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MASS ORAL (SABIN) POLIOMYELITIS VACCINATION

VIROLOGICAL AND SEROLOGICAL SURVEILLANCE IN CZECHOSLOVAKIA, 1958–9 AND 1960

BY

KAREL ŽÁČEK, M.D. ERVÍN ADAM, M.D. VLASTA ADAMOVÁ, M.D.
VÁCLAV BURIAN, M.D. DAGMAR ŘEZÁČOVÁ, M.D. ELIŠKA SKŘÍDLOVSKÁ
NINA VANĚČKOVÁ, M.D. AND VLADIMÍR VONKA, M.D.*

From the Institute of Sera and Vaccines, Prague, and the Infectious Diseases Clinic, Faculty of Paediatrics, Charles University, Prague

Extensive epidemiological, virological, and clinical studies have been carried out in Czechoslovakia since 1957, when mass vaccination against poliomyelitis was initiated in this country. At first, following antibody surveys in various regions, *Salk-type vaccine* was given, and the effectiveness of this procedure was evaluated by means of serological surveys (Škovránek *et al.*, 1958a, 1958b; Žáček *et al.*, 1960, 1961). A field trial with *oral vaccine* was begun in 1958–9, using Sabin's strains of attenuated polioviruses (Škovránek and Žáček, 1959, 1960; Škovránek, 1960a, 1960b; Žáček *et al.*, 1960, 1961); based on the success of this project, a nation-wide oral vaccination campaign was carried out in 1960. Preliminary results of virological and serological studies made in connexion with the administration of live attenuated virus vaccine have been published elsewhere (Škovránek, 1961; Škovránek and Žáček, 1961); it is the purpose of the present communication to summarize our recent experiences, with emphasis on serological results using different dosage schedules, and virological surveillance to determine how long attenuated polioviruses persist in communities in which large-scale oral vaccination has been carried out.

History of Poliomyelitis in Czechoslovakia

This story was reviewed in detail by Škovránek *et al.* (1958a). Briefly, the disease had a low endemic rate until the first large epidemic occurred in 1939, more than 2,000 cases being reported. As shown in the Chart, there has been a rise in the endemic rate beginning in the early 1940s, with severe outbreaks in 1948 and 1953. Between 1930–3 and 1950–3 the morbidity rate rose steadily from 1.52 to 10.03 per 100,000. A shift toward a higher incidence in older age-groups accompanied the rising morbidity in Bohemia but not in Slovakia, where the disease has remained primarily an infantile one.

In 1957 vaccination with Salk-type vaccine was begun, using the intradermal route. More than half (55.8%) of the children under 15 years received three doses of vaccine, and, although there were still 245 virologically confirmed cases in 1958, the vaccine apparently did have a considerable impact on the incidence of the disease. It was on this background that work with the live attenuated poliovirus vaccine (Sabin) was begun in 1958.

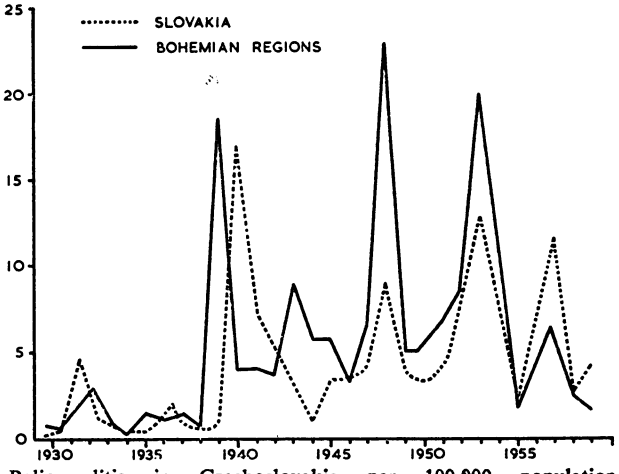
*Credit is due to many other valued co-workers, some of whom are acknowledged later.

Organization of Oral Vaccination Programmes, 1958–60

Both the 1958–9 field trial, which was limited to four regions, and the 1960 nation-wide programme were carried out on a voluntary basis. Parents were informed of the availability of vaccine through a health education campaign, and were requested to bring their children at appointed times to designated vaccination centres. The vaccine was administered by nurses under the supervision of a paediatrician. The entire programme was under the direction of epidemiologists of the Hygiene and Epidemiological Service, and was carried out through the ordinary health services.

Selection of Vaccinees.—The age-groups vaccinated were from 2 to 8 years in the field trial, and from 2 months to 14 years in the 1960 campaign. The percentage of children in each category who received oral vaccine is shown in Table I. By May, 1960, a total of 3,371,980 children, representing 92.9% of the population in the designated age-groups, had been immunized.

Schedules of Vaccine Administration.—Three dosage schedules were used: in the 1958–9 field trial the three types were given separately at monthly intervals in the order type 1, type 3, type 2; in the 1960 nation-wide campaign type 1, followed by types 2 and 3 together,



Poliomyelitis in Czechoslovakia per 100,000 population (Škovránek, 1960b).

were given to one group, and trivalent vaccine to the other group. The two 1960 groups each contained a large number of children in each age category (Table I). A certain number of children (approximately 100,000) who had received three types separately during the 1958–9 field trial were revaccinated a year later with trivalent vaccine, as part of the 1960 programme.

