Contents

Poliomyelitis

Poliomyelitis is an acute viral infection which ranges in severity from a nonspecific illness to paralysis with permanent disability. Worldwide, WHO estimates that some 140 000 new cases of paralytic poliomyelitis occurred in 1992. The cumulated number of children and adults with paralysis due to poliomyelitis is estimated at 10 to 20 million persons.

This document reviews the nature of immunity to polioviruses, techniques to measure protection against polioviruses, and the response to infection with wild and vaccine viruses. Emphasis has been placed on studies which provide data on children in developing countries.

1. The Virus

Polioviruses are classified into three distinct serotypes (type 1, type 2, and type 3) based on their reaction with reference panels of neutralizing antisera *(Bodian et al. 1949)*. They belong to the genus enterovirus in the family picornaviridae.

Polioviruses are stable at acid pH and can survive for weeks at room temperature and for many months at 0°C to 8°C. As with other enteroviruses, polioviruses are resistant to ether, 70% alcohol and other laboratory disinfectants. Treatment with 0.3% formaldehyde, 0.1 N HCl, or free residual chlorine at a level of 0.3 to 0.45 parts per million rapidly inactivates polioviruses, as does exposure to a temperature of 50°C or higher or to ultraviolet light *(Minor & Bell 1990)*.

Early work identified two distinct types of antigens in harvests from virus infected cells, which were designated as D antigen and C antigen. D antigen is largely but not exclusively associated with infectious virus and C antigen with empty capsids *(Minor 1990)*.

More recent investigations have revealed the complexity of the antigenic structure of polioviruses. The poliovirus virion is small, with a diameter of 27 to 30 nm, and contains a single stranded molecule of RNA. A thin 20-sided shell composed of four virion proteins (VP1, VP2, VP3, VP4) surrounds the RNA *(Hogle et al. 1985)*. Several sites involved in virus neutralization have been identified on the surface of the poliovirus. For example, a site composed of amino acids 89 to 100 of VP1 is a major immunogenic site for type 2 and type 3 polioviruses, as judged by monoclonal antibodies induced in mice *(Minor et al. 1986b)*.

2. The Nature of Immunity Against Poliomyelitis

2.1 Response to natural infection

Humans are the sole reservoir for poliovirus. Wild polioviruses are spread directly or indirectly from person to person. Virus dissemination is facilitated by poor sanitation. In all countries, children under two years of age create a microenvironment of less than optimal hygiene within the family and within daycare settings, readily facilitating fecal-oral and oral-oral (mouth-fingers-mouth) transmission. Feces can serve as a source of contamination of water, milk, or food, and houseflies can passively transfer poliovirus from feces to food *(Gear 1952)*.

Wild poliovirus enters through the mouth, attaches to receptors on the epithelium of the throat and intestine, and replicates inside these cells. Newly synthesized poliovirus is shed from infected cells; it can be cultured from the pharynx for the first week after onset of paralysis and from feces for several weeks and sometimes months after onset (Figure 1). From these sites the virus spreads to cervical and mesenteric lymph nodes. Poliovirus enters the blood stream via the lymphatics. Virus from the blood stream can invade the central nervous system unless sufficiently high levels of neutralizing antibodies are present to block it. Within the central nervous system, the virus spreads along nerve fibers and in the process of its intracellular multiplication it destroys motor neurons, resulting in flaccid paralysis. Sensory neurons are not affected.

The majority of wild poliovirus infections are asymptomatic. A type 1 polio outbreak in 1948 allowed direct assessment of the number of subclinical infections for each paralytic case using results of serological tests and virus isolations from stools *(Melnick & Ledinko 1953)* (Table 1). In a total

Figure 1. Time course of events in infection with poliovirus.
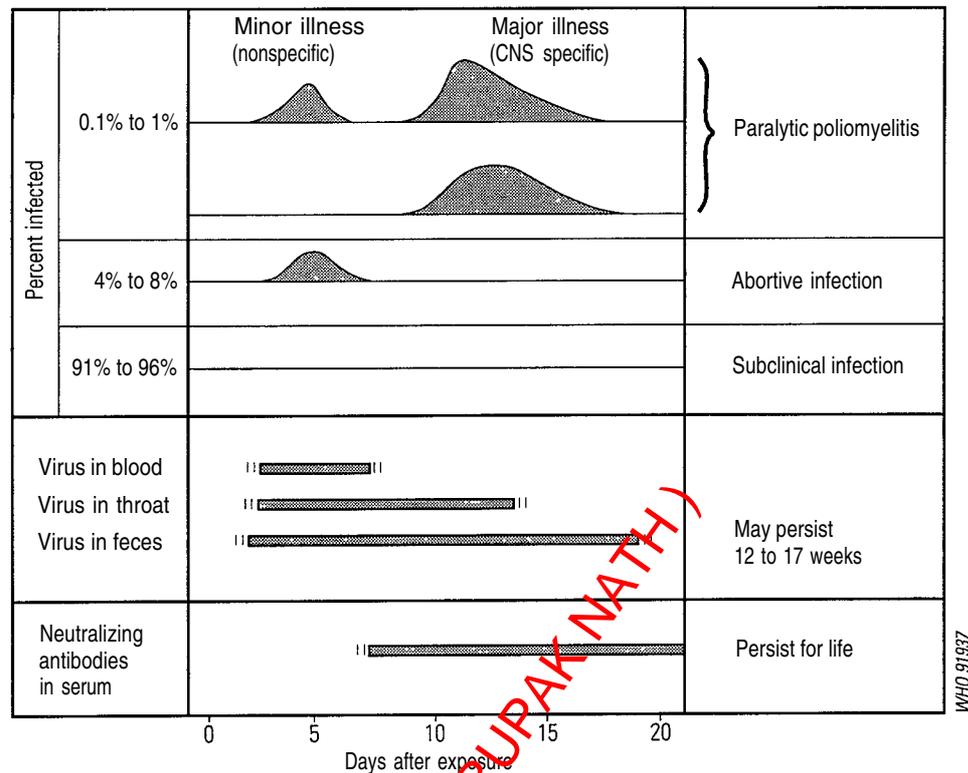
population of more than 80 000 persons aged 0 to 20 years, fewer than 1% developed paralysis. About one-quarter of children aged 0 to 14 years were infected subclinically, with somewhat higher rates in younger children. Among children aged 1 to 14 years, about 100 were subclinically infected for each paralytic case; among infants, about 200 were subclinically infected for each paralytic case.

Direct neural spread of poliovirus may also occur in certain situations, such as during tonsillectomy with subsequent bulbar paralysis or following injec-

tion of an irritating substance into a limb leading to subsequent paralysis of that limb *(Wyatt 1990)*. Recently, the cellular receptor for poliovirus has been identified *(Mendelsohn et al. 1989)* and there is optimism that molecular methods will lead to greater understanding of the pathophysiology of polio infection.

Following natural exposure, IgM and IgG appear in the serum about 7 to 10 days after infection. Sufficiently high levels can block poliovirus entry into the central nervous system. Initially, the IgM re-

Table 1. Ratio of subclinical infections per paralytic case during a poliovirus type 1 outbreak, Winston-Salem, North Carolina, 1948 *(Melnick and Ledinko 1953).*

Age group (years)	No. paralytic cases	Population in age group	No. paralytic cases per 1000 population	Subclinical infections Ratio*	Subclinical infections (%)	No. subclinical infections per paralytic case
< 1	3	1 800	1.6	5/18	28	175
1 to 2	10	3 900	2.6	10/3	26	100
3 to 4	12	3 600	3.3	7/29	24	73
5 to 9	25	7 300	3.4	8/38	21	62
10 to 14	13	6 300	2.1	5/25	20	95
15 to 19	9	6 200	1.6			
20+	6	58 100	0.1			
Total	78	87 200	0.9			

* Number with antibody 6 months after start of epidemic/number without antibody at start of epidemic, where a neutralizing antibody titer of 10 or higher is considered positive.

sponse is 2- to 8-fold greater than the IgG response. IgM levels peak at about 2 weeks after exposure and disappear from the serum within about 60 days. IgG levels increase steadily and persisting serum antibody belongs to this class. IgA antibody appears in the serum 2 to 6 weeks after exposure and remains at low levels; in some individuals there is no rise in serum IgA. Serum antibodies are type specific. There may be a low degree of heterotypic antibody induced by infection, especially between type 1 and type 2 polioviruses *(Ashkenazi & Melnick 1962)*. It is believed that serum neutralizing antibodies (primarily IgG) persist for life. A survey carried out in an isolated Eskimo village showed that IgG antibodies produced from subclinical infection with wild virus persisted for at least 40 years without subsequent exposure *(Paul et al. 1951)*.

Passive immunity is transferred from mother to fetus via the placenta. The concentration of type 1 and type 2 IgG neutralizing antibody in the newborn is approximately equal to that of the mother. Type 3 titers are somewhat lower than those of the mother, suggesting differential transplacental transfer of this serotype *(Ananthakrishanan et al. 1988, A. Cohen-Abbo and P. Wright personal communication 1991, Gelfand et al. 1960)*. The rate of decay of maternal antibody is constant; its half-life is estimated at about 30 days (range 21 to 50 days) and these data have been confirmed in recent studies in developing countries *(M. Pallansch personal communication 1991)*.

Poliovirus infection also induces development of secretory IgA antibody *(Ogra et al. 1968)*. Secretory antibody is produced by plasma cells originating in gut-associated lymphoid tissues, mainly Peyer's patches. These cells localize in mucosal sites, including the intestine, the pharynx, and the mammary glands *(Walker & Isselbacher 1977)*.

The persistence of secretory IgA antibody may be related to the virulence of the infecting virus and to the number of virus particles presented to the intestinal and nasal mucosa. Appreciable levels of secretory antibody have been detected in the nasopharyngeal secretions of individuals 10 to 15 years after natural infection with wild type 1 poliovirus *(Ogra & Karzon 1971)*.

2.2 Risk factors

A number of factors may affect the potential for infection with poliovirus or the severity of clinical poliomyelitis.

2.2.1 Immune deficiency

Infection with poliovirus poses an increased risk for persons with primary B cell immunodeficiencies. In these persons, infection with wild virus or vaccine strains may develop in an atypical manner, with an incubation period longer than 28 days, a high

mortality rate after a long chronic illness, and unusual lesions in the central nervous system *(Davis et al. 1977, Wyatt 1973)*. Among vaccine-associated cases in immunologically abnormal persons in the United States types 2 and 1 were the polioviruses most commonly isolated from stool specimens *(Strebel et al. 1992)*.

