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RESULTS OF A STUDY OF THE REACTOGENIC AND IMMUNOGENIC PROPERTIES OF LIVE ANTI-POLIOMYELITIS VACCINE

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SYNOPSIS

The authors have studied the harmlessness and immunogenic properties of live poliomyelitis vaccine made in Leningrad from Sabin strains of low pathogenicity for monkeys. More than 20 000 children of pre-school (6 months to 3 years) and school age (7-14 years) were each given 100 000 tissue-culture infective doses of virus of types 1, 2 and 3, injected either in three stages at monthly intervals in the form of monovaccines, or in two stages, a monovaccine of type 1 being followed after a month's interval by injection of a divalent vaccine of types 2 and 3. The vaccination caused no symptoms of lesions of the central nervous system or other organs. In the blood of the inoculated children there was a regular build-up of virus-neutralizing antibodies to the serotypes mentioned, the intensity of which depended on the antibody level before vaccination and was in a constant relationship to the multiplication of the virus in the intestinal canal. The antibody titre was maintained at high levels for 6-9 months after immunization and fell a little after 12-18 months. The vaccinal virus is easily transferred from vaccinated children to contact groups, which are gradually vaccinated by this natural means.

Lengthy and numerous passages of vaccinal strains through the intestinal canal of normal, susceptible children showed that strains may periodically appear which have a higher neurotropic activity for monkeys. This activity, however, did not increase in subsequent passage and returned to the initial level.

The active immunization of the susceptible population with killed or live vaccines is the only real method of effectively preventing poliomyelitis.

The basic features of the pathogenesis of poliomyelitis are such that vaccination poses two problems, one more complex than the other:

The first problem is the establishment in the blood of a protective barrier of specific antibodies, preventing the spread of the virus from its primary

and main reservoir in the intestinal canal into the central nervous system. The injection into susceptible children of the Salk inactivated vaccine successfully solves the problem of creating specific humoral immunity, which suppresses the danger of viraemia developing and of the causative agent then becoming generalized in the central nervous system. It does this the more effectively, the higher and more stable is the level of virus-neutralizing antibodies in the blood of the vaccinated persons.

The use of the killed vaccine in the period 1955-58 on more than 100 000 000 children has established its undoubted value for solving the most important and urgent task, i.e., the reduction of the number of paralytic, bulbar and fatal cases of poliomyelitis. The very important question how long post-vaccinal immunity lasts after injection of the Salk inactivated vaccine still requires further elucidation.

The second problem is the formation of local immunity of the intestinal tract in addition to humoral immunity, as this would ensure not only that the spread of the infectious process to the central nervous system would be stopped but that the multiplication of the virus in the main organ which is sensitive to it could be suppressed, thereby preventing the circulation of the poliovirus in the human community.

This more difficult and fundamental task of poliomyelitis prevention can be solved only through use of an attenuated living-virus vaccine, since the injection of the Salk inactivated vaccine does not increase the resistance of the intestinal canal, does not protect inoculated children against the development of slight and symptomless forms of poliomyelitis, has no effect on the circulation of the virus in the human community, and apparently confers only a short-term immunity.

According to the data obtained by Koprowski and Sabin (1955, 1958), who put forward the first low-pathogenicity strains for active immunization against poliomyelitis, enteral administration of the live vaccines concerned leads to the development of a local intestinal infection, which reproduces a symptomless form of poliomyelitis and encourages the development of an intense humoral immunity.

So far, however, the main problems of the safety of the live vaccine, both for the vaccinated themselves and, especially for persons coming into contact with them, remain unsolved.

It is quite admissible to suggest that attenuated strains of the poliomyelitis virus entering into the composition of the live vaccine may intensify their invasive and neurotropic properties both in multiplying in the intestinal canal of the vaccinated persons and, particularly, during their further long-term natural circulation through the bodies of healthy children who come into contact with vaccinated persons.

Vaccinal strains of the poliovirus in the live vaccine should possess the following properties:

(*a*) They should be harmless for *Macaca rhesus* or *Macaca cynomolgus* monkeys when 10^6-10^7 tissue-culture infective doses ($TCID_{50}$) per ml of live virus are injected intracerebrally.

(*b*) This property should remain highly stable not only after the virus has multiplied in the intestinal canal of vaccinated children but also after its further circulation through the intestinal canal of unvaccinated child contacts.

Stability of the hereditary characters of vaccinal strains does not necessarily mean that strains must exhibit exactly the same levels of neurotropic activity initially and after lengthy multiplication in the intestinal canal. It is only necessary that in these conditions the vaccinal strains should continue to display sufficiently marked quantitative differences from the average levels of neurotropic activity shown by the " street " strains (isolated from paralytic cases).

(*c*) They should be harmless for children inoculated with the live vaccine and also for children in contact with those inoculated.

(*d*) They should show that high capacity for multiplication of the virus in the intestinal canal which is necessary to establish local resistance to repeated infection.

(*e*) They should develop an intense general humoral immunity in the vaccinated.

The artificially attenuated strains of poliomyelitis of low pathogenicity for monkeys obtained in laboratory conditions by Koprowski and Sabin (1955) satisfy the last two requirements so far as their immunogenicity is concerned. However, the important problem of the stability and the harmlessness for human beings of the vaccinal strains has still not been solved and requires further research.

It is known that Sabin (1956, 1957) studied the clinical safety of the strains he obtained, which were attenuated for monkeys, on a group of more than 300 adults and also on a small group of children some of whom had antibodies to one or two serotypes of the poliovirus. The harmlessness of enteral administration of the vaccine as established in this series of observations is not an exhaustive proof of its safety for children under school age who do not possess antibodies against types 1, 2 and 3 of poliovirus (triply negative children). The different poliomyelitis morbidity rates among persons of different age-groups are related not only to the different levels of specific immunity in the various age-groups but also to the fact that different age-groups do not possess identical susceptibility to the causative agent of the disease.

In our observations and study of the live attenuated vaccine, special attention was paid to solving the problem of the harmlessness and the immunogenicity of the vaccine in enteral immunization of sufficiently large

groups of children of pre-school age in whom antibody findings were triply negative and who thus had the maximum susceptibility to poliomyelitis.

Research was carried out in stages as follows:

(1) the preparation of experimental batches of the live vaccine from the Sabin strains and the study of their neurotropic qualities on monkeys;

(2) the testing of monovalent vaccines on 127 children of pre-school age (0.5-3 years of age);

(3) the testing of the monovalent vaccines on 2010 children of the same pre-school age ; [1]

(4) the testing of the live vaccine for harmlessness on a further and larger group of children of pre-school and school age (up to 14 years), made up of about 20 000 vaccinated children and an internal control group (contact group) of 10 000.

