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# Characterization of poliovirus isolates in Japan after the mass vaccination with live oral poliomyelitis vaccine (Sabin) *

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*From 1962 to 1968, 127 poliovirus isolates (92 from clinical specimens and 35 from healthy subjects) were subjected to intratypic serodifferentiation by the modified Wecker and McBride techniques. The two techniques gave concordant results for 103 strains, 91 of which were classified as vaccine-like, 9 as nonvaccine-like, and 3 as intermediate; 20 were classified as vaccine-like by one technique and as intermediate by the other, and 4 as non-vaccine-like by one technique and as intermediate by the other. The origin of the non-vaccine-like strains is unknown, but it is unlikely that they had been circulating in the community long. An rct/40-marker test of the isolates revealed a higher positivity rate than that found in field trials of Sabin vaccine. The results indicate that wild poliovirus has been almost completely eradicated from Japan.*

After the mass vaccination with live oral poliomyelitis vaccine (Sabin), carried out in Japan in 1961, the incidence of paralytic poliomyelitis decreased markedly (Takatsu et al., 1972). The numbers of poliomyelitis cases notified annually since 1962 have ranged from one-tenth to one-hundredth of those reported annually before the mass vaccination. However, a few cases of disease clinically resembling paralytic poliomyelitis have been reported every year. Poliovirus strains isolated from clinical specimens have been characterized as a routine surveillance activity. Strains isolated from specimens collected for the investigation of poliovirus transmission among children, and from other sources, were also included.

## MATERIALS AND METHODS

### *Tissue culture and media*

The preparation of primary cultures from cynomolgus monkey kidney cells (MKTC); growth and maintenance media; and the plaque technique have been described elsewhere (Kitahara et al., 1967).

### *Virus*

The stock viruses of Sabin vaccine strains used as the reference were lots 1002-4 (type 1, LSc 2 ab), 2002-1 (type 2, P 712 CH 2 ab), and 3002-1 (type 3, Leon 12a 1 b) provided by the Connaught Medical Research Laboratories.[1] The strains Mahoney (type 1), MEF 1 (type 2), Saukett (type 3), and Suwa (a type-3 poliovirus strain isolated from a paralytic case in Japan in 1958) were used as the reference virulent viruses. The stock virus of these strains was prepared in this laboratory in cynomolgus MKTC.

The strains examined were isolated during the period 1962–68 from either healthy carriers (mostly children) or persons with diseases of the central nervous system (CNS) and certain other diseases of a minor nature (Tables 1 and 2). Some of the strains were sent by other laboratories in Japan. They did not cover all polioviruses isolated in the country, but included most of the strains isolated from clinical specimens (mostly faeces) from persons with CNS diseases. Other strains were isolated in our laboratory from clinical specimens. Isolates from healthy carriers were limited to those from faecal specimens

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Table 1. Polioviruses isolated (1962–1968)

| Year | Source of virus isolated | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | healthy carriers | | | | clinical cases | | | |
| | type 1 | type 2 | type 3 | types 1 & 2 | type 1 | type 2 | type 3 | types 2 & 3 |
| 1962 | 0 | 0 | 0 | 0 | 0 | 1 | 7 | 0 |
| 1963 | 2 | 7 | 2 | 0 | 0 | 1 | 5 | 0 |
| 1964 | 0 | 3 | 2 | 0 | 0 | 3 | 4 | 5 |
| 1965 | 0 | 7 | 1 | 1 | 2 | 1 | 1 | 2 |
| 1966 | 0 | 0 | 1 | 0 | 0 | 3 | 4 | 6 |
| 1967 | 1 | 0 | 0 | 0 | 3 | 5 | 5 | 6 |
| 1968 | 1 | 3 | 1 | 1 | 4 | 4 | 1 | 0 |
| Total | 4 | 20 | 7 | 2 | 9 | 18 | 27 | 19 |

Table 2. Strains examined, according to type and source

| Source of virus | Strains examined | | | |
|---|---|---|---|---|
| | type 1 | type 2 | type 3 | Total |
| healthy carriers | 6 | 22 | 7 | 35 |
| clinical cases | 9 | 37 | 46 | 92 |
| Total | 15 | 59 | 53 | 127 |

collected not less than 2 months after the routine vaccination with live oral vaccine.

