Memoranda

EVIDENCE ON THE SAFETY AND EFFICACY OF LIVE POLIOMYELITIS VACCINES CURRENTLY IN USE, WITH SPECIAL REFERENCE TO TYPE 3 POLIOVIRUS *

EPIDEMIOLOGY

INCIDENCE OF POLIOMYELITIS IN COUNTRIES WITH TEMPERATE CLIMATES

A progressive and rapid increase in the incidence of poliomyelitis occurred in the first half of the 20th century in numerous countries, mainly in those well developed socially and economically and with temperate climates. After 1955 when inactivated poliovirus vaccine was introduced, and especially after 1959 when live attenuated vaccines became available on a large scale, most countries in Europe, North America and Oceania and some countries in other regions of the world, experienced a reduction in the incidence of poliomyelitis of an order seldom if ever achieved so quickly and dramatically in the control of other diseases by vaccination.

In 1955, some 17 364 cases of poliomyelitis were reported in the USSR, 27 343 in 23 other European countries, and 31 582 in the United States of America, Canada, Australia and New Zealand—a total of over 76 000. In these same countries in 1967 only 1013 cases were recorded—a reduction of 98.7% in 12 years.

Most of the countries use live vaccine, but in Australia and in Scandinavia, where inactivated vaccines are mainly employed, the reduction in incidence has been similar to that observed in countries using the live vaccines.

INCIDENCE OF POLIOMYELITIS IN COUNTRIES WITH WARM CLIMATES

The information on the status of poliomyelitis in tropical and semi-tropical countries is usually less complete than that for the countries already mentioned but the data which are available lead to the conclusion that in many of the countries in Africa, Central and South America and Asia a progressive increase in morbidity is being observed. In a WHO survey of the information from 71 tropical and semi-tropical countries it was found that in 45 of them the over-all incidence in 1966 was 3 times greater than the annual average for 1951–55.[1] These data and the occurrence of large outbreaks in tropical countries in recent years indicate the increasing public health importance of poliomyelitis in those countries in warm climates where comprehensive regular vaccination of susceptible persons is not yet being carried out.

TYPE DISTRIBUTION OF POLIOVIRUSES

Before mass vaccination with poliovirus vaccines, type 1 was by far the most frequent cause of the paralytic disease. After the use of vaccines became widespread several reports showed that, as the incidence of poliomyelitis decreased, the proportion of cases caused by type 3 poliovirus increased.

In Hungary (Table 1) a comparison has been made between the 5 years (1955–59) before the introduction of live vaccine (Dömök & Molnar, 1961) and the 8 years (1960–70) since its introduction (I. Dömök, personal communication). The proportion of type 1 strains has fallen from 92% to 32% and the proportion of type 3 strains has risen from 4.5% to 52%. In other countries the percentage changes have usually been smaller. In the USA, for example, 16% of the strains isolated in the 3 years before live vaccine was introduced belonged

* This memorandum was prepared by the signatories listed on page 942, who took part in a consultation on poliomyelitis arranged by WHO. A previous memorandum, prepared following the same consultation and dealing with USOL-D bac (type 3 poliovirus) vaccine studies, appeared in *Bull. Wld Hlth Org.*, 1969, **40**, 295-300.

[1] Unpublished working document WHO/VIR/68.1 Rev. 2: a limited number of copies of this document is available to persons officially or professionally interested on request to Distribution and Sales, World Health Organization, 1211 Geneva, Switzerland.

TABLE 1

TYPE DISTRIBUTION OF POLIOVIRUS STRAINS ISOLATED FROM POLIOMYELITIS CASES BEFORE AND AFTER THE USE OF LIVE POLIOVIRUS VACCINE IN HUNGARY

Year	Type 1		Type 2		Type 3	
	No.	%	No.	%	No.	%
5-year period before use of live vaccine						
1955	7	22	10	31	15	47
1956	3	43	3	43	1	14
1957	69	87	8	10	2	3
1958	8	80	1	10	1	10
1959	535	97.6	2	0.4	11	2
5-year total	622	92	24	3.5	30	4.5
8-year period after use of live vaccine						
1960	5	31	2	13	9	56
1961	1	25	0	0	3	75
1962	0	0	1	100	0	0
1963	0	0	0	0	3	100
1964	1	33	0	0	2	67
1965	0	0	2	50	2	50
1966	4	80	1	20	0	0
1967	1	50	0	0	1	50
8-year total	12	32	6	16	20	52

to type 3 compared with 27% in the 8 subsequent years.

From the information obtained in a WHO reporting system for virus diseases diagnosed in laboratories in some 30 countries it is apparent (Table 2) that where poliovirus vaccines are not used, or are used on an inadequate scale, type 1 virus is still responsible for about 80% of the paralytic cases. In contrast, in well-vaccinated countries almost equal numbers of the different types are now being isolated both from persons with and from persons without disease of the central nervous system.

VACCINE-ASSOCIATED CASES OF POLIOMYELITIS

Below is given a list of countries for which data have been taken from already published material or from which information has been obtained by WHO (in connexion with the consultation following which this memorandum was prepared) on the occurrence and methods of investigation of cases of poliomyelitis temporally associated with the administration of live vaccine:

Country in which temporally associated cases observed	*Some cases believed to be vaccine-related*
Belgium	No evidence
Canada	Yes
Denmark *	Yes
England and Wales	Yes
France	Not sufficient evidence
Hungary **	Yes
Italy	Not sufficient evidence
Japan **	Yes
Poland **	Yes
Romania **	Yes
USA	Yes
USSR	Yes
Yugoslavia	No

* Type 3 live vaccine given once only.

** Countries in which vaccination given over a limited period annually.

Vaccines wholly prepared from the Sabin strains were used with the following exceptions: *Poland*—type 1 Chat, type 2 P712 CH2ab, type 3 W-Fox; *Romania*—Sabin strains except in 1967 when the WM-3 type 3 strain was used; *Yugoslavia*—in Croatia, type 1 Chat, Type 2 W-2, type 3 WM-3.

Vaccine-associated cases in vaccinated persons are usually defined as those which occur within a month after the vaccine has been given, and vaccine-associated cases in contacts as those which occur within 2 months after vaccination. All 13 countries tabulated above reported that cases typical of poliomyelitis had occurred either in vaccinated persons or in their contacts within a short time after vaccine had been administered. Since the two events (administration of vaccine and onset of a disease typical of poliomyelitis), even if independent, could well occur at about the same time it is not surprising that such a relationship was observed in all the countries listed. However, authorities in 9 of the 13 countries believed that some of the cases were vaccine-*related*. Of the others, 2 did not consider that they had sufficient evidence, and 2 believed that there was no evidence of vaccine-related cases.

The intensity of the search for such cases and the throughness of the investigation differed greatly between countries. Since the number of possible vaccine-related cases in any one country is very small and since complete proof of causal relationship

TABLE 2

NUMBERS OF POLIOVIRUS STRAINS ISOLATED AND IDENTIFIED IN CERTAIN WORLD AREAS, 1964–67

Area	Virus type	Source of specimen							Total
		Paralytic cases (1)	CNS disease, non-paralytic (2)	All isolations from persons with CNS disease (1+2)	Pyrexia and/or gastrointestinal symptoms (3)	Symptomless excreters or unrelated illnesses (4)	All isolations from persons without CNS manifestations (3+4)	No information	(1+2+3+4)
North America	1	15	14	29	5	38	43	7	79
	2	7	15	22	19	50	69	10	101
	3	9	20	29	14	78	92	7	128
	Total	31	49	80	38	166	204	24	308
Latin America (mainly Mexico)	1	950	1	951	17	12	29	65	1 045
	2	15	0	15	1	1	2	0	17
	3	43	0	43	1	1	2	3	48
	Total	1 008	1	1 009	19	14	33	68	1 110
Europe: France	1	89	30	119	19	110	129	10	258
	2	30	9	39	2	27	29	4	72
	3	18	4	22	6	29	35	0	57
	Total	137	43	180	27	166	193	14	387
Europe: other countries	1	69	51	120	45	238	283	23	426
	2	24	42	66	76	309	385	31	482
	3	50	37	87	70	301	371	20	478
	Total	143	130	273	191	848	1 039	74	1 386
South Africa	1	472	32	504	12	13	25	143	672
	2	38	1	39	6	5	11	17	67
	3	98	11	109	8	10	18	27	154
	Total	608	44	652	26	28	54	187	893
Asia: Thailand	1	105	0	105	2	2	4	0	109
	2	10	1	11	0	3	3	0	14
	3	2	0	2	0	4	4	0	6
	Total	117	1	118	2	9	11	0	129
Asia: Japan	1	1	1	2	0	3	3	0	5
	2	1	3	4	7	7	14	3	21
	3	11	1	12	5	6	11	0	23
	Total	13	5	18	12	16	28	3	49
Asia: Indonesia, Hong Kong and Singapore	1	124	3	127	5	0	5	2	134
	2	3	0	3	5	2	7	9	19
	3	5	1	6	1	0	1	1	8
	Total	132	4	136	11	2	13	12	161
All areas	1	1 825	132	1 957	105	416	521	250	2 728
	2	128	71	199	116	404	520	74	793
	3	236	74	310	105	429	534	58	902
	Total	2 189	277	2 466	326	1 249	1 575	382	4 423

