses was greatly increased in all classes, therefore these figures were not used for comparison.

The majority of the boys at the school live at home, but a proportion are boarders, especially among the older boys. In our sample eleven boarders were included, but only one of them was below Standard 3. It was notable throughout that there was a significantly higher absence rate among day boys than boarders.

While it is quite likely that some of the minor illness during the epidemic period was due to polio-virus, the total amount was no greater than would have been expected in comparable periods when the virus was not known to be present.

Antibodies to Types 2 and 3 viruses

The results with these viruses are summarized in Table 6. Differences in the results with Type 2 virus were very slight. Two subjects who had been negative in 1955 were positive in 1956 and one positive had become negative. There were also a few doubtful changes.

Table 6. *Antibodies to Types 2 and 3 polio-virus (grouped by age)*

Age group	No. tested	Type 2		Type 3	
		+ ve 1955	+ ve 1956	+ ve 1955	+ ve 1956
5–7	12	4	5	3½*	2½*
8–9	22	11½*	11	3	4
10–14	18	8	7½*	11½*	9
15+	27	7	8½*	11½*	9
Totals	79	30½*	32	29½*	24½*

* A doubtful positive is recorded as ½.

In the case of Type 3, fewer sera were recorded as positive in 1956 than in 1955; five 1956 sera being found negative, when the earlier specimen was positive. Again there were some doubtful changes. All the complete changes from positive to negative were among the twenty-eight sera tested from subjects over 13 years of age.

DISCUSSION

Since the post-epidemic tests reported here have been performed in a different laboratory from the earlier series, the question of the comparability of the two series of results necessarily arises. In the case of Type 2 virus the results from two series of serum specimens collected a year apart are very similar and only three

pairs of sera showed major differences in reaction. Sabin (1952) reported the transitory appearance of Type 2 neutralizing antibody in patients infected with Type 1 polio-virus, but the titre had fallen to a low level within three months and since our sera were collected approximately 6 months after the epidemic, much evidence of such a heterotypic rise could not be expected. The results suggest that the sensitivity and reliability of the tests made in the two laboratories are comparable. In the case of Type 3, sera from five patients who had been positive in 1955 were negative a year later. This might either mean a true fall in titre or that the test used in Dunedin was less sensitive than that in Johannesburg. Snow, Raad, Woolcott, Miles, Ames & Stokes (1955) investigating two specimens of serum from a series of school-children, the first taken towards the end of a Type 1 poliomyelitis epidemic and the second 6 months later, found no difference in the number of Type 3 positives between the two series of specimens. In that investigation both tests were made in the same laboratory. However, in the present series, none of the changes were found in fifty-one subjects under the age of 14; all were found in twenty-eight 14 years old or more and it seems likely that this may represent a true fall in titre of neutralizing antibody, in people who have not had an effective antigenic stimulus for a long time.

We therefore think it reasonable to treat the two series of results as comparable and to regard the apparent development of antibody to Type 1 virus in twenty-two of our subjects as real. The results then may be seen to be of considerable interest.

All except one of twelve children between the ages of 5-7 years developed neutralizing antibody against Type 1 polio-virus. There were no clinical cases of poliomyelitis in these school classes and the amount of absence due to minor illness during the epidemic period was not significantly different from that in comparable periods in other years. All except one of these boys lived at home and only two came from the same household. These twelve small boys, probably infected at school, carried the virus into ten homes, including the boarding house at the school. Although a high proportion of those tested in all groups were susceptible to Type 1 virus before the epidemic, in the older age groups, even in the class in which a paralytic case of poliomyelitis occurred only a relatively small proportion gave serological evidence of infection. Among the eleven boarders tested, seven were susceptible, but only two developed antibody, one being the 6-year-old already mentioned. The other susceptible boarders were 10 years or more old.

