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ENTEROVIRUS SURVEY IN CHILDREN AFTER MASS VACCINATION WITH LIVE ATTENUATED POLIOVIRUSES

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In Hungary three campaigns of oral immunization with Sabin's attenuated poliovirus strains were carried out in the period November, 1959, to June, 1960. (1) During November and December, 1959, the children aged 3 months to 14 years were twice fed with the trivalent vaccine in a single county (Győr-Sopron county). (2) During December, 1959, to February, 1960, the children of the same ages were immunized in the whole country separately with types 1, 3, and 2 at monthly intervals. (3) The children who had escaped vaccination and the infants born between September 1, 1959, and February 29, 1960, were twice fed with the trivalent vaccine during May and June, 1960. The results of the serological and virological examinations carried out in several laboratories of specimens collected during the above campaigns were favourable (Weissfeiler, 1961).

In this paper we present further data on the effectiveness of the mass oral vaccinations. The enterovirus excretion of the youngest healthy children was tested during the summer and early autumn of 1960—that is, during the season when the excretion rate of enteroviruses is usually the highest. We wished to see whether polioviruses were circulating in the population. In addition, the immune status of young children was examined six to eight months after vaccination.

Materials and Methods

Specimens.—Faecal samples were collected during August 24–31 and during October 26–31, 1960. The serum samples were collected during August 24–31. The samplings were organized by the local Public Health and Epidemiological Stations as ordered by the Ministry of Health according to a plan proposed by us. The specimens were sent in by ordinary mail. In August 300 healthy children aged 11 months to 4 years were tested. The children live in 10 counties (Baranya, Bács-Kiskun, Csongrád, Fejér, Győr-Sopron, Pest, Szabolcs-Szatmár, Vas, and Zala) and in the town of Budapest. Each had been immunized with the live poliovirus vaccine. Only one or two of them belonged to the same child community. Two stool samples and a serum sample were taken from each child. In October faecal specimens were collected from all the 24 administrative districts of Hungary (19 counties, Budapest, and 4 towns). We wished to examine specimens from 10 children aged 3 to 8 months and 10 children aged 9 to 23 months per 100,000 population from each county and town. The children under 9 months of age had not been vaccinated against poliomyelitis whereas those aged 9 months or more had been included in one of the three campaigns. From the same nursery only one child was selected. Faecal samples from 2,017 healthy children were suitable for testing. Of these children, 1,010 were under 9 months of age—that is, non-vaccinated. The ratio of tested children per 100,000 population ranged between 13.6 and 25.8 by geographical area.

Preparation of Specimens.—The specimens were prepared as described previously (Dömök *et al.*, 1961). The two samples taken from each child in August were mixed before testing. The 10% faecal suspensions and the inactivated sera were stored at −25° C. until the study was completed.

Isolation of Viruses.—The techniques have been published in detail elsewhere (Dömök *et al.*, 1960). Primary rhesus-monkey-kidney-cell cultures were used throughout. Each faecal suspension was inoculated into three tube cultures. The tubes were observed for 14 days.

Typing of the Isolates.—Hyperimmune sera to poliovirus 1–3, E.C.H.O. 1–15, 17, and 19–21, and Coxsackie A 9 and B 1–5 were used in the neutralization tests. The preparation of the sera and the origin of the type-strains have been published in detail (Dömök *et al.*, 1960). A strain was regarded as " non-typable " if it could not be neutralized by any of the sera listed used either alone or in combinations.

Neutralization Tests in Human Sera.—The sera were tested against the strains polio 1 Mahoney, polio 2 MEF_1, and polio 3 Saukett. Virus suspension containing 200 CPD50 per 0.1 ml. was added to an equal volume of each of the serum dilutions 1:2, 1:8, 1:32, 1:128, and 1:512. The virus–serum mixtures were kept at 37° C. for an hour and subsequently at +4° C. for 12 hours before being inoculated into monkey-kidney cultures, 0.1 ml. per tube. The tubes were examined microscopically for cytopathic changes on the fourth and seventh days. The titre was expressed in terms of the highest dilution showing neutralization on the seventh day in at least two of the three cultures inoculated.