HIV-infected persons could potentially be at risk of wild or vaccine-associated poliomyelitis when B cell function decreases late in the clinical course of the disease. However, based on global data reported to WHO as of October 1992, only four cases of paralytic poliomyelitis have been reported in HIV-infected persons. A case-control study conducted in 1988-1989 in Zaire did not find an elevated risk of paralytic poliomyelitis among HIV-infected children *(Vernon et al. 1990)*. Prospective and retrospective studies in both developing and industrialized countries report no serious adverse events in over 400 HIV-infected children who received live attenuated oral polio vaccine (OPV) *(Onorato & Markowitz 1992)*.

2.2.2 Injections

See section 9.2.

2.2.3 Malnutrition

Data on the risk of infection with wild poliovirus in malnourished children are not available. Following a dose of OPV, serum neutralizing antibody titers were similar in malnourished and well-nourished children; however, in malnourished children, secretory IgA antibody has been detected significantly less often, at lower levels, and with a delayed appearance *(Chandra 1975, 1981)*.

2.2.4 Physical activity

Early studies showed that for persons who developed paralytic poliomyelitis, the intensity of physical activity in the first 48 hours after the onset of paralysis correlated with the severity of paralysis *(Horstmann 1950)*. In contrast, physical activity prior to the onset of paralysis did not relate to subsequent paralysis.

2.2.5 Pregnancy

Outbreaks in industrialized countries in the period when large numbers of cases occurred in adults allowed assessment of pregnancy as a risk factor for paralytic poliomyelitis. Among adults aged 15 to 44 years, pregnant contacts of a polio case had an increased risk of paralysis compared with other female or male contacts *(Paffenbarger & Wilson 1955)*.

Poliovirus can cross the placenta; however, there is no evidence that the fetus is affected either by maternal infection with wild poliovirus or by maternal immunization with live attenuated vaccine. A pro-

spective study conducted in New York City from 1949 to 1953 found no evidence of an increase in congenital defects among 87 infants born to mothers infected with poliovirus during their pregnancies *(Siegel & Greenberg 1956)*. In 1985 in Finland, mass immunization with OPV was used to control a polio outbreak. An estimated 5000 pregnant women were included in the mass immunization. Subsequent follow-up demonstrated no increased rate in congenital malformations or in central nervous system defects *(Harjulehto et al. 1989)*.

2.2.6 Tonsillectomy

Aycock reported in 1942 that tonsillectomy in a person incubating poliovirus was likely to lead to bulbar poliomyelitis; later studies indicated that previous tonsillectomy at any time increased the risk of bulbar poliomyelitis *(Bodian & Horstmann 1965)*. Studies based on the immune response to OPV provided further clarification. Among children 3 to 11 years old previously immunized with OPV, IgA was present in the nasopharynx pre-tonsillectomy. After tonsillectomy, mean IgA titers declined abruptly and remained low for several months; serum antibody levels remained unchanged. Compared with children who had intact tonsils, seronegative children who had had their tonsils removed had a lower level of secretory antibody response in the pharynx when immunized with OPV *(Ogra & Karzon 1971)*.

3. Different Techniques for Measuring Immunity

Halsey and Galazka *(1985)* describe several ways of assessing immunity against poliomyelitis:

- measurement of serum neutralizing antibodies;

- measurement of secretory antibodies in feces, duodenal secretions, nasopharyngeal secretions, or breast milk;

- examination of previously immunized persons for the absence of poliovirus in the stool or throat following natural challenge with wild type virus or following challenge with a dose of attenuated oral polio vaccine; and

- measurement of protective efficacy, e.g. prevention of paralytic disease in immunized persons as compared to unimmunized persons in exposed populations, using epidemiologic methods.

3.1 Serum neutralizing antibodies

Tests for serum neutralizing antibodies are considered to be the most specific for determining the protective antibody response to poliovirus infections. Current methods do not allow differentiation between antibodies to wild or vaccine strains. Immunity to poliovirus is measured by determining the ability of serum to neutralize the infectivity of each of the three types of poliovirus for cell cultures. A standard dose of virus is incubated with dilutions of serum. The level of neutralizing antibody present is expressed as a titer, which is the reciprocal of the lowest dilution at which antibody is detected. For example, if antibodies are detected at a dilution of 1:8, the titer is 8.

3.1.1 Standardizing neutralizing antibody tests

Measurement of neutralizing antibody is dependent on the use of cell culture techniques, and it is expensive, time consuming (3 to 7 days per test), and requires technically skilled staff. Methodological differences such as virus strain, cell type, incubation time and temperature, and serum starting dilution can influence results. A collaborative study in 20 laboratories from 12 countries found a 10-fold difference in serum neutralizing antibody levels *(Albrecht et al. 1984)*. Other studies have also shown that unless proper techniques are followed, the sensitivity of the test is poor *(Kyriazopoulou & Bell 1972, Sabin 1983)*.

Recent efforts by WHO to standardize polio virology methods led to the publication in 1990 of a WHO Manual for Virological Investigation of Poliomyelitis *(Expanded Programme on Immunization and Division of Communicable Diseases 1990)*, which recommends a standardized technique for measurement of neutralizing antibodies, involving standard cell lines, and other standard reagents. International standard anti-poliovirus sera for types 1, 2, and 3 should be used *(Wood & Heath 1992)*. Results should be expressed in international units of neutralizing antibody.

Cell culture assays are technically more demanding than other methods of measuring antibody-antigen binding, such as agglutination, immune precipitation, and ELISA* However, these latter techniques have not been found to be generally suitable for polioviruses as they measure both neutralizing and non-neutralizing antibody. The value of the latter in protection against poliomyelitis is unknown, and may be irrelevant.

3.1.2 Definition of seroconversion

To assess response to vaccine in the research setting, serum specimens are obtained prior to immunization (usually on the day of immunization) and 30 days after each vaccine dose. Seroconversion is de-

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* Considerable efforts are being directed toward development of new methods for measuring protective antibody against poliovirus *(Ghendon 1992)*. Ideally, such methods should be inexpensive, rapid, reliable, able to distinguish vaccine-induced antibody from that induced by wild virus, and require only small amounts of serum.

fined as a fourfold rise in neutralizing antibody titer or a change from seronegative to seropositive. It is best if serum specimens from the same individual are analyzed in the same laboratory at the same time using appropriate reference and control sera.

During the first few months of life, most infants have circulating IgG antibodies acquired from the mother before birth. There are no practical techniques to distinguish these passively acquired antibodies from antibodies that the infant has made in response to immunization. Therefore, most investigators compare the antibody titers in cord blood or venous blood obtained prior to immunization with titers observed after immunization. Based on an estimated half-life of approximately 30 days (range 21 to 45 days), the expected level of passively acquired antibody is determined *(Halsey & Galazka 1985)*. If the titer obtained after immunization is fourfold greater than the expected titer of passive antibody, it is concluded that the infant has responded to the vaccine.

3.2 Secretory antibodies

The measurement of secretory antibodies has proved technically demanding. Most investigators have used a direct enzyme-linked immunosorbent assay (ELISA) or an indirect sandwich ELISA method *(Inouye et al. 1984, Losonsky et al. 1988, Nishio et al. 1988)*. To preserve secretory antibody prior to testing, specimens should be immediately frozen at -20°C. Specimens contaminated by blood cannot be analyzed.

3.3 Challenge studies

Failure to detect viral multiplication in the intestine (by excretion of virus in the stool) following challenge with poliovirus indirectly demonstrates the presence of intestinal immunity. Conversely, fecal shedding of virus comparable to that seen in a nonimmune individual suggests absence of intestinal immunity and "take" of the virus. A challenge can occur naturally during an outbreak (contacts of cases) or artificially with OPV.

For artificial challenge, most investigators have used monovalent type 1 OPV in preference to trivalent OPV in view of the work involved. The size of the challenge dose is critical, and at least 10^6 $TCID_{50}$ (dose which infects 50% of tissue cultures) of type 1 OPV is recommended. Excretion of poliovirus after administration of a dose of OPV is an indication of viral multiplication in the intestine, or "take". Persons administered OPV may briefly excrete low titers ($<10^2$ $TCID_{50}$ per gram) of vaccine virus in the stool during the subsequent 48 hours. This probably represents passive transit of the vaccine virus through the intestinal tract. However; when vaccine virus is de-

tected three or more days after immunization with OPV, it is present in much higher titers (10^3 to 10^7 $TCID_{50}$ per gram), indicating multiplication of the virus in the intestinal tract *(Halsey & Galazka 1985)*.

3.4 Protective efficacy

Measurement of vaccine efficacy is the ultimate test of protection, e.g. prevention of paralytic poliomyelitis in immunized persons as compared to unimmunized persons. The most conclusive pre-licensure efficacy study is the prospective, randomized, double-blind, placebo-controlled clinical trial. However, a pre-licensure trial may not provide information applicable on a wider basis since such trials are conducted in selected populations under optimal conditions. In practice, the vaccine may be used in different age groups, different schedules, and among different populations that may not have the same immunologic response.

Considerable work has gone into defining epidemiologic methods appropriate for post-licensure assessment of vaccine efficacy *(Orenstein et al. 1985, 1988a)*. Such studies can only be conducted under circumstances of persistent circulation of wild poliovirus. Most studies have been conducted during outbreaks. Epidemiological methods measure the relative risk of disease among the vaccinated compared with the unvaccinated. Assessments of vaccine efficacy have been conducted in the field setting in developing countries using either case-control or cohort methods. This is possible when there is a high retention rate of immunization cards documenting the dates of immunization. However, these studies are subject to a number of potential biases, including problems in case definition, incomplete ascertainment of cases, inappropriate control groups, and potential lack of comparability of vaccinees and nonvaccinees. Nevertheless, epidemiologic methods have provided useful estimates of the clinical protective efficacy of polio vaccines.

4. Protective Levels of Polio Antibodies

Persons are presumed to be protected against disease caused by a particular type of poliovirus if they develop type-specific serum neutralizing antibody; however, the level of serum neutralizing antibody which protects against clinical illness has not been determined. In animal experiments, passively administered antibody which provides moderate serum antibody levels (titers of 20 or higher) will protect against clinical illness, but this cannot be compared to the natural situation where challenge with wild or vaccine strains occurs *(Bodian & Nathanson 1960)*.

A study from the 1950s indicates that persons with low serum neutralizing antibody titers can be reinfected by wild virus. Among 237 naturally immune persons with neutralizing antibody titers of 40 or lower observed during family episodes of wild poliovirus infection in Louisiana during 1953 to 1957, 98% were reinfected as determined by a fourfold or greater rise in serum antibody titer *(Gelfand et al. 1959)*. In contrast, among 36 individuals with neutralizing antibody titers of 80 or higher; only 33% were reinfected.

