J. Hyg., Camb. (1968), **66**, 513
Printed in Great Britain

Genetic marker studies on poliovirus type 1 strains from the Blackburn poliomyelitis outbreak in 1965

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(*Received* 22 *February* 1968)

In July 1965 an outbreak of poliomyelitis developed in Blackburn, a town in north-west England with a population of about 100,000. At the time some 52% of the inhabitants aged less than 40 years had received three or more inoculations of inactivated poliomyelitis vaccine (Salk). No live attenuated vaccine had been used (Ardley, 1966).

The first patient was a girl of 18 who died of encephalitis on 8 July after an illness lasting 12 days. Poliovirus type 1 was isolated from the brain and faecal material collected at autopsy. Ten days after the death of the first patient a 4-year-old child became ill and in the next 2 weeks five more paralytic cases developed. On 3 August vaccination of the entire community with trivalent oral vaccine was begun.

The epidemiological picture then became less clear. Notification of cases continued but these were largely non-paralytic. On clinical grounds 53 patients were considered to have had poliomyelitis.

Specimens of faeces for virus investigation were collected from many of the cases, suspect cases and their contacts. In the first instance they were examined in the Preston Public Health Laboratory (Robertson *et al.* 1966). Poliovirus type 1 alone was isolated from six of the 53 patients, Coxsackie B 5 virus alone in two and poliovirus other than type 1 alone from 12, the remaining 33 being negative. In addition, many faecal specimens from other suspect cases or contacts of cases were examined, and 160 of these contained poliovirus type 1 alone or in combination with other viruses. Two specimens contained Coxsackie B 5 virus, two others Coxsackie B 3 virus and one echovirus type 8. All of these specimens were collected after the beginning of the mass vaccination campaign.

The faecal extracts from which poliovirus type 1 had been isolated were sent from the Preston Public Health Laboratory to the Virus Reference Laboratory for fuller examination with a view to differentiation into naturally occurring and vaccine strains by marker tests.

Marker tests. Three genetic marker tests were used:

(*a*) The reproductive capacity temperature marker (R.C.T.) which compares the growth of a strain at 39·8° C. with that at 36° C.

(*b*) The dextran inhibition test which measures the degree of inhibition of the strain by 0·05% dextran sulphate.

(*c*) The modified Wecker test which compares the degree of neutralization of

the strain by 'specific' anti-Sabin I serum with that by anti-Mahoney serum in a plaque reduction assay.

The values expected for naturally occurring and vaccine prototype strains are given in Table 1.

Table 1. *Results of marker tests on prototype polio* 1 *strains*

(Average of 5 determinations; range given in parentheses.)

Strain	R.C.T. 39·8° C. $\log_{10}$ reduction	Dextran inhibition $\log_{10}$ reduction	Serological index
Sabin 1	6·0 (6·5–5·5)	2·5 (1·5–3·0)	1·9 (1·0–∞)
Mahoney	0·5 (1·0–+0·5)	0·3 (+0·5–−0·5)	0·1 (0–0·7)

$$\text{Serological index} = \frac{\%\text{ plaque reduction under anti-Sabin 1 serum}}{\%\text{ plaque reduction under anti-Mahoney serum}}.$$

Table 2. *Results of marker tests on cases from which polio* 1 *was isolated*

Case no.	Strain no.	Titre 36° C. $\log_{10}$ TCD 50/0·1 ml.	R.C.T. 39·8° C. $\log_{10}$ reduction	Dextran inhibition $\log_{10}$ reduction	Serological index
1	944 (ex brain)	5·5	5·0	0·5	0·8
	967 (ex faeces)	5·5	5·0	0·5	0·5
2	983	6·0	6·0	0	0·5
3	966	6·0	6·0	0	0·5
4	982	7·0	5·5	0	0·7
5	Failed re-isolation	—	—	—	—
6	1848	6·0	5·0	+0·5	0·6

Table 3. *Results of marker tests where polio type* 1 *alone was isolated from faecal extract*