Results

Virus Excretion

Of the 300 healthy children aged 11 months to 4 years who were tested in August, 79 proved to excrete virus. The isolation rates by age are shown in Table I. The lowest rate was found in those under 2 years of age, the highest in children aged 4 years. Coxsackie B 4 strains were isolated most often. Among the E.C.H.O. viruses types 1 and 14 were most extensively spread. Polioviruses (type 1 and type 3) were isolated from two children both previously vaccinated with the homotypic monovalent vaccine. Both strains proved to be of rct/40⁻ character (Sabin, 1961).

The survey in August showed a remarkable geographical variance. The isolation rate ranged from 12 to 16% for the counties of Csongrád, Tolna, and Vas; from 35 to 43% for Györ-Sopron, Pest, and Szabolcs-Szatmár; and from 21 to 31% elsewhere. The type 1 poliovirus was isolated from Szolnok county, the type 3 virus from Baranya county.

Table II presents the results of the virological survey of October, 1960. Of the 2,017 healthy children tested 320 excreted virus. The isolation rate being 12.6% for infants aged 3 and 4 months, increased parallel with age and reached its peak (19.1%) at 13–18 months of age. Virus was isolated from 142 (14.1%) of the 1,010 children under 9 months of age and from 178 (17.7%) of the

TABLE I.—*Virus Excretion by Age, August,* 1960

	Age of Children Tested (Years)				Total
	≤1	2	3	4	
No. of children tested	48	85	111	56	300
Excreters No.	9	23	30	17	79
Excreters %	19	27	27	30	26
Type of isolates:					
Polio 1				1	1
3			1		1
E.C.H.O. 1	1	1	3	3	8
2	1				1
4		1			1
6			2		2
7		2	2	1	5
8		2	3		5
11	2	2		1	5
12		2	4		6
13		1		1	2
14	1	2	3	2	8
15		1		1	2
20				1	1
Coxsackie A 9		1		1	2
B 2		1			1
B 4	1	5	8	5	19
Unidentified	3	2	4		9

TABLE II.—*Virus Excretion by Age, October,* 1960

	Age of Children Tested (Months)						Total
	3–4	5–6	7–8	9–12	13–18	19–23	
No. of children tested	246	454	310	250	481	276	2,017
Virus excreters No.	31	64	47	43	92	43	320
Virus excreters %	12·6	14·1	15·1	17·2	19·1	15·5	15·9
Type of isolates:							
E.C.H.O. 1	5	6	7	9	13	4	44
2	1		1	1	5	3	11
3			2				2
4			2	1	2	3	8
5			1		2	1	4
6	1	4	3	1	7	2	18
7	2	2	2	4	1	2	13
8	3	4	3	1	4	2	17
9					1		1
11	1	10	3	3	7	6	30
12	1	1		1	4	2	9
13	3	6	6	4	11		30
14	5	9	6	5	6	6	37
15				1	2		3
19			2		1		3
20		2		2	1		5
21		1					1
Coxsackie A 9	1	4	1		1		7
B 1		1					1
B 2		1		1			2
B 3				1			1
B 4	3	7	4	3	9	7	33
B 5		1	1				2
Unidentified	5	5	3	5	15	5	38