More recent studies in Japan and England indicate that persons with low serum neutralizing antibody titers post immunization can be reinfected when challenged with vaccine virus. In Japan, among a group of 67 children followed annually for 5 years after immunization with two doses of trivalent OPV, 19 were found to have a type 1 antibody titer of 8 or lower. In 18 of these 19 children, a challenge dose of trivalent OPV led to reinfection, as measured by virus excretion in the stools *(Nishio et al. 1984)*. In England a total of 97 children who had been vaccinated with 3 doses of trivalent OPV in infancy were studied 8 to 16 years later, before and after administration of a challenge dose of trivalent OPV. Seventeen of these children had pre-challenge antibody to all three types of poliovirus at low levels (geometric mean antibody titers ranging from 9 to 36). Although this group is too small to establish statistical significance, it is worth noting that of eight children who failed to respond, seven had neutralizing antibody titers of 32 or higher, whereas those who showed at least a fourfold antibody rise post-challenge had lower pre-challenge titers *(Magrath et al. 1981)*.

These findings are consistent with earlier studies which showed that children with low serum antibody levels can be reinfected with vaccine virus *(Gelfand et al. 1959, McKay et al. 1963)*. These studies suggest that persons with low but detectable serum antibody are probably not in danger of developing clinical poliomyelitis. However, they may be reinfected with poliovirus and possibly provide a source of infection for others who have not been vaccinated.

A local barrier to poliovirus infection is provided by secretory IgA antibody. The level of secretory IgA antibody that provides protection is not known. The relationship between serum antibody levels and secretory antibody levels is also unclear. Children may be resistant to reinfection in the absence of serum antibody if the level of secretory antibody is sufficiently high *(Ogra et al. 1968)*.

In 1955 Salk introduced the concept of "heightened immunologic reactivity," which could protect from death by polio even with marginally adequate vaccines *(Alexander 1984)*. As this concept developed further, it was suggested that when the titer of neutralizing antibody fell below detectability, immunological memory would persist irreversibly so that restimulation by vaccine or infection would result in a rapid, high antibody rise. This secondary response to infection was postulated to be rapid enough to protect against paralytic disease.

Salk has argued that lifelong immunity to poliomyelitis can be induced with a single dose of inactivated polio vaccine (IPV) administered at 5 or 7 months of age *(Salk 1984)*. Since then, however, cases of paralytic polio have been described in persons who have received one or more doses of enhanced-potency IPV (eIPV) *(Hovi et al. 1986, Petersen 1991, Robertson et al. 1988, Slater et al. 1990)*. Moreover, the protective efficacy of a single dose of eIPV (39%) was found to be almost the same as the level of neutralizing antibodies obtained with a single dose of this vaccine *(Robertson et al. 1988)*.

5. Seroepidemiology in the Pre-vaccine Era

Because there are 100 to 200 subclinical infections for every case of paralytic poliomyelitis, seroepidemiology is especially important for polio as compared with the other EPI diseases. It should be emphasized, however, that the first level of defense provided by intestinal immunity is not reflected in serological data.

Serological profiles were completed in a number of countries prior to the introduction of vaccine (Egypt, *Paul et al. 1952;* Ghana, *Isomura et al. 1987, Pasca & Afoakwa 1971;* Iran, *EPI 1984;* Liberia, *Gelfand & Miller 1956;* Morocco, *Paul & Horstmann 1955)*. These population-based studies show a rapid decay in maternal antibodies in the first few months of life and a gradual increase in serum antibodies over the first five years of life (Figure 2). Relatively flat persistence of antibody may reflect repeated infection with wild virus. The age distribution of cases of paralytic poliomyelitis in these countries paralleled

Figure 2. Polio neutralizing antibodies to types 1,2, and 3, present at dilutions of 1:5 or higher, Morocco, 1953 *(Paul & Horstmann 1955)*.
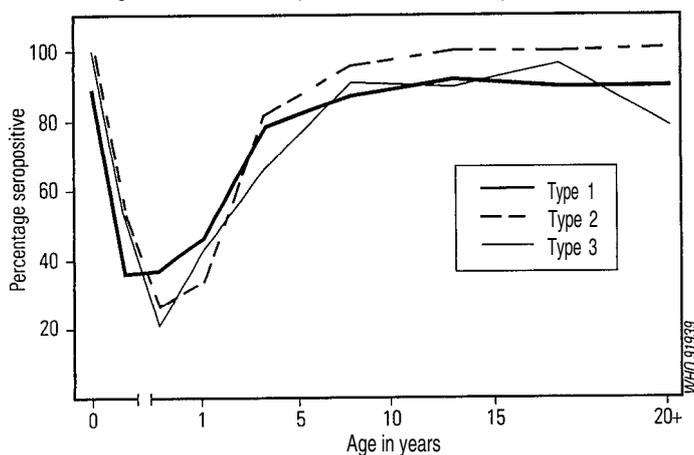
the age distribution of persons lacking antibody. Cases were rarely seen before 6 months of age; most cases occurred in children 6 months to 4 years of age.

Serological methods did not become widely available for polio until the 1950s *(Paul & White 1973)*. However, the above pattern was probably typical for the United States and the European countries at the end of the 19th century, when case reports indicated that 80% or more of paralytic cases occurred in children under 5 years of age. From the 1920s to the 1950s the urban industrialized parts of northern Europe and the United States experienced epidemics of paralytic polio that grew larger over time. By 1950, more than 20 000 new paralytic cases were reported annually in the United States. It is thought that improvements in community sanitation, including provision of sewage disposal systems and clean water supplies, led to fewer opportunities for infection among infants and young children. Exposure to wild poliovirus was therefore delayed until late childhood or adult life. By 1950, the peak age incidence in the United States shifted from infants to children aged 5 to 9 years, and about one-third of the cases were reported in persons over 15 years of age *(Melnick 1990)* (Figure 3).

As developing countries improve sanitation and raise immunization coverage levels, it is possible that a similar shift in the age distribution of cases could occur. This will depend in large part on how rapidly these changes occur and how quickly nonimmune persons accumulate among the population. The age distribution of cases reported in several outbreaks occurring since 1978 indicates that in most developing countries polio remains a disease of the very young (Figure 4).

6. Immunity Induced by Oral Polio Vaccine

6.1 Oral polio vaccine (OPV)

Candidate strains of attenuated poliovirus suitable for immunizing humans were developed independently by scientists at three different institutions in the United States: the Children's Hospital Research Foundation, Cincinnati (A.B. Sabin), Lederle Laboratories (H.R. Cox), and the Wistar Institute, Philadelphia (H. Koprowski). Because they provided good antibody levels and were less neurotropic for monkeys, the strains developed by Sabin were selected for widespread application. OPV began to be used in several countries during the spring of 1960. Initially, each serotype was given separately as a monovalent vaccine, with sequential administration of types 1,3, and 2. Trivalent vaccine came into use a few years later, although a few countries have continued to use

Figure 3. Age distribution of reported cases of poliomyelitis, by 5 year periods, Massachusetts, USA, 1912 to 1952 *(Nathanson & Martin 7979).*
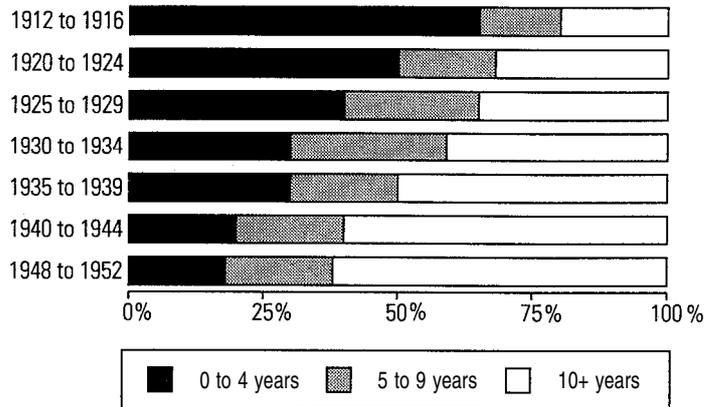
Figure 4. Age distribution of reported cases of paralytic poliomyelitis, various outbreaks *(Robertson & Oostvogel1989).*
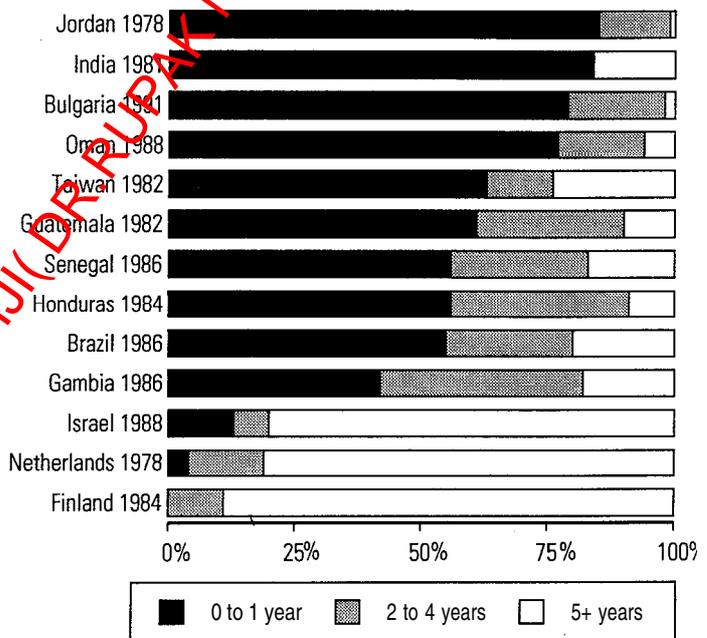
monovalent OPV up to the present. Since 1973 WHO has been directly responsible for the custody and distribution of the Sabin strains of OPV and has exercised strict supervision over production laboratories in cooperation with national control authorities *(Cockburn 1988)*. Sufficient quantities of the master seed have been prepared to supply the global requirements for the next 200 years. The passage history of the Sabin strains is detailed in a recent review *(Melnick 1988)*.