Horstmann, McCollum & Mascola (1955) found 100% infection in susceptible family contacts under the age of 15 years during a Type 1 poliomyelitis epidemic, but only two out of seven older susceptible family contacts became infected. 87% of susceptible daily contacts under 15 years of age were infected. In an earlier investigation of three families with clinical cases of poliomyelitis, Brown & Ainslie (1951) isolated virus from all eleven family contacts under 12 years of age, but from only two of eleven older persons. Brown, Francis & Ainslie (1948) had previously found that while children under 12 years of age excreted virus for 4-5 weeks in subclinical infections, adults usually excreted virus for only a week or less. Eklund & Larson (1956) investigated a Type 3 epidemic occurring in the winter of 1953-54 on St Paul Island in the Pribilofs. Fifty children under the age of 10 years

all gave serological evidence of infection and 49/52 between the ages of 10–19 years as against 24/37, 20 years or more old. Miles, Stokes & Ames (1955), on the basis of serological studies, suggested that the commonest time for poliomyelitis infection to take place was during the first years at school. Miles (1957) with regard to the spread of poliomyelitis has again emphasized the importance of the young school-child at an age when his personal hygiene is still poor. Our results reinforce this point by showing that nearly all boys under the age of 8 years became infected. The infection rate among susceptibles of 8–9 years was 44 %, of 10–14 years 25 %, and only one of fifteen susceptibles 15 years or more old gave evidence of infection. In this last group, eleven were medical students in their clinical years and one of them had developed antibody. This low infection rate compares with Wehrle's (1956) failure to find any evidence of infection in seventy-five hospital personnel approximately one-third of whom were susceptible to the strains of virus isolated, during a season in which they were exposed to cases of poliomyelitis in the wards.

It had seemed possible that the low rate of infection in the older age groups might be due to the presence of heterotypic antibodies, but no evidence could be obtained that the presence or absence of heterotypic antibodies in any way affected the development of Type 1 antibody during this epidemic. It is more likely that the younger children whose personal hygiene standards are still low and who excrete the virus for a long time, are the most efficient transmitters of the virus both to their contacts in the same age group and to all members of their families.

Table 3 compares the percentage of conversions from sero-negative to sero-positive in 5-year age groups with the percentage of notified cases of poliomyelitis during the epidemic in the same age groups. The contrast is striking, for whereas 63 % of susceptibles under the age of 10 years developed immunity only 22 % of the notified cases were in that group; while 43 % of notified cases were over the age of 14 but only one (7 %) of the susceptibles in this group tested developed immunity. If our figures could be taken as representative of the province as a whole, they would mean that the chance of an infection with polio-virus during this epidemic leading to clinical disease was eighteen times greater in adolescents and adults than in the 5–9 years age group. In view of the small size of our samples, this observation should be treated with some reserve.

SUMMARY

1. Sera taken from seventy-nine individuals who had been examined for poliomyelitis antibodies shortly before a Type 1 epidemic, were examined for the development of antibodies to polio-virus 6 months after the epidemic.

2. Ten of eleven (91 %) susceptibles aged 5–7 years, 44 % of those aged 8–9 years, 25 % of those aged 10–14 and only one of fifteen (7 %) 15 years or more old had developed neutralizing antibodies to Type 1 polio-virus.

3. In the school which the younger subjects attended, one boy developed paralytic poliomyelitis while the school was in session, but the amount of minor illness in the epidemic period was not significantly different from the 3-year average.

4. The comparison between these findings and the notification rate for the

province suggested that the chance of an infection with polio-virus during this epidemic, leading to a clinically recognizable attack of the disease, might have been as much as eighteen times higher in adolescents and adults than in the 5-9 age group.

5. The epidemiological significance of these results is discussed.

We have pleasure in acknowledging our indebtedness to Dr J. H. S. Gear for the report on the sera collected before the epidemic, to Dr W. Murphy, Medical Officer of Health, for data about notifications, to Dr A. M. Douglas for assisting by collecting some of the bloods, to the Principal and staff of John McGlashan College for their readiness to assist us at all times and to all those subjects who allowed us to take a further specimen of their blood for this investigation.

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