TABLE 3

POLIOMYELITIS INCIDENCE IN POLAND WITHIN 42 DAYS BEFORE AND AFTER FEEDING ATTENUATED POLIOVIRUS TYPE 1 (CHAT), TYPE 3 (W-FOX) OR TYPE 2 (P712)

Vaccine	Type 1			Type 3			Type 2		
Year	Cases within 42 days before vaccination	Number of vaccinated persons	Cases within 42 days after vaccination	Cases within 42 days before vaccination	Number of vaccinated persons	Cases within 42 days after vaccination	Cases within 42 days before vaccination	Number of vaccinated persons	Cases within 42 days after vaccination
1959–60	84	7 522 900	41	46	7 085 100	97	No vaccination		
1961	5	757 700	6	6	699 400	29	No vaccination		
1962	1	986 800	5	No vaccination			2	7 581 534 [a]	15
1963	2	785 600	4	No vaccination			2	882 100	2
1964		772 200	1	No vaccination				795 400	
1965	2	878 800	5	No vaccination			2	917 321	

[a] 1 948 400 vaccinated in November and December 1961.

in individual cases is impossible to obtain, the absence of reports of vaccine-related cases in some countries may be the result of lack of adequate methods of ascertainment and study. It would be much easier to overlook cases when vaccination is carried out through the year than when it is concentrated into a defined period.

The 9 countries in which it was believed that vaccine-related cases occurred were divisible into two groups—those in which vaccine was usually administered over a short period annually and those in which vaccine was given over a longer period.

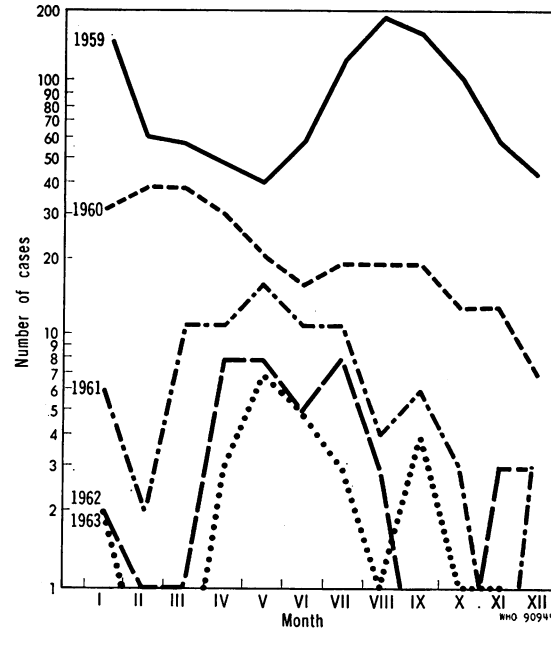
CASES IN COUNTRIES WHERE VACCINES ARE ADMINISTERED ANNUALLY OVER A SHORT PERIOD

In four countries (see tabulation, p. 926) vaccine was given regularly over a brief period, usually in the winter or early spring.

Poland was the first country to show (in 1959–61) the seasonal change in the incidence of poliomyelitis and its relation to oral vaccination with type 3 virus (W-Fox vaccine strain). A similar relationship was observed in connexion with type 1 (CHAT) and type 2 (P712) oral vaccines in 1962–63 (Kostrzewski, Kulesza & Załeska, 1962; Kostrzewski & Kulesza, 1963). But the number of cases reported during

FIG. 1

MONTHLY POLIOMYELITIS INCIDENCE IN POLAND, 1959–63 [a]



[a] Reproduced, by permission, from Kostrzewski (1966).

TABLE 4

QUARTERLY DISTRIBUTION OF POLIOMYELITIS CASES REGISTERED IN HUNGARY, 1961–67

Year	Quarter-year [a]			
	1st	2nd	3rd	4th
1961	[P3], [P3], [P3], [P1+E14], Neg.	Neg.		
1962		(P2)		
1963	[P3], [P3], [P3]			Ad6
1964	[P3], [P3]			(P1)
1965	[P3], [P3]	Neg.	(P2), (P2) [b]	Neg.
1966	P1 [c]		P1 [c]	P1,[c] [P1+P2], Ad3, Neg.
1967	(P3)	P1 [c]		

[a] P1, P2, P3, E14, Ad3 and Ad6 indicate the poliovirus, echovirus or adenovirus types excreted. Entries within square brackets [ ] indicate vaccine-related cases; those within parentheses ( ) indicate vaccine-related cases in contacts; " Neg. " indicates negatives in virus isolation experiments.

[b] Transient paresis only; not included in Table 6.

[c] Strains from cases which occurred in an epidemic in a gipsy population within a circumscribed area.

FIG. 2

SEASONAL DISTRIBUTION OF POLIOMYELITIS CASES IN HUNGARY IN 1961–67 COMPARED WITH 1950–59

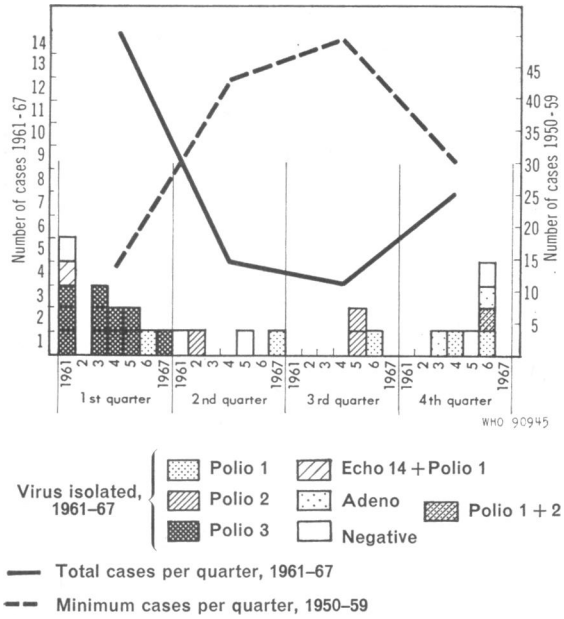


TABLE 5

QUARTERLY DISTRIBUTION OF POLIOMYELITIS CASES IN ROMANIA BEFORE AND AFTER THE WIDESPREAD USE OF LIVE POLIOVIRUS VACCINES [a]

Year or period	Percentage distribution of cases in indicated quarter-year				Total no. of cases
	1st	2nd	3rd	4th	
Before use of live vaccine					
1949–54	6	16	56	22	5 914
1955–60	7	15	49	29	15 953
After use of live vaccine					
1962	27	24	29	20	97
1963	22	43	19	16	86
1964	28	33	22	17	60
1965	14	47	23	16	51
1966	14	32	32	22	41
1967	24	43	21	12	42
1968	21	33	33	12	48
1962–68	22	36	25	17	425

[a] Cases clinically typical of poliomyelitis but not always notified as such.

the 42 days after oral vaccination with type 1 or type 2 virus was much lower than after type 3 (Table 3 and Fig. 1). Evidence that since 1961 the seasonal incidence of poliomyelitis has altered and that it now occurs early in the year is illustrated for Hungary in Table 4 and Fig. 2 and for Romania in Table 5. A similar picture was given in the report from Japan. In Denmark live type 3 vaccine was given on one occasion only over a period of one month in 1966 and 4 cases due to type 3 occurred a month afterwards in persons fed with the vaccine. In Hungary, Poland and Romania, the oral vaccines were usually fed separately as monovalent vaccines with an interval of at least 4 weeks between the different types.