Most early trivalent preparations of OPV contained the three poliovirus types in equal proportions; however, in 1961 a study in Canada evaluated a "balanced" formulation of trivalent OPV which contained 10^6, 10^5, and $10^{5.5}$ $TCID_{50}$ of Sabin types 1,

2, and 3, respectively *(Robertson et al. 1962).* Whereas there had been relatively lower seroconversion rates to types 1 and 3 when administered in equal proportion, the "balanced" preparation resulted in detectable levels of neutralizing antibody against all three types in almost all subjects. The "balanced" formulation was adopted in Canada in 1962 and a similar formulation was adopted in the USA in 1963. Since studies of monovalent preparations in developing countries (most of these studies were with non-Sabin strains) had shown serological responses in children similar to those seen in industrialized countries, the "balanced" trivalent formulation was adopted for use in developing countries without further testing.

6.1.1 Potency of OPV

The WHO requirements for OPV state that the virus concentration in the final vaccine should be determined in cell cultures in terms of infective units per dose *(WHO Expert Committee on Biological Standardization 1990).* For many years the potency recommended by WHO was not less than 10^6, 10^5, and $10^{5.5}$ TCID$_{50}$ of poliovirus types 1, 2, and 3, respectively, per single human dose of trivalent OPV. In 1986 the Region of the Americas began to use a trivalent formulation with $10^{5.8}$ TCID$_{50}$ of poliovirus type 3 *(de Quadros et al. 1991),* following a study in Brazil which demonstrated improved immunogenicity when the amount of type 3 virus in the trivalent vaccine was increased *(Patriarca et al. 1988).* The subsequent success in controlling poliomyelitis in the Americas using this formulation led the EPI Global Advisory Group to recommend a formulation of trivalent OPV with 10^6, 10^5, $10^{5.8}$ TCID$_{50}$ per dose for types 1, 2, and 3, respectively, on a global basis *(EPI 1991).*

6.1.2 Vaccine-associated paralysis

Following the introduction of OPV, it became clear that rare cases of paralytic poliomyelitis were temporally associated with administration of the vaccine. Studies of these cases strongly implied that they were caused by the Sabin strains, which had regained neurovirulence after replicating in the intestine of the vaccinee. Type 3 was the most common isolate associated with paralysis in vaccine recipients; type 2 was associated with paralysis mostly among contacts of cases. A WHO collaborative study during 1980 to 1984 found that the number of cases among OPV recipients and contacts of vaccine recipients was about one case per 3.3 million doses of trivalent OPV distributed or administered in 8 countries *(Esteves 1988).* These data are in agreement with other such studies. In the USA the overall frequency of vaccine-associated poliomyelitis has remained stable since the mid-1960s, with one case per 2.5 million doses of trivalent OPV distributed during 1980 to 1989. However, the relative frequency of paralysis associated

with the first dose in the OPV series was one case per 700 000 doses compared with one case per 6.9 million subsequent doses *(Strebel et al. 1992).*

These data suggest that as OPV coverage levels increase for infants, who are likely to attain protection while they are still under the umbrella of maternal antibody, the incidence of vaccine-associated paralysis in recipients can be expected to decline *(Hovi 1991).* Likewise, as well-immunized cohorts become adults, the incidence of vaccine-associated paralysis in contacts can be expected to decline.

The molecular basis for the neurovirulence of polioviruses has been studied by a number of investigators *(Chumakov et al. 1991, Evans et al. 1985, Minor et al. 1986a, Omata et al. 1986, Skinner et al. 1989).* This work may lead in the future to vaccines with greater genetic stability of the attenuation phenotype *(Lemon & Robertson 1991).*

6.2 Serum antibodies

During the 1970s less-than-optimal responses to trivalent OPV in developing countries became apparent when reports of low rates of seroconversion to poliovirus types 1 and 3 began to appear in the medical literature *(Ghosh et al. 1970, John & Jayabal 1972, Oduntan et al. 1978).*

A recent review examines data accumulated in developing countries during the past 25 years *(Patriarca et al. 1991).* Thirty-two studies in 15 developing countries reported the response of at least 20 children to three doses of trivalent Sabin-derived OPV which contained at least 10^6, 10^5, and $10^{5.5}$ TCID$_{50}$ of types 1, 2, and 3 poliovirus, respectively. After three doses of trivalent OPV, there was wide variation in the percentage of children seroconverting with rates of 73% (range 36% to 99%) for type 1, 90% (range 71% to 100%) for type 2, and 70% (range 40% to 99%) for type 3.

Additional studies of children who received trivalent OPV in developing countries have become available since this review was prepared and are presented in Table 2. These data, all based on documented histories (dated records) of immunization, continue to show a wide range of response to trivalent OPV in tropical countries. Rates of seropositivity and/or seroconversion are lowest for type 3, followed by type 1.

The precise cause of lower seroconversion rates to types 1 and 3 in some parts of the developing world is not clear. Available data suggest that type 2 vaccine virus and enteric pathogens often interfere with the response to types 1 and 3, but this interference may be partially overcome by modifying the absolute and relative dosage of the three Sabin vaccine virus types *(Patriarca et al. 1988).* The interval between doses may also be important, in view of prolonged excretion of vaccine virus and the potential for interference

Table 2. Summary of recent studies in developing countries on serologic response to 3 or 4 documented doses of Sabin-derived trivalent OPV in infants as measured at 7 to 12 months of age.

Country	Neutralizing antibody (%) after 3 or 4 doses			Schedule (months)	No. of infants	Lowest dilution tested	Reference
	Type 1	Type 2	Type 3				
Brazil	100	100	96	0/2/4/9	27	1:5	Weckx et al. 1992
	96	100	74	2/4/6	27	1:5	
China	99	100	99	2/3/4	92	1:8	Beijing Epidem. Station 1990
Ghana	100	100	97	0/2/3/4	51	1:10	Osei-Kwasi 1988
Korea	100	100	100	2/4/6	26	1:8	Shin 1989
Oman							Sutter et al. 1991
Khasab	97	97	74	3/5/7	35	1:8	
Khasab	91	100	83	3/5/7	35	1:8	
Rustaq	94	86	43	3/5/7	35	1:8	
Rustaq	86	91	51	3/5/7	35	1:8	
Pakistan (1988)	89	92	94	0/3/5/7	36	1:8	EPI 1990b
Pakistan (1989)							Pakistan Ministry of Health 1990
Northwest	89	84	88	0/3/5/7	82	1:8	
Punjab	93	94	86	0/3/5/7	152	1:8	
Saudi Arabia	77	84	72	3/4/5	64	1:8	Abanamy et al. 1992
Singapore	100	100	100	3/4/5	30	1:8	Yin-Murphy et al. 1992
Togo	90	100	82	0/2/3/4	30	1:5	EPI 1990b
	90	100	80	0/2/3/4	30	1:5	
Uganda	90	98	62	3/4/5	60	1:8	EPI 1990b
Zimbabwe	100	100	100	3/4/5	28	1:8	Tswana & Berejena 1988

with response to subsequent doses. Continuing intestinal infection (manifested by fecal excretion of one strain of OPV) could potentially interfere with the immune response to a subsequent dose. These and other factors which may influence the serological response of children in developing countries to OPV are now under study in WHO-sponsored prospective clinical trials in different parts of the world.

6.2.1 Persistence of serum antibodies

Duration of immunity is best examined in long-term prospective studies in which children who have received vaccine of known potency are followed for a number of years post immunization. So far, few prospective studies have been conducted.

In the United States 57 infants received 3 doses of trivalent OPV in 1968 and all had titers of 10 or higher for all 3 types at that time. Serum obtained from these children 5 years later showed the proportion with neutralizing antibodies at a titer 2 or higher was 98% for type 1 poliovirus, 98% for type 2, and 84% for type 3. A second group of 58 children received 4 doses of trivalent OPV in 1968 and all had antibody at a titer of 10 or higher for all three types. Five years later the proportion with neutralizing antibodies at a titer of 2 or higher was 98%, 98%, and

87% for types 1, 2, and 3, respectively. All sera from this study that were negative with the standard microneutralization technique were tested again with a more sensitive plaque reduction method. Four of 5 sera without type 1 antibody on the microneutralization test demonstrated antibody on the plaque reduction test. Two sera negative for type 2 antibodies and 15 of 17 sera negative for type 3 antibodies by the neutralization method were positive by plaque reduction *(Krugman et al. 1977).*

In Italy a group of 276 children were followed for 4 years after receipt of 3 doses of trivalent OPV. More than 94% maintained neutralizing antibody titers of 4 or higher against type 1 and type 2; however, only 84% and 75% had a type 3 titer at this level 2 and 4 years post immunization *(Trivello et al. 1988).*

Data from a prospective study conducted in Japan suggest that during the 5 years after two doses of trivalent OPV (at 3 and 6 months of age) neutralizing antibody titers for types 1 and 2 decline gradually, whereas a more rapid drop and statistically significant is seen with type 3 *(Nishio et al. 1984)* (Table 3). The same study found similar results in a group of children who received a booster dose of OPV at 5 years of age and were followed annually for the next 4 years.

Table 3. Polio neutralizing antibodies (at a titer of 4 or higher) and geometric mean titers (GMT, log 2) in 67 Japanese children five years after receipt of two doses of OPV at 3 and 6 months *(Nishio et al. 1984).*

Years after immunization	Type 1		Type 2		Type 3	
	Percent positive	GMT	Percent positive	GMT	Percent positive	GMT
1	93.7	5.40	100.0	7.10	93.7	5.02
2	90.6	5.00	98.1	6.91	87.0	4.28
3	91.0	4.72	98.5	6.45	82.1	3.85
4	92.5	4.69	98.5	6.19	77.6	3.39
5	88.1	4.25	94.0	6.51	80.6	3.66

Prospective data on persistence of antibody in the developing countries is lacking. Given the lesser serological response in some countries, it will be important to assess antibody persistence in these settings in the future.

6.2.2 Seroepidemiology after long-term use of OPV

The introduction of OPV in 1960 and its subsequent widespread use has had a dramatic impact on the incidence of poliomyelitis. In many countries, OPV has virtually eliminated poliomyelitis from the time the vaccine was fully introduced until today.

In countries where high levels of OPV coverage have been maintained for 15 years and longer, the age-specific pattern of immunity has changed (Figure 5). The impact of immunization in increasing serum antibody levels in the very young is striking, particularly since these were the age groups at the highest risk in the pre-vaccine era. Although limited episodes of poliomyelitis due to (imported) wild virus continue to occur in countries where OPV coverage is high, a strong immune barrier appears to have been created in the population, inhibiting widespread transmission of wild poliovirus.

