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Vaccination against Poliomyelitis in Poland with Types 1 and 3 Attenuated Viruses of Koprowski *

1. Virological Studies of the Vaccine Strains and Serological Studies of the Vaccinated Population

F. PRZESMYCKI,[1] H. DOBROWOLSKA,[2] B. MIRSKI,[3] R. STAŃCZYK,[4] H. WIÓR[5] & H. ZAŁESKA[6]

Mass vaccination of the juvenile population with live poliovirus vaccine was carried out in Poland in 1959 and 1960. In all, some 7 239 000 children 6 months to 14 years old were given type 1 (CHAT) virus, and nearly 6 818 500 children of the same age-group received type 3 (W-Fox) virus.

A serological conversion rate (from $<1:4$ to $\geqslant 1:4$) of 91.7% was achieved with type 1 and of 91.0% with type 3—a satisfactory demonstration of the immunogenicity of the strains used. Although type 2 virus was not administered, seroconversion with this type occurred in 65.4%. This may have been caused by antigenic elements common to the three poliovirus types, and previous vaccination with Salk vaccine may also have been a contributory factor.

The authors conclude that the mass vaccination described in this paper has created a highly immune population in Poland.

The first phase of the investigations in Poland on live attenuated vaccine prepared from Koprowski's strains (Przesmycki et al., 1960a, 1960b) was completed in 1958.

The first vaccinations, using type 1 vaccine, were carried out in a small town and three neighbouring villages having a total population of 8716 persons. Here 2888 individuals aged 6 months to 16 years were vaccinated; they constituted 95% of the registered population in this age-group. No symptoms of disease were observed among the vaccinated subjects. On the basis of virological and serological investigations, it was established that the vaccine has sufficiently strong antigenic properties and induces the appearance of antibodies. From the time the vaccinations were begun on 20 October 1958, not a single case of poliomyelitis infection has been observed in these localities.

On the basis of an analysis of these results, a special committee appointed in 1959 by the Minister of Health ordered that mass vaccinations against poliomyelitis using live attenuated vaccine be organized throughout Poland, according to the following plan:

(1) Children aged 6 months to 7 years must be vaccinated at least twice with inactivated vaccine before the administration of live vaccine.

(2) Children 7 years of age or older might be vaccinated with live vaccine without previous vaccination with inactivated vaccine.

The conduct of the vaccinations was assigned to the Sanitary and Epidemiological Station located in each province. The type 1 (CHAT) and type 3 (W-Fox) strain was provided by Dr H. Koprowski.

MATERIALS AND METHODS

Investigation of neuropathogenic properties in monkeys

Throughout the entire vaccination period, a single pool of type 1 vaccine and a single pool of type 3 vaccine, obtained in several batches, were used.

* From the Department of Virology, State Institute of Hygiene, Warsaw, Poland (Director: Professor Dr F. Przesmycki). This material has also been published in Polish in *Przegl. epidem.*, 1961, **15**, 213.

[1] Chief, Department of Virology, and Director, State Institute of Hygiene, Warsaw, Poland.

[2] Chief, Poliomyelitis Laboratory, Department of Virology, State Institute of Hygiene, Warsaw, Poland.

[3] Senior Assistant, Poliomyelitis Laboratory, Department of Virology, State Institute of Hygiene, Warsaw, Poland.

[4] Assistant Professor of Pathology, School of Medicine, Warsaw, Poland.

[5] Chief, Department of Communicable Disease, Ministry of Public Health and Social Welfare, Warsaw, Poland.

[6] Chief, Section of Communicable Disease Control, Ministry of Public Health and Social Welfare, Warsaw, Poland.

Every batch of vaccine employed was investigated at the State Institute of Hygiene's Department of Virology for its neuropathic activity in monkeys.

In accordance with the recommendations of the WHO Study Group on Requirements for Poliomyelitis Vaccine (Oral) (1962), the neuropathogenic activity of the vaccine was investigated both in intracerebrally and intraspinally inoculated monkeys and in intramuscularly inoculated monkeys. The vaccine was inoculated intracerebrally into the monkey in the region of the thalamus in a dosage of 1 ml; 0.1-0.3 ml of various dilutions were also inoculated into the lumbar enlargement of the spinal cord. A separate group of monkeys was inoculated intramuscularly (in the gluteal muscle) with a 10-ml dose of several batches of type 1 vaccine. After a period of 18-21 days under clinical observation every monkey investigated was sacrificed under anaesthesia and necropsy performed.