TABLE I.—*Oral Poliomyelitis Vaccination in Czechoslovakia, 1958–9 and 1960*

Group	Schedules for Administration of Vaccine	Date	Age-groups	No. of Children Vaccinated and Percentage of Estimated Population	
				No.	%
1*	1 3 2	15–20/12/58 12–16/1/59 9–13/2/59	2–6 years‖ 7–8 ,,‖	90,386 24,124	49·6† 24·1†
			Total	114,510	40·5†
2§	1+2+3	12–20/4/60	2–6 months 7 months–6 years 7–14 years	12,401 253,133 373,824	84·0† 93·9† 96·7†
			Total	639,358	95·3†
3§	1 2+3	28/3–11/4/60 2–11/5/60	2–6 months 7 months–6 years 7–14 years	73,195 1,357,591 1,941,194	85·3‡ 91·2‡ 94·5‡
			Total	3,371,980	92·9‡

* Vaccine given in four Czech regions only in 1958–9.
† Percentage of children in four Czech regions vaccinated in 1958–9.
‡ Percentage of children in the remaining 16 Czechoslovak regions primarily vaccinated in 1960.
§ Nation-wide administration of oral vaccine.
‖ All these children received all three types of virus.

Dose of Vaccine.—The dosage of each type was approximately 100,000 TCD50 regardless of schedule, except for infants 2–6 months of age, who received 500,000 TCD50 of each type. Vaccine used in the field trial was provided by Dr. A. B. Sabin,† and represented aliquots of the 1956 original lots. The bulk of the vaccine used in 1960 was prepared at the Institute of Sera and Vaccines, Prague, using Sabin's strains. Some type 2 and 3 vaccine was kindly supplied by Professor M. P. Chumakov, of Moscow.

Collection of Specimens for Serological and Virological Surveillance

Blood specimens were collected from a total of 917 children immediately before and approximately four weeks after vaccination. Representative samples were taken from individuals included in each of the three dosage schedules, as shown in Tables II–IV. In addition, repeated serological surveys were carried out in the general population in two Czech regions before and at different times after oral vaccination and/or revaccination. Specimens were obtained from both vaccinated and non-vaccinated individuals of different age, chosen at random in proportion to the density of the population and indicated age-groups. Detailed information on the sampling procedure used has been reported elsewhere (Žáček *et al.*, 1958a; Škovránek, Žáček, *et al.*, 1959). Blood specimens were collected by teams of physicians and nurses who travelled from Prague to the areas under study (Žáček *et al.*, 1958a; Škovránek, Žáček, *et al.*, 1959). The blood was taken aseptically and immediately put on wet ice and transported to Prague. Within 48 hours the sera were separated, inactivated, and, after the addition of antibiotics, stored at −20° C. until tested.

Faecal Specimens. — In order to determine the prevalence of polioviruses and other enteric viruses

†We are grateful to Dr. A. B. Sabin, Cincinnati, U.S.A., for making this material available to us.

TABLE II.—*Poliomyelitis Antibody Conversion Rates after Oral Vaccination with Approximately* 100,000 *TCD*50 *of Type* 1, 2, *and* 3 *Viruses, Each Given Separately in December–February,* 1958–9, *to* 85 *Children aged* 2–8 *Years*

Pre-vaccination			Post-vaccination									
Negative to Type*	No. of Persons	Total No. of Negs.	Negative to Type*							Total No. of Negs.	Complete Conversions to Triple Positive		Seronegative Conversion Rates
			1	2	3	1+2	1+3	2+3	1+2+3		No.	%	
1	9	9	1							1	8	89·0	
2	1	1								0	1	100·0	Type 1: 32/33 (97·0%)
3	7	7								0	7	100·0	,, 2: 14/15 (93·4%)
1+2	2	4								0	2	100·0	,, 3: 31/32 (97·0%)
1+3	13	26			1					1	12	92·0	
2+3	3	6								0	3	100·0	Total : 77/80 (96·3%)
1, 2, and 3	9	27		1						1	8	89·0	
Totals	44	80	1	1	1					3	41	93·2	
Seronegative index		31·4								1·18			

* Titre of <1 : 4.

TABLE III.—*Poliomyelitis Antibody Conversion Rates after Oral Vaccination with Approximately* 100,000 *TCD*50 *of Type* 1 *Virus Given Separately in March,* 1960, *and Type* 2 *and* 3 *Given Together in May,* 1960, *to* 456 *Children aged* 6 *Months to* 14 *Years*

Pre-vaccination			Post-vaccination									
Negative to Type*	No. of Persons	Total No. of Negs.	Negative to Type*							Total No. of Negs.	Complete Conversions to Triple Positive		Seronegative Conversion Rates
			1	2	3	1+2	1+3	2+3	1+2+3		No.	%	
1	47	47	5							5	42	89·4	
2	3	3								0	3	100·0	Type 1: 186/194 (96·0%)
3	78	78			3					3	75	96·2	,, 2: 81/81 (100·0%)
1+2	4	8								0	4	100·0	,, 3: 209/232 (90·0%)
1+3	80	160			8		1			9	71	88·8	
2+3	11	22			2					2	9	81·8	Total: 476/507 (92·7%)
1, 2, and 3	63	189	1		8		1			10	53	84·1	
Totals	286	507	6		21		2			29	257	93·4	
Seronegative index		37·6								2·12			

* Titre of <1 : 4.