The Preparation of Experimental Batches of Live Vaccine in Monolayer Monkey-Kidney Tissue-Culture for Observations on Human Beings

Taking into account Sabin's data concerning the possibility of an increase in the neurotropic activity of attenuated strains in the intestinal canal of healthy susceptible persons, between 24 and 30 additional passages of the Sabin strains mentioned below were made in monolayer monkey-kidney tissue-cultures. We used the rapid-passage technique recommended by Sabin (inoculation of the cultures with massive doses of the virus, the pH of the culture fluid being maintained at about 7.4-7.6). By adopting this method we counted on further attenuating the initial vaccinal strains and on producing more stable hereditary characters of the strains in the course of further adaptation of the virus to extraneural tissue.

The experiments were carried out with variants of the virus tested by Sabin in his trials on human subjects (type 1 LSc of 22.12.55; type 2 P 712-10 ab. of 5.9.56; and type 3 Leon 14 ab. of 5.4.56). The strain of type 1 was received from Sabin in March 1956; and strains of type 2 P 712-10 ab. and type 3 Leon 14 ab. were received in July 1956. We did not have at our disposal at that time all the latest variants of Sabin's attenuated strains, which he had obtained in the form of pure lines passed three times through single plaques by the Dulbecco method.

In January 1957 the laboratory prepared experimental samples of live vaccine in monolayer monkey-kidney tissue-cultures from attenuated vaccines, in a quantity of about 5 litres for each serotype. The virus-containing suspension obtained on synthetic medium 199 showed a titre corresponding to the level of pathogenicity of the original Sabin strains.

[1] The main series of observations was carried out in infant homes and on groups of children under school age in children's homes in Leningrad. In these homes we vaccinated about 60% of the children, the remaining 40% serving as an internal control (contact) group.

TABLE 1. PATHOGENICITY * FOR *MACACA RHESUS* MONKEYS OF VACCINAL STRAINS OF POLIOVIRUS INJECTED INTO THE BRAIN OR SPINAL CORD IN AN INITIAL CONCENTRATION OF 6.6-7.4 LOG_{10}

Undiluted tissue-culture	Method of infection	Virus type		
		1	2	3
Vaccinal strains according to Sabin's data	Intracerebral	0/12	0/12	0/12
	Intraspinal	3/15	2/24	0/29
Live vaccine prepared in Leningrad	Intracerebral	0/10	0/10	0/10
	Intraspinal	1/10	1/12	0/13

* Expressed as the number of monkeys paralysed over the number tested.

Table 1 shows the results of tests on monkeys of monovalent vaccines of types 1, 2 and 3 as compared with the data given in Sabin's paper concerning the pathogenicity for monkeys of the latest variants he has obtained. The vaccines were tested by separate injection of monovalent undiluted vaccines in the brain and spinal cord of monkeys. We demonstrated the high degree of attenuation of the live vaccine obtained. There was a complete absence of clinical symptoms on intracerebral injection of 0.5 ml of the initial (undiluted) material into the thalami. When initial undiluted viruses of the first and second type were injected intraspinally there developed in a small proportion of the monkeys, on the fourth to the eighth day, pareses and paralyses of the lower extremities which followed a mild course. Recovery from these motor lesions depended on their severity. In the case of paresis the functions were restored in the majority of the animals within three weeks after infection. When paralysis developed these lesions became persistent and remained unchanged, whereas the general condition of the animals was quite satisfactory. Among the 12 additional monkeys who, in the course of experiments in 1957-58, developed paralysis after infection with vaccines of types 1 and 2, no deaths occurred. Vaccine of type 3 did not cause any clinical symptoms when the undiluted material was injected intraspinally.

Histopathological examination of monkeys infected with the vaccinal strains was carried out by O. A. Svyatukhina (Laboratory of Normal and Pathological Morphology of the Nervous System; Director, Professor Y. M. Zhabotinski) and showed that the severity of histological changes in the spinal cord was in direct relationship to the markedness of the clinical symptoms. When there were no clinical symptoms, histopathological examination also gave negative results in intraspinally injected monkeys. This requires further verification in view of the limited number of sick animals we investigated.

All the monovalent vaccines were tested for bacterial sterility according to the instructions for the testing of Salk vaccine, including special tests for

tuberculosis through the infection of guinea-pigs. The same live vaccines were tested for the absence of the virus herpes B by intracutaneous and subcutaneous infection of rabbits, and for absence of the virus of lymphocytic choriomeningitis by intracerebral infection of white mice. The vaccine was also tested in monolayer tissue-cultures of monkey kidney after neutralization of the poliovirus with homologous rabbit immune serum. This made possible the exclusion of the presence in the vaccine of any other cytopathogenic viruses.

Testing for Harmlessness and Immunogenicity in Trials on Children of Pre-school Age

As a preliminary measure the live vaccine in polyvalent form (a mixture of equal volumes of the monovalent vaccines) was administered enterally in the quantity of one million tissue-culture doses to eight members of the staff of the Virology Department and to 67 children aged between 4 and 15 years. None of those inoculated showed local or general reactions to the inoculation during the following two months. In inoculated children with low or medium antibody levels before immunization, an increase in antibodies to all three types of virus was established (Table 2). Virological examination of this group one month after immunization gave negative findings.

At the end of April 1957 we received permission to try out on 150 children in infants' homes in Leningrad the reaction-causing and immunogenic properties of the live vaccine prepared in the Virology Department of the Institute of Experimental Medicine. What was envisaged was the testing

TABLE 2. CHANGES IN TITRE OF VIRUS-NEUTRALIZING ANTIBODIES IN THE BLOOD OF 68 CHILDREN, AGED 4-15 YEARS, INOCULATED WITH POLYVALENT LIVE VACCINE OF TYPES 1, 2 AND 3

Antibody titre after vaccination	Antibody titre before vaccination																	
	type 1						type 2						type 3					
	0	4	16	64	256	1024	0	4	16	64	256	1024	0	4	16	64	256	1024
1024	1	3	3	1	—	1	2	3	3	—	1	3	—	1	2	5	1	1
256	1	4	8	1	5	—	7	7	2	3	1	—	2	6	7	3	2	—
64	3	6	5	7	—	—	6	10	7	3	—	—	6	6	8	8	—	—
16	4	7	4	—	—	—	4	4	2	—	—	—	2	4	4	—	—	—
4	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Number of children	9	24	20	9	5	1	19	24	14	6	2	3	10	17	21	16	3	1

of the clinical harmlessness of the vaccine, the study of virological and serological findings in those who had been inoculated by enteral administration of polyvalent or monovalent vaccines, the study of the duration of acquired immunity from the data obtained by serological tests or from investigation of the dynamics of virus multiplication in the intestinal canal after a repeated administration of live attenuated vaccine, and the investigation of possible changes in the neurotropic activity for monkeys of the vaccinal strains used, in the course of their natural circulation in the intestinal canal of healthy susceptible persons.