For microscopic examination, sections were taken from the spinal medulla at the level of the cervical (C5, C7), thoracic (T1), and lumbar (L3, L5) segments; from the medulla oblongata, basal nucleus, and pons (brain stem); as well as sections from the anterior, parietal, and occipital cortex, and the cortex of the cornu Ammonis, medulla oblongata, and cerebellum.

The sections were fixed in paraffin, prepared in series (of about 25-30 preparations from each level), and stained with haematoxylin and eosin.

The following criteria were set up (Stańczyk) to determine the degree of intensity of the occasional changes observed:

0 = No change observed.

1 = Chromatolysis of single motor cells, satellitosis of these cells, and slight perivascular infiltration.

2 = Neuronophagia limited to a few cells, pronounced perivascular and intramuscular infiltration.

3 = Extensive neuronophagia, cell loss, abundant perivascular and interstitial infiltration, and glial proliferation.

$\pm$ = Changes which cannot be characterized as clear evidence of poliomyelitis.

The CHAT (type 1) and W-Fox (type 3) vaccines were also investigated for the presence of virus B and lymphocytic choriomeningitis virus, according to the rules laid down for inactivated vaccines. It was found that neither virus was present.

Organization of the vaccinations and procedures used

The conduct of the vaccination project was entrusted to the provincial sanitary and epidemiological stations. Vaccinations in the individual provinces were carried out over a period of 6-12 days. At definite times, 1-ml or 2-ml ampoules containing vaccine were packed in dry-ice and sent by car to the individual provinces by the Institute of Virology. In the provincial sanitary and epidemiological stations, the vaccine was diluted in normal saline: 1/500 for type 1, which corresponds to 200 000 infective doses ($TCID_{50}$); and 1/1000 for type 3, corresponding to about 100 000 infective doses ($TCID_{50}$). From the provincial stations, the diluted vaccine was packed in ordinary ice containers and sent by car to the vaccination points, so that it could be used within a few hours of dilution.

This vaccine was preserved throughout the whole vaccination period in the refrigerator (+4°C) or in ice, and used up to 48 hours from the time of dilution. The infectious titre of the dilute vaccine samples administered locally was verified in the laboratory. In every investigation it was established that the titre of the vaccine remained at the same level as at the time of dilution.

Virological and serological studies

Virological and serological investigations were undertaken with the vaccination programme.

To determine the infectiousness for the human intestines of the virus contained in the vaccine, stool specimens collected before vaccination, 10-14 days after vaccination with type 1, and 1 and 14 days after vaccination with type 3 were investigated. Each of the four centres participating was to investigate 200 complete stools, obtained from children in the youngest group, i.e., from 6 months to 7 years old.

In order to determine the immunizing properties of the vaccine, blood samples were investigated for their content of neutralizing antibodies. For this purpose, blood samples were collected before vaccination and 30 days after vaccination with each type. Altogether, investigations were to be carried out in each centre before and after the vaccination of 500 children in various age-groups from 6 months to 15 years.

RESULTS

Investigation of neuropathogenic properties in monkeys

The infective titre ($TCID_{50}$) of several batches of the CHAT strain (type 1) ranged from log $10^{-8.3}$

to $10^{-6.8}$; that of the W-Fox strain (type 3) amounted to log $10^{-8.3}$ per 1 ml. In general, no changes in the internal organs of monkeys inoculated intracerebrally, intraspinally or intramuscularly were seen at necropsy. None were found in the central nervous system except for vestiges of the intervention in the form of haemorrhagic foci and scars faintly coloured with haemosiderin.

The results of the neurovirulence tests are given in Tables 1-3: Table 4 describes the histological lesions observed in each affected monkey.

As is evident from Table 1, type 1 (CHAT) vaccine was injected intracerebrally into 67 monkeys, among which no clinical symptoms were observed during the period of observation, although pronounced histopathological changes were noted in three.

Table 1 also gives the results of CHAT vaccine injected intraspinally into 24 monkeys. Typical clinical symptoms were noted in seven monkeys and histopathological changes in nine.

As shown in Table 4, in three monkeys which received a dosage of $10^{6.65}$ $TCID_{50}$, changes appeared chiefly in the lumbar segment, the site of the introduction of the virus. In other segments, the changes were not very pronounced. In five monkeys which received a dose of $10^{5.65}$, the changes were manifested chiefly in the lumbar segment. In one monkey, changes of slight intensity were observed in the thoracic segment only, and they must be characterized as doubtful changes. In one of the monkeys which received a dose of $10^{5.2}$ $TCID_{50}$, changes were found in other segments besides the lumbar.