TABLE IV.—*Poliomyelitis Antibody Conversion Rates after Oral Vaccination with Approximately* 100,000 *TCD*50 *of Types* 1, 2, *and* 3 *Given Together in April,* 1960, *to* 376 *Children Aged* 6 *Months to* 14 *Years*

Pre-vaccination			Post-vaccination										
Negative to Type*	No. of Persons	Total No. of Negs.	Negative to Type*							Total No. of Negs.	Complete Conversions to Triple Positive		Seronegative Conversion Rates
			1	2	3	1+2	1+3	2+3	1+2+3		No.	%	
1	50	50	21							21	29	58·0	
2	4	4								0	4	100·0	Type 1: 88/137 (64·2%)
3	43	43			5					5	38	88·4	,, 2: 47/54 (87·0%)
1+2	0									0			,, 3: 114/142 (80·3%)
1+3	49	98	7		3		5		1	16	33	67·3	
2+3	12	24			3					3	9	75·0	Total: 249/333 (74·9%)
1, 2, and 3	38	114	12		1		3	7		23	15	39·5	
Totals	196	333	40		12		8	7	1	68	128	65·3	
Seronegative index		29·5								6·03			

* Titre of $<1:4$.

after oral vaccine was introduced on a large scale, a controlled study was set up in vaccinated and non-vaccinated regions, beginning in 1958. Specimens were collected predominantly from the child population under 10 years of age, on a random basis in proportion to the population density (Burian *et al.*, 1960; Záček *et al.*, 1960). Collections were made by the Regional Virus Laboratories, where the specimens were stored frozen (at −20° C.), and shipped in batches to our laboratory in Prague at intervals of 14 to 30 days, either in the frozen state or at least on wet ice. The first collections were made in November, 1958, before oral vaccine was introduced. Subsequently, at intervals of two to four months, repeated random collections were made throughout 1959: 2,322 specimens were collected in the four vaccinated regions, and 2,964 in 10 non-vaccinated provinces. The virological surveillance was continued in 1960 in the four original vaccinated regions and in 10 others where vaccine was given in 1960. Some of these latter had served as control areas in the 1958–9 survey.

Surveillance of Cases of C.N.S. Disease. Collection of Specimens.—During 1957–60 an effort was made to collect specimens from all patients in Czechoslovakia with acute C.N.S. disease, whether paralytic or non-paralytic. These included both serum specimens and stools. In 1960, in order to make the laboratory diagnosis as complete as possible, five faecal and five blood specimens were obtained (including specimens from cases of isolated seventh-nerve paralysis). Blood specimens were collected on admission to the hospital and at weekly intervals thereafter; stools were obtained at intervals of 1 to 2 days, beginning as early as possible. From cases of aseptic meningitis two stool and two blood specimens were obtained—the first specimens on admission, the second stool within a few days, and the second serum at four weeks.

Laboratory Methods

Virus Isolation.—The techniques employed were the standard ones that had been in use in this laboratory for a number of years (Žáček *et al.*, 1958b). For each specimen three to five monkey-kidney monolayer tube cultures were inoculated with 0.2 ml. of 10% stool extract which had been treated with antibiotics. The cultures were observed for cytopathic effect (C.P.E.) over a period of 10 to 14 days. Those showing C.P.E. were harvested, passed, and identified by means of tube neutralization tests with suitable antisera.

Antibody Determinations.—Serum-neutralizing antibodies were determined by the colorimetric (*p*H) test in plastic panels, as outlined in the 1958 report of the W.H.O. Expert Committee on Poliomyelitis (1958). Sera were diluted in fourfold steps from 1:4 to 1:1,024, and approximately 100 TCD50 of virus was added. The mixtures were held at room temperature for one hour before inoculation. Positive and negative serum controls were included in every series of tests performed on the same day. Paired samples from an individual were always run in the same test. In our results the original serum dilutions are recorded and a change from $<1:4$ to 1:4 is regarded as significant and indicative of antibody conversion.

The complement-fixation test was used in testing sera from patients with C.N.S. disease. The Black and Melnick method was used (Expert Committee on Poliomyelitis, 1958).

Antibody Responses. Conversion Rates in Vaccinees According to Dosage Schedules

Observations on the immunizing capacity of oral poliovirus vaccine after various schedules of its administration are given in Tables II to IV, and V and VI, respectively.

Table II demonstrates the antibody responses of 85 children (group 1) aged 2–8 years, who received each type of virus separately in 1958–9. It can be seen that 41 out of 44 or 93% of children lacking antibody to one or more types of poliovirus prior to vaccination converted to triple positives. The total seronegative conversion rate was 96.3% without any significant differences between individual types. The so-called seronegative index,‡ which according to Embil *et al.* (1960) is intended to express the relative hazard of paralytic disease or "poliomyelitis potential," was reduced from 31.4 before vaccination to the very low level of 1.18 after virus feeding.

The number of triple negatives was unfortunately low in this group of children, and does not permit a significant evaluation and comparison with antibody responses in single- or double-negative children; nevertheless it may be of interest that only one gap of type 2 antibody was noted among nine triple-negative children under investigation.

Table III concerns group 2, the largest of the study groups, which consisted of 456 children, aged 6 months to 14 years. These children were given type 1 virus in the spring of 1960 and a mixture of types 2 and 3 one month later. Included were 286 (62.7%) who were without antibody to one or more types before

‡The seronegative index is calculated as follows:

$$\frac{\text{Observed total of type-specific seronegatives}}{\text{Number in population sample} \times 3} \times 100 = \text{Index.}$$

vaccination. Of these, 257 (93.4%) developed antibodies to all three types and the total seronegative conversion rate was 92.7%. These total ratios are approximately the same as those observed in the first group of children who received each type of virus separately. In terms of responses according to pre-vaccinal antibody status, there were no striking differences in the conversions with types 1 and 2 viruses among children who lacked only type 3 antibody prior to vaccination.