Serological surveys carried out after 15 years or more of national coverage with OPV have indicated at least 95% antibody prevalence against all three types of poliovirus in persons 2 years of age and older in Italy *(Santoro et al. 1984, Volpi et al. 1976),* Singapore *(Goh & Yamazaki 1987),* and the USA *(Mayer 1984, Orenstein et al. 1988b).* However, in these studies serum dilutions for the neutralizing antibody test started at 1:2 or 1:4. A study by Linnemann demonstrates that when antibody status appears excellent with a titer of 2 or higher; the same sera considered at a titer of 8 or higher may reveal significant gaps in immunity *(Linnemann et al. 1974)* (Figure 6). Similar data examining the same sera at starting dilutions of 1:8 and 1:2 were obtained in a carefully performed serological study of 304 children aged 1 to 15 years in Barbados *(Evans et al. 1979).* In this study the percentage of children lacking antibody was two- to four-fold greater at 1:8 than at 1:2 serum dilutions. This information poses an epidemiological dilemma since the level of neutralizing antibody that affords protection is not known (see section 4).

Serological surveys which have used a titer of 8 or higher as the measure of seropositivity show relatively lower levels of immunity (below 80%) in some age groups against individual poliovirus types in Australia (Menser et al. 1980), Belgium *(Lamy et al. 1979),* Germany *(Maass & Doerr 1986),* Northern

Figure 5. The prevalence of neutralizing antibodies against poliovirus types 1, 2, and 3 in Poland in 1958 during the pre-vaccine era and in 1979 after long-term use of OPV *(Galazka 1988).*
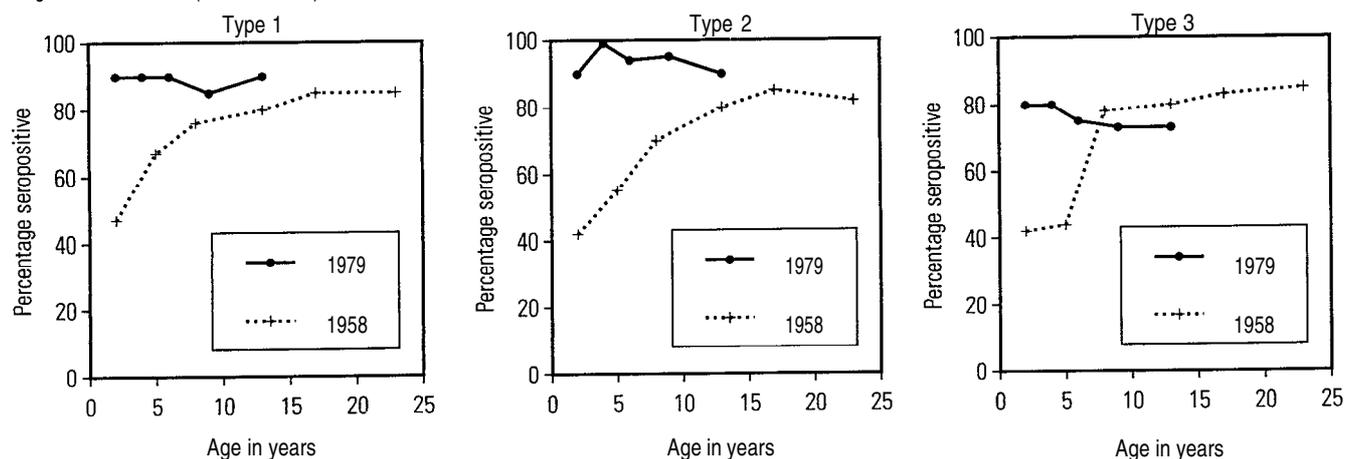
Ireland *(Rooney et al. 1986),* United Kingdom *(White & Greene 1986).* In most surveys the antibody levels are lowest for type 3, followed by type 1. These gaps raise concerns of either primary vaccine failure (lack of initial antibody response) or secondary vaccine failure (waning of vaccine-induced neutralizing antibody). Periodic monitoring of serum antibody patterns by age is useful in setting an optimal immunization schedule *(EPI 1990c).* It can be anticipated that with further reductions in the transmission of polioviruses, the chance for natural boosting of antibodies will be reduced, with possible subsequent reduction in the duration of vaccine-induced immunity.

Serosurveys in developing countries are more complicated to interpret than in countries where immunization coverage has been 90% or higher for a long time. A survey in Jamaica obtained sera from a population based sample of more than 2500 children and adolescents aged 1 to 19 years *(Ashley et al. 1989).* Overall, 81%, 95%, and 72% had antibody titers of 8 or higher against polio types 1, 2, and 3, respectively. Among children 1 to 4 years who had documentation of receipt of 3 or more doses of OPV, 85%, 99%, and 81% were seropositive. Among children 1 to 4 years who had never received vaccine 53%, 77%, and 55% were seropositive, probably reflecting circulation of both wild virus and vaccine virus.

6.3 Secretory antibodies

The immune response to OPV closely parallels that to natural infection. Virus multiplies in the same alimentary tract sites and in related lymphoid tissues and is excreted in the feces for several weeks and, usually after large doses, in pharyngeal secretions for up to 10 to 12 days *(Fox 1984).* The administration of OPV results in the development of secretory IgA antibody in the nasopharynx and intestine approximately one to three weeks after immunization (see Figure 7). Secretory antibody activity has been observed to persist for as long as 5 to 6 years *(Ogra 1984).* Local secretory IgA antibody induced by OPV is considered to be important in protecting the individual and in reducing the rate of transmission of wild type polioviruses by immune persons.

Colostrum produced in the first three days after childbirth contains secretory IgA antibody, which might interfere with the immune response to OPV. Nevertheless, several studies show that among breastfed infants who are fed OPV in the first three days of life, 20% to 40% develop serum antibodies and 30% to 60% excrete vaccine virus *(Halsey & Galazka 1985).* Lower levels of secretory IgA are present in breast milk produced after the fourth day. There is no significant effect of breast-feeding on the response of older infants to OPV *(Deforest et al. 1973, John et al. 1976).*

Figure 6. Polio neutralizing antibodies, by type and titer, among 296 children age 6 years, Cincinnati, USA, 1972 *(Linnemann et al. 1974).*
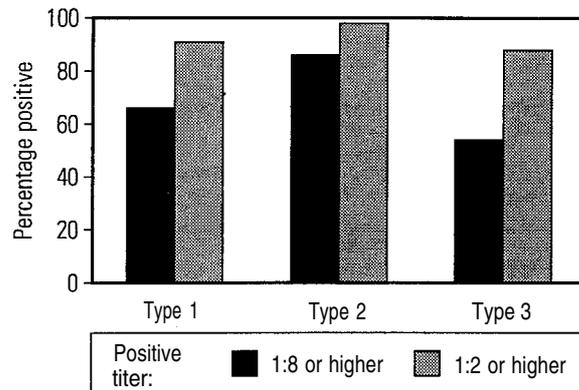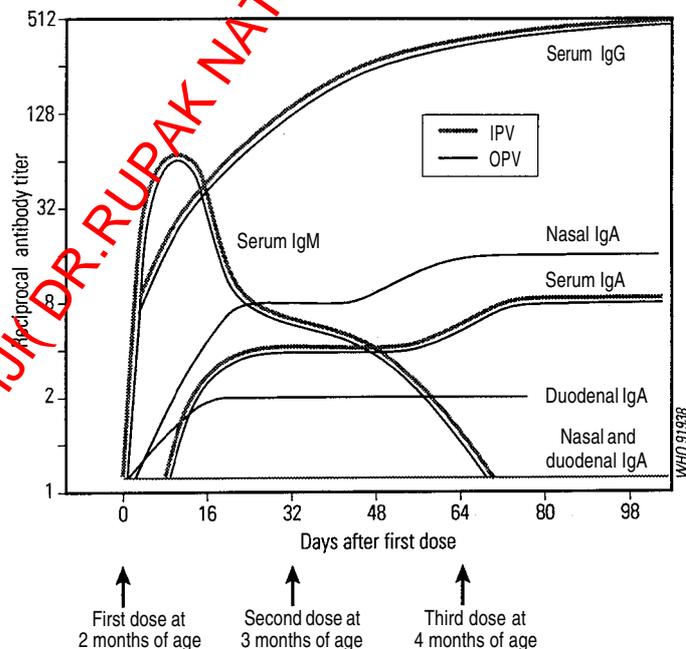
Figure 7. Serum and secrectory antibody resposes to 3 doses of OPV or 3 doses of IPV at 2, 3, and 4 months of age. *(Ogra et al. 1968).*

6.4 Challenge studies

6.4.1 Challenge with OPV

The most comprehensive challenge study was one of the earliest *(Ghendon & Sanakoyeva 1961)* (Table 4). This study used a challenge dose of monovalent type 1 OPV of 10^6 TCID$_{50}$ (the same dose of type 1 in the current trivalent vaccine) and provided quantitative data on duration of virus excretion and titer of virus per gram of stool. Among children previously immunized with 3 doses of monovalent OPV, 37% excreted challenge virus, but the time of excretion was short (mean 4.6 days) and the virus titer per gram of stool was low. In contrast, among children immunized with two doses of IPV 74% excreted virus. The period of virus excretion was three times as long in IPV vaccinees compared with OPV vaccinees and the quantity of virus per gram of

Table 4. Response to challenge with a dose of 10^6 TCID$_{50}$ of type 1 monovalent OPV among children aged 1 to 3 years *(Ghendon & Sanakoyeva 1961).*

Study group	No.	Shed virus in stool		Mean excretion (days)	Mean virus titer TCID$_{50}$ per gram of stool
		No.	%		
Not vaccinated and triple seronegative	30	24	80	20.4	141 000
Vaccinated with IPV: 2 doses	31	21	74	12.3	13 000
Not vaccinated and recent shedding of type 1	19	7	37	5.0	140
Vaccinated with OPV: monovalent 1,3,2	33	33	37	4.6	150
Not vaccinated and triple seropositive	32	11	34	5.1	110
Not vaccinated and post paralytic polio*	18	0	0	-	-

* Post-polio patients were 12 children aged 7 to 15 years and 6 adults.