Table 2 gives the results of intramuscular injection of the virus. Doses of $10^{7.85}$ to $10^{8.65}$ $TCID_{50}$ in a volume of 10 ml were given to 35 monkeys. Blood was taken after 24, 48, 72 and 96 hours. In nearly every monkey investigated, the presence of virus in the blood was established on the first and second days after vaccination, yet the titre of virus in the blood was low and remained within the range of $10^{-3.75}$ on the first day after vaccination (maximum), through $10^{2.75}$ to $10^{1.75}$ on the second day, to an inappreciable titre on the third day after vaccination. In this method, the peak level of virus in the blood (in repeated counts on the total volume of blood of the monkey) was at least 100 times lower than the level of virus introduced. It must also be emphasized that the day after vaccination the level of virus had already begun to fall, and by the fourth day the virus had already disappeared completely from the peripheral blood. The virus introduced in the vaccine had apparently remained in the blood without multiplying.

After intramuscular administration, symptoms of clinical disease appeared in four of the 35 monkeys vaccinated (Table 2). The histopathological studies (Table 2 and Table 4) showed mild or moderate changes in the central nervous system in six of the 35 monkeys vaccinated intramuscularly, which supports the results obtained by Melnick & Benyesh-Melnick (1960) and by Kirschstein et al. (1960).

Table 3 gives the results of type 3 (W-Fox) vaccine injected intracerebrally into 19 monkeys. No symptoms of clinical disease were observed, although typical histopathological changes were found in one monkey.

The same vaccine injected intraspinally into 29 monkeys (Table 3) produced typical clinical symptoms in six, and histopathological changes in seven

TABLE 1

NEUROVIRULENCE FOR MONKEYS OF TYPE 1 POLIOVIRUS (CHAT) VACCINE GIVEN BY THE INTRACEREBRAL AND INTRASPINAL ROUTES

Exp. No.	Log_{10} TCD_{50} inoculum	Species of monkey	Ratio of monkeys developing:	
			Paralysis	Polio lesions
		Intracerebral		
1	7.65	*Rhesus*	0/4	0/4
2	7.45	„	0/10	2/10
3	8.65	*Cynomolgus*	0/4	0/4
4	7.75	*Rhesus*	0/4	0/4
	6.75	„	0/4	0/4
	5.75	„	0/4	0/4
	4.75	„	0/4	0/4
	3.75	„	0/4	0/4
5	7.75	„	0/5	0/5
	6.75	„	0/5	0/5
6	6.85	(*Rhesus* and *Cynomolgus*)	0/10	1/10
	5.85		0/9	0/9
Total			0/67	3/67
		Intraspinal		
7	6.65	*Rhesus*	2/9	1? & 3/9
	5.65	„	4/10	5/10
8	5.20	*Cynomolgus*	1/5	1/5
Total			7/24	1? & 9/24

TABLE 2

NEUROVIRULENCE FOR MONKEYS OF TYPE 1 POLIOVIRUS (CHAT) VACCINE GIVEN BY THE INTRAMUSCULAR ROUTE

Exp. No.	Log_{10} TCD_{50} inoculum	Species of monkey	Ratio of monkeys developing:		Ratio of monkeys showing Viraemia on post-inoculation day:				
			Paralysis	Polio lesions	1	2	3	4	5-8
1	8.65	*Cynomolgus*	0/5	1? & 1/5	ND [a]	ND	ND	1/5	0/5
2	7.85	*Rhesus*	2/5	2/5	5/5	3/5	1/5	0/5	0/5
3	7.85	*Cynomolgus*	2/5	1/5	5/5	5/5	4/5	0/5	0/5
4	7.85	*Cynomolgus* and *Rhesus*	0/10	2/10	10/10	9/10	5/10	0/10	0/10
5	8.35	"	0/10	0/10	9/10	9/10	3/10	0/10	0/10
Total			4/35	1? & 6/35	29/30	26/30	13/30	1/35	0/35

[a] ND = Not done.

monkeys. Among the 10 monkeys which received a dose of $10^{7.5}$ or $10^{6.9}$ $TCID_{50}$ changes appeared in three, chiefly in the lumbar and cervical segments. Among the five monkeys which received a dose of $10^{5.9}$ $TCID_{50}$, doubtful changes appeared only in one, and these only at the site of the injection of the virus. Among the four monkeys which received a dose of $10^{4.9}$ $TCID_{50}$, pronounced changes appeared in only one, and in a second there were very slight changes. Among the five monkeys which received a dose of $10^{3.9}$ $TCID_{50}$, changes developed in only one monkey, and these were of relatively slight intensity (Table 4).

