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Genetic marker studies of poliovirus

II. Strains isolated from cases of poliomyelitis associated with the administration of live attenuated vaccine

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The experience of a decade of vaccination against poliomyelitis demonstrates that the disease can be prevented by either formalin-inactivated Salk-type vaccine or live attenuated Sabin vaccine. The practical advantages of live vaccine have led to its use for mass vaccination in many countries, and epidemiological studies have shown that its large-scale use is very safe (Galbraith, 1963; Gelfand, 1963).

However, studies of the viruses excreted by recipients of live vaccine have revealed that the laboratory characters of the Sabin strains, especially type 3, revert towards those of the naturally occurring strains after quite short periods of intestinal passage (Magrath, Boulger & Hartley, 1964).

The theoretical risk to those vaccinated and to their contacts is thus not entirely negligible. For this reason the Public Health Laboratory Service Poliomyelitis Surveillance Committee has sought all cases of poliomyelitis associated with the administration of vaccine (Miller & Galbraith, 1965). The criteria adopted for vaccine association are administration of oral vaccine to the patient himself in the 28 days preceding the onset of symptoms, or to a household contact within 60 days of onset.

During 1962, the first year of live virus vaccination in England and Wales, 18 paralytic cases were notified and faecal specimens were examined from 17 of these. Strains of poliovirus were obtained from 15 and of these 13 were available for the present study. The remaining 2 faecal extracts failed to yield virus when re-isolation was attempted after storage. In addition a number of other strains were tested: from household contacts of the above cases, from non-paralytic reactions attributed to vaccination, and sporadic strains apparently unrelated to vaccination. Tables 1–3 list the strains and the clinical details.

An attempt has been made to group these strains into wild and vaccine categories on the results of genetic marker tests. No marker test so far proposed corresponds exactly to the monkey neurovirulence test while the exact relation of even the latter to human virulence is not finally decided (Pavilanis *et al.* 1964). It seemed desirable then to choose tests to cover as wide a range of virus properties as possible. On the other hand it was necessary to limit the tissue culture and labour used for the tests.

The reproductive capacity temperature-marker test (R.C.T.$_{40}$) (Lwoff, 1962) and

the intratypic serodifferentiation test (Wecker, 1960) have been applied to strains of all three serotypes; and the dextran sulphate inhibition test (Cossart, 1966) has in addition been used for type 1 strains. The R.C.T.$_{40}$ test measures a feature of the growth cycle of the virus strains while the serological and dextran tests depend on the properties of the protein coat of the particles themselves.

Table 1. *Poliovirus type* 1 *strains*

Origin	Laboratory no.	Vaccine history	Clinical details
Colindale	284	Nil	Paralytic poliomyelitis
	285	Nil	Aseptic meningitis
	286	Nil	Aseptic meningitis
Bristol	308	Self: 14th day before onset	
Leicester	312	Nil	
Cardiff	315	Self: day of onset	
	316	Nil	
	318	Nil	
	319	Self: 4th day before onset	Paralytic poliomyelitis
	427A		
	427B		
	427C		
	320		
	321		
	323	Self: 14th day before onset	
	430	Self: 1st day before onset	
	431A	Self: 5th day before onset	
	431C	Self: 5th day before onset	
	431D	Self: 5th day before onset	
Guildford	568	Sibling: 22nd day before onset	Fatal encephalitis
	569	Self: 22nd day before onset	Healthy
	570	Sibling: 22nd day before onset	Healthy

Table 2. *Poliovirus type* 2 *strains*

Origin	Laboratory no.	Vaccine history	Clinical details
Leicester	399	Sibling: 40 days before onset	Weakness in arm following smallpox vaccination to self
Cardiff	426A	Self: 3rd day before onset	Paralytic poliomyelitis
	B		
	C		
	D		
	E		
Colindale	287	Self: 1st day before onset	Diarrhoea

MATERIALS AND METHODS

The R.C.T.$_{40}$ test, intratypic serodifferentiation and the dextran sulphate test were performed by the techniques which have been described fully in the previous paper (Cossart, 1967).