Strain no.	Titre 36° C. $\log_{10}$ TCD 50/0·1 ml.	R.C.T. 39·8° C. $\log_{10}$ reduction	Dextran inhibition $\log_{10}$ reduction	Serological index
1833	6·5	6·5	−0·5	1·0
1842 C	5·0	5·0	−1·0	n.d.
1846 A*	6·0	5·5	+1·0	0·1
1847 A	7·0	7·0	−0·5	1·4
B	4·5	4·5	−0·5	1·3
C	5·5	5·5	−1·0	1·2
1848†	5·0	5·0	+0·5	0·6
1852	4·0	4·0	0	1·1
1874	6·0	6·0	−0·5	1·1

* Contact of case no. 7.
† Additional strain from case no. 7.
n.d. = not done.

https://doi.org/10.1017/S0022172400028254 Published online by Cambridge University Press

Table 4. *Marker tests on strains isolated from family contacts of the index cases*

Case no.	Contact strain no.	Type	Titre 36° C. $\log_{10}$ TCD50/0·1 ml.	R.C.T. 39·8° C. $\log_{10}$ reduction	R.C.T. 39·2° C. $\log_{10}$ reduction	Dextran inhibition $\log_{10}$ reduction	Serological index
1	None traced	—	—	—	—	—	—
2	(*a*) 1877 A	Mixture	4·0	n.d.	4·0	—	—
	1877 B	Mixture	4·0	n.d.	4·0	—	—
	1877 C	Mixture	4·0	n.d.	4·0	—	—
	963	Polio 1	6·0	5·0	n.d.	−0·5	0·8
	(*b*) 1878	No growth	—	—	—	—	—
	964	Polio 1	6·0	5·0	n.d.	0	0·8
	222	Mixture	5·0	n.d.	5·0	—	—
3	(*a*) 1863	Mixture	6·0	n.d.	5·0	—	—
	(*b*) 1965	Polio 1	6·0	5·0	n.d.	0	0·6
4	(*a*) 1860	Mixture	5·0	n.d.	5·0	—	—
	(*b*) 1867	Polio 1	6·0	6·0	n.d.	+0·5	n.d.
	(*c*) 1884	Mixture	5·0	n.d.	4·0	—	—
	223	Mixture	5·0	n.d.	5·0	—	—
	(*d*) 205	Mixture	5·5	n.d.	5·0	—	—
	(*e*) 1875	Mixture	5·0	n.d.	5·0	—	—
5	(*a*) 1883	Mixture	5·0	n.d.	4·0	—	—
	(*b*) 1885	Mixture	4·0	n.d.	4·0	—	—
6	225	Mixture	5·0	n.d.	5·0	—	—
7	(*a*) 1866	Mixture	5·0	n.d.	5·0	—	—
	(*b*) 1846	Polio 1	6·0	6·0	n.d.	+1·0	0·1
	(*c*) 1869	No growth	—	—	—	—	—
	(*d*) 228	Polio 1	6·0	6·0	n.d.	0·7	0·7

n.d. = not done; — = not applicable.

RESULTS

The six strains from paralytic cases were all similar and of naturally occurring type (Table 2). They share the peculiarity of being severely inhibited at 39·8° C., a temperature at which most naturally occurring strains grow readily. At 39·2° C., however, the Blackburn strains grow to within 10^{-1} TCD50 of their titre at 36° C. This feature has also been observed in a proportion of type 1 strains isolated from paralytic cases before the introduction of vaccination against poliomyelitis (Cossart, 1967*a*).

From six further cases mixtures of polioviruses, including type 1, were isolated. Since the titres of all these mixtures were reduced by 10^4 TCD 50/0·1 ml. or more at 39·2° C. they are considered to be of vaccine origin.