Vaccine dosage determination

The minimum permissible dosage of vaccine from the immunological standpoint was determined in previously published small-scale studies using triply seronegative children (Przesmycki, 1960a).

On the basis of the above virological and serological observations, it was established that for mass vaccinations, 200 000 $TCID_{50}$ must be used for type 1, and 100 000 $TCID_{50}$ for type 3, as monovalent vaccinations.

The vaccinations were carried out in three stages. In the first stage, in 1959, the population of a small town and three neighbouring villages (Wyszków) was vaccinated. In the second stage, in June 1959, a total of 643 252 persons in two provinces, Kraków and Opole, were vaccinated. In the third stage, from October 1959 to April 1960, the population in the age-group 6 months to 14 years in all the remaining provinces was vaccinated.

After carrying out the vaccinations in Wyszków and determining the vaccination doses in children, a population of children aged 6 months to 15 years was vaccinated in two provinces, Kraków and Opole, during the period 11-17 June 1959. In Kraków province, 421 322 persons were vaccinated,

TABLE 3

NEUROVIRULENCE FOR MONKEYS OF TYPE 3 POLIOVIRUS (W-FOX) VACCINE GIVEN BY THE INTRACEREBRAL AND INTRASPINAL ROUTES

Exp. No.	Log_{10} TCD_{50} inoculum	Species of monkey	Ratio of monkeys developing:	
			Paralysis	Polio lesions
		Intracerebral		
1	7.85	*Cynomolgus*	0/9	0/9
2	7.45	"	0/5	0/5
	6.45	"	0/5	1/5
Total			0/19	1/19
		Intraspinal		
3	7.5	*Cynomolgus*	4/5	2/5
	6.5	"	2/5	0/5
4	6.9	*Rhesus*	0/5	1? & 1/5
	5.9	"	0/5	2? & 1/5
	4.9	"	0/4	2/4
	3.9	"	0/5	1? & 1/5
Total			6/29	4? & 7/29

TABLE 4

HISTOPATHOLOGY OF POLIOMYELITIS LESIONS IN MONKEYS INOCULATED WITH TYPE 1 (CHAT) OR TYPE 3 (W-FOX) POLIOVIRUS STRAINS

Monkeys from:		Strain inoculated	Route of inoculation	Log_{10} TCD_{50} inoculum	Severity of lesions [a] in CNS [b]					
Table No.	Exp. No.				C5	C7	T1	L3	L5	T6
1	2	CHAT	Intracerebral	7.45	3	3	—	2	2	0
			"	"	2	2	—	3	3	+
	6		"	6.85	3	3	0	3	0	0
	7		Intraspinal	6.65	2	2	1	3	3	+
			"	"	1	1	±	2	2	+
			"	"	1	1	1	2	2	+
			"	5.65	0	0	1	2	2	+
			"	"	0	0	1	0	0	0
			"	"	2	2	2	3	3	+
			"	"	0	0	0	2	2	0
			"	"	0	0	0	2	2	0
	8		"	5.20	2	2	1	3	3	+
2	1		Intramuscular	8.65	0	0	0	2	2	0
	2		"	7.85	0	0	0	3	3	0
			"	"	0	0	0	2	2	0
	3		"	"	1	1	1	3	3	0
	4		"	"	1	1	1	1	1	0
			"	"	0	0	0	2	2	0
3	2	W-Fox	Intracerebral	6.45	2	2	1	3	3	+
	3		Intraspinal	7.5	2	2	1	2	2	0
			"	"	3	3	1	3	3	+
	4		"	6.9	2	2	1	2	2	0
			"	5.9	0	0	0	1	1	0
			"	4.9	1	1	1	1	1	0
			"	"	2	2	0	3	3	+
			"	3.9	2	2	0	1	1	+

[a] Lesions graded for severity as follows:

0 = No lesion.
1 = Chromatolysis of single motor cells, satellitosis of these cells, and slight perivascular infiltration.
2 = Neuronophagia limited to a few cells, pronounced perivascular and interstitial infiltration.
3 = Extensive neuronophagia, cell loss, abundant perivascular and interstitial infiltration, and glial proliferation.
± = Changes which cannot be characterized as undoubtedly poliomyelitic.

[b] C = Cervical, T = Thoracic, L = Lumbar; C5 = 5th cervical segment, etc.
TC = Recovery of virus from the cord in tissue culture.

and in Opole province, 221 930 persons, making a total of 643 252 persons. These vaccinations did not cause any complications or increase in deaths from poliomyelitis.