Typing was performed with undiluted culture fluid of infected MTKC and the tests were carried out with virus from the 1st to the 4th passages after isolation. Some strains had been passaged in the original laboratories through HeLa, KB, FL, HEp-2, or Vero cell cultures. These viruses were passaged once in MKTC, identified or confirmed as being of the serotype reported, and then used for the tests.

*Reproductive capacity temperature (rct) marker test*

The growth medium contained in the 56-g bottle used for MK cultures was replaced by the maintenance medium 1 day before virus inoculation. On the day of inoculation the medium was again discarded and 0.2 ml of each dilution of the test virus were inoculated into bottles (8 bottles per dilution). After adsorption of the virus at 36°C for 60 min, the cultures were overlaid with 5 ml of nutrient agar. The bottles were then divided into 2 groups. Those of one group were incubated at 35°C. The others were placed in a water-bath, at either $40\pm0.1$°C (for the strains of types 1 and 2) or $40.3\pm0.1$°C (for type-3). Plaques were counted on the 5th day. The reference virulent and attenuated strains were included in each test. Strains with the difference of log PFU (plaque forming units) at low and high temperatures equal to or more than $10^5$ were classified as negative; those with the difference equal to or less than $10^2$, as positive; and others as intermediate.

*Intratypic serodifferentiation test*

*Modified Wecker technique.* The preparation of antisera against attenuated poliovirus strains and the procedures used in this technique have been described elsewhere (Kitahara et al., 1967; Soda et al., 1969). Rabbit antiserum against LSc 2 ab, R-Pool-W, was used at a dilution of 1 : 12 800; monkey antiserum against P 712 CH 2 ab, M-2151-5, at a dilution of 1 : 6 400; and rabbit antiserum against strain Leon 12a 1 b, N-R-35-2, at a dilution of 1 : 2 400. The same criteria as applied previously (Kitahara et al., 1967; Soda et al., 1969) were used to evaluate the relation of an isolate with the vaccine virus. In addition, immune sera prepared against a domestic type-1 wild virus (strain Hokkaido 1) isolated in 1960, and against two vaccine-derived type-1 strains with a marked antigenic drift were used. The reference wild viruses were strains Mahoney (type 1), MEF 1 (type 2), and Suwa (type 3) (Soda et al., 1969).

*McBride technique.* The preparation of antisera; the modification of the McBride (1959) test; and

the criteria for the evaluation of the results are described elsewhere (Nakao, 1969). As the reference wild viruses, Mahoney (type 1), MEF 1 (type 2), and Saukett (type 3) strains were used (Nakao, 1969).

## RESULTS

### *Poliovirus strains*

Among the strains isolated from healthy carriers, type-2 virus was dominant (22/35 or 63%), whereas type-3 virus, either alone or mixed with type 2, was more frequently isolated from clinical specimens (46/92 or 50%), Type-1 poliovirus has been isolated less frequently since the mass oral vaccination.

Table 3 shows the type distribution of the isolates from various clinical cases. Fifty-one strains were derived from 38 clinically typical poliomyelitis cases. Type-3 strains were the most frequently isolated from these cases (28/51, 55%), followed by type 2 (22/51, 43%); only one type-1 virus was isolated. Most of these cases (28/38) were temporally vaccine-associated; 7 had no history of vaccination against poliomyelitis. Although contact with a vaccinated person (in a day-nursery) was confirmed in only one case, the others may well have been in contact with one or more vaccinated persons, since the routine vaccination of newborn infants is carried out in most districts twice a year (Takatsu et al., 1972). Type-2 and type-3 viruses were evenly distributed among the isolates from persons with other CNS diseases.