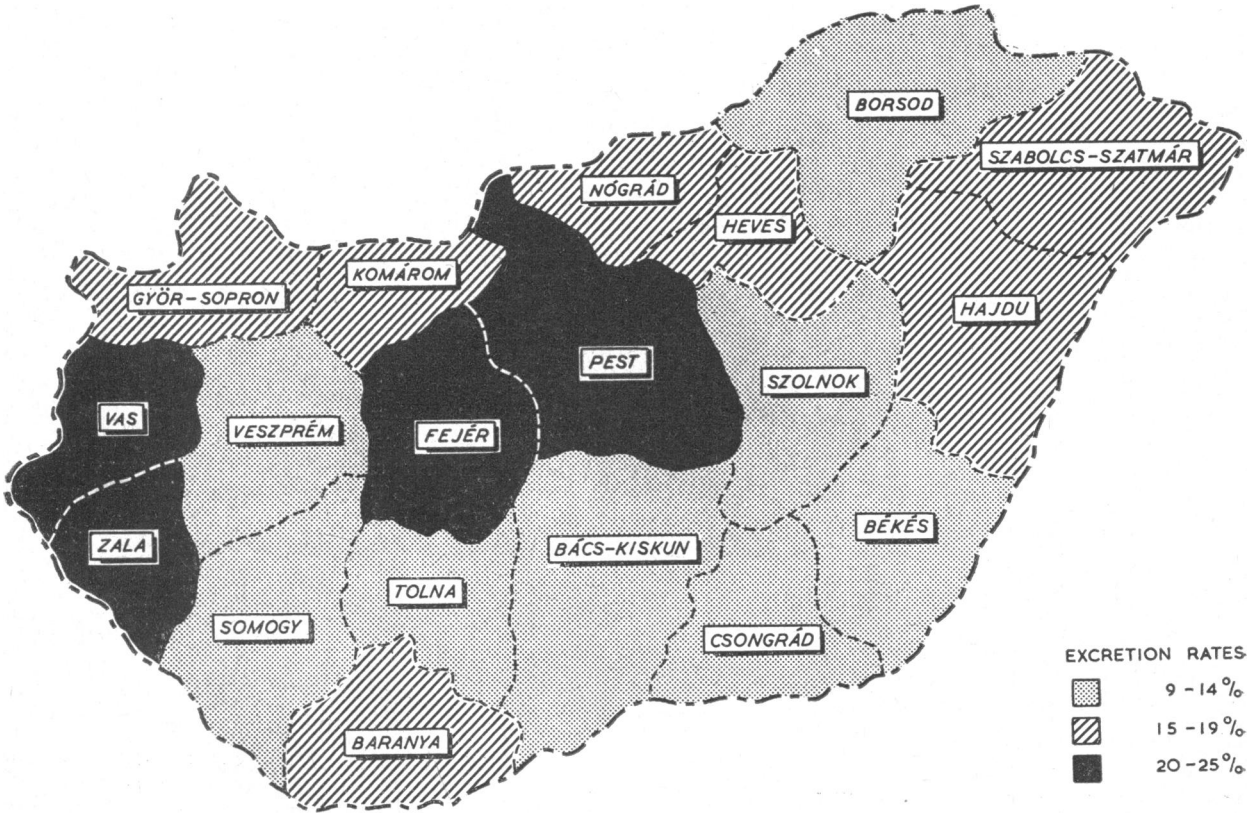

FIG. 1.—Virus excretion rates in October, 1960, in various counties of Hungary.

1,007 children aged 9 to 23 months. No poliovirus was isolated, neither from the vaccinated children nor from the non-vaccinated ones. Of the isolates 236 (73.7%) proved to be E.C.H.O. virus, 46 (14.4%) Coxsackie virus, while 38 (11.9%) could not be identified. In the infants under 9 months of age 10.4%, in the older children 13.2%, excreted E.C.H.O. virus. The excretion rates for Coxsackie viruses were 2.4 and 2.2% respectively.

As in August, also in October, E.C.H.O. 1 and 14 and Coxsackie B 4 strains were excreted most often, although the frequency of the Coxsackie B 4 virus had declined. On the other hand, the percentage of the E.C.H.O. 11 and 13 strains had increased. Fig. 1 illustrates the isolation rates by geographical areas. The circulation of enteroviruses was most intensive in Fejér, Pest, Vas, and Zala counties. The most frequent types (E.C.H.O. 1, 11, 13, and 14, and Coxsackie B 4) were present in almost every area. During the two months from August to October the virus circulation had remarkably changed in some areas. This change was favourable in Györ-Sopron and Szabolcs-Szatmár counties and unfavourable in Vas county.

Serological Survey

Among the 300 children tested for virus excretion in August, 237, 236, and 234 were tested also for neutralizing antibodies to poliovirus types 1, 2, and 3, respectively. Of the 237 children, 201 had been immunized with the complete series of the monovalent vaccines, whereas 36 (in Györ-Sopron county) had received two doses of the trivalent vaccine. Regardless of this, antibodies to types 1, 2, and 3 were demonstrated in the 1:4 or over diluted sera in 94.5%, 96.6%, and 94.4%, respectively, of the children tested. The respective percentages for the children immunized with monovalent vaccines were 95.5, 96.6, and 94.4; and for those immunized twice with the trivalent vaccine, 89, 100, and 100. It should be noted that the number of children in the latter group was too small for comparison.

The percentage distribution of sero-negative children and of children with different titres as a function of age are illustrated in Figs. 2, 3, and 4.

Regardless of the type of virus, the greatest number of negative children were found in the youngest group. The divergence between the children under and over 2 years of age was most pronounced in relation to the type 1 virus. In the younger group 22% and in the older groups 1.6–5.5% had no demonstrable type 1 antibodies. The frequency of high titres to the type 1 virus was closely related to the age of the children. Such a correlation could not be demonstrated for the other two types of virus.