The occurrence of typical cases soon after vaccination programmes have been carried out over brief periods under non-epidemic conditions and the small number or (often) the virtual absence of such cases at other periods of the year offer strong evidence that some of the cases are directly related to the vaccine. The data do not in themselves, however, provide the means of assessing the extent of the risk, or of assessing the relative risks associated with the different strains.

CASES IN COUNTRIES WHERE VACCINES ARE ADMINISTERED OVER LONGER PERIODS

In four countries in the tabulation on page 926 (Canada, England and Wales, USA and USSR) vaccination is carried on over longer periods of the year. No general epidemics have occurred in these countries recently and wild polioviruses are seldom isolated. In these countries also some of the vaccine-associated cases reported were considered to be vaccine-related, but the evidence was much more difficult to interpret epidemiologically.

DISTRIBUTION OF VACCINE-ASSOCIATED CASES ACCORDING TO TYPE OF POLIOVIRUS

In Table 6 a summary has been prepared of the type distribution of the strains isolated from some of the paralytic cases among persons vaccinated with Sabin strains or their contacts in the countries for which data were available. The numbers in the table are considerably fewer than the total numbers reported because only cases in which a single vaccine type was fed and the same single virus type was subsequently isolated have been included. In some countries this information was available for only a small proportion of the cases. In others the picture was obscured by the use of trivalent vaccines which make it more difficult to interpret the significance of the virus types isolated or of the antibody responses measured because they are often multiple. The age and sex distribution of the patients is given in Table 7.

TABLE 6

DISTRIBUTION OF HOMOTYPIC VIRUS TYPES ISOLATED FROM STOOLSI N PARALYTIC CASES AMONG VACCINATED PERSONS FED MONOVALENT SABIN-STRAIN VACCINE OR THEIR CONTACTS

Country	Type 1	Type 2	Type 3	All types	Period covered
			Vaccinated persons [a]		
Denmark	0	0	4	4	1966
Hungary	0	0	7	7	1961–67
Japan	0	0	2	2	1962–67
Romania	0	1	9	10	1963–68
USA	12	0	25	37	1961–68
Total	12 (20 %)	1 (2 %)	47 (78 %)	60	
			Contacts [b]		
Hungary	1	2	1	4	1961–67
Romania	0	0	3	3	1963–68
USA	1	3	1	5	1961–68
Total	2	5	5	12	

[a] Interval between ingestion and onset, 1 month.
[b] Interval between ingestion and onset, 2 months.

TABLE 7

AGE AND SEX DISTRIBUTION OF VACCINE-ASSOCIATED CASES LISTED IN TABLE 6

Country	Age	Sex		Total cases
		M	F	
Denmark	5–25 years	2	2	4
Hungary	6–12 months	3	4	7
Japan	5 and 6 months	2	0	2
Romania	7 months–2 years	—[a]		10
USA	0–20 years	14	6	20
	⩾ 21 years	12	5	17

[a] Data not available.

Each of the countries listed has recorded more cases associated with type 3 than with types 1 and 2 in vaccinated persons. Though the numbers were smaller, a similar preponderance of type 3 cases was observed when the analysis (not reproduced in the tables) was restricted to cases where, in addition to a monovalent vaccine being fed and the same type isolated from stools, there was evidence of increase of antibody to this single type.

In contacts type 2 and type 3 strains were equally represented and type 1 was isolated least often. The number of observations on contacts suitable for inclusion in this table was, however, too small to permit conclusions to be drawn on the significance of the type distribution.

It has been repeatedly emphasized, with good reason, that the virus isolated may not be the virus causing the paralysis, and that the isolations may simply be an indication of the relative capacity of the vaccine strains to multiply in the gut, or of the length of time excretion continues after implantation of the virus.

The quality of the information from different observers varies greatly and the data available are seldom complete in all respects. It is stated in the Report to the Surgeon General of the USA by the group which made a special investigation in that country (Special Advisory Committee on Oral Poliomyelitis Vaccine, 1964) that " The Committee recognizes that it is not possible to prove that any individual case was caused by the vaccines and that no laboratory tests available can provide a definitive answer ".[1] It is therefore essential to consider as a whole the clinical, epidemiological and virological patterns of the observations and to take into account the intensity of the investigations which are made before reaching a conclusion that there is a relationship, other than purely temporal, between the vaccination and the disease.

It is believed that the data presented here, taken as a whole, provide evidence that vaccine-related cases of poliomyelitis have occurred and that type 3 has been more often associated with them than have types 1 and 2.[2] Information on the incidence of vaccine-associated cases in relation to quantities of vaccine distributed or numbers of persons vaccinated is given in the Annex. From the data available it is impossible to make an accurate estimation of the risk but it is clear that it is very slight. It in no way diminishes the paramount need for the establishment of regular and effective programmes in all countries for the vaccination with live vaccine of persons presumed susceptible to the disease, but it emphasizes the need for the full investigation of cases of poliomyelitis which occur soon after vaccine has been administered and the need for an international scheme for the collection from different countries of information from which valid comparisons can be made and any untoward risk in a particular situation can be determined and dealt with.

LABORATORY OBSERVATIONS

In view of the epidemiological evidence that type 3 is more often related to post-vaccination paralysis than types 1 and 2, the laboratory data on the immunogenicity, stability and neurovirulence of the Leon $12a_1b$ strain (Leon) and the WM-3 strain of type 3 are reviewed below and the results are reported of special studies carried out in connexion with the consultation following which the present memorandum was prepared.

IMMUNOGENICITY AND GENETIC STABILITY OF THE LEON STRAIN

The immunogenicity and genetic stability of the attenuated poliovirus strains with special reference to the Leon virus have been reviewed recently by Vonka and his colleagues (1967), who give an extensive list of references which should be consulted by those who wish to have more complete information than is provided by the selected data given here.

Immunogenicity

Duration of virus excretion. In spite of numerous extensive studies on virus excretion after feeding oral

[1] However, strong evidence of a causal relationship may be obtained in a fatal case by the isolation of a vaccine-like strain from the tissues of the central nervous system.

[2] Dr A. B. Sabin did not find it possible to accept that the data presented constituted adequate evidence of a causal relationship between vaccination and the occurrence of spinal paralysis clinically characteristic of poliomyelitis. He prepared a separate statement which is published on page 947 of this issue.

poliovirus vaccine, not many data are available in which the relative duration of excretion of types 1, 2 and 3 is compared. This is due to factors such as the successive administration at relatively short intervals of all 3 viruses to the same subjects, the use of divalent or trivalent vaccines, differences in the immunity status and age of the vaccinees and interference by non-poliomyelitis viruses. With monovalent vaccines the findings on the length of excretion of the 3 types are at variance. For example, Smorodincev and his colleagues (1959) observed no marked differences between the types, but Gelfand and his colleagues (1959) reported that both the pharyngeal and the faecal excretion of type 3 virus persisted for a longer period than the excretion of type 1 or type 2, and they found a higher percentage of family contacts infected with type 3 than with the other types. Benyesh-Melnick et al. (1967) observed that 5 weeks after the feeding of monovalent vaccines 37% of children were still excreting type 2 virus compared with 14% excreting type 1 and 7% type 3.

Experience with trivalent vaccine indicates that type 2 infects the alimentary tract better than do types 1 and 3 and gives the highest excretion rates (Vorošilova et al., 1960; Benyesh-Melnick et al., 1967).

Alimentary tract resistance. Successful implantation of the attenuated polioviruses in the human alimentary tract decreases the susceptibility of the intestine to reinfection with homotypic viruses. Several observations show that the state of intestinal resistance following type 3 infection may be lower than that following type 1 or type 2. Drozdov and his colleagues (1961) showed that children with antibody after natural infection with type 3 could be readily reinfected with the homologous vaccine virus. Sabin (1959b) rechallenged groups of children with homologous viruses 2 years after the first feeding and detected the strongest resistance to type 2 and the weakest to type 3. Although all children re-fed with type 3 were reinfected, the duration of excretion was shorter than after the first feeding, but the peak titres of virus per gram of stool were the same as or only slightly lower than after the first feeding. There was some evidence in these studies that the multiplication of the type 1 and type 3 viruses given originally had been interrupted by the administration of the succeeding dose of virus whereas the type 2 virus, which was given last, multiplied for 8 weeks or longer. Sabin (1960) also found that in a few individuals there was evidence of growth of the type 3 poliovirus for 3–4 weeks without development of antibody and that when one such subject was challenged 2 years later with homologous virus the intestinal tract showed marked resistance to reinfection.