The range of values obtained with the prototype strains are given in Table 4.

Table 3. *Poliovirus type 3 strains*

Origin	Laboratory no.	Vaccine history	Clinical details
Southend	263	Child: 13th day before onset	Paralytic poliomyelitis (mother of 265, 6, 8 and 9)
	264	Child: 13th day before onset	
	265	Self: 13 days before onset	Children of 263
	266	Sibling: 13 days before onset	
	268	Sibling: 13 days before onset	
	269	Sibling: 13 days before onset	
Guildford	357	Self: 6 weeks before onset	Bulbar poliomyelitis
Manchester	382	Self: 14 days before onset	Paralytic poliomyelitis
Bradford	386	Self: 17 days before onset	Paralytic poliomyelitis
	388	Self: 24 days before onset	Child of paralytic poliomyelitis
Colindale	395	? H.C.* ? interval	Fatal encephalitis
	414	Sibling: 21st day before onset	Paralytic poliomyelitis

* H.C. = household contact.

Table 4. *Results of marker tests on prototype strains given as the average of five determinations with the range of values shown in brackets*

Strain	R.C.T.$_{40}$ ($\log_{10}$ reduction*)	Serological index†	Dextran marker ($\log_{10}$ reduction)
Sabin 1	6·0 (6·5–5·5)	1·9 (1·0–∝)‡	−2·5 (1·5–3·0)
Mahoney	0·5 (1·0–+0·5)	0·13 (0–0·7)	0·3 (+0·5–0·5)
Sabin 2	6·5 (6·5–6·5)	1·6 (1·31–1·8)	—
YSK	1·0 (1·5–0)	0·20 (0·12–0·5)	—
Sabin 3	5·5 (6·0–5·0)	75 (100–65)	—
Saukett	0·5 (1·5–0)	20 (11–25)	—

* $\log_{10}$ reduction at 39·8° (types 1 and 2) or 40·3° C. (type 3).
† See method for derivation of the values.
‡ ∝ omitted from calculation of average.

RESULTS

Tables 5–7 list the results of the three marker tests. The number of each serotype isolated from the paralytic vaccine-associated cases is: Type 1, 8; Type 2, 1; Type 3, 4. In the United States in a similar series Gelfand (1963) reported the isolation of 8 type 1 strains, no type 2 strains and 13 type 3 strains while Furez, Armstrong, Moreau & Nagler, (1964) found that 12 of 17 vaccine-associated cases in Canada in 1962 excreted type 3 viruses only. The predominance of type 1 in England and Wales may be due to the use of oral vaccine for controlling an outbreak of type 1 poliomyelitis in South Wales. Seven of the eight type 1 strains were from this area.

These seven strains closely resembled each other and the two strains (316 and 318) from unvaccinated cases of paralytic poliomyelitis in the same area. The four other type 1 strains (284, 285, 286 and 312) with no association with vaccine are all similar to the South Wales strains. The most distinctive feature of their behaviour is the depression of growth at 39·8° C. in many instances to values in the vaccine range.

As both the serological and dextran markers gave results distinctly of the wild type it was decided to repeat the reproductive capacity temperature test using 39·3° C. as the upper temperature. The results are shown in Table 8.