Table IV shows the results of the third group, fed a single dose of trivalent vaccine in April, 1960. Here the total antibody response was significantly lower when compared with the previous two groups: only 128 out of 196 or 65.3% of individuals who were without antibodies to one or more types before vaccination converted to triple positives, and the total seronegative conversion rate was 74.8%; the rates were 64.2%, 87.0%, and 80.3% for types 1, 2, and 3 respectively. The seronegative indices dropped from 29.5 before feeding to 6.03 after feeding.

The response to trivalent vaccine in terms of pre-vaccinal antibody status—that is, different combinations of single, double, and triple negatives—indicated that for types 2 and 3 there were fewer conversions in children who lacked more than one antibody than in those in whom only one type was missing. The responses in triple negatives were particularly low. With type 1, however, the greatest number of failures occurred in single negatives, 21 out of 50 or 42% of whom failed to convert (Table IV). There is no obvious explanation for this latter finding.

Tables V and VI further indicate the number of single, double, and triple negatives by age-groups and their responses to oral vaccine given on two dosage schedules: (*a*) type 1 separately, followed by types 2 and 3 together (schedule 2—see Table III); and (*b*) one single dose of trivalent vaccine (schedule 3—see Table IV). As can be seen, there were no significant differences in the responses of the children by age-groups after vaccination with type 1 followed by types 2 and 3 (schedule 2). Table VI demonstrates that, with respect to type 1 antibody response, similar results were obtained in single, double, and triple negatives by age-groups, fed one single dose of trivalent vaccine (schedule 3); in contrast, type 2 and/or 3 responses were significantly lower in double and/or triple negatives aged 6–12 months, compared with conversions among double and/or triple negative children older than 1 year of age.

Antibody Patterns in Normal Children Before and After the Introduction of Oral Vaccine

Table VII summarizes the results of serological surveys in two of the regions in which the field trial was carried out in 1958–9 among children 2–8 years of age, some of the same children being revaccinated in 1960. Both vaccinated and non-vaccinated children were sampled. In the primary course of vaccination the three

TABLE V.—*Poliomyelitis Antibody Response of Children by Age-groups. Children Orally Vaccinated with Type 1 Separately and Types 2 and 3 Together (See Table III)*

Age-groups	Pre-vaccination		Post-vaccination						
	Neg. to Type	No. of Children Tested	Negative to Type						
			1	2	3	1+2	1+3	2+3	1+2+3
6–12 months	1	5							
1–4 years		30	3						
5–9 ,,		5							
10–14 ,,		7	2						
	Total	47	5						
6–12 months	3	8	1						
1–4 years		46	1						
5–9 ,,		8							
10–14 ,,		16	1						
	Total	78	3						
6–12 months	1+3	18			2				
1–4 years		54			5		1		
5–9 ,,		6			1				
10–14 ,,		2							
	Total	80			8		1		
6–12 months	1+2 +3	23			2				
1–4 years		34	1		4				
5–9 ,,		3			1		1		
10–14 ,,		3			1				
	Total	63	1		8		1		

TABLE VI.—*Poliomyelitis Antibody Response of Children by Age-groups. Children Orally Vaccinated With one Single Dose of Trivalent Vaccine (See Table IV)*

Age-groups	Pre-vaccination		Post-vaccination						
	Neg. to Type	No. of Children Tested	Negative to Type						
			1	2	3	1+2	1+3	2+3	1+2+3
6–12 months	1	4	1						
1–4 years		18	7						
5–9 ,,		22	9						
10–14 ,,		6	4						
	Total	50	21						
6–12 months	3	2							
1–4 years		14			3				
5–9 ,,		19			1				
10–14 ,,		8			1				
	Total	43			5				
6–12 months	1+3	5			2				1
1–4 years		28	3		1		2		
5–9 ,,		13	5				2		
10–14 ,,		3							
	Total	49	8		3		4		1
6–12 months	1+2 +3	21	8				3	6	
1–4 years		12	4						
5–9 ,,		4	1		1				
10–14 ,,		1							
	Total	38	13		1		3	6	

TABLE VII.—*Poliomyelitis Antibody Status of Children aged 2–8 Years, Estimated Before and at Different Times After Initiation of Oral Vaccination Programme in Usti and Jihlava Regions, 1958–60*

Children	Date Serum Survey	No. of Sera Tested	Percentage of					Seronegative Indices
			Negatives to Type*			Triple		
			1	2	3	Neg.	Pos.	
Non-vaccinated	Nov., 1958†	390	31·8	12·6	36·2	7·4	49·5	25·4
	March, 1959	303	28·7	13·6	28·7	6·6	45·9	23·4
	,, 1960	374	28·9	14·2	36·1	7·0	52·0	26·4
Vaccinated Dec.-Feb., 1958–9. Each type given separately	,, 1959	257	1·9	1·9	4·5	0	92·0	2·85
	,, 1960	261	7·3	3·8	18·4	1·5	78·0	9·84
Revaccinated April, 1960. One dose of trivalent vaccine	May, 1960	176	0·6	0	1·7	0	98·0	0·76

* Titre of <1 : 4. † Before vaccine introduced.

types were given separately, while the revaccination, approximately one year later, was with the trivalent preparation.