A single dose of trivalent vaccine, which frequently results in only limited replication of type 3 virus, also induces a lower alimentary tract resistance to challenge with type 3 than to challenge with types 1 and 2. For example, Vorošilova and her colleagues (1960, 1961) studied naturally immune children as well as those previously fed with trivalent vaccine, and observed that, when challenged with trivalent vaccine, they excreted type 3 more frequently than types 1 and 2.

The very important question of the possible differences in the alimentary tract resistance to homologous and heterologous viruses *of the same type* has been almost neglected. In the single study reported Janda and his colleagues (1967) challenged children, fed previously with Leon type 3, with homologous virus (Leon) and heterologous virus (USOL-D bac strain). The heterologous virus was excreted more frequently, in higher titres, and for longer periods of time than the homologous virus.

Humoral immunity. An indication of the serological efficiency of attenuated polioviruses is given by the seroconversion rates obtained, by the ability of the viruses to boost pre-existing antibody, by the levels of the titres of antibodies that develop, and by the persistence of antibody. Differences between the 3 types have been reported, but it is often difficult to draw definite conclusions, the data having been obtained after different vaccination schedules, in different epidemiological conditions, and in subjects who differed in their natural immunity or in their previous history of Salk vaccination. Also, the techniques of the tests for antibody have varied from laboratory to laboratory. In some instances the results of the neutralization test seem to have been influenced by the antigenic relationship between the vaccine virus and the virus used for the assay. It has been reported that antibody detectable by the metabolic inhibition (pH) test but not by the test for cytopathic effect (the CPE test) develops more frequently against type 3 than against type 1 or type 2 (Sabin, 1959a); that is, antibodies against types 1 and 2 may be detected in both the pH and the CPE test, but antibodies against type 3 may be detected in the pH test only.

Selected examples from the literature of responses to different types of vaccine viruses are summarized below:

Authors	Seroconversion rate (%) Type 1	Type 2	Type 3	Vaccine
Ramos Alvarez, Bustamante & Alvarez Alba (1960)	84	81	73	Monovalent vaccines
Smorodincev et al. (1960)	97	100	96	Monovalent vaccines
Skovránek et al. (1959)	95	83	85	Monovalent vaccines
Rathbun et al. (1962)	89	80	61	Monovalent vaccines
Sabin et al. (1961)	100	100	100	Monovalent vaccines
Japan ... Vaccine Administration Subcommittee (1966)	96	81	88	Monovalent vaccines
Benyesh-Melnick et al. (1967)	93	100	74	Monovalent vaccines
Vorošilova et al. (1960)	65	80	57	Trivalent vaccine (1 dose only)
Sabin et al. (1960)	68	82	43	First dose, trivalent vaccine in area of high enterovirus infection *
Smorodincev et al. (1960)	97	75	46	Trivalent vaccine (1 dose only)
Gsell & Wiesmann (1962)	84	83	72	First dose, trivalent vaccine **

* After a second dose, seroconversions reached 96% for type 1, 96% for type 2 and 72% for type 3.

** After a second dose, seroconversions reached 100% for type 1 and type 2 and 95% for type 3.

These results indicate that when monovalent vaccines were used the seroconversion rate for type 3 was often lower than for types 1 and 2. But monovalent type 3 vaccine when fed alone has sometimes given a conversion rate of 100% (Sabin et al., 1961). A single dose of trivalent vaccine usually gives a poor seroconversion rate for type 3 but this is an indication that type 3 often fails to infect when in competition with types 1 and 2 in the gut. When trivalent vaccine is given and type 3 virus is excreted in the stools the antibody responses are as frequent to type 3 as to types 1 and 2. In practice trivalent vaccine is always administered in multiple doses and usually gives seroconversion rates of 90% or more.

Persistence of antibodies. The most important data on the differences in immunological activity of the three viruses come from studies on the persistence of antibodies, particularly when tests were done in the same laboratory using the same serological techniques. Several of the reports below indicate that the titres of type 3 antibody may drop to low or undetectable levels earlier than the type 1 or type 2 antibody titres:

Authors	Time of sampling after feeding Type 3	Percentage of subjects without antibody to: Type 1	Type 2	Type 3	Vaccine and vaccinees
Záček et al. (1962)	2 months	2	2	4	Monovalent vaccines;
	14 months	7	4	18	age of vaccinees, 2–8 years
Melnick et al. (1969)	½ year	15	15	7	Monovalent vaccine;
	5 years	28	9	46	age of vaccinees, 1–3 years
	½ year	2	5	14	Monovalent vaccine;
	5 years	17	7	41	age of vaccinees, 5–9 years
Sabin (1965)	8 years	0	0	0	Monovalent vaccines
Melnick (unpublished)	Variable	8	4	37	Students who had been repeatedly immunized with Salk and Sabin vaccines before entering medical college

Genetic stability

Rct/40 and d *marker characteristics.* Leon type 3 poliovirus is genetically less stable than the Sabin type 1 and type 2 viruses, both *in vitro* and *in vivo*. This has been a general experience of manufacturers of poliovirus vaccine and also of investigators studying the stability of the attenuated polioviruses under experimental conditions. Some of the data on the rct/40 and *d* characters of the vaccine strains after passage in the human alimentary tract are summarized below:

Authors	Percentage reversion to + character: rct/40 marker Type 1	rct/40 marker Type 2	rct/40 marker Type 3	d marker Type 1	d marker Type 2	d marker Type 3
Benyesh-Melnick & Melnick (1959)	0	0	42	26	72	91
Verlinde & Wilterdink (1960)	0	0	50	—	—	—
Gendon et al. (1961)	0	7	41	—	—	—
Toyoshima et al. (1963)	0	0	78	—	—	—
Benyesh-Melnick et al. (1967)	0 *	0 *	47 *	36 *	25 *	68 *
	0 **	0 **	13 **	18 **	30 **	50 **
Japan ... Marker Test Subcommittee (1968)	1	6	25	56	63	65

* After monovalent vaccine.

** After trivalent vaccine.

In all studies a much higher reversion rate to the rct/40+ character for type 3 than for type 1 or 2 was observed. Benyesh-Melnick and her colleagues (Benyesh-Melnick & Melnick, 1959; Melnick & Benyesh-Melnick, 1960; Benyesh-Melnick et al., 1967) also observed a higher reversion rate for the *d* + marker for type 3, although the Japan Live Poliovaccine Research Commission, Marker Test Subcommittee (1968) found high rates for this marker in all 3 vaccine strains.

Antigenic stability

As shown in Table 8 the antigenic stability of the type 3 Leon strain is in marked contrast to the antigenic lability of the type 1 LSc2ab strain.

Thus, intratypic serodifferentiation should be useful for the identification of Leon progeny. However, wild type 3 viruses have been found which are indistinguishable from the vaccine virus in their reaction with antiserum. Some of these viruses could be differentiated from the vaccine virus in reciprocal tests. It is therefore advisable to use reciprocal tests when investigating viruses suspected of inducing paralytic disease and having Leon characteristics. If wild type 3 viruses are present in the population before the administration of type 3 vaccine, their antigenic character should be established.

IMMUNOGENICITY AND GENETIC STABILITY OF THE WM-3 STRAIN

The WM-3 strain has also been thoroughly studied and has been used for vaccine production for several years in Croatia, Yugoslavia. It was derived from the W-Fox strain by passage 27 times at 23°C in monkey-kidney cells followed by 10 passages at 37°C. (Plotkin et al., 1961). It was found by these authors to be more stable than the W-Fox or the Leon strain. Böttiger (1968) has found that isolates of virus from children fed WM-3 are less able to replicate at 39.2°C than the virus isolated from children fed Leon virus. Similar results have been obtained by Ikić (1966).