Table 5. *Results of marker tests: type* 1

Laboratory no.	Titre at 36° C.	R.C.T.$_{40}$ $\log_{10}$ difference at 39·8° C.	Serological index	Dextran marker $\log_{10}$ difference
284	7·0	−5·5	0·63	+1·0
285	7·0	−6·0	0·61	+0·5
286	6·0	−4·5	0·64	0
*308	5·5	−5·5	1·05	0
312	6·5	−4·5	0·35	0
*315	6·0	−3·0	0·66	0
316	6·0	−3·5	0·35	0
318	6·0	−3·5	0·72	−0·5
*319	6·0	−2·0	1·0	−1·0
*427A	7·0	−5·5	0·66	0
*427B	7·0	−5·5	0·28	0
*427C	6·5	−1·5	0·50	0
*320	6·5	−5·0	0·80	+0·5
*321	6·5	−5·0	0·60	+0·5
*323	6·0	−3·0	0·78	0
*430	6·0	−6·0	0·72	−0·5
*431A	6·0	−6·0	0·93	−0·5
*431C	5·5	−5·5	1·21	0
*431D	7·0	−6·5	0·66	−0·5
568	7·5	−7·5	∝	−2·0
569	6·5	−6·5	19·0	−2·0
570	6·0	−6·0	∝	−1·5

* Vaccine associated cases of paralytic poliomyelitis.
Strains isolated from a single patient are grouped together by a brace.

Table 6. *Results of marker tests: type* 2

Laboratory no.	Titre at 36° C.	R.C.T.$_{40}$ ($\log_{10}$ difference at 39·8° C.	Serological index
399	6·5	−6·5	2·3
*426A	5·5	−6·0	1·2
*426B	6·0	−5·5	∝
*426C	6·5	−5·0	1·6
*426D	6·0	−6·0	2·2
*426E	6·0	−5·5	3·4
287	6·0	−4·5	1·5

* Vaccine-associated cases of paralytic poliomyelitis.
Strains isolated from a single patient are grouped together by a brace.

The reduction of only 0·5° C. restored the growth of most of the strains almost to the titre at 36° C. Similar behaviour has also been found by Magrath (1966) with some wild type 1 strains isolated from cases of paralytic poliomyelitis in 1953 and 1957. In contrast, however, strains 568, 569 and 570 remain almost

completely inhibited as does Sabin type 1 virus. These three strains were thought to be of vaccine origin on clinical grounds, and both serological and dextran markers also suggest this. Strains 430 and 431, however, fail to give clear-cut results and have been classed as intermediate on the basis of the three tests used.

All the type 2 strains are of vaccine type in both marker tests. This was expected

Table 7. *Results of marker tests: type* 3

Laboratory no.	Titre at 36° C.	R.C.T.$_{40}$ ($\log_{10}$ difference) at 40·3° C.	Serological index
*263	6·0	−1·5	40
*264	6·5	−3·5	28
265	6·0	−1·0	50
266	6·5	−4·5	92
268	5·5	0	54
269	7·5	−2·0	33
357	5·5	−4·0	44
*382	5·5	−1·5	21
*386	6·0	−2·0	25
388	5·0	−2·5	66
395	5·5	−2·5	8
*414	6·0	−2·0	11

* Vaccine associated cases of paralytic poliomyelitis.
Strains isolated from a single patient are grouped together by a brace.

Table 8. *Comparison of two different upper temperatures in the* R.C.T.$_{40}$ *test for type* 1

Laboratory no.	Titre ($\log_{10}$) at 36° C.	Log_{10} reduction at 39·8° C.	Log_{10} reduction at 39·3° C.
284	7·0	5·5	0
285	7·0	6·0	0·5
286	6·0	4·5	0
308	5·5	5·5	3·0
312	6·5	4·5	0·5
315	6·0	3·0	2·0
316	6·0	3·5	1·5
318	6·0	3·5	2·0
319	6·0	2·0	0
427A	7·0	5·5	0
427B	7·0	5·5	2·0
427C	6·5	1·5	1·5
320	6·5	5·0	0·5
321	6·5	5·0	2·5
323	6·0	3·0	2·0
430	6·0	6·0	4·0
431A	6·0	6·0	3·0
431C	5·5	5·5	2·5
431D	7·0	6·5	4·0
568	7·5	7·5	4·5
569	6·5	6·5	4·0
570	6·0	6·0	4·0
Mahoney	6·5	0·5	0
Sabin 1	6·5	6·0	4·0

from the clinical diagnosis for 399 and 287, but 426 is a case of paralytic poliomyelitis in the same South Wales outbreak from which most of the type 1 strains were derived. The persistent excretion of type 2 virus is unexpected. If the illness was really due to a wild type 1 strain it does not seem likely that it would be displaced so thoroughly by a single Sabin strain given 3 days before the onset of symptoms (Feldman, Halquin & Gelfand, 1964). No serological evidence is available to clarify the position.