The results seem to indicate that under field conditions the administration of all three types of attenuated polioviruses to 40% of the total child population aged 2–8 years did not significantly influence the immunological status of the non-vaccinated children in the same age-group, even though a relatively large proportion of such children were susceptible to one or more types of poliovirus. Thus the general spread of attenuated polioviruses administered during the winter season was apparently insignificant in the age-group 2 to 8 years in these two provinces.

The impact of the vaccine on the immunity of children who ingested the attenuated poliovirus strains is indicated by the high percentage (92) of triple-positive children one month after the 1959 programme was completed. One year later this was reduced to 78%, largely as a result of lack of type 3 antibody in 18.4% of children tested. Type 1 and 2 antibodies, however, were still detected in 93 to 96% of children after a lapse of one year.

Prevalence of Enteroviruses as Determined by Virological Survey

Results of tests on faecal samples collected in 1958–9 in four vaccinated and 10 non-vaccinated regions are given in Table VIII. It will be seen that a certain number of poliovirus isolations were made in the immediate post-vaccination period (after each type was given), primarily from vaccinees. However, the number decreased steadily, so that by five months very few and by 10 months no polioviruses were detected. The picture in the control non-vaccinated regions during the same period was not very different: there was some activity of all three types of poliovirus in two Czech regions (Č. Budějovice and Gottwaldov) chiefly between April and September, 1959; but considerably more poliovirus strains were isolated in Slovakian regions.

The results of enterovirus surveillance in 1960, after the nation-wide oral vaccine programme had been completed, are given in Table IX. In the one to two months following the period of vaccination polioviruses were isolated from up to 16% (type 3) of specimens. However, several months later, during September and October, only three isolations of poliovirus were made from 2,347 stool samples. Two of these were type 2, both from non-vaccinated children, and one type 3, from a vaccinated child. These results are in marked contrast to the expected poliovirus isolation rate at this season of the year, and suggest, first, that there was no significant tendency for attenuated strains to persist in the population after community-wide vaccination of the children; and, secondly, that after the nation-wide vaccination programme wild polioviruses were virtually absent at the time of the usual seasonal rise in poliomyelitis. This absence of polioviruses, which correlates well with the absence of clinical cases during this period, is commented on below.

Laboratory Investigation of Clinical Cases

The results of virus isolation from cases reported in 1957–60 are given in Table X (for Czech regions only). The poliovirus isolation rate by type of disease was between 51 and 73% for paralytic cases, between 12 and 24% for children with isolated facial palsy, and only 7 to 10% for the aseptic meningitis cases. A number

TABLE VIII.—*Virological Surveys in Orally Vaccinated and Non-vaccinated Regions of Czechoslovakia in* 1958 *and* 1959

Regions	No. Regions Surveyed	Date of Sampling	Stool Specimens		Virus Strains Isolated Polioviruses (Percentage of Type)‡			Enteroviruses Other than Polioviruses (%)
			No. Tested‡	Percentage Positive for Poliovirus	1	2	3	
Czech (vaccinated)*	2	Dec., 1958†	0/242	0/0·8	0/0·8	0	0	2·5
	2	Feb., 1959	47/198	44·6/4·0	8·5/0·5	0/0·5	36·0/3·0	2·1/0
	3	April	106/450	12·3/2·2	0	7·5/1·6	4·7/0·7	0·9/0·7
	4	June	141/545	1·4/0·9	0	0·7/0·7	0·7/0	0·7/1·7
	4	Sept.-Oct.	140/451	0/1·1	0/0·2	0/0·7	0/0·2	2·9/4·4
Czech (not vaccinated)	3	Dec., 1958†	293	1·2	0·7	0·3	0	2·7
	3	Feb., 1959	243	0·4	0·4	0	0	0
	2	April	251	3·6	2·0	0·4	1·2	3·6
	6	June	986	0·7	0·6	0·1	0	2·7
	4	Sept.-Oct.	557	2·8	1·1	0·2	0·9	2·5
Slovak (not vaccinated)	3	June-July, 1959	634	8·0	2·7	3·3	1·9	11·5

* Vaccination carried out December, 1958, to February, 1959. † Before vaccination. ‡ Numerator = Viruses recovered from vaccinated individuals. Denominator = Viruses recovered from non-vaccinated individuals.

TABLE IX.—*Virological Surveys in Czechoslovakia by Random Sampling in* 1960

Regions	No. Regions Surveyed	Date of Sampling	Stool Specimens		Virus Strains Isolated Polioviruses (Percentage of Type)§			Enteroviruses Other than Polioviruses (%)
			No. Tested§	Percentage Positive for Poliovirus	1	2	3	
4 Czech regions (orally vaccinated already in 1958–9)	4	Jan. 1960	106/360	0/0·3	0	0/0·3	0	0·9/1·1
	4	March*	153/313	0/0·6	0	0	0/0·6	1·3/1·3
	2	April†	194/24	49·0/42·0	9·3/12·5	11·0/12·5	28·3/16·7	3·1/0
	4	June	385/80	10·7/2·5	1·4/1·3	3·6/0	6·0/1·3	0·8/1·3
	1	July	79/26	2·5/0	0	1·3/0	1·3/0	5·0/0
	3	Sept.-Oct.	459/138	0·2/0	0	0	0·2/0	8·7/13·0
Remaining Czechoslovak regions (not vaccinated in 1958–9)	6	Jan. 1960*	0/794	0/4·7	0/1·3	0/0·1	0/3·3	0/0·9
	7	March*	0/851	0/5·5	0/2·2	0/0·9	0/2·4	0/1·3
	10	June	1,051/36	30·6/50·0	0·6/0	9·7/13·9	20·4/36·0	3·5/2·8
	6	July	630/57	4·8/19·3	0	1·9/7·0	2·9/12·3	4·4/7·0
	10	Sept.-Oct.	976/774	0/0·3	0	0/0·3	0	8·1/67