Both children excreting poliovirus were triple-positive, having 1:1,024 titres to the homotypic strains.

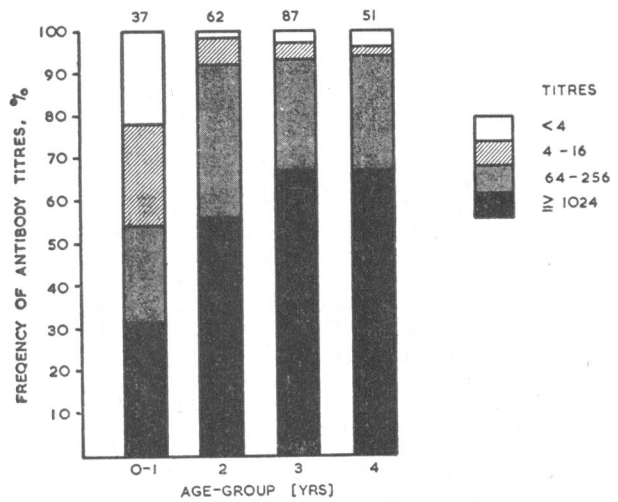

FIG. 2.—Polio 1 antibody titres by age in August, 1960. The figures at the head of the columns indicate the number of children tested.

Discussion

As has already been stated (Rudnai, 1960; Dömök and Molnár, 1961), Hungary experienced an intensive poliomyelitis epidemic in 1959 in spite of the two-year compulsory immunizations with the Salk vaccine (Bakács, 1960; Rudnai and Barsy, 1961). The young healthy children were still found to be heavily infected by poliovirus at the end of the epidemic (Dömök *et al.*, 1961). In Györ-Sopron county 65 healthy children under 4 years of age were tested for virus excretion late in October, 1959. Of these, 13 excreted poliovirus, and the total number of enterovirus excreters was 32. Poliovirus excretion was common even in the older children. Of 95 healthy children aged 4 to 15 years, 12 proved to excrete poliovirus. Subsequently, in December, 1959, 13 strains of poliovirus and 37 strains of other entero-

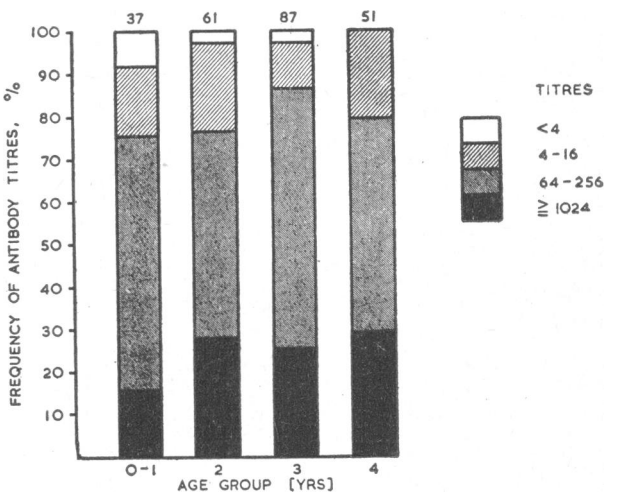

FIG. 3.—Polio 2 antibody titres by age in August, 1960.

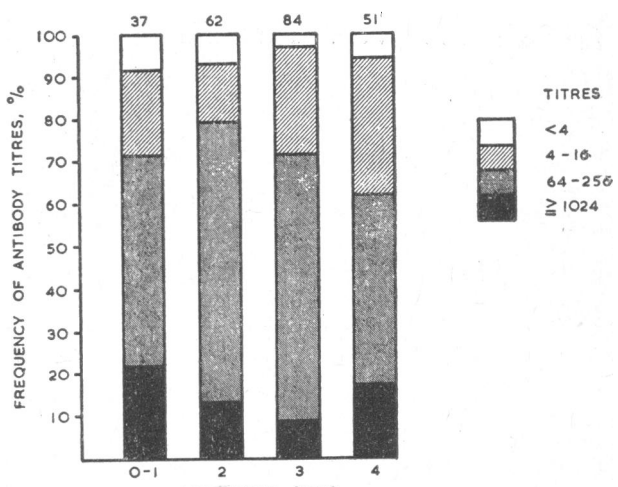

FIG. 4.—Polio 3 antibody titres by age in August, 1960.

viruses were isolated from 290 healthy children under 4 years of age. These children lived in 10 administrative districts of Hungary.