STUDIES ON NEUROVIRULENCE

Neurovirulence of the Leon strain

Gendon et al. (1961), Čumakov et al. (1961), Tyufanov et al. (1963) and Winter & Boulger (1964) have shown that passage of the Leon virus in cell cultures gives progeny of increased neurovirulence. Some batches of Leon vaccine which have undergone 3 *in vitro* passages have been discarded as having unacceptably high neurovirulence. Similar results obtained with virus isolated in tissue culture from vaccinated persons confirm that the increase of neurovirulence for type 3 progenies is greater than for type 1 or type 2 progenies. Some authorities are cited in the following tabulation, which shows, except where otherwise indicated, the number of isolates inducing clinical poliomyelitis/the number of viruses tested:

Authors	Observation: Type 1	Type 2	Type 3
Sabin (1959a)	2/10	4/12	9/11
Verlinde & Wilterdink (1960)	1/18	0/16	10/20
Gendon et al. (1961)	1/40	4/28	8/17
Egashira, Uchida & Shimoje (1967)	4/5 *	0/3 *	3/7 *
Benyesh-Melnick et al. (1967)	0/28 **	1/20 **	13/32 **

* When based on lesion-index, type 3 progenies most virulent.

** No. of monkeys with clinical signs/No. of monkeys inoculated (usually monkeys inoculated with each isolate).

Only Egashira and his colleagues (1967), who examined a small number of isolates, reported a higher level of neurovirulence for type 1 than for type 3; however, on the basis of histological studies these authors concluded that the type 3 virus progenies were the more neurovirulent.

TABLE 8

STABILITY OR LABILITY OF INTRATYPIC CHARACTERS OF TYPE 3 LEON AND TYPE 1 LSc2ab STRAINS DURING HUMAN PASSAGE

Stability of type 3 Leon		Lability of type 1 LSc2ab	
Author	Observations	Author	Observations
Nakano, Gelfand & Cole (1966)	(a) Of 164 vaccine-derived viruses, 155 (94%) were classified as vaccine-like by both the Wecker and the McBride techniques. None was classified as non-vaccine-like by both techniques. (b) No change in intratypic antigenic character was observed in isolates collected between 2 and 28 days after infection of 5 children. (c) 108 wild type 3 viruses were tested. Of 32 viruses isolated from paralysed patients before 1962, 4 were vaccine-like by both techniques. Of 30 strains isolated from paralysed patients after 1962, 6 were vaccine-like by both techniques. Of 46 wild viruses isolated from healthy subjects, 3 were vaccine-like by both techniques. 11 wild "vaccine-like" viruses were tested in reciprocal tests: 6 of these could be differentiated from the vaccine virus in this way.	Gelfand, Nakano & Cole (1962)	An antigenic shift during human passage was observed with both the Wecker and the McBride techniques. Nearly all the isolates in the first week after vaccine administration were vaccine-like. Thereafter the percentage of "non-vaccine" viruses increased with time after vaccination.
		Vonka, Janda & Šimon (1962)	About half of the 22 viruses isolated from the vaccinees or their contacts within 14 days after the time of vaccine administration were classified as "non-vaccine-like" using the Wecker technique.
		Woods, Weiss & Robbins (1962)	Using agar diffusion and McBride tests, the authors observed changes in the intratypic antigenic marker. The virus isolated on Day 6 after vaccine was fed was vaccine-like; the viruses isolated on Days 13 and 44 were antigenically different.
Furesz et al. (1964)	7 type 3 viruses isolated from paralysed patients with known contact with the vaccine were investigated using the McBride technique; 2 of these were antigenically different from Leon virus. The authors consider one of them as wild virus, because of its high neurovirulence.	Wasserman & Fox (1962)	Instability of the intratypic antigenic marker was demonstrated by the McBride technique. Isolates recovered up to 10 days after vaccination were vaccine-like. From 10 days onwards, the isolates differed antigenically from the LSc2ab virus with increasing frequency. After 6 weeks, no vaccine-like viruses were recovered.
Furesz et al. (1966)	After 7 passages in the human alimentary tract, the virus retained its intratypic antigenic character.		
Deibel & Macdonald (1968	(a) Of 45 viruses from persons with known contact with the vaccine, 40 were vaccine-like. (b) Of 40 strains from persons with no known recent contact with the vaccine, 1 was vaccine-like in reciprocal test. (c) Serial specimens from vaccinees were investigated; a slight antigenic shift was observed in 2 out of 6.	Ozaki et al. (1965)	At 1 week after vaccination, strains were vaccine-like, but at 4 to 9 weeks they had shifted antigenically. The elution marker also shifted in the course of vaccine-virus multiplication in the vaccinated children, but not as much as the antigenic marker. (While type 1 vaccine and virulent strains were readily distinguished by their elution patterns, no differences were noted for vaccine and virulent strains of types 2 and 3.)

Comparisons of seed viruses at different passage levels, and of vaccines. Employing the intraspinal method of inoculating monkeys, a special study was carried out for this consultation in one laboratory in which the neurovirulence of Leon seed viruses of different passage levels from 18 vaccine producers and of some vaccine lots were tested. The WM-3 vaccine strain and the USOL-D bac and Glenn candidate strains were also tested.

The passage history in cell cultures of the Leon seed strains is shown in Table 9. The variations in the passage history of these seed lots should be noted. Only some of the strains were included in the tests (they are listed in Table 11 below).

Test method. In the intraspinal neurovirulence tests in cynomolgous monkeys (Boulger & Perkins, 1966, 1967), a range of vaccine dilutions is used in order to obtain a dose-response curve from "maximum effect" to "no effect", and a quantal response is used to construct the activity curve. The percentage of monkeys in each group which show viral activity is recorded and the 50% end-point of effect is calculated from the steepest part of the dose-response curve because this gives the most sensitive index of variation. Thus the neurovirulence of a strain is expressed as the number of tissue culture infective doses of poliovirus required to produce histological poliomyelitis in 50% of mon-

TABLE 9

PASSAGE LEVELS OF LEON SEED VIRUS USED TO PRODUCE SABIN POLIOVIRUS VACCINE

Producer	Passage level and period of use of seed of indicated type [a]		
	Type 1	Type 2	Type 3
1	Not known	Not known	SO + MAN
2	SOM +1 (1959-63)	SOM +1 (1959-63)	SOM +1 (1959-62) SOM+4 (1962-63)
3	SOM +1 (1961-)	SOM +1 (1961-)	SOM +1 (1961-)
4	SOM +1 (1961-)	SOM +1 (1961-)	SOM +1 (1961-)
5	SOM +1 (1960-)	SOM +1 (1960-)	SOM +1 (1961-64) SO + MAN (1964-)
6	SOM +2 (1960-62)	SOM +2 (1960-63)	SOM +2 (1960-62)
7	SOM +2 (1960-)	SOM +2 (1961-)	SOM +1 (RNA extract)
8	SOM +1 (1960-)	SOM +1 (1960-)	SOM +1 (1960-)
9	SOM +1 (1963-)	SOM +1 (1963-)	SOM +1 (1963-)
10	SOM +1 (1960-)	SOM +1 (1960-)	SOM +1 (1960-)
11	SOM +1 (1962-64) SOM +2 (1964-66)	SOM +1 (1962-63)	SOM +1 (1963-) SO + MAN (1965-66)
12	SOM +1 (1961-)	SOM +1 (1962-)	SOM +1 (1962)
13	SOM +1 (1964-)	SOM +1 (1964-)	SOM +1 (1964-)
14	SOM + 2 (1963-)	SOM + 2 (1963-)	SO + MAN + 1 (1963-)
15	SOM + 3 (1961-)	SOM + 3 (1961-)	SOM + 2 (1961-)
16	SOM + 1 (1966-)	SOM + 1 (1967-)	SO+(Led.1+1)+1(1967-)
17	SOM + 5 or 6	SOM + 6	SOM + 5 or 6
18	SOM+5 or 6+1(1968-) SO+MAN+2 (1969-)	SOM + 6 + 1 (1968-) SO+MAN+2 (1969-)	SOM + 5 or 6 + 1 (1968-) SO + MAN + 2 (1969-)

[a] SO = Sabin original seed virus.
SOM = 25-litre lot prepared from SO by Merck, Sharpe & Dohme in one passage and used in world-wide large-scale field trials.
SOM + 1 = Seed lots prepared by individual manufacturers to free SOM from SV40.
SO + MAN = SO given directly by Dr Sabin to manufacturers other than Merck, Sharpe & Dohme and seed prepared in one passage.
SO + (Led 1+1) + 1 = SO given to Lederle and after 2 passages by Lederle supplied to other manufacturer who made seed by one further passage.
SOM +2 etc. = Number of passages from SOM.

keys; the lower the dose, the higher the neurovirulence.