By 1 February 1958 the investigations on 127 vaccinated children were finished, and by 1 June 1958 a further group of 2010 persons, most of them aged 3 years or under, had been vaccinated with official permission. Vaccination consisted of oral administration of 1 ml of the monovalent vaccines of types 1, 2 and 3 together with a spoonful of milk or milk mixture (for infants), in an amount of 100 000 tissue-culture doses each time. In the trials on infants under 1 year, vaccination was carried out on two successive days to ensure the passage of the virus into the intestinal canal.

Clinical observations were carried out under the guidance of the Nervous Diseases Clinic of the Leningrad Medical Paediatric Institute (Director, Professor E. F. Davidenkova) and the Infectious Diseases Clinic of the Leningrad Institute of Sanitation and Hygiene (Director, Professor V. V. Kosmachevski) according to the following plan.

The children to be vaccinated were chosen by paediatricians permanently supervising communities of children from among somatically healthy children whose temperature had been normal for a fortnight before vaccination. The children's temperatures were taken regularly and the general condition of the respiratory tract and intestines was kept under observation. Before vaccination and for six months after vaccination, detailed neurological examinations were carried out, special attention being paid to the development of symptoms of irritation of the meninges and spinal nerve roots and to the condition of muscle tone and reflexes. Where necessary, experts in connected branches of medicine, such as ear, nose and throat specialists, surgeons, etc. were drawn into the work. The general blood picture and the erythrocyte sedimentation rate were determined before and repeatedly after vaccination. Observations carried out up to the time of writing have shown the complete harmlessness of the use of live attenuated vaccine, which has not caused local or general reactions or changes in the nervous system. Individual cases where there were rises in temperature among the inoculated children were found to be unconnected with vaccination (influenza outbreak in 1957, Coxsackie virus, tonsillitis, etc.).

The conclusion that the live vaccine used was harmless for 2010 inoculated children of pre-school age becomes particularly convincing in view of the fact that no less than 70% of the inoculated children were triply negative reactors, i.e., had the maximum susceptibility to poliomyelitis.

The same conclusion was drawn by clinicians in regard to the group of children, nearly equal in number and of identical age and immunological condition, which was in constant and close contact with the inoculated children. Despite the swift involvement of the overwhelming majority of the children in the contact group in the circulation of the vaccinal strains, they remained healthy, and according to clinical observation did not develop abortive or apparent symptoms of poliomyelitis, lesions of the intestines or other disorders during the next 6-12 months after immunization.

Quantitative Changes in Multiplication of Attenuated Virus in Intestinal Canal of Inoculated Children on Primary and Repeat Administration

During 1957-58, V. I. Ilyenko, B. P. Alekseyev and N. E. Gorev carried out repeat sample tests on 369 children divided into groups of 20-30 and inoculated with monovalent vaccines of types 1, 2 and 3. Changes were studied in the quantity of virus present in faecal specimens taken after various periods had elapsed from the time of immunization, the investigations lasting two months.

Faecal specimens were collected twice before vaccination and 1, 4, 8, 12, 16, 24, 28, 32, 42, 49, and 60 days after vaccination. The specimens were mixed after receipt with five times their quantity by weight of Hank's solution to produce a 20% suspension which was centrifuged twice, for 30 minutes each time, at 3000 and 6000 revolutions per minute. The pH of the supernatant fluid was tested with phenol red, and where a yellow colouring occurred the fluid was alkalinized with a 5% solution of sodium bicarbonate until a dark-pink colour was obtained. Until the test was carried out the original faecal material or the 20% centrifugate was kept frozen at −20°C. The supernatant fluid obtained was diluted to 10^{-1} to 10^{-6} in mixture No. 199 and inoculated into monolayer fibroblast cultures prepared from trypsinized human embryonic tissue. First of all the same material was tested in parallel on monolayer monkey-kidney tissue, which showed the virus in substantially the same titres as the fibroblast cultures. (There was a divergence of not more than 0.2-0.5 $\log_{10}$ in favour of the monkey-kidney tissue.) This enabled us to use for our basic investigations monolayer fibroblast cultures, which are more readily available in our laboratory.

Preliminary evaluation of the results of titration was carried out 8-10 days after inoculation of the faecal matter investigated. The final dilutions which caused cytopathogenic changes were passaged further on fresh monolayer cultures under the control of homologous antisera for identification of the vaccinal strain administered to the children.

Thus the concentration of virus per gram of faeces at various times of test after immunization was determined.

FIG. 1. TYPE 1 POLIOVIRUS MULTIPLICATION IN INTESTINAL CANAL OF CHILDREN INOCULATED WITH LIVE VACCINE

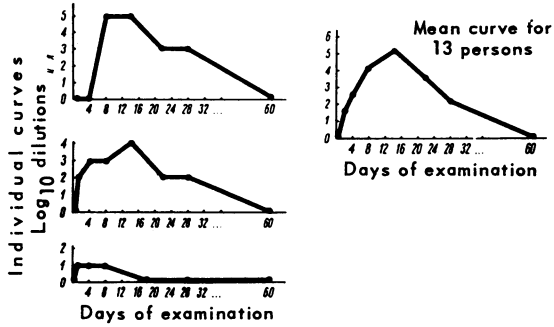


FIG. 2. TYPE 2 POLIOVIRUS MULTIPLICATION IN INTESTINAL CANAL OF CHILDREN INOCULATED WITH LIVE VACCINE

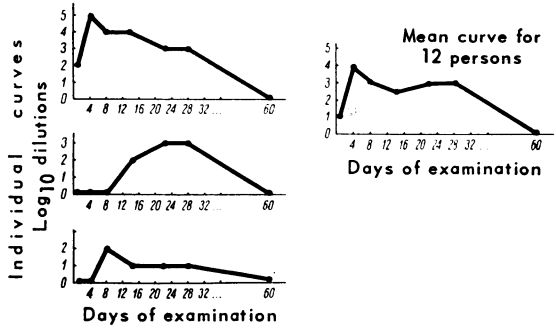
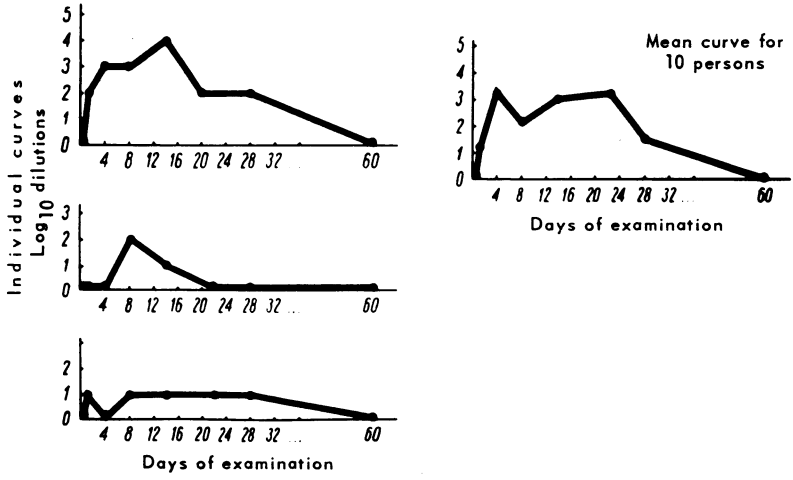