* Before nation-wide oral vaccination in 1960. † 14–21 days after vaccination with one dose of trivalent oral vaccine (vaccination carried out April 12–20, 1960). ‡ Vaccination carried out: type 1 March 28–April 11, 1960; types 2+3 May 2–11, 1960. § Numerator = Viruses recovered from vaccinated individuals. Denominator = Viruses recovered from non-vaccinated individuals.

of the latter were found to be associated with various E.C.H.O. and Coxsackie viruses. During 1957 and 1958 type 1 poliovirus was responsible for 99% and 92% of the cases respectively, but in 1959 the figure was only 75% and the other 25% were divided equally between types 2 and 3. As to cases reported in 1959 in children who had received oral vaccine in the field trial, there were four of these (including one case of facial palsy), but no polioviruses were isolated from them.

More detailed results for 1960 are given separately in Table XI (for the whole of Czechoslovakia). Although the total number of paralytic cases reported (including children with facial palsy) was not strikingly different from the figure for 1959, the time of year at which the 1960 cases occurred was unusual. Thus 46 of the 48 strains of poliovirus isolated from patients with paralytic disease were recovered between January 1 and the end of May, and no poliovirus was isolated from the five paralytic cases reported between August 1 and November 1. Isolation of poliovirus from individuals with either facial palsy or aseptic meningitis followed a similar seasonal pattern, with 48 isolations in June, 1 in July, and none thereafter. However, the reported incidence of these illnesses continued at a relatively high level through October, and several different enteroviruses were isolated from the later cases.

Discussion

The immunizing capacity of the Sabin oral vaccine when used under field conditions has been found to be of a high order as indicated by the serological data reported above. Thus the three strains given separately in winter-time produced an immune response in over 90% of children. Results almost as good were obtained with the combined schedule—type 1 followed by types 2 and 3—but the trivalent vaccine produced conversions in only 64%, 87%, and 80% for the three types respectively. Presumably this failure to achieve adequate infection and immunity with trivalent vaccine is associated with intertypic interference, as others have also found (Sabin *et al.*, 1960; Krugman *et al.*, 1960; Sabin, Ramos Alvarez, *et al.*, 1960; Smorodintsev *et al.*, 1960; Chumakov *et al.*, 1960; the type 2 strain is the dominant one in this particular vaccine, and apparently crowds out the other types. This is well illustrated by the fact that, as shown in Table IV, only 39.5% of triple negatives given trivalent vaccine converted to all three types, while such conversion occurred in 89% fed each type separately (Table II) and 84% of those given type 1, followed by types 2 and 3 (Table III). In general, responses to type 3 were poorest no matter what the schedule, as has been observed by others (Verlinde and Wilterdink, 1959; Chumakov *et al.*, 1960; Smorodintsev *et al.*, 1960). That unexplained variability of response sometimes occurs, however, is illustrated by the failure of 21 out of 50 children lacking only this type to become infected when fed type 1 virus in a trivalent vaccine (Table IV).

The question of the durability of antibodies acquired by ingestion of oral vaccine was examined by means of a serological survey of 261 children aged 2–8 years who had been vaccinated with the three types separately one year previously (Table VII). The percentage of children positive for each type was lower than it had

TABLE X.—*Number and Type Distribution of Polioviruses Isolated from Clinical Cases in Czech Regions in* 1957–60

Year	Clinical Form	No. of Cases Reported	No. of Cases Tested	Percentage Yielding Polioviruses	Poliovirus Type Recovered			Poliovirus Isolated						
								Total No.	Type					
									1		2		3	
					1	2	3		No.	%	No.	%	No.	%
1957	P.		305	73	219	0	3							
	FP	639	73	23	16	1	0	291	287	99·0	1	0·3	3	1·0
	AM	725	750	7·0	52	0	0							
1958	P	221	163	58	88	6	0							
	FP		58	24	13	1	0	143	132	92·3	11	7·7	0	0
	AM	684	480	7·3	31	4	0							
1959	P	122	108	51	43	4	8							
	FP		73	12	8	1	0	105	79	75·2	13	12·4	13	12·4
	AM	658	434	9·7	28	8	5							
1960	P	49	47	66	20	3	8							
	FP	129	129	13	4	7	6	87	33	37·0	20	23·0	34	39·0
	AM	4,824	449	8·7	9	10	20							

P = Paralytic. FP = Facial paralysis. AM = Aseptic meningitis.