Mass preventive vaccinations for those 6 months to 14 years old were therefore instituted throughout the whole country in October 1959 and completed in April 1960. Altogether, 7 239 007 persons were immunized with type 1, which constitutes 80.9% of the registered population in this age-group; and 6 818 490, or 76% of the registered population, with type 3.

TABLE 5

ISOLATIONS OF ENTERIC VIRUSES FROM STOOLS COLLECTED BEFORE AND AFTER VACCINATION

Laboratory	No. persons tested	Percentage virus isolations of polioviruses or non-poliovirus cytopathogenic agents (CP)						Cell system
		Before vaccination				After vaccination		
		Type 1	Type 2	Type 3	C P	Type 1	Type 3	
Warsaw	160	1	0	3	3	55	67	Monkey kidney
Lublin	215	2	0	0	2	27	25	Monkey kidney
Gdańsk [a]	A. 1 544 B. 682 C. 160	11	3	2	13	29	43	Monkey kidney HeLa
Kraków	105	11	0	4	4	23	57	HeLa

[a] A = Before vaccination.
B = After Type 1.
C = After Type 3.

Virological and serological studies

Regional virological investigations were carried out before mass vaccination. The results are given in Table 5. The difference in incidence of enteroviruses may be attributable to the different seasons of the year in which the material was obtained. It is known that enteroviruses occur less frequently in winter, causing the recovery rate to be high in Gdańsk in the fall[1] but low in Warsaw during the winter. Attention should also be drawn to the low incidence of type 2 poliomyelitis virus in every regional investigation.

Significant regional differences are also observed in the results of the isolation of type 1 virus after vaccination. These may be caused by the times at which the stool specimens were collected, since many stools from the Lublin district were collected during the 21st to the 26th days. Other factors are the high quantity of non-poliomyelitis enteroviruses disseminated in the Gdańsk region, which may have interfered with the poliomyelitis virus given in the vaccine, and the use of tissue culture materials of varying sensitivity to poliomyelitis virus.

In the results of vaccination with type 3, a significantly higher percentage isolation of strains was found compared with type 1. This is probably caused by the higher intestinal infectivity of type 3 virus. An exception is the low percentage of isolation in the Lublin investigations, caused, as already mentioned, by the collection of the samples at too late a time after vaccination.

The percentage of positive results of stool investigations was analysed after vaccination and related to the serological picture obtained before vaccination in these same age-groups. As shown by the serological survey conducted before the vaccine was administered, about 47% of the children in the age-group 6 months to 3 years already had antibodies against type 1 poliomyelitis virus. It is known that the virus multiplies in a significantly lower percentage of individuals possessing antibodies. The result of the investigations in Warsaw (55% isolations) may largely represent, therefore, the 53% of individuals lacking antibodies against type 1 virus.

The findings are similar for type 3. Before vaccination 55% of the Warsaw children in this age-group did not possess antibodies against type 3 virus, while after vaccination type 3 virus was isolated from 67% of the children.

The results of investigations of the serological response to vaccination are given in Table 6, showing the transition (conversion) of negative serum to positive serum for each separate type. Seroconversion occurred in 91.7% of type 1 and 91% of type 3 homotypic negatives.

Even though type 2 virus was not administered in the vaccine, conversion of type 2 also occurred in the sera of vaccinated persons, averaging 65.4%. This phenomenon may be caused by common antigenic elements in the individual types of polio-

[1] Virus was fed in Gdańsk in the fall.

TABLE 6

RESPONSE OF HOMOTYPIC SERONEGATIVES TO TYPES 1 AND 3 LIVE POLIOVIRUS

Laboratory	Conversion from < 1:4 to ⩾ 1:4						Tissue culture and end-point of test
	Type 1		Type 2		Type 3		
	Pos./Total	%	Pos./Total	%	Pos./Total	%	
Warsaw	72/78	92.3	36/54	66.7	114/116	98.3	Monkey kidney cytopathogenic
Lublin	87/97	89.7	33/90	36.7	79/98	80.6	Monkey kidney colour test (ph)
Gdańsk	146/158	92.4	145/190	76.3	181/198	91.4	Monkey kidney or HeLa colour test (ph)
Kraków	26/28	92.8	22/27	81.5	31/33	93.9	HeLa colour test (ph)
Total	331/361	91.7	236/361	65.4	405/445	91.0	

myelitis virus. Another contributory factor may be previous vaccination with Salk vaccine.

Although these investigations were conducted in four independent centres employing somewhat different methods, the results approximate one another very closely, which confirms their validity.

Attention should also be called to the results obtained in Gdańsk.