FIG. 3. TYPE 3 POLIOVIRUS MULTIPLICATION IN INTESTINAL CANAL OF CHILDREN INOCULATED WITH LIVE VACCINE

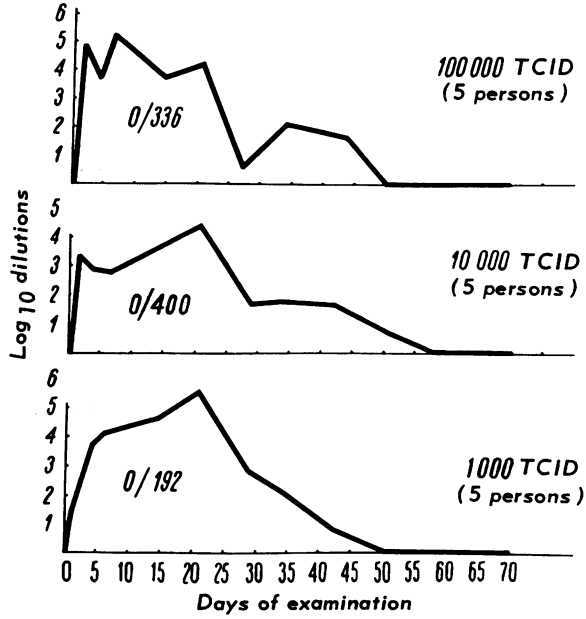


The results of the virological examination of individual children of pre-school age and the average figures for the concentration of virus in groups of between 10 and 13 children triply negative at the moment of vaccination are given in Fig. 1, 2 and 3.

Viruses of all three types appear in the faeces in the first days after vaccination, and reach high titres in the majority of the children 4-8 days after injection of the virus (10^4 to 10^6 per g of faeces). The maximum quantities of virus are found between the fourth and the twenty-eighth day, after which excretion of the virus gradually decreases and ceases altogether after 45-60 days.

In the overwhelming majority of children whose blood contained no antibodies to any of the three types, the excretion of the virus took on a protracted, intense and regular character. In a smaller number of children the excretion of virus was short-lived and ended after 16 or 20 days, or continued longer but in smaller quantities (10^1 to 10^2). There were no cases with negative findings during the whole period of examination, which points to the high capacity of the Sabin strains for multiplication in the intestinal canal of children susceptible to poliomyelitis. The elimination

FIG. 4. MULTIPLICATION OF TYPE 2 POLIOVIRUS IN INTESTINAL CANAL OF VACCINATED CHILDREN IN RELATION TO DOSE OF VACCINE



The figures inside each curve represent the mean antibody titre for the group examined in the first sera (numerator) and in the second sera (denominator), taken two months after vaccination.

of neurotropic properties achieved during attenuation of types 1, 2 and 3 viruses was not reflected in their infectivity for the intestinal canal.

The intensity of virus excretion was higher in the case of type 1 and somewhat lower in the case of types 2 and 3. In children inoculated with 10 000 TCID of virus of types 1, 2 and 3, the virus multiplication noted was also regular and intensive. When doses of 1000 TCID were given, multiplication was much less intensive and regular. This shows that the dose of 100 000 TCID recommended by Sabin (1957) is quite reliable and ensures a solid reserve of excess virus (Fig. 4).

We did not observe any stable relationship between the capacities for multiplication of the various types of vaccinal virus and the frequency of contact infections caused by them among 340 susceptible children of 1-3 years of age who were in contact with the inoculated children. On some occasions type 1 virus was most frequently isolated from contact children and on others type 2. These observations were paralleled by the immunological changes which were found to take place in children of the contact group living with the children inoculated with monovalent vaccines of types 1, 2 and 3 (Table 3).

TABLE 3. IMMUNOLOGICAL CHANGES IN 14 UNINOCULATED CHILDREN IN CONSTANT CONTACT WITH CHILDREN SIMULTANEOUSLY INOCULATED WITH LIVE VACCINE OF TYPES 1, 2 AND 3

Children in uninoculated (control) group	Antibody titre after contact with inoculated children for periods shown (in days)											
	type 1				type 2				type 3			
	0	21	60	90	0	21	60	90	0	21	60	90
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	4	64	64	64	0	0	0	0
3	0	0	0	256	0	0	4	4	0	4	4	4
4	0	0	0	16	0	0	0	0	0	0	0	0
5	0	0	256	256	4	256	256	256	64	256	256	256
6	0	0	256	256	0	0	0	16	0	0	0	0
7	0	0	64	256	0	16	16	16	0	0	0	0
8	0	16	16	16	0	0	0	0	0	0	0	64
9	0	0	16	64	0	0	0	0	0	0	0	0
10	0	0	16	256	0	0	0	0	0	0	0	0
11	0	0	16	256	0	0	0	0	0	0	0	0
12	0	0	16	16	0	0	0	0	0	0	0	0
13	0	0	16	64	0	0	0	0	0	0	0	0
14	0	0	64	64	0	0	0	0	0	0	0	0

TABLE 4. COURSE OF SPREAD OF VACCINAL VIRUSES OF POLIOMYELITIS FROM VACCINATED CHILDREN TO CONTACT GROUPS

Virus type	Number of children		Percentage of children in contact group excreting virus after following number of days:								Cumulative percentage of virus carriers (contact group) after following number of days:								
	vaccinated	contact	10	20	30	40	50	60	80	130	10	20	30	40	50	60	80	130	Total
1	33	55	16.5	11	11	38	31	10	2	2	16.5	21.9	27.3	47.3	54.6	54.6	54.6	54.6	54.6
2	59	31	35.4	38	45	29	27	13	13	6	35.4	54.4	60.8	64.0	67.2	70.4	76.8	80	80
3	25	71	8.4	34	31	45	65	15	18	14	8.4	26.7	42.2	62.0	71.8	73.2	73.2	76	76