TABLE XI.—*Incidence of Polioviruses Among Clinical Cases in Czechoslovakia in* 1960

Month	Paralytic Poliomyelitis						Facial Palsy (Up to 14 Years)					Aseptic Meningitis				
	No. of Cases Reported	No. of Cases Tested	No. of Polioviruses Isolated				No. of Cases Tested	No. of Polioviruses Isolated				No. of Cases Tested	No. of Polioviruses Isolated			
			Types			Total		Types			Total		Types			Total
			1	2	3			1	2	3			1	2	3	
Jan. ..	11	9	5	1	1	7	4	—	—	—	—	5	—	—	1	1
Feb. ..	13	11	8	—	1	9	4	1	—	1	2	5	—	—	1	1
March ..	23	19	12	—	4	16	4	—	—	—	—	13	5	2	2	9
April ..	19	15	5	2	4	11	21	4	1	1	6	29	5	4	6	15
May ..	13	9	1	—	2	3	20	—	3	5	8	35	—	5	10	15
June ..	4	4	—	—	—	—	18	—	2	2	4	40	1	1	2	4
July ..	5	4	1*	1*	—	2	17	—	1	—	1	132	—	—	—	—
Aug. ..	2	2	—	—	—	—	27	—	—	—	—	250	—	—	—	—
Sept. ..	1	1	—	—	—	—	22	—	—	—	—	11	—	—	—	—
Oct. ..	2	2	—	—	—	—	19	—	—	—	—	9	—	—	—	—
Nov. ..	—	—	—	—	—	—	7	—	—	—	—	—	—	—	—	—
Dec. ..	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
Total	93	76	32	4	12	48	164	5	7	9	21	529	11	12	22	45

* These two cases became ill one and six days after returning from Yugoslavia (type 1) and Egypt (type 2) respectively, and are therefore presumed to be "imported" cases.

been in a survey conducted one month after vaccination; particularly was this true for type 3, and to a less extent for type 1. Several explanations of this are possible: (1) there was a loss of antibody to type 1 in approximately 5% and to type 3 in approximately 15% of children, probably among those whose titres had never been high; (2) in calculating the percentage of conversions, a shift from <1:4 to 1:4 was regarded as significant and implying infection. Such low levels may in fact have been due to heterotypic responses, or possibly to unreliability of the colorimetric neutralization test in this range.

We are inclined to believe that the first explanation is the more likely, for rarely were levels of 1:4 or 1:8 encountered with types 1 and 2, but such titres appeared fairly often in response to type 3, for which the greatest discrepancy in the two surveys was noted. If this is correct, and a small percentage of children lose their antibodies during a year, it might be reasonable to consider revaccination with trivalent vaccine after one year. This would cover any negatives who had been missed on the first round, and also bolster the immunity of those who had lost their antibodies during the year. Our results with such a revaccination procedure resulted in the presence of antibody to all three types in close to 100% of children (Table VII).

As to the question of spread and persistence of attenuated polioviruses, the findings in our virological surveillance programme indicate that, under field conditions such as prevailed in the studies reported, spread to the general population by contact infection was minimal (field trial, 1958–9). These results are in line with those reported previously from this laboratory (Škovránek, Žáček, *et al.*, 1959; Burian *et al.*, 1960) and to those of Fox *et al.* (1960), who showed that, although spread of attenuated polioviruses among intimate familiar contacts was considerable, there was little evidence of extension outside the family to the community. Both Gard (1960) and Paul (1962) have emphasized the importance of the age of the infected individual in terms of spread of virus, by far the most effective spreader being the child under 2 years old. Our present studies on this point were confined to children aged 2 to 8 years. We have previously (Žáček *et al.*, 1960) reported, however, that in one highly industrial region where the oral vaccine field trial was carried out (Ustí) there was apparently no significant spread from vaccinees aged 2 to 8 years to children aged 12–23 months; on the other hand, in another more rural region (Jihlava) antibody conversions occurred in a considerable number of children aged 12–23 months, most of whom had been triple negatives before the vaccination trial was carried out. Whether these conversions were associated with spread of attenuated viruses or to infection with wild polioviruses is not known, but the evidence would seem to favour the latter interpretation.

Our present results also indicate that the attenuated polioviruses do not tend to persist in a community after being introduced on a large scale, even though, as was the case in 1959, a number of susceptibles are present in the population (Table VIII). The striking reduction in the prevalence of polioviruses after the nation-wide vaccination programme (Table IX) suggests that by means of the oral vaccine it may be possible to reduce the circulation of wild polioviruses to an insignificant minimum. These results are in accord with those of Sabin *et al.* (1961).

Summary

In connexion with oral poliomyelitis vaccination campaigns carried out in Czechoslovakia in 1958–9 and in a nation-wide extent in 1960 extensive virological and serological studies were accomplished. The results of these investigations indicate several points.

Immunological Efficacy.—(1) In a group of children who received each type of attenuated virus (Sabin) separately, 93.2% of those who lacked antibody to one or more types of poliovirus prior to vaccination converted to triple positives. The total seronegative conversion rate was 96.3% in this group, without any significant differences between individual types. (2) In another group of children who were fed type 1 virus separately and a mixture of types 2 and 3 one month later, 257 out of 286, or 93.4%, of those who lacked antibodies to one or more types before vaccination became positive to all types, and the total seronegative conversion rate was 92.7%. Only in children who were double or triple negative prior to vaccination was the antibody response to type 3 found to be lower—81.8 to 88.8% of these children developed antibodies to this type as compared with type 3 single-negative children, 96.2% of whom converted. (3) In a third group of children who were fed a single dose of trivalent vaccine the antibody response was significantly lower when compared with the preceding two groups. Only 128 out of 196, or 65.3%, of children who were without antibodies to one or more types before vaccination converted to triple positives, and the total seronegative conversion rate was found to be 74.8% (64.2, 87.0, and 80.3% for types 1, 2, and 3, respectively). The weakest response was demonstrated to type 1 virus by 42% gaps in children who lacked only type 1 antibody before feeding, and by 39.9, 18.4, and 29% negatives to types 1, 2, and 3, respectively, in children triply negative before vaccination.