Despite the fact that high enterovirus circulation was noted, poliomyelitis virus of both type 1 and type 3 was able to act on the organism, shown by the percentage of individuals in whom antibodies developed. (Sabin et al. (1960) observed a similar phenomenon in investigating vaccinated subjects in Mexico.)

Also worthy of attention is the conversion of the sera of children lacking antibodies against all three types of virus, so-called " triple-negative " subjects (Table 7). These children converted to positive over 95% of the time for each type.

As has been pointed out already, the receptivity of the alimentary canal to the virus depends to a great extent upon the organism's previous contact with the virus; therefore, we analysed the conversion of sera of individuals in different age-groups (Table 8). A marked difference is evident between conversion among the youngest and oldest age-groups investigated in that the 1-3-year-olds responded better. This is probably dependent upon an altered intestinal receptivity to the virus in older individuals who have had previous experience with poliovirus.

Attention must also be called to conversion of type 2 in the different age-groups. It is quite apparent that accompanying an increase in age is a decrease in the percentage of positive conversions of type 2 virus, which was not administered in the vaccine. It is possible that the high percentage of conversion in the 1-7-year-old age-group is related to previous vaccination with Salk vaccine. This

TABLE 7

RESPONSE OF TRIPLE-NEGATIVE CHILDREN TO TYPES 1 AND 3 LIVE POLIOVIRUS

Laboratory	Triple-negatives before vaccination	Conversions to positive			Possible conversions achieved after vaccination	
		Type 1	Type 2	Type 3	No.	%
Warsaw	13	12	12	13	37/39	95
Lublin	15	13	9	15	37/45	82
Gdańsk	87	85	83	85	253/261	97
Kraków	10	9	9	9	27/30	90
Total	125	119	113	122	354/375	94

TABLE 8

SEROLOGICAL RESPONSE OF HOMOTYPICALLY NEGATIVE CHILDREN TO VACCINATION WITH TYPES 1 AND 3 POLIOVIRUSES IN WARSAW AND LUBLIN, BY AGE-GROUP

Age-group (years)	Subjects converting from < 1:4 to ≥ 1:4 after vaccination					
	Type 1		Type 2		Type 3	
	Pos./Total	%	Pos./Total	%	Pos./Total	%
1-3	49/51	96.1	26/36	72.2	80/83	96.4
4-7	72/81	88.9	44/69	63.8	91/96	94.8
8-14	38/43	88.4	17/44	38.6	31/36	86.1

vaccine may not have induced the formation of antibodies, but may have contributed to the " readiness " of the organism for their production, even after the introduction into the organism of heterologous types of virus.

In Table 9 are shown the results of investigations determining the antibody level developing in subjects who were seronegative before vaccination.

As is evident, an antibody concentration in the range of 1: 64 to 1: 512 and over for type 1 developed in 70% of the vaccinated subjects. For type 3, an antibody concentration over this same range appeared in about 80% of the vaccinated subjects.

TABLE 9

POST-VACCINATION ANTIBODY IN SUBJECTS SERONEGATIVE BEFORE VACCINATION WITH TYPES 1 AND 3 LIVE POLIOVIRUS VACCINE

Poliovirus type	Laboratory	No. negative before vaccination	Percentage distribution of reciprocal after vaccination			
			0	4-32	64-256	≥ 512
1	Warsaw	78	7.8	15.3	29.4	47.4
	Lublin	97	10.3	26.8	36.0	26.8
	Kraków	26	7.6	15.5	53.8	23.1
2	Warsaw	55	32.7	25.4	25.4	16.2
	Lublin	90	43.3	30.0	15.5	11.1
	Kraków	22	22.7	45.5	18.2	13.6
3	Warsaw	116	1.7	6.0	31.9	60.4
	Lublin	99	11.1	22.2	28.2	38.3
	Kraków	31	6.4	6.4	32.3	54.9
1	Total	201	9.0	20.9	35.8	34.3
2	Total	167	37.1	30.5	19.2	13.2
3	Total	246	6.1	12.6	30.5	50.8

In Lublin, lower antibody titres were obtained for type 3 virus. We are not in a position to account for this phenomenon. It is possibly related to the conditions under which the vaccinations were carried out.

In order to ascertain whether the type 2 antibody response was heterologous or caused by natural infection, serological studies were performed (Table 10) on subjects who had developed titres of >1: 16 against all three virus types. Eight-and-a-half months after vaccination, only three out of 14 subjects had type 2 titres of >1: 16; whereas such high levels were found for type 1 in six out of eight cases and for type 3 in 12 out of 14 cases.