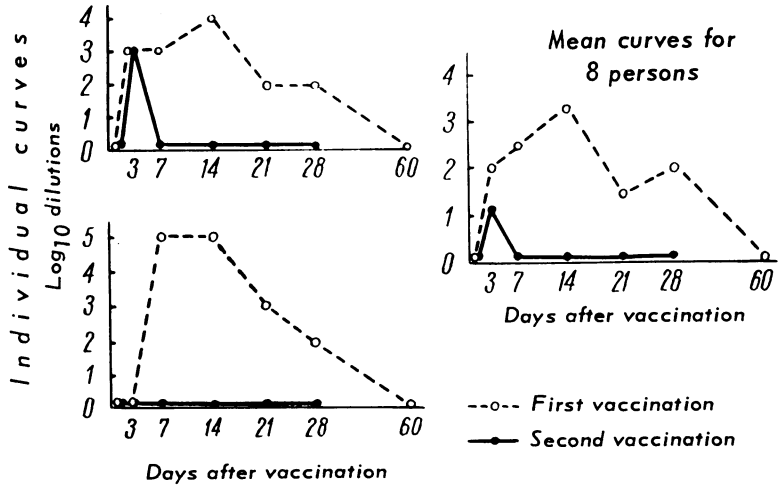
The high degree of contagiousness of type 1 vaccinal virus was noted also in children who had been inoculated against type 2 or type 3, and who had been in contact with children inoculated with vaccines of the two other serotypes. In the course of three months of contact the overwhelming majority of those children acquired an immunity to type 1 virus, whereas natural immunization against type 2 and type 3 viruses under the same conditions occurred in less than half of the contact group of susceptible children. Table 4 shows the course of spread of vaccinal viruses of types 1, 2 and 3 from inoculated children to uninoculated groups between 1 and 3 years of age consisting of 31-71 children in contact for five months with children vaccinated with one type of virus. In these observations the total percentage of virus carriers among children in the contact group varied from 54.6 (type 1) to 76 (type 3) and 80 (type 2); i.e., the percentage was very high.

If a homologous type of live vaccine was injected again, 3-6 months after the first immunization, the virus was either not detected at all in the examination carried out at various periods after the repeat immunization, or else was found in very small quantities and only sporadically (observation on more than 60 children). This bears witness to the formation in children inoculated with live vaccine of a local type-specific immunity of the intestinal canal, the duration of which needs further investigation (Fig. 5).

Immunological Changes in Blood of Children Vaccinated with Monovalent Vaccines

Children inoculated with monovalent vaccines of types 1, 2 and 3 (the three serotypes being administered in different orders in different cases) were examined by L. M. Kurnosova to determine the content of virus-neutralizing antibodies in the blood before vaccination and in samples taken 3, 8, 12, 15, 23 and 28 weeks after vaccination. The sera were heated for 30 minutes at 56°C and were stored frozen until tested. The majority

FIG. 5. MULTIPLICATION OF TYPE 1 POLIOVIRUS IN INTESTINAL CANAL AFTER FIRST AND SECOND VACCINATIONS



of the sera collected at various times were tested in a single-stage experiment by neutralization of 100 TCID of the virus of each serotype. Indication of neutralization was taken to be the elimination of cytopathogenic effect or the inhibition of the metabolism of the tissue-culture in a Salk colour test. Parallel experiments with the two methods regularly showed higher titre values with the colour test than when the cytopathogenic effect method was used, the difference ranging from twofold to fourfold. Because the colour test is more simple and its results are easily reproducible, we carried out the majority of our titrations by that method and used as a source of cells human fibroblasts, which gave results identical with those obtained by the use of cells from monkey-kidney tissue.

Our investigations showed that about 70% of the children aged between 6 months and 3 years whom we examined had absolutely no antibodies to any of the three types of virus in their serum diluted 1:4; 10% of the children had antibodies to one or two types of virus in a 1:4 dilution of their serum but very rarely in a 1:16 dilution. The immunological characteristics of our children were therefore extremely favourable for an evaluation of the harmlessness and effectiveness of the live vaccine.

All the children examined responded between 21 and 60 days after enteral administration of the live vaccine with an intensive increase in antibody titre, reaching an average of 1:256-1:1024, this being maintained for 6-9 months, after which there was a gradual decrease during the 15 months they were kept under observation after immunization. Fig. 6 and 7 show the mean titres of antibody to viruses of types 1, 2 and 3 in a constant group of 70 children of pre-school age. It is not impossible that a stimulating

FIG. 6. VIRUS-NEUTRALIZING ANTIBODY TITRES IN CHILDREN AFTER PRIMARY IMMUNIZATION WITH CORRESPONDING MONOVALENT VACCINES

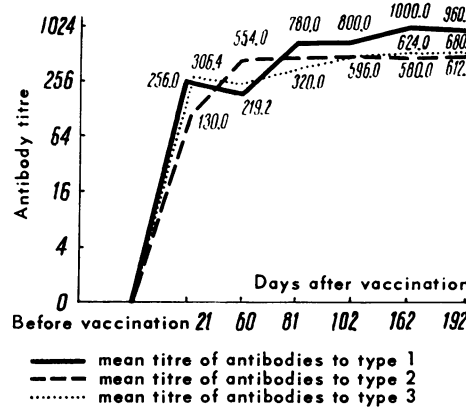
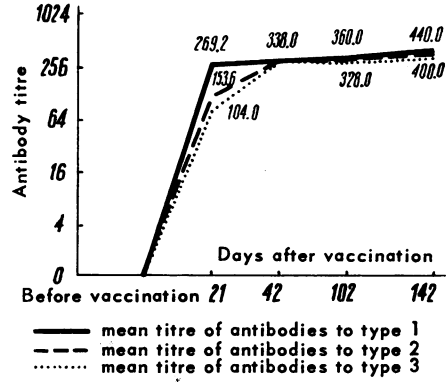


FIG. 7. VIRUS-NEUTRALIZING ANTIBODY TITRES IN CHILDREN AFTER SECOND OR THIRD IMMUNIZATION WITH CORRESPONDING MONOVALENT VACCINES

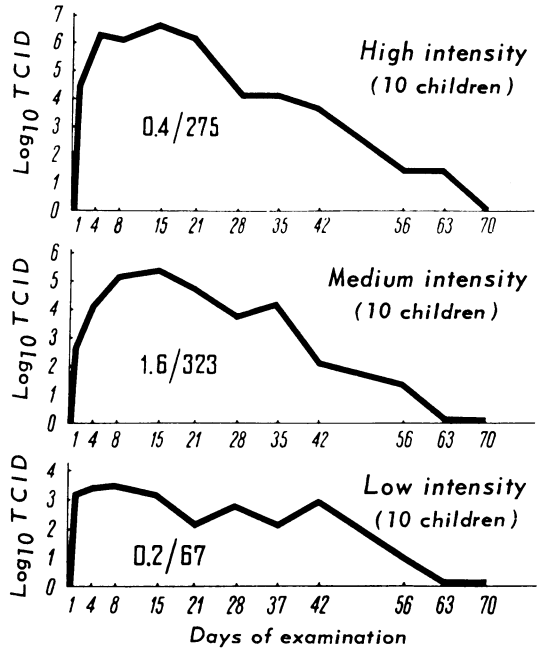


effect, leading to the maintenance of high titres of humoral immunity in children inoculated with the live vaccine, is produced by repeat contact infections caught from uninoculated children who, in the following months, maintain the circulation of the vaccinal virus in the children's community concerned.