The 50% end-points of activity are used to ensure that the results of a single test of a vaccine virus do not differ by more than twice the standard deviation from the arithmetic mean of tests made on the reference homotypic Sabin strain.

Reproducibility of the test. In order to estimate the reproducibility of the test the data from 8 tests in cynomolgous monkeys made over a period of 8 years in the laboratory with a single preparation (SOM) [1] were collated and are shown in Table 10. The results were so similar that the arithmetic mean for all tests could be given and the 95% confidence limits calculated. It was not possible to compare the incidence of clinical poliomyelitis between tests because of the absence of specific weakness or paralysis. This low incidence with the Leon virus is sufficiently constant to differentiate it from the Sabin type 1 and 2 strains (Boulger & Perkins, 1967). As the paralytogenic activity of strains increases,

[1] Defined in the footnote to Table 9.

TABLE 10

RESULTS OF 8 NEUROVIRULENCE TESTS ON THE LEON SEED VIRUS (SOM) [a] USING 222 CYNOMOLGOUS MONKEYS INOCULATED INTRASPINALLY

Year of test	No. of monkeys	Injection trauma [b]	No. of monkeys with clinical poliomyelitis	Lumbar lesion ratio [c]		Brain lesion ratio [c]	
				Glial	Neuronal	Glial	Neuronal
1961	30	0.48	2	4.2	5.3	5.3	6.0
1962	29	0.30	1	3.7	5.0	5.3	5.8
1963	23	0.39	1	3.1	5.3	5.6	6.0
1964	30	0.29	2	3.1	5.0	5.0	5.9
1965	28	0.28	1	4.2	5.8	5.6	5.8
1966	26	0.37	0	4.0	5.1	5.6	5.8
1967	28	0.33	1	4.4	5.1	5.3	6.0
1968	28	0.39	4	3.5	4.5	5.1	⩾6.4
Arithmetic means		0.35	1.5	3.8	5.1	5.4	6.0
95 % confidence limits		0.21–0.49	0.3–2.7	2.8–4.8	4.4–5.8	4.9–5.9	5.6–6.4

[a] See footnote to Table 9.
[b] Proportion of the 14 sections of lumbar cord per monkey showing correct inoculation.
[c] Ratio between histological 50 % lesion dose and infectivity titre ($TCID_{50}$) in cell culture; mean virus dose: 6.4 $\log_{10} TCID_{50}$.

decreased lumbar neuronal lesion ratios [1] are obtained. In other words, the lower the virus dose giving a 50% activity, by histological criteria, the higher the neurovirulence of the strain.

Comparison of neurovirulence of the Leon progeny. From the results of the relatively small number of tests reported in the upper part of Table 11 there is some evidence of a tendency to produce virus of increasing neurovirulence when the number of passages of the virus in tissue culture is increased. Exceptions are the RNA-derived seed virus and vaccine, but this vaccine, when fed to children, is known to revert towards neurovirulence.

In order to provide a clearer picture of the relation between increase in the number of passages and increasing neurovirulence, the results of the tests in the upper part of Table 11 have been added to the results of 29 tests of Leon strain seeds or vaccines produced at various passage levels. They are shown in Fig. 3. The results of tests with the WM-3, Glenn and USOL-D strains of type 3 virus are included. For the Leon strains the passage level is shown simply as SO (Sabin original) plus the subsequent number of passages. Seeds are shown separately from vaccines. Each symbol in Fig. 3 represents one test.

[1] Defined in the footnotes to Table 10.

The data in this figure confirm the trend seen in Table 11—namely, that increasing the number of passages increases the neurovirulence of the strains. The conclusion from these studies, all made in one laboratory, is that neurovirulence for monkeys is enhanced by passage of the strains. It does not necessarily follow, however, that increased simian neurovirulence is an indication of an increased risk of inducing paralysis in human beings fed with these strains.

Results in other laboratories. These findings are supported by other studies.

1. SO+2 seed was used by one manufacturer for the production of 8 batches of vaccine (i.e., the vaccine virus had undergone 3 passages). Three of the batches were rejected because the neurovirulence had increased.

2. In a laboratory which uses the intrathalamic route of inoculation of rhesus monkeys more than 1000 monkeys (30 monkeys per test) were given undiluted vaccine. It was found that with the reference preparation (type 1 poliovirus NA-2) 6.2% of monkeys showed histological evidence of poliomyelitis and none showed clinical disease; 3.8%, 1.3%, 0.1% and 0.0% showed histological lesions graded 1, 2, 3 or 4 respectively (Kirschstein et al., 1960). In

TABLE 11

RESULTS OF COMPARATIVE NEUROVIRULENCE STUDIES ON TYPE 3 POLIOMYELITIS VIRUSES INJECTED INTRASPINALLY INTO CYNOMOLGOUS MONKEYS

Seed or vaccine [a]	No. of monkeys	Virus dose ($TCID_{50}$)	Injection trauma [b]	No. of monkeys with clinical poliomyelitis	Lumbar lesion ratio [c]		Brain lesion ratio [c]	
					Glial	Neuronal	Glial	Neuronal
Studies on Leon virus (518 monkeys)								
SO+MAN	29	6.5	0.27	0	4.7	6.0	6.5	6.5
SO+MAN+1 [d]	26	6.7	0.28	1	4.6	5.8	5.8	6.4
	24	6.1	0.38	2	3.8	4.7	5.7	5.9
RNA seed (SOM+4)	34	6.8	0.40	0	3.6	6.0	6.8	6.8
RNA vaccine (SOM+5) (arithmetic mean of 12 tests)	363	6.6	0.36	0.7	4.0	5.6	6.4	6.6
SOM+6 [e]	22	5.6	0.39	2	3.2	4.6	4.9	5.5
	20	5.6	0.32	2	3.4	4.2	4.5	5.3
Studies on type 3 viruses other than Leon								
WM-3	35	6.4	0.22	5	3.1	3.6	4.2	4.6
Glenn seed	33	4.8	0.36	0	3.8	>4.8	>4.8	>4.8
Glenn vaccine	27	5.3	0.39	0	4.0	>5.3	>5.3	>5.3
USOL-D bac seed [f]	26	6.3	0.42	2	4.0	5.1	>6.3	>6.3
USOL-D bac vaccine								
Test 1	25	6.3	0.35	3	4.2	5.6	6.0	>6.3
Test 2	27	6.3	0.47	2	4.3	5.4	6.0	>6.3
Test 3 [g]	27	7.2	0.23	4	4.1	5.0	5.9	6.3
Reference study								
Reference SOM	222	6.4	0.35 (0.21–0.49)	1.5 (0.3–2.7)	3.8 (2.8–4.8)	5.1 (4.4–5.8)	5.4 (4.9–5.9)	6.0 (5.6–6.4)

[a] For definition see footnote to Table 9.
[b] Proportion of the 14 sections of lumbar cord per monkey showing correct inoculation.
[c] Ratio between histological 50 % lesion dose and infectivity titre ($TCID_{50}$) in cell culture.
[d] Tests with vaccines produced by 2 different manufacturers.
[e] 2 tests on seed from same manufacturer.
[f] After being freed from SV40 virus.
[g] Original Vonka seed + 1 passage; SV40 virus present.

contrast, 24 consecutive lots of type 3 attenuated poliovaccine made from seed virus SO+2 were tested and it was found that 12.6% of monkeys had histological lesions, 5% had clinical disease and 9.7% had lesions with severity of grades 3 or 4. All these batches were rejected as having an unacceptably high neurovirulent activity. When vaccine was made from SO+1 seed 22 consecutive lots were found to be less neurovirulent than the reference type 1 preparation (1.5% showed histological lesions and none clinical disease).