Table 5. *Comparison of strains from Blackburn with those from Hyde*

Strain no.	Diagnosis	Titre 36° C. $\log_{10}$ TCD50/0·1 ml.	R.C.T. 39·8° C. $\log_{10}$ reduction	Dextran inhibition $\log_{10}$ reduction	Serological index
		Blackburn			
944	Fatal bulbar	5·5	5·0	−0·5	0·8
967	palsy	5·5	5·0	−0·5	0·5
963	Contact	6·0	5·0	−0·5	0·8
964	Contact	6·0	5·0	0	0·8
965	Contact	6·5	5·0	0	0·6
966	Paralytic	6·0	6·0	0	0·5
981	Non-paralytic	6·5	4·0	−0·5	0·5
982	Non-paralytic	7·0	5·5	0	0·7
983	Non-paralytic	6·0	6·0	−0·5	0·8
		Average	5·0	−0·3	0·7
		Hyde			
1226	Contact	6·0	5·5	−1·0	0·3
1227	Contact	6·5	5·5	−0·5	0·3
1228	Contact	6·5	2·0	−0·5	0·4
1229	Contact	6·5	3·0	−0·5	0·6
1230	Fatal case	6·0	0·5	−0·5	0·4
1231	Contact	6·5	2·0	−1·0	0·7
1232	Non-paralytic	6·5	0	0	0·6
1233	Paralytic	5·5	0·5	0	0·1
1234	Contact	7·0	0·5	0	0·7
1235	Contact	7·0	0·5	0	0·6
1236	Contact	6·5	2·5	−0·5	0·8
1237	Fatal case	6·5	2·0	−0·5	0·8
		Average	2·0	−0·3	0·5

The 160 other faecal samples which had been found to contain poliovirus type 1 were examined. Of these seven were finally found to contain only type 1 virus, and marker tests performed on them showed five to be of vaccine type and two of the same naturally occurring type as was isolated from the index cases (Table 3). On consulting the key these were found to be an additional strain from one of the index cases and a strain isolated from one of his family contacts.

Mixtures of strains from 42 of the 153 remaining specimens were tested for their capacity to grow at 39·2° C. Of these 29 failed to grow at all, eight reached titres of between 10^2 and 10^4 TCD50/0·1 ml., while the growth of the other four mixtures was unaffected. These four contained type 3 poliovirus. Since Sabin 3 vaccine strain multiplies readily at 39·2° C. it is probable that all these strains are of vaccine origin.

All strains from the family contacts of the index cases were examined (Table 4). It seems likely that most of these specimens were collected after the administration of vaccine as the proportion of wild strains is low. As was also observed in the 1962 outbreak in Cardiff (Cossart, 1967*b*) attenuated vaccine does not appear to displace an established wild strain even in an asymptomatic subject.

A point of some interest is that a small outbreak of poliomyelitis due to type 1 virus occurred in the nearby town of Hyde almost simultaneously. The strains from this outbreak could be differentiated from the Blackburn strains by their ability to grow at 39·8° C. (Table 5).

DISCUSSION

The most striking feature of this study is that strains of naturally occurring type were found only in the index cases and their immediate contacts. This is probably because few samples could be collected before the area was saturated with attenuated vaccine, and it emphasizes the effectiveness of this blanketing technique in limiting the spread of wild virus. However, the emergence of other enteroviruses in these circumstances is unexpected and suggests that the production of high titres of homotypic antibody in the community is a component in the mode of action of the vaccine blanket, which may be as significant as its competitive effect.

The R.C.T. test with the upper temperature adjusted to the highest level at which growth of the prototype strain for the outbreak is unaffected seems a useful approach to the problem of mixtures, but some growth of type 3 vaccine strains must be expected up to about 40° C.

These findings illustrate the usefulness of marker tests, not only in differentiating naturally occurring and vaccine strains, but also in characterizing strains from different outbreaks.

SUMMARY

Genetic marker tests were performed on 61 strains of poliovirus isolated during the Blackburn outbreak from cases or suspected cases and their contacts. The results were correlated with the epidemiological data and good agreement was found for the serological and dextran inhibition tests. The reproductive capacity temperature marker, however, showed inhibition at 39·8° C. of strains otherwise judged to be of naturally occurring type. These strains could be differentiated from those of vaccine origin because they grew readily at 39·2° C. This feature also distinguished the strains isolated in Blackburn from those obtained in the nearby town of Hyde in a simultaneous outbreak.

Dr J. Ardley, Medical Officer of Health, Blackburn, kindly provided a key which enabled the contacts of cases to be traced; and Dr L. Robertson, Public Health Laboratory, Preston, made available his extensive records as well as the many strains isolated in his laboratory.

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