Figure 8. Response to challenge with $10^{5.8}$ TCID$_{50}$ of type 1 monovalent OPV among children immunized with OPV or eIPV at 2, 4, and 18 months, Baltimore, USA *(Onorato et al. 1991).*
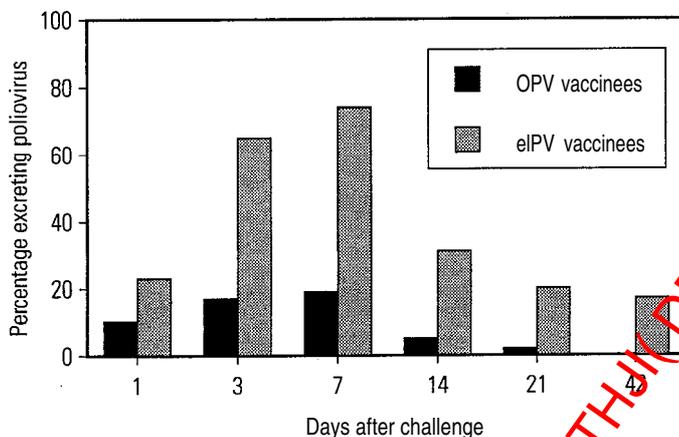
stools was 100 times higher. This study also included children never vaccinated (both triple seronegative and triple seropositive children) as well as a group of children and adults who had had paralytic poliomyelitis. Based on these benchmarks, it could be seen that the response to challenge of the OPV-immunized children was comparable to that of children naturally immune to all three types. However, neither the OPV-immunized children nor the triple seropositive group were as resistant to challenge as the group who had had paralytic poliomyelitis.

A study in Kenya compared the response to a low challenge dose of monovalent type 1 OPV (3000 to 7000 TCID$_{50}$) in children immunized with OPV or enhanced potency inactivated polio vaccine (eIPV) containing 40 D antigen units of type 1 poliovirus). This low challenge dose did not produce a statistically significant difference between the two vaccines in their ability to induce intestinal immunity; however, more eIPV immunized children than OPV-immunized children excreted virus at 1 week post-challenge and only eIPV-immunized children continued to excrete challenge virus from 2 to 4 weeks after challenge *(Kok et al. 1992).*

A study conducted in the United States compared mucosal immunity produced by OPV and eIPV. After a low challenge dose of type 1 monovalent OPV (500 to 800 TCID$_{50}$) only 18% of children previously immunized with 3 doses of OPV excreted the challenge virus compared with 46% of children previously immunized with three doses of eIPV. An increase in the challenge dose to about 600 000 TCID$_{50}$ led to an increase in the proportion of children excreting challenge virus to 31% in the OPV group and 82% in the eIPV group *(Onorato et al. 1991).* The duration of virus shedding was prolonged in eIPV-immunized children (mean 15.5 days) compared with OPV recipients (mean 6.4 days). At 42 days post-challenge 9% of eIPV vaccinees still shed virus (see Figure 8).

6.4.2 Challenge with wild virus

A major reason for the success of OPV is its community effect. That is, enteric multiplication of vaccine virus leads to its dissemination beyond the individual being immunized. The spread of OPV virus from vaccinees to unimmunized persons is a particular advantage in areas where vaccine coverage levels are low. The ability of vaccine virus to spread is enhanced by crowding and poor hygiene, but spread occurs readily both within households and to a lesser degree via community contact under conditions of better hygiene *(Gelfand et al. 1959).*

A study conducted in Houston during 1960 examined the spread of polioviruses to the siblings and their extra-familial contacts for 105 index children aged 2 to 18 months vaccinated with trivalent Sabin-strain OPV *(Benyesh-Melnick et al. 1967).* Seventy-seven percent of index children excreted poliovirus at one week after receipt of vaccine, 39% of the siblings (aged 0 to 59 months) at 2 weeks, and 20% of contacts of the siblings (aged <1 to 9 years) at 5 weeks (Figure 9). Examination of the types of poliovirus excreted showed type 1 in 12 index children and 16 siblings; type 2 in 28 index children and 49 siblings; and type 3 in 25 index children and 30 siblings. Among contacts of siblings, 5 excreted type 1, 53 type 2, and 9 type 3.

Prospective studies in the United States and Japan have shown that antibody boosting occurs, presumably as a result of community spread of OPV viruses *(Krugman et al. 1977, Nishio et al. 1984)*. Community dissemination of vaccine virus probably accounts for the striking decrease in polio incidence frequently observed after the introduction of OPV, a reduction much greater than would be expected based on immunization coverage levels alone *(Heymann et al. 1987)*.

Empirical evidence suggests that secretory antibody induced by OPV blocks wild virus replication in the intestine. Despite circulation of wild polioviruses, outbreaks are rare in countries where coverage with 3 doses of OPV has been 80% or higher for many years.

During recent years, polio outbreaks have been investigated in countries where the level of trivalent OPV coverage has been reported to be high, suggesting that the immune barrier provided by OPV was insufficient. However, in several of these outbreaks the reported coverage was for two doses only, or when investigated with coverage surveys, the OPV coverage was not high and the outbreak could be attributed to failure to vaccinate rather than to vaccine failure. For example in Taiwan, where an outbreak with more than 1000 paralytic cases occurred in 1982, coverage with two doses of OPV was 80% among children aged 1 to 4 years *(Kim-Farley et al. 1984)*. Epidemiologic investigations in Taiwan showed that the most important risk factor was failure to receive vaccine - 66% of the cases had received no vaccine and 19% only a single dose. In The Gambia in 1986 a type 1 polio outbreak led to 305 paralytic cases; however, coverage with three doses of OPV was only 64% among children 1 to 2 years of age and only 51% among children 3 to 7 years of age *(Otten et al. 1992)*.

Several outbreaks appear to have broken through the barrier provided by high OPV coverage. In 1988 to 1989 a type 1 outbreak with 118 cases occurred in Oman, a country where coverage with 3 doses of OPV was 86% among children 1 to 4 years of age

Figure 9. Intra and extra family poliovirus fecal excretion after receipt of a dose of trivalent OPV by the index child, Houston, USA, 1960 *(Benyesh- Melnick et al. 1967)*.
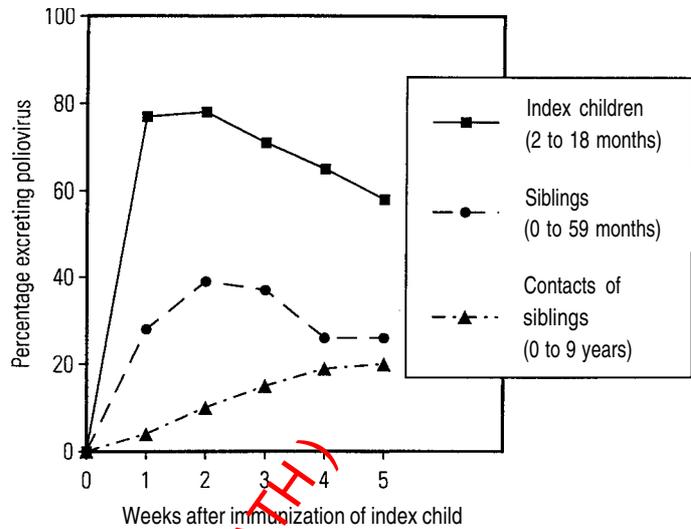
(Sutter et al. 1991). Because of the high OPV coverage at the time of the outbreak, the investigators postulated that intestinal protection may have been overwhelmed by large quantities of wild virus. Another type 1 outbreak in South Africa in 1987 to 1988 resulted in 412 paralytic cases in a population where 90% of 2 year old children sampled in a cluster survey had neutralizing antibodies at titers of 10 or greater to type 2 and 3, suggesting high levels of immunization coverage *(Schoub et al. 1992)*. The authors suggest that massive flooding in the outbreak area, with subsequent disruption of sewage and water services, facilitated spread of large quantities of wild virus.

6.5 Protective efficacy

In recent years, use of epidemiological methods has allowed retrospective assessment of the protective efficacy of polio vaccines in the outbreak setting (Table 5). The efficacy for three doses of OPV was above 70%, except for the study in Honduras where

Table 5. Studies of the clinical protective efficacy of polio vaccines conducted in the outbreak setting.

Country	Year	Wild virus type	Age group (months)	Method	Type of polio vaccine	Point estimate of efficacy (%) (95% confidence interval)			Reference
						3 doses	2 doses	1 dose	
Brazil	1986	3	24-35	cohort	OPV	94			Patriarca et al. 1988
Gambia	1986	1	12-35	case-control	OPV	72 (53,83)	68 (34.84)	68 (27,86)	Deming et al. 1992
Honduras*	1984	1	0-71	case-control	OPV	50 (0,79)			EPI Americas 1985
Oman	1988	1	5-24	case-control	OPV	91 (0,99)	80 (5,97)	30 (0,85)	Sutter et al. 1991
Senegal	1986	1	4-91	case-control	eIPV		89 (62,97)	36 (0,67)	Robertson et al. 1988
Taiwan	1982	1	12-35	cohort	OPV	98	96		Kim-Farley et al. 1984

Cold chain failure identified.

low efficacy was associated with problems in the cold chain. The efficacy estimate in Taiwan may be high because of the inability to adjust for confounding factors.

Although the ability of a polio vaccine to prevent paralysis can be considered the ultimate proof of its protection, results of retrospective studies of vaccine efficacy should be compared with caution since study design, methods of case ascertainment, and criteria for a confirmed case of paralytic poliomyelitis differed among these studies. Case-control studies are better suited' for vaccine efficacy measurement since adjustment for confounding may be built in and confidence limits are readily calculated (Table 5). Factors that influence vaccine efficacy have been discussed elsewhere *(Orenstein 1985, 1988a).*

7. Immunity Induced by Inactivated Polio Vaccine

7.1 Inactivated polio vaccine (IPV)

In 1949 Enders, Weller and Robbins described the successful. cultivation of the Lansing strain of poliovirus in cultures of non-nervous human tissues *(Enders et al. 1949).* This was the breakthrough that allowed development of polio vaccines. The first inactivated polio vaccine (IPV) was produced by Salk using virus grown on monkey kidney cells and inactivated with: formalin. After extensive field testing, IPV was licensed in the United States in 1955. The strains of virus used in the vaccine were Mahoney (type l), MEF-I (type 2) and Saukett (type 3). The same strains: are used by all manufacturers of IPV today, except in Sweden where the Brunenders strain is used for type 1 *(Salk & Drucker 1988).*

Shortly after IPV became widely available in the United States; cases of paralytic disease were reported

in recipients. Epidemiological and laboratory investigation revealed that active virus was present in several lots of vaccine from one manufacturer, Cutter. As a result, new filtration steps were introduced in the production process to remove aggregated, possibly poorly inactivated virus particles and safety tests were improved.

IPV is standardized in D antigen units. The D antigen content of IPV is measured *in vitro* by ELISA or by a double immunodiffusion assay. These tests need to be correlated with an *in vivo* system, usually in rats or chickens *(Minor 1990).* The original IPV contained 20, 2, and 4 D antigen units of poliovirus types 1, 2, and 3, respectively, although the potency varied considerably. In 1978 the Rijks Instituut in Holland introduced a new culture technique using cells on microcarriers to produce a more potent IPV containing 40, 8, and 32 D antigen units of types 1, 2, and 3, respectively *(van Wezel et al. 1984).* Vaccine of this potency is known as enhanced potency IPV, or eIPV. DPT vaccine has been combined with eIPV with good serological response to both vaccines and the convenience of a single injection.