Table 3. Number and type of polioviruses isolated from clinical cases

| Type of poliovirus isolated | Clinical polio-myelitis [a] | Other CNS diseases [b] | Other minor diseases | Total |
|---|---|---|---|---|
| type 1 | 1 | 2 | 6 | 9 |
| type 2 | 9 | 7 | 2 | 18 |
| type 3 | 15 | 8 | 4 | 27 |
| types 2 and 3 | 13 | 6 | 0 | 19 |
| Total | 38 | 23 | 12 | 73 |

[a] Paralytic cases as classified in typical clinical poliomyelitis (clinical category A) by the Poliomyelitis Surveillance Committee (28 cases within 30 days of live oral poliovaccine, 3 cases more than 30 days after vaccination, and 7 cases without vaccination).

[b] Including other paralytic diseases (categories B and C according to the Poliomyelitis Surveillance Committee) and aseptic meningitis.

### *Serological characterization of poliovirus strains*

The results of the intratypic serodifferentiation tests are summarized in Table 4. Out of 127 strains examined, 91 (72%) were classified by both techniques as vaccine-like and 9 (7%)—7 of type 1 and 2 of type 3—as nonvaccine-like. Two type-2 strains were classified as intermediate by both techniques; with 24 other strains (19%), the classifications diverged. The results suggest that wild poliovirus has been almost completely eradicated from Japan since the mass vaccination with Sabin vaccine.

Table 5 presents a summarized description of the strains classified as nonvaccine-like. Three strains of type-1 virus isolated in 1963 and 1965 are from the same district in the northern part of the main island, and 4 type-2 strains isolated in 1965 are from the southern part of the country (Shikoku island). The 7 type-1 strains were further examined by means of a rabbit antiserum against type-1 wild poliovirus and two rabbit antisera, each against a type-1 vaccine-derived strain with antigenic shift. The results indicated that these strains were antigenically different from the wild type-1 viruses that were prevalent before the introduction of Sabin vaccine into Japan as well as from the type-1 vaccine-derived strains that showed antigenic shift from the original vaccine strain.

The exact nature of these nonvaccine-like strains remains unknown. In any case, it is unlikely that they are still circulating in the community, because they have been isolated only rarely, in spite of extensive surveys.

As shown in Table 6, most of the viruses (45/51, 88%) from clinical poliomyelitis cases were classified as vaccine-like, whereas 4 other strains were classified as vaccine-like by the McBride technique and as intermediate by the Wecker technique; one type-2 strain as vaccine-like by the Wecker technique and as intermediate by the McBride technique; and only one type-1 strain as nonvaccine-like by both techniques. The 4 strains mentioned above were isolated from 3 temporally vaccine-associated cases, and the 2 last-mentioned strains were from 2 paralytic cases without a history of vaccination against poliomyelitis. I. A., a boy 1.5 years old, contracted paralytic poliomyelitis in July 1964, when he and his parents were visiting relatives in another prefecture. The type-2 poliovirus strain isolated from his stool was classified as vaccine-like by the McBride technique and as intermediate by the Wecker technique. The onset of this child's illness occurred about 1½ months after the routine spring vaccination in his district of

Table 4. Results of McBride and Wecker tests of poliovirus strains isolated (1962–1968)

| Classification | | Type 1 | | Type 2 | | Type 3 | | Total |
|---|---|---|---|---|---|---|---|---|
| | | healthy carriers | clinical cases | healthy carriers | clinical cases | healthy carriers | clinical cases | |
| complete agreement by the two techniques | vaccine-like | 2 | 1 | 11 | 30 | 6 | 41 | 91 |
| | intermediate | 1 | 0 | 1 | 1 | 0 | 0 | 3 |
| | nonvaccine-like | 1 | 6 | 0 | 0 | 0 | 2 | 9 |
| vaccine-like by McBride test, intermediate by Wecker test | | 0 | 1 | 2 | 3 | 1 | 3 | 10 |
| vaccine-like by Wecker test, intermediate by McBride test | | 2 | 1 | 4 | 3 | 0 | 0 | 10 |
| nonvaccine-like by McBride test, intermediate by Wecker test | | 0 | 0 | 4 | 0 | 0 | 0 | 4 |
| nonvaccine-like by Wecker test, intermediate by McBride test | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | | 6 | 9 | 22 | 37 | 7 | 46 | 127 |