The magnitude of the increase in virus-neutralizing antibodies did not depend on whether the susceptible children had been inoculated with the vaccine of the type concerned first (Fig. 6) or second or third (Fig. 7). There were also no noticeable differences in the intensity and duration of humoral immunity in children inoculated against various types of administered virus. This bears witness to the absence of any strict interdependence between the quantities of virus in the intestinal canal and the immunological

changes which they cause in the blood, since the concentration of antibodies to the more active virus of type 1 did not differ from the concentration of antibodies to the less active virus of type 3, nor did it differ in groups of children with different degrees of intensity of virus multiplication in the intestinal tract (Fig. 8).

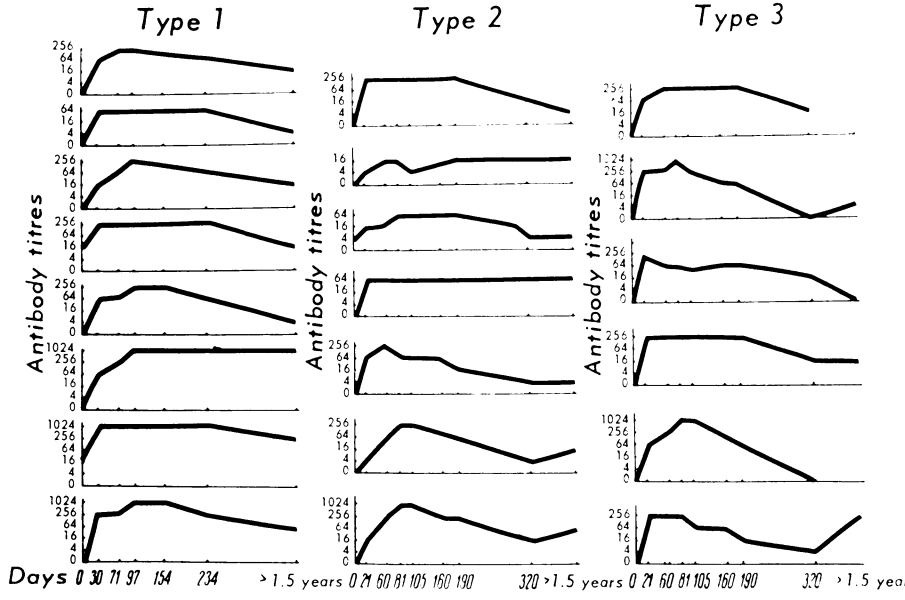
FIG. 8. CORRELATION BETWEEN INTENSITY OF MULTIPLICATION OF TYPE 1 POLIOVIRUS IN INTESTINAL CANAL OF VACCINATED CHILDREN AND ANTIBODY LEVEL 70 DAYS AFTER IMMUNIZATION



The figures inside each curve represent the mean antibody titre for the group examined in the first sera (numerator) and in the second sera (denominator).

The results obtained from these immunological examinations of children between 6 months and 3 years of age inoculated with monovalent live vaccines of types 1, 2 and 3 showed a completely regular increase in the number of virus-neutralizing antibodies for the first 3-8 weeks and the maintenance of the level of humoral immunity achieved for the subsequent 6-9 months (Fig. 9) and for a further 15 months. The titres obtained are not less but, on the contrary, considerably greater than the optimal changes in humoral immunity obtained by triple immunization with the Salk inactivated vaccine, and thus also guarantee a more effective elimination of the paralytic, bulbar and fatal forms of the infection than can be obtained by the use of killed vaccine.

FIG. 9. ANTIBODY TITRES IN INDIVIDUAL CHILDREN, AGED 1-3 YEARS, INOCULATED WITH LIVE POLIOMYELITIS VACCINE OF TYPES 1, 2 AND 3



Quite marked humoral changes occur also in children with partial immunity before vaccination and in children in contact with vaccinated persons.

TABLE 5. IMMUNOLOGICAL CHANGES IN BLOOD OF CHILDREN, AGED 1-3 YEARS, INOCULATED WITH MONOVALENT VACCINE OF POLIOVIRUS TYPE 1 AND DIVALENT VACCINE OF POLIOVIRUS TYPES 2 AND 3

Antibody titre 3 months after vaccination	Antibody titre before vaccination																	
	type 1						type 2						type 3					
	0	4	16	64	256	1024	0	4	16	64	256	1024	0	4	16	64	256	1024
1024	5	—	—	—	—	—	3	—	—	—	—	1	—	—	—	—	—	—
256	2	1	—	—	—	—	7	1	—	—	—	—	7	1	—	1	—	—
64	2	—	—	2	—	—	—	—	—	1	—	—	2	—	1	—	—	—
16	1	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Number of children	10	1	—	2	—	—	10	1	—	1	—	1	10	1	1	1	—	—

The quantitative indices of immunological changes in vaccinated children are similar whether the immunization has been carried out with three separate monovalent vaccines or by the injection first of a type 1 monovaccine and then of a divalent vaccine of types 2 and 3 (Table 5).

In view of this it is possible either to use the three-stage separate immunization procedure with monovaccines of types 1, 2 and 3 or to use a two-stage procedure more convenient for mass immunization by administering first a monovaccine of type 1 and then a divalent vaccine of types 2 and 3. The intervals between injections should be from one to one-and-a-half months.

The simultaneous development of local immunity in the intestinal canal must lead to a further limitation of morbidity and must have an important effect in inhibiting the circulation of the virus in communities inoculated with the live vaccine.

Changes in Neurotropic Properties of Vaccinal Strains on Repeated Passage through the Intestinal Canal of Healthy Persons

An important problem in the study of the reaction-causing properties of live vaccine is to discover the range of any possible intensification of the neurotropic properties of the vaccinal strains during their multiplication in the intestinal canal of inoculated children and particularly in the course of their further circulation through the intestinal canal of healthy children coming into contact with those inoculated.

The basic criterion for assessing changes in the neurotropic qualities of vaccinal strains isolated after lengthy multiplication in the intestinal canal of healthy children was to test them on *Macaca rhesus* monkeys by intracerebral and intraspinal titration of the isolated strains in dilutions of 1:1, 1:10 and 1:100.

In the majority of tests we took two or three healthy *Macaca rhesus* monkeys for each dilution, using for this only animals not previously subjected to experiment.

The material for study was:

(1) The vaccinal virus strains isolated from inoculated children from 21-28 days after inoculation.