Serological Surveys in General Population.—The results seem to indicate that, under field conditions, the separate administration of all three types of attenuated polioviruses to 40% of the total child population aged 2–8 years did not significantly influence the immunological status of the *non-vaccinated* children in the same age-group. The impact of the vaccine on the immunity of children aged 2–8 years who ingested attenuated poliovirus strains is indicated by the high percentage (92) of triple positives one month after the vaccination was completed. One year later this was reduced to 78%, largely as a result of lack of type 3 antibody in 18.4% of children tested. Type 1 and 2 antibodies, however, were still detected in 93% to 96% of children after a lapse of one year.

Laboratory Investigation of Clinical Cases.—As to cases reported in 1959 in children who had received oral vaccine in the field trial of 1958–9, there were four of these, and no poliovirus was isolated from them. In 1960 a total of 240 paralytic cases (including 164 cases of facial palsy) were virologically tested and a total of 69 polioviruses were isolated from them, all being recovered from cases which occurred between January 1 and the end of July, 1960. Between August 1 and the end of the year no poliovirus was isolated from five paralytic cases, 76 patients with facial palsy, and 270 aseptic meningitis cases reported and/or tested during this period of time.

Spread of Enteroviruses.—The surveillance of the spread of enteric viruses in orally vaccinated and non-

vaccinated regions was initiated in November, 1958, and repeated random collections of stool specimens were made throughout 1959 and 1960. In 1958–9 a total of 2,322 specimens was collected in the four vaccinated regions, and 2,964 specimens in 10 non-vaccinated provinces; in 1960 a total of 7,486 stool specimens was collected from healthy children throughout the whole country. At least three important observations can be made from the results gained hitherto. (1) The initial spread of viruses which follows their mass introduction into the child population is self-limited, and the attenuated strains (Sabin) do not appear to persist in a community longer than a few months. (2) There is no evidence that the attenuated viruses spread beyond the regions into which they had been introduced. (3) An extraordinarily reduced spread of polioviruses could be demonstrated at the peak of the season, after the nation-wide use of oral vaccine in the spring of 1960.

We are indebted to many co-workers without whose help this programme, completion of surveillance, could not have been carried out. Special acknowledgment is made to the staff of the Epidemiological Department of the Ministry of Health of Czechoslovakia; epidemiologists and virologists from the Hygiene and Epidemiological Service in many regions, particularly Drs. Anna Mayerová, Jaroslav Pešek, Irena Poledníková, Vladislav Potužník, Mojmír Suchánek, Michal Tarabčák, Milada Tesaříková, Jaroslav Valihrach, Helena Vojtová, and others; and to the staff of the Virus Departments of the Institute of Sera and Vaccines, Prague.

Addendum

Since this paper was prepared for publication a new nation-wide oral vaccination was carried out in 1961 in Czechoslovakia. In April, 1961, approximately the same number of children of the same age-groups as in 1960 received type 1 live poliovirus followed in June by a mixture of types 2 and 3. This means that about 90% of the child population have been revaccinated.

As regards the incidence of poliomyelitis, not a single virologically confirmed case of paralytic poliomyelitis was revealed in Czechoslovakia up to December 20, 1961.

In our virological surveys, carried out in the same manner as in previous years, not a single poliovirus strain was recovered from about 2,800 stool specimens collected at random from healthy children in March as well as approximately 600 samples collected in September, 1961, and tested up to the time of writing.

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ANTIBODY RESPONSE IN INFANTS TO THE POLIOMYELITIS COMPONENT OF A QUADRUPLE VACCINE

BY

IRENE B. HILLARY, M.B., D.P.H., D.C.H.

P. N. MEENAN, M.D.

Department of Medical Microbiology, University College, Dublin

A. P. GOFFE, M.B., Dip.Bact.

G. J. KNIGHT

A. D. KANAREK, B.Sc., Ph.D.

The Wellcome Research Laboratories, Beckenham

AND

T. M. POLLOCK, M.B., Ch.B.

Medical Research Department, the Wellcome Foundation

The two regimes recently recommended by the British Ministry of Health (*Brit. med. J.*, 1961b) for the active immunization of children against diphtheria, tetanus, pertussis, and poliomyelitis during the first two years of life involve either three or four injections of combined diphtheria, tetanus, and pertussis vaccine (triple vaccine) and three injections of Salk poliomyelitis vaccine, thus making a total of six or seven injections. The number of injections would, of course, be considerably reduced by replacing Salk vaccine by the Sabin oral vaccine, and the Ministry of Health has already put out a scheme for such a replacement (*Brit. med. J.*, 1962).

Although oral vaccine may eventually prove to be the vaccine of choice throughout the world, one must not lose sight of the fact that Salk vaccine may serve a useful purpose in many instances, especially if it were combined as a quadruple preparation with diphtheria, tetanus, and pertussis vaccines. The use of such a preparation would likewise reduce the number of injections necessary during early childhood. Already quadruple vaccine has been used in the U.S.A. (Bordt *et al.*, 1960) and in Canada (Wilson *et al.*, 1960), but there has been no routine use of it in the Republic of Ireland or in the United Kingdom. It was therefore considered advisable to carry out a study to investigate its value, and a comparison was made of the poliomyelitis antibody levels obtained in infants after immunization with quadruple vaccine with those after triple vaccine and poliomyelitis vaccine given at the same time but in separate injection sites.