The type 3 strains show a graduation of results throughout the range. Numbers 263 and 264 are from an adult case of severe paralytic poliomyelitis from whom, however, no satisfactory serological data are available. One of her children (265) was given oral vaccine 13 days before the onset of her own illness. Strains 266, 268 and 269 were isolated from the stools of the three other children who were not vaccinated. One child was excreting a typical vaccine strain (266) but the strains from the others, including the vaccinated child and the patient herself, show varying degrees of reversion to values well within the wild range. This provides a fairly clear example of a vaccine strain reverting to virulence. The three other vaccine-associated cases of paralysis (382, 386 and 414) have values in the wild range for both markers.

Number 388, the strain from a vaccinated child of an adult case of paralysis from whom no virus was isolated, has intermediate values, as has 357, a clinically doubtful case of bulbar poliomyelitis occurring 6 weeks after vaccination. A case of encephalitis (395) with only a possibility of contact with vaccine is of wild type in both tests.

DISCUSSION

It is seen that only one case of vaccine-associated paralytic poliomyelitis (no. 426) yielded a strain with *in vitro* vaccine characters, and there is a suspicion that this type 2 strain may not have been responsible for the illness.

The type 1 strains can be grouped with some confidence into wild and vaccine groups, only 2 of the 18 cases yielding intermediate strains. The South Wales strains form a homogeneous group, whether from vaccinated cases or not, and their distinctive behaviour in the R.C.T.$_{40}$ test suggests they should all be regarded as of wild origin.

It would also seem that the upper temperature used in the R.C.T.$_{40}$ test should be reduced to 39·3° C. when testing type 1, since above this temperature some wild strains may fail to grow. Variation in this temperature over the range 39–40° C. may reveal differences between wild strains from different sources and could be a useful epidemiological tool.

The type 3 strains studied include some from a single family in which reversion of the vaccine strain to clinical and laboratory virulence probably occurred. The possibility that the unvaccinated siblings and parent were harbouring wild type 3 strains with such a range of properties seems remote; no other type 3 strains were isolated in the area over the period studied. With the tests used the probable origin of the three type 3 strains from vaccine-associated cases which behave like wild strains in the marker test remains in doubt.

Of the strains from 13 patients obtained through epidemiological surveillance, *in vitro* marker tests were able to class 6 as being unrelated to vaccine and 3 others as also wild but of uncertain origin. One strain was of vaccine type, two had intermediate characters and reversion to virulence seemed probable in the strains from the remaining case and her family.

SUMMARY

Strains of poliovirus were obtained from 13 of the 18 persons in England and Wales with paralytic episodes after administration of oral vaccine in 1962. They have been studied using three marker tests: the R.C.T.$_{40}$ test, intratypic serodifferentiation and inhibition by dextran sulphate. For comparison a number of strains from subjects with non-paralytic vaccine-associated reactions and from patients with paralytic poliomyelitis not related to vaccine were also tested.

Of the eight patients excreting type 1 strains seven came from South Wales where an outbreak was in progress. They all resemble naturally occurring strains from the outbreak in growing at 39·3° but not at 39·8° C.

Only one subject excreted type 2 virus which was of vaccine type.

The type 3 strains included a series from a family group where a range of results from vaccine to the wild range was obtained. Three other patients with vaccine-associated paralysis excreted type 3 strains with the characteristic of naturally occurring strains.

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