(2) Strains isolated from healthy children in contact with inoculated children in long-term repeated virological examination. In view of the fact that the average length of time which the vaccinal virus spends in the intestinal canal is 30 days, we estimated the number of possible additional passages made by the virus among the children in contact with the inoculated children by calculating the period after inoculation in days, subtracting 45 days, and dividing the result by 30. If, for example, the virus was isolated from the contact, uninoculated, group 140 days after the introduction into that community of children of the vaccinal strain corresponding to it in

type, we considered that the strain isolated had undergone (140 minus 45) ÷ 30 = 3, i.e., not less than three additional passages through the intestinal tract of children in the group investigated.

Detailed tests of 14 virus strains of types 1, 2 and 3, isolated during four to five natural passages through the intestinal canal of children under three years of age, have shown the possibility of a small increase over the initial neurotropic activity for monkeys. However, the actual range of these changes is very small and did not exceed a dilution of 1:10 of the initial virus culture (containing not less than 6.2-7.4 $\log_{10}$ tissue cytopathogenic doses per ml) in intracerebral infection of monkeys or 1: 100 in intraspinal infection.

(3) Artificial passage of the virus through the intestinal canal of three to five children, triply negative according to blood antibodies.

In these investigations the cytopathogenic viruses isolated after 14-28 days from the intestinal canal of inoculated children were identified with the introduced vaccinal strain, after which the viruses from two or three different children were pooled. The pool was titrated on monkeys to determine its intracerebral and intraspinal activity, and was then again administered orally to a small group of susceptible children, etc.

The children were given virological examinations after 1, 3, 7, 14 days and so on at 7-day intervals up to 70 days, in order to study the changes in the quantity of viruses of the passaged strains in the intestinal canal.

Up to the present time we have carried out eight successive passages with viruses of types 1, 2 and 3. The results are given in Table 6.

As will be seen from Table 6, in the course of consecutive passages of vaccinal strains through the intestinal canal of inoculated children we periodically isolated viruses with a higher level of neurotropic activity than that of the initial live vaccine. In the cases noted, illness occurred and paralysis developed in monkeys on the intracerebral injection of the isolated strains of types 1, 2 and 3 (containing in the initial liquid 6.2-7.4 $\log_{10}$ tissue cytopathogenic doses of virus) both undiluted and in a dilution of 1: 10. Still more regular were the similar results obtained through intraspinal injection of the passaged viruses, which caused illness in the monkeys after their infection with tissue fluid in a dilution of 1:10 and, in one case, 1:100.

The figures for the pathogenicity for monkeys of the passaged strains nevertheless remained quite low and were quite sharply different from the average figures of pathogenicity of the street strains from paralytic patients, for which a regular paralytogenic dose, in intracerebral infection of *Macaca rhesus* monkeys with freshly isolated tissue-cultures of the virus, is a dilution of 1: 100 000 or more.

It is also important to realize that neurotropic activity, while it does increase within small limits, does not grow progressively more intense in successive passages but shows periodical relapses, after which it again

TABLE 6. CHANGES IN NEUROTROPIC ACTIVITY OF VACCINAL STRAINS OF POLIOVIRUS (INITIAL CONCENTRATION, 6.2-7.4 LOG_{10}) FOR *MACACA RHESUS* MONKEYS AFTER PASSAGE THROUGH INTESTINAL CANAL OF SUSCEPTIBLE CHILDREN

Type of virus	Method of infection	Activity of the strains after indicated number of passages through intestinal canal								
		initial	1	2	3	4	5	6	7	8
1	Intracerebral	—	—	—	—	+	—	—	—	—
	Intraspinal	±	++	—	+	+++	+++	+	++	+
2	Intracerebral	—	—	—	—	++	—	—	++	—
	Intraspinal	±	+	—	—	++	—	+	++	—
3	Intracerebral	—	+	—	—	—	—	—	—	—
	Intraspinal	—	+++	—	+	—	—	+	—	+

— = Monkeys healthy
\+ = Monkeys fall ill on injection of virus in dilution 1:1
++ = " " " " " " " " " 1:10
+++ = " " " " " " " " " 1:100

returns to its initial low level. Thus, passages through the intestinal canal do not cause a constant increase in the neurotropic activity of vaccinal strains but evidently show up the small residue of pathogenic properties still present in the initial strains and which remained hidden when those strains were cultivated under test-tube conditions. Lengthy passage of a vaccinal virus through the intestinal canal leads to some decrease (1.0-2.0 log_{10}) in the mean figures for its multiplication, particularly in the case of the low-titre viruses types 2 and 3, but has no effect on the duration of virus excretion which, in children of this group, does not exceed 45-60 days.

Between November 1958 and March 1959 we vaccinated with the live vaccine an additional 20 000 children aged between 1 and 14 years, some of them by the three-stage method (monovaccines of types 1, 2 and 3) and some by the two-stage procedure (type 1 followed by divalent vaccine of types 2 and 3 after one to one-and-a-half months); immunological examinations have shown that both procedures, which involve the injection of 100 000 TCID of each type in each vaccination, are highly effective. About 40% of the children in the children's establishments concerned were not vaccinated and served as a control (contact) group.

Clinical observations have established a complete absence of neurological or general infectious symptoms among the vaccinated and the contact groups, which amount at the moment to about 30 000 children.

In view of this it seems obvious that the observations made of the neurotropic activity of the vaccinal strains rising to a concentration of

3.5-4.5 $\log_{10}$ are of no practical significance, since even at this level of pathogenicity for monkeys the vaccinal strains remain completely harmless for human beings.

Conclusion

1. In observations on 22 201 children of pre-school age, 30% of whom were infants of 1-3 years of age and triply negative in regard to antibodies to the polioviruses, no neurological or general clinical reactions were noted to the enteral administration of a live vaccine prepared from Sabin's strains.

2. Similar results were obtained in a group of uninoculated children, comprising 40% of the total number in the children's establishments and of the same composition, who were in close and protracted contact with the inoculated children. During the period of this contact fairly regular infection of these uninoculated children with the vaccinal strains was established.

3. In children of pre-school age inoculated and examined virologically, quite regular multiplication of the vaccinal strains in the intestinal canal was noted, and this reached its highest intensity between 8 and 21 days after immunization (10^4-10^6 TCID of virus per g of faeces); the intestinal canal was thereafter quickly cleared of the viruses between 45 and 60 days from inoculation. Virus of types 1 and 2 multiplied most intensively, and virus of type 3 less intensively. The frequency of contact infections among uninoculated children was similar for all three types.

4. The repeated administration of live vaccine of homologous type between three and six months after enteral immunization does not cause vaccinal infection at all or is accompanied by only very meagre and short-lived multiplication of the virus introduced. This proves the development in those vaccinated with live vaccine of a local immunity of the intestinal canal, which is capable of limiting the circulation of the virus among the inoculated population.

5. Enteral immunization with live vaccine stimulates the development of humoral immunity, the intensity, duration and frequency of which are greater than those obtained by the threefold injection of Salk-killed vaccine.