DISCUSSION

It is generally agreed that vaccine injected into the thalamus of the monkey should rarely, if ever, produce the clinical symptoms and histopathological changes characteristic of poliomyelitis. Inability of the virus to spread *via* the central nervous system to the most susceptible motor cells is considered to indicate adequate attenuation and strains with this characteristic were considered to be suitable sources for the production of live vaccines. On the other hand, attenuated viruses introduced into the spinal cord produced, in quite a large percentage, both symptoms of paralysis and typical histopathological changes. This was explained by the fact that the virus was introduced into the most susceptible cells and was therefore in the most favourable conditions for development.

The three groups of strains (Koprowski's, Sabin's, Cox's) investigated by Murray and co-workers

TABLE 10

PERSISTENCE OF ANTIBODIES TO POLIOVIRUS TYPES 1, 2 AND 3 AFTER VACCINATION WITH TYPES 1 AND 3

Reciprocal antibody titre	Before vaccination			One month after vaccination [a]			8½ months after vaccination [b]		
	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
⩾ 1 024				3	4	9	2		2
512				1	1	1	2		2
256				3	4	2	1		1
128				1	3	2	1	1	5
64					1			2	2
32					1				
16	5	1	1				1	2	2
8		2	1				1	4	
4		2						1	
< 4	3	9	12					4	

[a] One month after type 3; two months after type 1.
[b] 8½ months after type 3; 9½ months after type 1.

(1959) caused symptoms of paralysis and histopathological changes, depending on the dose, when introduced intraspinally. On the average, histopathological changes after intraspinal vaccination of monkeys developed in 75% using the type 1 CHAT (Koprowski), in 76% with the LSc-2ab strain (Sabin), and in 79% with the SM strain (Cox). After vaccination with the type 3 W-Fox strain (Koprowski), changes occurred in 69% of the monkeys; the Leon, 12ab strain (Sabin) caused changes in 65%, and the Lederle-Cox strain in 75% of the monkeys.

In our tests, neither CHAT nor W-Fox produced clinical symptoms of poliomyelitis when inoculated intracerebrally. Histological lesions were noted in about 5% of monkeys inoculated with each virus. On the other hand, intraspinal inoculation of CHAT and W-Fox produced illness in 29% and 20%, and lesions in 37% and 23% respectively of the monkeys.

The intramuscular inoculation of virus was proposed as a test of attenuation by Melnick & Benyesh-Melnick (1960). CHAT virus given intramuscularly in 10-ml amounts paralysed 11% of monkeys and caused lesions in 17%. Kirchstein et al. (1960) found that 10 ml of Sabin's LSc-2ab type 1 strain produced lesions in three out of four inoculated monkeys. Results with CHAT, therefore, approximate those obtained with LSc-2ab. In addition, we demonstrated that viraemia gradually declined after intramuscular injection, without evidence of multiplication of virus in extravascular tissues.

As is evident from every investigation carried out to date and from the opinions of the WHO Expert Committee on Poliomyelitis (1960), no strain exists entirely devoid of neurovirulence for monkeys. Besides, it is not known whether attenuated strains neuropathogenic for monkeys after intraspinal introduction are neuropathogenic for humans after oral administration.

The view is beginning to prevail that one can evaluate a vaccine only on the basis of investigations on groups of the order of several hundreds of thousands of persons, taking into consideration the safety of the strain and the degree of immunity it confers from an epidemiological standpoint.

If we compare the conversion results obtained in our investigations with the conversion obtained by various investigators after the administration of vaccines prepared from the Sabin and Lederle-Cox strains, we see that the results are very similar. For example, Chumakov and co-workers (1960), after administering Sabin's vaccine to an age-group 6 months to 25 years old, obtained a conversion with type 1 ranging from 76.2% to 90.8%; of 97.7% with type 2; and of 89.1% with type 3. Vaccinations carried out by Kleinman and co-workers (1960) with

the Lederle-Cox strains, in children around 3 years old in Minnesota, obtained 100% conversion for type 1, 90.9% for type 2, and 97.4% for type 3.

It is generally admitted that conversion after the administration of live vaccine should amount to about 90%. In our investigations, conversion with respect to both types 1 and 3 was approximately 91%.

It must be pointed out that conversion also developed in 65% of vaccines with type 2, which was not included in our vaccination programme. Repeat investigations on antibody concentration carried out in these same persons after the lapse of a year showed disappearance of the antibodies or a fall of their titre to the initial level. The appearance of type 2 antibodies therefore was a transient phenomenon. It is interesting to note that epidemiological observations after the mass vaccination was completed suggested a protection rate of 88%, which is close to our figures for seroconversion.