The surveys in August and October, 1960, showed that the situation has changed remarkably. In these periods the circulation of enteroviruses in general was at least as intensive as in 1959, *but the polioviruses had practically disappeared.* The reality of this conclusion was proved particularly by the October survey, when 1.6% of the infants aged 3 to 8 months and 0.6% of the children aged 9 to 23 months were tested everywhere in Hungary. The weight of these data is increased by the facts that (1) only one child was selected from the same nursery, and (2) that the children under 9 months of age had not been immunized against poliomyelitis. Thus if poliovirus has been present this completely susceptible age-group would have been particularly favourable for its circulation.

Another finding also makes it more probable that the polioviruses had disappeared in Hungary. We could not isolate poliovirus from any of the specimens collected from 585 suspect cases of enterovirus infection during the second half of 1960. Other enteroviruses were isolated from 42 patients. The seven cases reported as poliomyelitis during the above period were included in the 585 cases tested. Most of the reported cases were atypical, and the clinical suspect could never be confirmed serologically. The facts that as few as seven cases were reported as poliomyelitis during the second half of 1960 and that only three of these occurred during the summer months (Kátay, 1961) are in accordance with the conclusions drawn from the 1960 surveys.

The two healthy children who excreted poliovirus in August are thought to be persistent excreters of the vaccine strains. This assumption is supported, but not proved, by the rct/40⁻ character of both strains. Both children had received the homotypic vaccine strain six to eight months earlier. Since such a long duration of excretion is improbable, we believe that the children might have picked up the virus from infants vaccinated in May and June, 1960. Persistent excretion of the vaccine strains occurs very rarely. Sabin *et al.* (1961) did not succeed in reisolating the vaccine strains except in a single case (type 2 poliovirus) from 680 vaccinated children under 16 years of age three months after the oral vaccinations in Cincinnati.

Enterovirus infections are very common in the youngest age-groups. Supposing that the excretion rates obtained in our October study were valid for the whole population of the respective age-groups, the number of the symptomless excreters under 2 years of age in Hungary could be estimated at 40,000. Consequently, the isolation of an enterovirus from a patient in itself should not be accepted as evidence of the aetiological role of the isolate. This principle is neglected in some publications.

We found a direct relation between age and enterovirus excretion in infants under 1 year of age. This should be emphasized, because it is generally accepted that the excretion rate gradually declines with rising age —for example, Gelfand (1961). This is true only for older children. Our data are in accordance with the findings of Vandeputte (1960), who continuously examined children under 2 years of age for virus excretion in the Congo during 1957 and 1958. However, Vandeputte found the peak of the age curve at 8 months, whereas our age curve has its peak between 13 and 18 months. The difference might be explained by the hot climate and poor hygienic condition of the Congo. The poorer excretion of the youngest children might be due to the special conditions of life in infancy and not to the maternal antibodies. The latter seem not to hinder the implantation of viruses in the intestinal tract, according to the available data.

The results of our serological data are in accordance with the data of Fornosi (1961) and of Váczi *et al.* (1961), who tested children during vaccination campaigns early in 1960.

Summary

Some months after the country-wide immunizations with live poliovirus vaccine in Hungary healthy young children were tested for enterovirus excretion and poliovirus antibodies.

In August, 1960, among 300 children under 5 years of age 79 were found to excrete enterovirus. Two isolates proved to be poliovirus (types 1 and 3). Both poliovirus strains were found to be rct/40⁻. We also tested 237 children for neutralizing antibodies to the three types of poliovirus: antibodies were demonstrated in 94.5%, 96.6%, and 94.4% to types 1, 2, and 3 respectively.

In October, 1960, 1,010 children aged 3 to 8 months, and 1,007 children aged 9 to 23 months, were tested for enteroviruses. The geographical distribution of the children was proportional to that of the total population (about 20 per 100,000 population in every district of Hungary). The children over 9 months had been immunized with the live vaccine, the younger children had not. No poliovirus was isolated, while 15.9% of the children excreted non-polio enteroviruses. In infants under 1 year of age a direct relation was found between age and enterovirus excretion rate.

During both periods of study Coxsackie B 4, E.C.H.O. 1, and E.C.H.O. 14 strains were isolated most often.

The present studies have supplied evidence of disappearance of the wild polioviruses from the population after mass vaccinations with live polio vaccine strains.

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