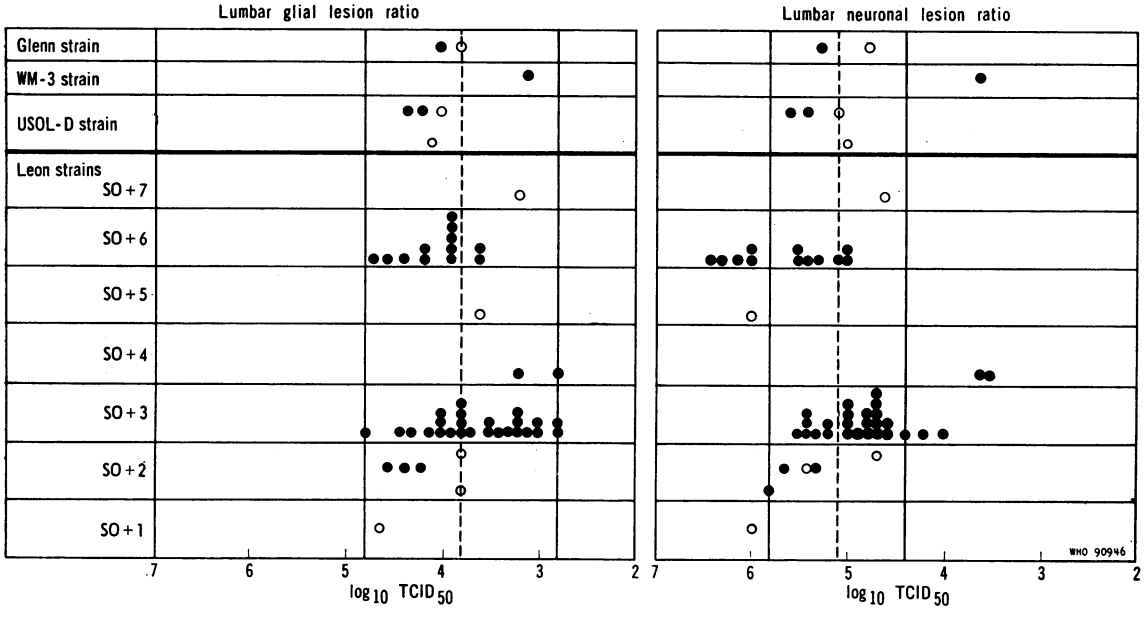
3. In another laboratory type 3 vaccines prepared from seed virus SO+6 or 7 were studied by inoculation of monkeys intrathalamically, intraspinally and intramuscularly. It is shown in Table 12 that 6.7% of monkeys had histological lesions and 2.5% clinical disease when injected with undiluted virus intrathalamically.

Comparison of neurovirulence of Leon with other attenuated type 3 strains

The other vaccine strain WM-3 and candidate type 3 strains USOL-D bac and Glenn were also titrated by the intraspinal test in the first-mentioned

FIG. 3

NEUROVIRULENCE OF LEON AND OTHER SEEDS AND VACCINE VIRUSES INJECTED INTRASPINALLY INTO CYNOMOLGOUS MONKEYS [a, b]



[a] SO + 5 and SO + 6 are RNA-derived virus.

[b] The vertical broken lines represent the arithmetic means and the vertical continuous lines the 95 % confidence limits of the 8 tests of strains used for reference and shown in Table 10.

o Seed viruses. • Vaccine lots.

laboratory. The results (see the middle portion of Table 11 and Fig. 3) show that the WM-3 strain is more neurovirulent than Leon but that the Glenn and USOL-D bac strains are not significantly different in neurovirulence from the Leon strain.

So far as the WM-3 strain is concerned, previous studies on neurovirulence in different laboratories are not in agreement. Pagano & Hoskins (1967) compared WM-3 with the Leon strain and concluded that the 2 strains were similar in neurovirulence but studies in 3 other laboratories have shown the WM-3 strain to be more virulent than the reference preparations or the Leon strain. Ikić (1965) found that WM-3 virus was excreted from the gut of vaccinees for a shorter period than the Leon virus, whereas MacLeod et al. (1967) showed that the neurovirulence of the excreted virus was increased to a degree similar to that of the Leon strain after human passage.

The vaccine has proved immunogenic and free from harmful effects; more than 10 million doses have been given.

Correlation of in vitro *and* in vivo *markers.* In one laboratory it was found that Leon type 3 strains with reverted genetic markers had greater neurovirulence than type 1 and type 2 strains which had reverted (Table 13). Sixteen strains that had reverted to "*d*+, rct/40+" were tested by giving only 100 $TCID_{50}$ of virus by the intrathalamic route to each of 2 monkeys. Of the 32 monkeys in the test, 13 developed paralysis and 20 developed characteristic lesions in the spinal cord. These observations were confirmed by the intraspinal inoculation of monkeys, using the faecal material obtained from vaccinated children. Since there was no tissue-culture passage, alteration of the viral genetic material by laboratory manipulation could not have occurred. There was an increase in virulence in strains that reverted in the *d*+ marker, and an even greater increase in virulence in those that reverted in both the *d*+ and the rct/40+ markers. This was found even though the dose of virus was very small, in some cases less than 10 $TCID_{50}$.

TABLE 12

COMPARATIVE DATA ON THE FREQUENCY OF POLIOMYELITIS IN MONKEYS INOCULATED BY DIFFERENT ROUTES WITH TYPE 3 LIVE POLIOVIRUS VACCINE PREPARED AND TESTED IN THE SAME LABORATORY

Virus type	Inoculation route	Undiluted			Dilution 10^{-1}			Dilution 10^{-2}			Dilution 10^{-3}			Dilution 10^{-4}			Total No. of monkeys	Those with poliomyelitis (%)	
		No. of monkeys	Poliomyelitis (%)		No. of monkeys	Poliomyelitis (%)		No. of monkeys	Poliomyelitis (%)		No. of monkeys	Poliomyelitis (%)		No. of monkeys	Poliomyelitis (%)			Clinically	Histologically
			Clinically	Histologically		Clinically	Histologically		Clinically	Histologically		Clinically	Histologically		Clinically	Histologically			
3	Intrathalamic	623	2.5	6.7	399	1.0	3.2	—	—	—	—	—	—	—	—	—	1 022	1.95	5.38
3	Intraspinal	40	25.6	36.0	69	10.1	40.5	36	11.1	27.7	24	8.3	33.3	21	0	18.1	190	12.1	33.15
3	Intramuscular	32	0	0	28	0	0	—	—	—	—	—	—	—	—	—	60	0	0

TABLE 13

GENETIC MARKERS AND VIRUS DOSE FOR INTRACEREBRAL NEUROVIRULENCE OF VACCINE VIRUS AFTER HUMAN PASSAGE FOLLOWING FIRST PASSAGE IN MONKEY KIDNEY TISSUE CULTURE [a]

Virus type and dose ($TCID_{50}$)	Markers	No. of animals tested [b]	No. of animals with positive response	
			Clinical signs	Pathological lesions
Type 1	*d*−T−	6	0	4
($10^{8.5}$)	*d*+T−	22	0	12
Type 2	*d*−T−	6	0	1
($10^{4.0}$)	*d*+T−	14	1	5
Type 3	*d*+T+	32	13	20
($10^{2.0}$)				

[a] Reproduced, by permission, from Benyesh-Melnick et al. (1967).

[b] Usually, 2 animals per strain.

CONCLUSIONS

In the report of the WHO Scientific Group on Human Viral and Rickettsial Vaccines (1966) it was stated that " it is abundantly clear that the oral [poliovirus] vaccines are among the safest of live antigens in use ". The present review lends added support to that conclusion and to the contention that regular programmes for the vaccination of susceptible persons should be continued in the countries which have already established them and should be developed in those countries which have not yet done so.

However, the accumulated evidence supports earlier observations that a few vaccine-related cases occur and that the type 3 vaccine strain is much more frequently associated with these cases than the type 1 and type 2 strains.

At present it is difficult to compare the epidemiological data on vaccine-related cases from different countries because of the variation in the intensity of investigation and the diversity of methods of collecting and recording the information. It is obviously important to facilitate the collection from different countries of data on which valid comparisons can be made. Agreed criteria for accepting a case as vaccine-associated or vaccine-related need therefore to be established and a uniform scheme developed for the investigation and reporting of cases of spinal paralysis in countries where live poliovirus vaccines are used extensively. This scheme

should include epidemiological, clinical and laboratory information and information on the type and quantity of vaccines used and on the time of year at which vaccine is given. Special attention needs to be paid to the problem of using live virus vaccines in persons with immunodeficiencies and similar rare diseases of the host.

In many reports on serological findings in the vaccinated the old notation of antibody titres is still employed. More uniformity in the results, both between studies in the same country and between studies in different countries, would be achieved if results were reported in International Units (Lyng & Weis Bentzon, 1963; WHO Expert Committee on Biological Standardization, 1963).