Table 5. Nonvaccine-like poliovirus strains isolated (1962–1968)

| Sero-differentiation | Type of virus | Month & year of specimen collection | Source of specimen [a] | | | LPV & interval [b] | rct-market [c] |
|---|---|---|---|---|---|---|---|
| | | | sex | age (years) | condition | | |
| nonvaccine-like by both techniques | type 1 | Aug. 1963 | male | <1 | healthy | no | ± |
| | | July 1965 | female | 4 | aseptic meningitis | yes, 4 m | + |
| | | July 1965 | female | 2 | fever | yes, 55 d | + |
| | | Sept. 1967 | male | 5 | fever | unknown | + |
| | | Sept. 1967 | male | 5 | fever | unknown | + |
| | | Oct. 1967 | male | 3 | paralysis (B) | yes, >1.5 y | + |
| | | March 1968 | male | 3 | paralysis (A) | no | + |
| | type 3 | May 1962 | male | 5 | paralysis (B) | yes, 3 d | − |
| | | Feb. 1963 | female | 8 | myalgia epidemica | yes, 6 m | + |
| nonvaccine-like by one of the two techniques | type 2 | June 1965 | female | 5 | healthy | yes, >1 y | + |
| | | Sept. 1965 | male | 44 | healthy | no | ± |
| | | Sept. 1965 | male | 5 | healthy | no | + |
| | | Sept. 1965 | female | 4 | healthy | no | + |

[a] paralysis (A) = clinically typical poliomyelitis; paralysis (B) = clinically atypical paralysis.
[b] d = days; m = months; y = year(s).
[c] rct/40 for type-1 and type-2 viruses; rct/40.3 for type-3 virus.

Table 6. Characteristics of poliovirus strains isolated from clinical poliomyelitis (category A) (1962–1968)

| Type of virus | Number of strains | Vaccine-like by both techniques | Vaccine-like by McBride; intermediate by Wecker | Vaccine-like by Wecker; intermediate by McBride | Non-vaccine-like by both techniques | rct-marker [a] | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | — | ± | + |
| type 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| type 2 | 22 | 19 | 2 | 1 | 0 | 1 | 13 | 8 |
| type 3 | 28 | 26 | 2 | 0 | 0 | 4 | 5 | 19 |
| Total | 51 | 45 | 4 | 1 | 1 | 5 | 18 | 28 |

[a] rct/40 for types 1 and 2; rct/40.3 for type 3.

domicile, and it is highly probable that he was infected with vaccine-originated type-2 poliovirus. F. T., a 3-year-old boy, contracted paralytic disease in March 1968, and type-1 poliovirus was isolated from his stool. In the area where he lived the last live poliovaccine administration had been carried out in November 1967 and the spring vaccination was scheduled for May 1968. No other paralytic case has been reported from this area, nor has poliovirus of similar serological characteristics been isolated there. The origin of the strain remains unknown; however, if this type-1 vaccine virus survived for 4 months in the community, it must have been transmitted mostly among those with immunity against poliovirus, acquiring antigenic drift in the meantime, since neither the patient nor his family had come into contact with recent arrivals from areas of poliomyelitis endemicity.