7.2 Serum antibodies

Early studies in Burkina Faso, Finland, Mali, and Sweden showed that eIPV could be expected to yield greater than 90% seropositivity against all 3 types after one dose and 100% seropositivity after two doses *(Bernier 1986).* More recent studies of the response of infants to two doses of eIPV, with the first given at 6 to 8 weeks of age and the second 4 to 8 weeks later, have been carried out in Brazil *(Schatzmayr et al. 1986),* India *(Simoes et al. 1985),* and Kenya *(Kok et al. 1992)* (Table 6). The study in India specifically examined the effect of interval between doses. Response to types 1 and 3 was good with either a 4 week or an 8 week interval; however,

Table 6. Studies assessing the serum antibody response to two doses of eIPV in developing countries.

Country	Neutralizing antibody (%) after two doses			Age at first dose (weeks)	Interval between doses (weeks)	No. of infants	Lowest dilution tested	Reference
	Type 1	Type 2	Type 3					
Brazil*	99	100	100	8	8	80	1:5	Schatzmayr et al. 1986
India**	95	75	97	6 to 7	4	64	1:4	Simoes et al. 1985
	95	83	96	6 to 7	8	75	1:4	
	94	88	100	8 to 12	4	17	1:4	
	100	95	100	8 to 12	8	21	1:4	
	100	90	90	13 to 45	4	19	1:4	
	100	100	100	13 to 45	8	18	1:4	
Israel*	80	98	71	0	24	49	1:4	Swartz et al. 1989
	100	100	100	8	32	61	1:4	
Kenya **	94	98	87	8	8	84	1:8	Kok et al. 1992

 * Seroprevalence in Brazil and Israel.
 ** Seroconversion in India and Kenya.

the neutralizing antibody response to type 2 was better with an 8 week interval. Infants given two doses starting at 8 weeks had a better response than those starting at 6 weeks, independent of interval between doses.

There is little information on the response to a dose of eIPV administered earlier than 6 weeks of age. This may reflect concerns that passive antibody could block serologic response to polio antigen. To study this question, one would ideally wish to compare infants with high and low levels of maternal antibody, as has been done with other inactivated virus vaccines such as hepatitis B vaccine. A study in Israel examined the effect of a birth dose of IPV *(Swartz et al. 1989)*. Groups of newborns received IPV with different D antigen contents ranging from 40, 8, and 32 (the standard formulation of eIPV) to 160, 32, and 80 for poliovirus types 1, 2, and 3, respectively. All infants had maternal antibodies at the time they received eIPV. At 3.5 and 6 months of age their neutralizing antibody titers were no higher than would be expected in children who had not received vaccine. When they were given a second dose of eIPV at 6 months of age, the proportion who responded was lower and the relative increase in geometric mean antibody titer was less in these children compared with another group of children who received doses of eIPV at 2 and 8 months of age (Table 6).

7.2.1 Persistence of serum antibodies

Concerns about the persistence of serum antibodies following immunization with IPV have prompted most countries using this vaccine to include booster doses in the immunization schedule. There are few prospective studies that examine the issue of antibody persistence after IPV.

In Sweden, Bottiger followed 65 IPV-immunized children for 18 years. They received doses of IPV at 9, 10 to 11, and 16 to 29 months and a booster at 6 or 10 years. Antibody titers fell markedly during the first 2 to 5 years after immunization, then the decline leveled off to a mean decrease in titer of 0.05 to 0.10 $\log_{10}$ per year *(Bottiger 1990)*. At age 18, all had neutralizing antibody titers of 4 or higher against all three types. Those 18 year olds who received a booster at 10 years of age had mean antibody levels against type 1 and type 3 poliovirus that were 4 to 5 times higher than 18 year olds who received a booster dose at 6 years of age. Since the method for producing IPV in Sweden differs in some steps from production methods in other countries, these data may not represent the situation in IPV-immunized populations outside Sweden *(D. Magrath personal communication 1991)*.

The more potent eIPV has been available since 1978. It will be important to follow prospectively children immunized with this vaccine (particularly those that live in polio-free countries) to assess whether their serum antibodies are long lasting. A prospective study in Israel followed 86 children who received doses of eIPV at 2, 4, and 10 months of age. One month after the third dose 100% of children were seropositive to all three types. Five years later all the children remained seropositive but their geometric mean antibody titers had declined considerably *(Bernier 1986)*.

7.2.2 Seroepidemiology after long-term use of IPV

As with OPV, there is empirical evidence that countries which achieve and sustain high immunization coverage with IPV enjoy a good level of protection against wild poliovirus. Such countries include Finland, France, Iceland, the Netherlands, Norway, and Sweden. The recommended schedule in most of these countries calls for 2 to 3 IPV doses in the primary series and 2 to 4 booster doses.

In Sweden serosurveys conducted in 1968 and in 1978 examined immunity across a wide age-range of the population *(Bottiger 1987)*. The Swedish immunization schedule calls for 2 doses of IPV 1 to 2 months apart in infants, followed by a booster 6 to 18 months later. Coverage is over 99% among persons born after 1940. The 1968 survey found that for most age groups 95% to 100% of persons were seropositive to all three types. The lowest levels of immunity were among persons born in 1948 to 1959. In this group, 88%, 100%, and 95% were seropositive for types 1, 2, and 3, respectively, at a titer of 4 or higher. These persons were subsequently offered a booster dose of IPV, and in the 1978 survey 99% or more were seropositive for all three types.

7.3 Secretory antibodies

In early studies, parenteral administration of IPV failed to induce a secretory antibody response in the nasopharynx and intestine (Figure 7). However, administration of a large dose of IPV directly into the nasopharynx or the intestine elicited a low level local IgA antibody response which lasted 60 to 90 days; there was no associated antibody response in the serum *(Ogra & Karzon 1971)*. More recent studies have used molecular methods to examine the secretory IgA antibody response to the virion proteins, VP1, VP2, and VP3 in the nasopharyngeal secretions of infants after immunization with OPV, eIPV, or both vaccines. The secretory antibody response to VP1 and VP2 was similar in all groups; however, the secretory antibody response to VP3 was significantly higher in children who had received OPV or both OPV and eIPV *(Zhaori et al. 1989)*.

7.4 Challenge studies

7.4.1 Challenge with OPV

Several early studies examined the response of IPV-immunized children to challenge with OPV. Most studies showed decreased pharyngeal shedding of poliovirus in IPV recipients compared with unimmunized children *(Dick et al. 1961, Glezen et al. 1966, Sabin 1959)*. In contrast, the studies did not consistently demonstrate decreased fecal shedding of virus. Some studies showed a decrease in rate of excretion, duration of excretion, and the absolute amount of virus present in the stool in IPV recipients compared with unimmunized children; however, other studies showed no difference *(Benyesh-Melnick et al. 1967, Ghendon & Sanakoyeva 1961, Henry et al. 1966, Sabin 1959)*. All studies which compared responses of IPV and OPV immunized children showed a far greater decrease in excretion of challenge virus among those immunized with OPV.

More recent studies in Kenya *(Kok et al. 1992)* and in the United States *(Onorato et al. 1991)* (described in section 6.4.1) also showed a greater decrease in excretion following challenge among OPV immunized children compared with children immunized with eIPV (Figure 8).

7.4.2 Challenge with wild virus

During a type 1 epidemic in the USA in 1960, 38 families of polio patients were studied. Previous immunization with IPV had no effect on intrafamilial spread of wild virus. Among IPV-immunized persons with neutralizing antibody titers 128 or higher the duration of fecal shedding was shorter, although the proportion shedding virus did not differ with titer *(Marine et al. 1962)*.

Outbreaks have occurred in populations well-immunized with IPV. In the Netherlands, from 1970 to 1980 the immunization schedule called for 5 doses of IPV in childhood and coverage with 3 or more doses was higher than 95%. In 1978 a type 1 epidemic with 80 cases of paralytic poliomyelitis occurred among unimmunized members of a religious group *(Bijkerk 1979)*. In affected schools, 21% of children immunized with IPV and 45% of nonimmunized children were found to be excreting wild poliovirus. Despite this, the outbreak did not spread to the general population, suggesting the importance of the IPV barrier. Through overseas contacts among members of the religious group, the outbreak spread to unimmunized persons in Ontario, Canada (which uses IPV) and the USA (which uses OPV). Again, the outbreak did not spread to the general population in either Canada or the USA.

Following the 1978 outbreak, the Netherlands changed to a schedule with 6 doses of IPV and coverage of 97% or more was achieved; however, members of the religious group continued not to accept immunizations. In 1992, a type 3 polio outbreak was reported among members of the same religious community *(EPI 1992c)*. Between September 1992 and February 1993, a total of 68 patients were reported. The outbreak did not spread to the IPV-immunized majority of the country *(A. van Loon personal communication 1993)*.

In Finland the immunization schedule calls for 6 doses of IPV and coverage has been more than 90% for many years. No cases of poliomyelitis were reported during two decades and Finland was cited as an example of a country which had used IPV to successfully eradicate poliomyelitis. In 1984 an outbreak occurred due to type 3 poliovirus *(Hovi et al. 1986)*. Nine cases of poliomyelitis were identified, of which at least two cases - 12 and 17 years of age - had received 5 doses of IPV in the past. Investigations of healthy contacts and other healthy persons showed type 3 poliovirus to be widespread. Factors contributing to the outbreak included a drop in the titer of the type 3 component of the vaccine leading to lower type 3 neutralizing antibody titers in persons immunized with this vaccine and minor antigenic differences in the epidemic strain compared with the type 3 strain in the IPV.

In Israel in 1988 a type 1 outbreak led to 15 cases mainly in young adults immunized in the distant past with OPV *(Slater et al. 1990)*. They lived in a subdistrict where the immunization schedule for infants had consisted of 3 doses of eIPV at 1, 3.5, and 10 months of age since 1982. Healthy infants tested at the onset of the outbreak were found to be excreting wild poliovirus. A major factor in this outbreak was the spread of wild poliovirus to susceptible persons via the intestinal tracts of eIPV vaccinated children.