The interference sometimes caused by enteric viruses (Plotkin et al., 1960; Sabin et al., 1960) was avoided by vaccinating in the winter months.

We judge, in view of these results, that the vaccination of Polish children with types 1 and 3 live virus has created a highly immune population.

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RÉSUMÉ

Pendant la période 1959-60, une campagne de masse a été poursuivie en Pologne afin de vacciner contre la poliomyélite tous les sujets du groupe d'âge 6 mois-15 ans. Administré par voie orale, le vaccin vivant procuré par le Dr Koprowski dérivait de souches du type 1 (CHAT) et du type 3 (W-Fox).

Cette opération a été précédée d'une étude du vaccin utilisé en vue de s'assurer de son innocuité pour le tissu nerveux. A cet effet, l'unité de virologie de l'Institut national d'Hygiène a fait toute une série d'essais sur des singes auxquels le vaccin était administré en injection intracérébrale et intramédullaire, voire intramusculaire (type 3). Les investigations ultérieures ont apporté la preuve que les souches en question étaient suffisamment atténuées. Avant d'entreprendre la vaccination de masse, il restait à déterminer les doses propres à conférer l'immunité: dans ce but, la population enfantine de la petite ville de Wyszków et de trois villages voisins a été soumise à la vaccination (1959). Les chiffres de $DICT_{50}$ ont été respectivement de 200 000 et 100 000 pour les types 1 et 3.

La campagne d'immunisation massive a comporté deux phases. Elle a intéressé tout d'abord (11-17 juin 1959) les provinces de Cracovie et d'Opole, où tous les enfants de 6 mois à 15 ans ont eu à ingérer le type 1 (CHAT): un total de 643 200 personnes ont été ainsi immunisées, soit 421 300 sujets dans la province de Cracovie et 221 900 dans celle d'Opole.

Ensuite, la campagne d'immunisation a été étendue à tout le territoire de la Pologne. Commencée en octobre 1959, elle s'est achevée en avril 1960: au total, 7 239 000 personnes ont ingéré le virus type 1, soit 80,9% de la population appartenant à ce groupe d'âge, et 6 818 500 (76%) ont reçu le virus type 3.

Trois laboratoires provinciaux et l'unité de virologie de l'Institut national d'Hygiène ont fait une enquête sérologique et virologique afin d'évaluer la capacité de prolifération des virus dans le milieu intestinal. A cette fin, des échantillons de selles ont été prélevés avant l'ingestion des vaccins des types 1 et 3, et 10-14 jours plus tard. Chaque laboratoire a examiné environ 200 prélèvements effectués chez des enfants de 6 mois à 7 ans.

Le pouvoir immunisant des vaccins a été étudié en mesurant la réponse sérologique d'échantillons de sang prélevés avant la vaccination avec chaque type de souche virale, et 30 jours plus tard. Chaque laboratoire a examiné au point de vue sérologique 500 enfants de 6 mois à 15 ans.

Après vaccination avec le type 1, le virus poliomyélitique a été isolé dans les fèces dans 23-55% des cas; le pourcentage était de 25-67% avec le type 3. A ce propos, il convient de signaler des différences régionales significatives en ce qui concerne l'isolement post-vaccinal respectif des types 1 et 3. Divers facteurs peuvent être invoqués pour expliquer ces différences: à Lublin, les prélèvements ont été effectués du 21e au 26e jour; à Gdansk, le tractus intestinal renfermait en abondance des entérovirus non poliomyélitiques concurrents (d'où l'intérêt de vacciner en hiver); enfin, la sensibilité au virus poliomyélitiques des cultures de tissu est variable.

Après vaccination avec le type 3, les pourcentages de virus découverts dans les selles ont été significativement plus élevés que ceux du type 1, en raison probablement du plus haut degré d'infectivité intestinale du type 3.

Le taux de conversion des titres d'anticorps (de $<1:4$ à $\geqslant 1:4$) a été atteint dans 91,7% des cas avec le type 1, contre 91,0% avec le type 3. Bien que le type 2 n'ait pas été utilisé, on a noté 65,4% de conversions sériques à ce type. Le phénomène serait dû en partie à l'existence d'éléments antigéniques communs aux trois virus, en partie à l'administration antérieure de vaccin Salk.

Chez 70% des vaccinés au type 1, les titres d'anticorps oscillaient entre 1: 64 et 1: 152; ils étaient plus élevés chez 80% des vaccinés au type 3. En conclusion, les auteurs estiment que la vaccination des enfants polonais avec des virus vivants atténués des types 1 et 3 a créé une population qui est efficacement immunisée.

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