*Results of rct-marker testing*

As shown in Table 7, 15 (43%) of 35 strains from healthy carriers and 48 (53%) of 90 strains from

Table 7. 2 Results of rct-marker testing[a] of poliovirus strains isolated (1962–1968)

| Source and type of virus | All isolates | | | Vaccine-like isolates | | |
|---|---|---|---|---|---|---|
| | negative | intermediate | positive | negative | intermediate | positive |
| *healthy carriers* | | | | | | |
| type 1 | 3 | 3 | 0 | 2 | 0 | 0 |
| type 2 | 6 | 5 | 11 | 3 | 3 | 5 |
| type 3 | 1 | 2 | 4 | 0 | 2 | 4 |
| Total | 10 (29%) | 10 (29%) | 15 (43%) | 5 (26%) | 5 (26%) | 9 (47%) |
| *clinical cases* | | | | | | |
| type 1 | 2 | 1 | 6 | 1 | 0 | 0 |
| type 2 | 5 | 16 | 14 | 4 | 14 | 12 |
| type 3 | 8 | 10 | 28 | 7 | 10 | 24 |
| Total | 15 (17%) | 27 (30%) | 48 (53%) | 12 (17%) | 24 (33%) | 36 (50%) |

[a] rct/40 for types 1 and 2; rct/40.3 for type 3.

clinical cases were rct-positive. Since some strains were classified as nonvaccine-like or as intermediate by one or both techniques, a comparison was made between strains of types 2 and 3 that had been classified as vaccine-like by both techniques. Thus it was shown that 17 (41%) of 41 type-2 strains from healthy carriers and clinical cases were positive, the corresponding figures for type-3 viruses being 28 (60%) of 47. These proportions are high compared with those obtained with isolates in an oral vaccination trial previously reported (Japan Live Poliovaccine Research Commission, 1967). Three vaccine-like type-1 isolates were rct/40-negative. The rct-marker test results for isolates from typical poliomyelitis cases are shown in Table 6. It is noteworthy that 28 (55%) of 51 isolates were positive.

## DISCUSSION

The survey of poliovirus circulation among children and the characterization of poliovirus isolates from clinical cases as well as from healthy carriers are among the most important activities related to the surveillance of poliomyelitis. In Japan, the mass vaccination with Sabin vaccine from 1961 to 1963 covered nearly all the susceptible age groups up to 12 years with 2 doses of each type of Sabin vaccine. The effect was dramatic: a marked decrease in the incidence of paralytic cases (Takatsu et al., 1972). From 1962 to 1968, 35 strains of poliovirus isolated from healthy persons and 92 strains isolated from clinical cases were studied. These included nearly all the poliovirus isolates from paralytic cases and most of those isolated from healthy persons at least 2 months after the routine vaccination. Serological investigation revealed that 91 strains (72%) were classified as vaccine-like by both the McBride technique and the modified Wecker technique. If 20 other strains that were classified as vaccine-like by one technique and as intermediate by the other are considered as vaccine-related, 88% of the poliovirus isolates were vaccine-related. However, 9 strains were classified as nonvaccine-like and 3 strains as intermediate by both techniques, and 4 strains as nonvaccine-like by one technique but intermediate by the other. These results seem to indicate that wild poliovirus has been almost completely eradicated from the community, although a few polioviruses antigenically different from Sabin vaccine viruses have occasionally been isolated. It may be impossible to trace the origin of such viruses, but it may be that they were imported from abroad or derived from vaccine virus with heavy antigenic drift while being transmitted among persons with intestinal immunity against poliovirus. It is suggested that such polioviruses, either wild or vaccine-derived, cannot survive long in the community, since no sign of the continuous circulation of such viruses in Japan has been observed. As reported earlier (Takatsu et al., 1972), 209 out of 621 reportedpoliomyelitis cases were classified clinically as typical paralytic poliomyelitis; 51 strains isolated from 38 such cases were examined and all except one were considered as vaccine-related. This does not necessarily mean that the illness was induced by these viruses, because most of the cases were temporally vaccine-associated. Of the 7 patients without any history of vaccination with live oral poliovaccine, one—an infant—became ill while at a day-nursery attended also by recently vaccinated infants. Although contact with vaccinated persons was not confirmed in the other 6 cases, it cannot be excluded that the polioviruses isolated must have been derived from vaccinated persons living in the same neighbourhood as the patients. The origin of a type-1 poliovirus isolated from a nonvaccinated boy—which was classified as nonvaccine-like by both techniques—remains unknown, but it was unlikely that this virus had been introduced from abroad. It was also serologically quite different from the type-1 wild poliovirus that had been prevalent in the area concerned 8 years before the mass vaccination.