6. Eightfold passage of vaccinal strains through the intestinal canal of susceptible children is accompanied by an occasional intensification of their neurotropic activity for monkeys. When infection is by the intracerebral route the level of this activity does not exceed a virus tissue-culture dilution of 1:10; when infection is by the intraspinal route, the figure is 1:100. This increase in neurotropic activity does not develop further in subsequent passages through the intestinal canal, in which the circulating vaccinal strains are maintained in a markedly attenuated condition.

7. The information set out in this paper shows the harmlessness and high level of immunogenicity of Sabin's live vaccine for the groups of children most susceptible to poliomyelitis and provides a basis for the further study of this important method in larger-scale epidemiological experiments.

RÉSUMÉ

Les auteurs ont étudié les propriétés réactogènes et immunogènes du vaccin antipoliomyélitique vivant obtenu à Léningrad par culture de souches atténuées de A. Sabin sur une couche monocellulaire de tissu rénal de singe. Le vaccin a été administré à raison de 100 000 doses infectantes, par voie buccale, à plus de 8000 enfants d'âge préscolaire (six mois à trois ans), et à plus de 12 000 enfants d'âge scolaire (7 à 14 ans), soit en trois fois (vaccin monovalents des types 1, 3 et 2, à des intervalles d'un mois), soit en deux fois (vaccin monovalent du type 1, suivi un mois après d'un vaccin bivalent des types 2 et 3). Les observations des pédiatres et des neurologues ont établi l'innocuité de la vaccination et l'absence de symptômes neurologiques ou infectieux, aussi bien chez les vaccinés que chez le groupe témoin (40% de l'effectif vacciné) qui était en contact constant et prolongé avec les vaccinés.

La plupart des enfants de six mois à trois ans qui ne possédaient pas d'anticorps des 3 types ont présenté une multiplication intense des sérotypes de virus inoculés, avec un maximum entre le 7e et le 20e jour, où la concentration de virus par gramme de fèces a atteint 10^4 à 10^6; le virus disparaissait du tractus intestinal au bout de 30 à 60 jours. On a observé des résultats analogues après administration de 10 000 doses infectantes.

Le virus-vaccin s'est transmis facilement des enfants vaccinés aux enfants non vaccinés avec une intensité variable selon le pourcentage de sujets vaccinés dans la collectivité et l'intensité des contacts. Dans les collectivités fermées où les vaccinations ont porté sur 50 à 70% des enfants de moins de trois ans, l'infection par contact a atteint, au bout de trois ou quatre mois, 70% de l'effectif. Les souches de vaccin vivant atténué utilisées ont des propriétés héréditaires suffisamment stables et ne donnent pas de réversion au type original.

L'étude expérimentale de la réversion au type des souches inoculées s'est faite par passages répétés (8 fois) dans le tractus intestinal des enfants vaccinés en utilisant également la circulation naturelle du virus dans les groupes d'enfants en contact étroit avec les vaccinés. Dans les deux groupes on a observé à plusieurs reprises l'excrétion de virus des types 1, 2 et 3 présentant pour les singes un pouvoir pathogène plus grand que le virus-vaccin original. Toutefois, le renforcement périodique de l'activité neurotrope des souches vaccinales dans le tractus intestinal des enfants réceptifs ne présentait aucun caractère stable ni progressif, car ces souches revenaient régulièrement à leur état original lors des passages suivants. On voit donc qu'il existe, chez les souches vaccinales, une certaine réserve de pouvoir pathogène pour les singes, qui se manifeste mieux dans le tractus intestinal humain que dans les cultures de tissu rénal de singe.

On a constaté des changements immunologiques tout à fait réguliers, intenses et suffisamment stables (pendant les 15 mois suivants) chez le groupe très réceptif à la poliomyélite d'enfants vaccinés de moins de trois ans dont 90% étaient négatifs aux trois types de virus avant la vaccination. Les chiffres d'augmentation des anticorps neutralisants, leur régularité et leur durée sont sensiblement plus élevées que chez les enfants ayant reçu trois injections de vaccin tué de Salk. Des modifications humorales très nettes ont lieu également chez les enfants qui présentent, avant la vaccination, une immunité partielle, ainsi que chez les enfants en contact avec les vaccinés.

Les indices quantitatifs des changements immunologiques produits chez les enfants vaccinés ne diffèrent pas suivant que l'on administre en trois fois des vaccins monovalents ou que l'on utilise un vaccin bivalent des types 2 et 3 pour la deuxième vaccination.

On peut donc utiliser non seulement l'immunisation en trois fois avec des vaccins des types 1, 3 et 2, mais aussi le procédé plus commode pour la vaccination de masse qui comporte deux injections (un vaccin du type 1 et un vaccin bivalent des types 2 et 3). Les intervalles entre les vaccinations sont de un mois à un mois et demi.

Le vaccin vivant provoque une immunité locale du tractus intestinal qui se manifeste par une multiplication nettement moins forte des types homologues en cas de réintroduction du virus un à six mois plus tard.

Les études à poursuivre sur le vaccin vivant devront consister principalement à accumuler des observations sur les propriétés réactogènes et immunogènes, et des données épidémiologiques sur l'efficacité du produit, la durée de l'immunité obtenue et le perfectionnement de la technique, en particulier des moyens de conservation du vaccin.

REFERENCES

Koprowski, H. et al. (1956) *J. Amer. med. Ass.*, **162**, 1281
Sabin, A. B. (1955) *Ann. N. Y. Acad. Sci.*, **61**, 924
Sabin, A. B. (1956) *J. Amer. med. Ass.*, **162**, 1589
Sabin, A. B. (1957) *J. Amer. med. Ass.*, **164**, 1216
Sabin, A. B. (1958) *Advanc. Pediat.*, **10**, 197
Smorodintsev, A. A. et al. (1958) [*Living vaccine against poliomyelitis*]. In: Institute of Experimental Medicine, USSR Academy of Medical Sciences [*Annual report . . . 1957*], Leningrad, p. 301

Postscript (June 1959)

By June 1959, the authors had completed the additional series of field observations carried out in different places in the USSR (where previous Salk vaccine application had not been intensive) on 1 421 000 children, aged 6 months to 14 years and immunized twice with live Sabin vaccine (type 1 followed by a divalent vaccine of types 2 and 3 after 1 month). More than 15% of the serologically tested vaccinated children were triply negative before immunization, which means that nearly 200 000 children belonged to the most susceptible group in regard to the polioviruses. No neurological or general clinical reactions were noted during these intensive field trials, and no increase in poliomyelitis cases was registered in the vaccinated or contact groups in the cities or villages where 70-90% of all the children were vaccinated. Thus, in the authors' opinion, the live vaccine from Sabin's strains can be considered completely safe for large-scale use against poliomyelitis.