There is no clear evidence that vaccines made from seed of a high passage level are more likely to be associated with paralytic cases in man than vaccines made from seed of a lower passage level. Nevertheless the neurovirulence test in monkeys can differentiate between such vaccines. It is necessary, therefore, to make vaccines, particularly type 3 vaccines, from seed lots with the lowest passage level from the original virus and to make available a satisfactory low passage seed lot to new producers and to those wishing to change the type 3 seed lot that they are currently using.

The study and evaluation of current marker tests and the development of new marker tests merit encouragement, not only for the control of vaccine production but also for investigation of excreted virus to determine genetic stability on passage in man.

A collaborative study, possibly through the WHO reference and research centres, could usefully be set up to obtain more uniformity in methods of carrying out marker tests. It is particularly important to have both positive and negative reference virus suspensions for inclusion in each test, and these need to be prepared and made available, especially when laboratory investigations on vaccine-associated cases are carried out.

A collaborative investigation of the neurovirulent characteristics of coded vaccines was initiated in 1966 by the Permanent Section on Microbiological Standardization of the International Association of Microbiological Societies. The laboratory work has now been completed and when the analysis of the data is available it may be necessary to make a reassessment of current neurovirulence tests.

Further studies are needed to find a type 3 vaccine virus which is genetically more stable, which gives higher and longer-lasting antibody response, and which provides a greater intestinal resistance to reinfection than Leon $12a_1b$.

Annex

INCIDENCE OF VACCINE-ASSOCIATED CASES

Based on the numbers of doses of vaccine distributed or administered, estimates of the proportions of vaccine-associated cases have been made for Canada, Denmark, Hungary, the United Kingdom and the United States of America (Table 14). These estimates are based on all cases typical of paralytic poliomyelitis occurring in vaccinated persons within a stated period (usually 5–30 days) after vaccine (Sabin strains) was fed. They overestimate the risk because they include all temporally associated cases, in some of which the association must be purely coincidental.

The estimates vary considerably from country to country but in each country the risk for type 3 vaccine virus is greater than that for types 1 and 2. It may be significant that in the USA the risk from trivalent vaccine (54 million doses of which were used between 1965 and 1968) is apparently lower than the risks observed with monovalent type 1 and type 3 vaccines in 1961–64 and 1965–68. It is difficult to be certain that valid comparisons can be made between countries but it may be noteworthy that the rate for type 3 in Hungary (2.82 cases per million persons vaccinated) was much higher than that reported by other countries.

The figures are based on estimates of total amounts of vaccine distributed or on total numbers of persons vaccinated and not on the numbers of susceptible persons vaccinated. An estimate based on a presumed susceptible population has been attempted in Hungary (I. Dömök, personal communication). The rates calculated by this method were 3.8 per million for type 1, 2.4 per million for type 2 and 13.6 per million for type 3. Whatever the true rates, the risk is in fact extremely small; 89 cases have been reported in association with over 370 million doses

TABLE 14
INCIDENCE OF VACCINE-ASSOCIATED CASES IN 5 COUNTRIES

Country	Period of observation	Live vaccine type	Estimated No. of doses (millions)	No. of cases	Rate per million doses
Canada (Nagler, 1963)	1962	Trivalent	3.8	4 (type 3)	1.05
Denmark (Tulinius, 1967; modified)	1966	Type 3	2.75	4	1.45
Hungary (I. Dömök, personal communication)	1961–67	Type 1 Type 2 Type 3	3.9 4.0 3.9	3 2 11	0.77 0.51 2.82
United Kingdom (Miller & Galbraith, 1965)	1962–64	Trivalent	18.0 (3 type 3, 1 types 2 and 3)	4	0.25
USA (Special Advisory Committee on Oral Poliomyelitis Vaccines, 1964)	1961–64	Type 1 Type 2 Type 3	84.9 90.0 89.0	15 2 36	0.17 0.02 0.40
USA (M. Gregg, personal communication)	1965–68	Type 1 Type 2 Type 3 Trivalent	6.6 5.0 5.4 53.5	1 0 4 3	0.15 0.0 0.74 0.06

of vaccine (Table 14) and, as already stated, some of these cases were certainly not causally related to the vaccine.

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RÉSUMÉ

DONNÉES RELATIVES À L'INNOCUITÉ ET À L'EFFICACITÉ DES VACCINS ANTIPOLIOMYÉLITIQUES VIVANTS ACTUELLEMENT EN USAGE, NOTAMMENT EN CE QUI CONCERNE LE POLIOVIRUS DE TYPE 3

Deux aspects primordiaux de la vaccination antipoliomyélitique, l'innocuité et l'efficacité des vaccins vivants, ont été discutés lors d'une consultation de spécialistes qui s'est tenue sous les auspices de l'OMS.

On a constaté que dans les pays qui bénéficiaient de programmes bien conçus d'immunisation par vaccins vivants l'incidence de la poliomyélite avait diminué de 98,7% en douze ans. Par contre, dans beaucoup de pays tropicaux ou semi-tropicaux, où de tels programmes n'avaient pas été mis en œuvre, l'incidence de la maladie était en augmentation. Lorsque les opérations de vaccination n'étaient pas d'une ampleur suffisante, on isolait dans la plupart des cas des souches de type 1. Dans les pays où la majeure partie de la population était protégée, les isolements obtenus chez des sujets présentant ou non une atteinte du système nerveux central montraient une fréquence quasi égale des trois types.

Des cas de poliomyélite survenus au cours des opérations de vaccination ont été signalés dans 13 pays, et dans 9 d'entre eux les autorités sanitaires ont estimé qu'il existait une *relation* entre l'apparition des cas et l'administration du vaccin. Il a cependant été impossible d'apporter la preuve irréfutable d'une relation causale dans un cas précis et il a fallu considérer les faits dans leur ensemble. Leur interprétation a été plus aisée dans les pays où les vaccinations n'étaient pratiquées que pendant une brève période (généralement en hiver) de chaque année.

Un fait particulièrement digne de remarque est la prépondérance des infections par des souches de type 3 parmi les cas survenus chez les personnes vaccinées ou leurs contacts après administration d'un vaccin monovalent. Il n'a pas été possible d'évaluer le risque réel découlant de la vaccination, mais ce dernier est manifestement très faible. D'après les informations centralisées par l'OMS — et présentées dans une annexe — le total des cas de poliomyélite observés chez des personnes vaccinées à l'occasion de l'administration de plus de 370 millions de doses de vaccin est très inférieur à 89.

Si l'on prend comme critères les taux de conversion sérologique et de persistance des anticorps, on constate que le pouvoir immunogène de la souche vaccinale de type 3 (souche Leon) est inférieur à celui des souches vaccinales des types 1 et 2. De même, la stabilité génétique de cette souche, comme l'indique l'étude des marqueurs classiques, est moindre que celle des souches des deux autres types. En revanche, la souche de type 3 montre une stabilité antigénique de loin supérieure à celle de la souche de type 1. Lors d'inoculations au singe par la voie intrarachidienne ou intrathalamique, la souche Leon fait preuve d'une neurovirulence plus élevée que les souches vaccinales des types 1 et 2. D'autre part, l'étude comparative de virus de semence ayant subi un nombre variable de passages et des lots de vaccin qu'ils ont servi à préparer donne à penser que la neurovirulence augmente en fonction de la répétition des passages.

En conclusion, les participants à la réunion ont estimé que les vaccins antipoliomyélitiques buccaux étaient parmi les plus sûrs des antigènes vivants utilisés. Ils ont recommandé que des programmes réguliers de vaccination soient poursuivis dans les pays qui les ont déjà mis en pratique et que des opérations analogues soient entreprises aussi rapidement que les circonstances le permettent dans les pays qui n'en ont pas encore bénéficié. Malgré l'absence de toute corrélation nette entre le nombre de passages auxquels a été soumis le poliovirus de type 3 et l'apparition de cas de paralysie chez des enfants, il est conseillé d'utiliser pour la préparation des vaccins des lots de semence qui n'ont subi qu'un minimum de passages. Il conviendrait que les pays adoptent des critères uniformes pour la reconnaissance des cas de poliomyélite pour lesquels on suspecte une influence de la vaccination, ce qui permettrait d'utiles comparaisons. Enfin, les participants ont recommandé que les techniques utilisant les marqueurs classiques fassent l'objet d'une mise au point en vue de leur normalisation.

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