Several authors have reported the intratypic serodifferentiation of poliovirus isolates before and after the introduction of Sabin vaccine (Vonka et al., 1962a, b; Wasserman & Fox, 1962; Nakano et al., 1963, 1966; Nakano & Cole, 1970; Furesz et al., 1964a, b, 1966; Kitahara et al., 1967; Deibel & Macdonald, 1968; Soda et al., 1969; Nakao, 1969; Rocchi et al., 1970). It was indicated that type-1 Sabin vaccine strain showed more or less antigenic drift after passages through the human intestinal tract, whereas strains of types 2 and 3 were relatively stable. Furthermore, a few wild poliovirus strains, chiefly of type 3, were rather difficult to differentiate from Sabin vaccine strains. Most earlier publications concern isolates obtained from vaccinated persons or their contacts in field trials or during epidemics, when Sabin vaccine was used. The characterization of poliovirus isolates from vaccine-associated or contact cases obtained by the extensive surveillance of poliomyelitis was reported by Hopkins et al. (1969), Chumakov et al. (1969), and Dömök et al. (1969). In the USA, from 1965 to 1967, 3 isolates from vaccine-associated cases and 13 isolates from contact cases were examined. All these isolates were

obtained from regions other than south-western border states where wild polioviruses were still prevalent. Only 1 of the 16 strains examined—a type-3 virus—was considered to be a wild-type virus; the remaining 15 strains (2 of type 1; 5 of type 2; and 8 of type 3) were considered as vaccine-like serologically, although 3 were described as being of mixed type on account of other markers. Chumakov et al. (1969) reported that, during the 5-year period 1964–1968, 26 vaccine-associated cases with residual paralysis were found in the USSR. Detailed data on the serological characterization of the poliovirus isolates from these cases were not presented, but two points appear interesting in view of poliomyelitis surveillance: first, 9 of these cases were excluded from the analysis because they were reported in southern, epidemiologically unfavourable, areas of the country where there is still circulation of type-1 poliovirus with the antigenic and rct/40 characteristics of a wild variant. Secondly, type-2 and type-3 poliovirus strains differing in their characteristics from Sabin vaccine strains were isolated from vaccine-associated cases in other regions of the country. It was also mentioned that, in Moscow (1965) and in Vilnius and Betoksary (1966), such strains were isolated from sewage. Dömök et al. (1969) reported that 6 out of 11 type-1 poliovirus strains isolated from vaccine-associated cases were not vaccine-like, although they were rct/40-negative and d-negative, whereas 1 was vaccine-like and 4 were doubtful. All the viruses of type 2 (6 strains) and type 3 (17 strains) were considered as vaccine-like. These data from the USSR and Hungary may be comparable to those for Japan reported here, since nation-wide vaccination of almost all susceptible persons was carried out with Sabin vaccine in all three countries, although the vaccination schedule and form of administration differed in each case. Type-1 virus was not isolated in Japan from vaccine-associated cases. However, in both the USSR and Hungary a limited region presented epidemiological problems in relation to serologic and other marker characterization of type-1-isolates from vaccine-associated cases. As regards viruses of types 2 and 3 isolated from vaccine-associated cases, our data appear to resemble those from Hungary, although some strains isolated from healthy children in Japan showed serological characteristics different from those of the vaccine strains described by Chumakov et al. (1969).

The results of rct-marker testing of the poliovirus strains classified as vaccine-like indicated that there was no difference in the extent of marker reversion between the strains isolated from healthy carriers and those from clinical cases. Although rct/40.3-positive virus was more frequently observed with type-3 isolates, a considerable number of type-2 isolates were also rct/40-positive. The higher rate of rct/marker reversion among isolates from healthy carriers may be accounted for by a long interval between specimen collection and routine vaccination. However, the reversion rate among the isolates from clinical cases was almost the same, which appears rather high compared with the data obtained in the previous trial considering that most of the present isolates, especially those of type-3 virus, were isolated from vaccinated persons within a month of oral vaccination. A certain host factor and/or elevated body temperatures as a symptom of illness in such clinical cases might be related to a higher rct-marker reversion rate of poliovirus isolates. Dömök et al. (1969) indicated that most of the type-1 and type-2 strains isolated from vaccine-associated cases were rct/40-negative and some of them gave intermediate results, whereas most type-3 strains were positive, with some intermediate results. These strains do not always correspond to those reported here. In the case of type-3 polioviruses, a difference in the temperature for the rct test (rct/40 according to Dömök et al. and rct/40.3 according to us) might have accounted for the difference in the results.

## RÉSUMÉ

### CARACTÉRISATION D'ISOLATS DE POLIOVIRUS AU JAPON APRÈS LA VACCINATION DE MASSE PAR LE VACCIN ANTIPOLIOMYÉLITIQUE VIVANT BUCCAL (SABIN)

On a entrepris de caractériser les souches de poliovirus isolées à partir d'échantillons recueillis chez des personnes atteintes de poliomyélite ou d'autres affections et chez des sujets en bonne santé après la campagne de vaccination antipoliomyélitique de masse menée au Japon en 1961.

Au total, 127 souches ont été isolées de 1962 à 1968: 92 chez des malades et 35 chez des sujets sains. Dans le 1er groupe, on comptait 51 souches provenant de 38 cas de poliomyélite, 29 souches provenant de 23 cas d'autres affections du système nerveux central et 12 souches provenant de 12 cas d'affections bénignes. On a utilisé pour

classer les souches les techniques modifiées de Wecker et de McBride. Les épreuves de sérodifférenciation ont permis de classer 103 souches de façon identique par l'une ou l'autre technique: 91 étaient semblables à la souche vaccinale, 9 en différaient et 3 étaient de type intermédiaire. Vingt souches ont été reconnues comme étant de type vaccinal par une technique et de type intermédiaire par l'autre; 4 ont été classées comme souches de type non vaccinal par une technique et comme souches de type intermédiaire par l'autre. Sur 51 souches isolées de cas typiques de poliomyélite, 45 étaient de type vaccinal selon les deux techniques, 5 étaient classées comme semblables à la souche vaccinale par une technique et comme de type intermédiaire par l'autre; une seule souche était identifiée comme d'un type différent de celui de la souche vaccinale par les deux techniques. De ces 51 souches, 44 provenaient de cas de poliomyélite associés dans le temps à la vaccination et 7 de cas survenus chez des sujets non vaccinés; 5 de ces dernières étaient de type vaccinal selon les deux techniques, une souche de type 2 était de type vaccinal (technique de Wecker) ou intermédiaire (technique de McBride) et une souche de type 1 était de type non vaccinal suivant les deux techniques. L'origine des souches de type non vaccinal demeure inconnue, mais rien n'indique que ce genre de souches était depuis longtemps en circulation dans la collectivité.

L'épreuve du marqueur crt/40 pratiquée sur tous les isolats a montré que parmi les souches isolées chez les malades 53% étaient positives et 17% négatives; parmi les souches isolées chez des sujets sains, on comptait 43% de souches positives et 29% de souches négatives. Ces différences étaient moins tranchées pour les souches de type vaccinal.

Selon les auteurs, ces résultats indiquent que les souches sauvages de poliovirus ont été presque complètement éliminées au Japon depuis la mise en œuvre de la campagne de vaccination de masse.

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