7.5 Protective efficacy of IPV

Before licensure, IPV (with approximately 20, 2, and 4 D antigen units of poliovirus types 1, 2, and 3, respectively) was assessed in a randomized, placebo-controlled field trial in 1954 which enrolled 1.6 million children in Canada, Finland, and the USA *(Francis et al. 1957)*. In this study, IPV was judged to be 60% to 70% effective in preventing paralysis due to type 1 poliovirus and 90% or more effective against paralysis due to types 2 and 3.

In 1986 to 1987 an outbreak due to type 1 wild virus in Senegal provided an opportunity to assess the clinical protective efficacy of eIPV in a region where it had been used since 1980 *(Robertson et al. 1988)*. A case-control study demonstrated that the efficacy of two doses of eIPV in the prevention of paralysis was 89%; the efficacy of one dose was only 39% (Table 5). Coverage with two doses of eIPV in the region was only 26% to 28%, indicating that the main cause of the outbreak was failure to vaccinate, rather than vaccine failure. Additional studies of the

clinical protective efficacy of eIPV would be useful to confirm these findings.

8. Combination Schedules

An immunization schedule combining both OPV and eIPV is presently not recommended by EPI. Such a schedule could potentially achieve both the high serum antibody levels provided by eIPV and the intestinal protection provided by OPV. To date, experience with a schedule employing both vaccines has been limited to Denmark, one province in Canada, Egypt, the West Bank, and Gaza.

8.1 Experience

The reasons for use of a combined schedule in Denmark are complex. IPV was introduced in Denmark in 1955, but due to a shortage of vaccine it was administered intradermally and seroresponse was less than satisfactory, especially for types 1 and 3. Since approximately half of the cohorts who had received IPV intradermally had no antibodies, it was decided to offer OPV to all children and adults below 40 years of age. OPV campaigns were carried out in 1963 (monovalent type 1) and 1966 (monovalent type 3 followed by trivalent OPV). Afterwards, the serological response was higher than than 95% for all three types. However, following the monovalent type 3 OPV campaign in 1966, a few cases (5 to 8) of OPV-associated poliomyelitis occurred *(I. Petersen and H. Zoffmann personal communication 1991).*

Since 1968 Denmark has used a combined schedule with doses of IPV (administered subcutaneously) at 5, 6, and 15 months of age and OPV at 2, 3, and 4 years of age *(Petersen 1991).* No further boosters are recommended. The acceptance of this schedule has been high and 95% of the population is fully immunized with both vaccines. Serological surveys conducted in 1973, 1979, and 1988 showed that more than 95% of the population has poliovirus neutralizing antibodies at titers of 8 or higher for all three types.

Since 1968 only two indigenous cases of paralytic poliomyelitis have occurred in Denmark (in 1969 and 1976). The case reported in 1969 occurred in a contact of an OPV recipient and was caused by a vaccine-like strain of poliovirus type 3. This child had received only one IPV injection and no doses of OPV. Although 1000 to 2000 fecal samples from hospitalized persons are examined each year in Denmark, only 3 wild poliovirus strains have been found since 1968. Two of these strains were imported; the last indigenous wild virus isolated was from the polio case in 1976. Although wild strains have undoubtedly been introduced in Denmark by persons visiting from polio endemic countries or by Danish travelers,

the population appears to have sufficient intestinal immunity to prevent circulation of such wild strains.

A combined schedule has been used since 1978 in the West Bank and Gaza *(Tulchinsky et al. 1989).* A combined schedule was selected because previous efforts at control with OPV alone had proved frustrating *(Melnick 1981).* In the West Bank OPV is given at 2, 3.5, 5, 6.5, and 12 months; eIPV is given at 3.5 and 5 months. In Gaza the schedule is more complex: OPV (type 1 only) is given at age one month, followed by OPV and eIPV given together at 2.5 and 4 months, and OPV alone at 5.5 and 12 months. Immunization coverage is 95% in both areas. The annual incidence of poliomyelitis has declined dramatically in these areas during the past 10 years, despite the fact that wild poliovirus has continued to circulate in neighboring countries.

8.2 Studies in industrialized countries

A recent study in the United States examined a variety of sequential immunization schedules, including eIPV-eIPV-eIPV, OPV-OPV-OPV, eIPV-OPV-OPV and eIPV-eIPV-OPV at 2, 4, and 12 months of age *(Faden et al. 1990).* Nearly all of the 123 children who completed the study developed serum neutralizing antibodies to all three types. Compared with OPV alone, children who received at least one dose of eIPV had higher geometric mean titers of serum neutralizing antibody. Nearly all of the children who received two or more doses of OPV had nasopharyngeal IgA antibody against all three types.

Another small study examined patterns of virus excretion in 21 children who had received polio vaccines in different sequential schedules (OPV at 2 months; OPV-OPV or eIPV-OPV at 2 and 4 months; or eIPV-eIPV-OPV at 2, 4, and 12 months) *(Ogra et al. 1991).* One month after the last dose of OPV, four children shed revertant poliovirus type 3 in their stools; all these children were in the group which had received eIPV prior to OPV The design of this study has been criticized; nevertheless, it has raised concerns that immunization with eIPV followed by OPV does not protect against the generation of nonattenuated virulent revertants in the vaccinees *(Murdin & Thipphawong 1992).* Other studies of sequential schedules are in progress in the United Kingdom and the United States.

8.3 Studies in developing countries

Logistic considerations have influenced the types of combination schedules which are being studied in developing countries. The most practical schedules will not require extra visits for immunization and they should be relatively easy to implement. A recent study in Côte d'Ivoire evaluated serum neutralizing antibody responses in 714 infants who had previ-

ously received three doses of OPV at 2, 3, and 4 months of age, and who were then randomized to receive a supplemental dose of OPV or eIPV at the time of measles vaccination. Although both vaccines increased seroconversion to all three poliovirus types, antibody responses were greater in the eIPV group. *(Moriniere et al. 1993)*

A WHO-sponsored randomized trial is being conducted in The Gambia, Oman, and Thailand to examine the serological response and the intestinal immunity provided when both eIPV (combined with DPT) and OPV are given at the same visit to children at 6, 10, and 14 weeks of age. This large-scale collaborative study has enrolled more than 1500 infants; results are expected in 1993.

9. Implications for Immunization Programmes

9.1 OPV is the polio vaccine of choice

Because of its low cost, ease of administration, superiority in conferring intestinal immunity, and the potential to infect household and community contacts secondarily, trivalent OPV is recommended by EPI as the vaccine of choice for developing countries.

In 1977, when the original EPI guidelines were formulated, a starting age of 3 months was chosen for the routine schedule in order to be consistent with the recommended guidelines used in Western Europe. The schedule recommended in 1977 called for doses of OPV at 3, 5, and 7 months of age.

Subsequent studies showed that infants at 6 to 8 weeks of age respond to immunization with OPV even in the presence moderate levels of maternal antibodies. Additional studies documented a reasonable response. to OPV given at intervals of 4 weeks *(Halsey & Galazka* 1985). In light of these findings, the EPI Global Advisory Group has recommended a

Figure 10. Age distribution of 2420 reported cases of poliomyelitis, Bombay, India, 1987 to 1989 *(Indian Council of Medical Research).*

schedule with doses of OPV at 6, 10, and 14 weeks of age *(EPI 1985)*. In 1990 the Global Advisory Group reaffirmed that trivalent OPV remains the vaccine of choice for developing countries *(EPI 1991)*.

9.2 Importance of a birth dose of OPV

A guiding strategic principle of any immunization programme is that protection should be achieved prior to the time infants are at risk from a disease. In developing countries, the majority of cases of paralytic poliomyelitis reported in outbreaks occur in children under 5 years of age (Figures 4 and 10). Community-based and hospital-based data in polio endemic areas show that more than three-quarters of the paralytic cases occur in children younger than 2 years of age *(Ananthakrishnan et al. 1988, Mahadevan et al. 1989, Onadeko & Familusi 1990)*. The importance of providing vaccine as early in life as possible before exposure to wild virus occurs was highlighted by type 1 polio outbreaks in 1988 in Oman *(Sutter et al. 1991)* and in 1990 in Bulgaria *(EPI 1992b)*. In these countries, a birth dose was not part of the national schedule and doses of OPV were routinely administered at 3, 5, and 7 months of age. Early immunization would have prevented cases in each of these outbreaks. Today, in both of these countries the national schedule includes a birthdose of OPV (in Bulgaria this is for high risk groups) and the next dose is given by two months of age.

Since 1984, the Global Advisory Group has recommended a birth dose of OPV in polio endemic countries *(EPI 1985)*. Among neonates who receive a dose of OPV, 70% to 100% will develop local immunity in the intestinal tract and 30% to 50% will develop serum antibodies to one or more poliovirus types *(Halsey & Galazka 1985)*. Most infants excrete the virus for less than four weeks; therefore, the administration of a single dose of OPV at birth should not interfere with the dose of OPV recommended at 6 weeks of age.

The beneficial effect of a dose of OPV given at birth has been demonstrated most clearly in studies conducted in China *(De-xiang et al. 1986)*. A higher percentage of infants fed a dose at birth had antibodies against all three types of poliovirus at younger ages (Figure 11). In studies carried out in India and Brazil, the serological response was as good in infants beginning immunization at birth or during the first 4 weeks of life as in older children *(John 1984, Weckx et al. 1992)*.

No harmful effects have been observed from the early administration of OPV. Infants who fail to respond with serum antibodies following a dose of OPV in the neonatal period respond normally to subsequent doses of OPV *(Halsey & Galazka 1985)*.

Injection-associated poliomyelitis provides an additional incentive for a dose of OPV at birth and for
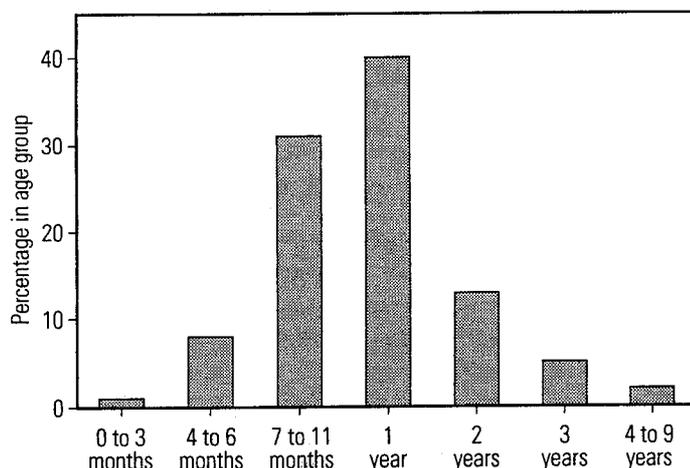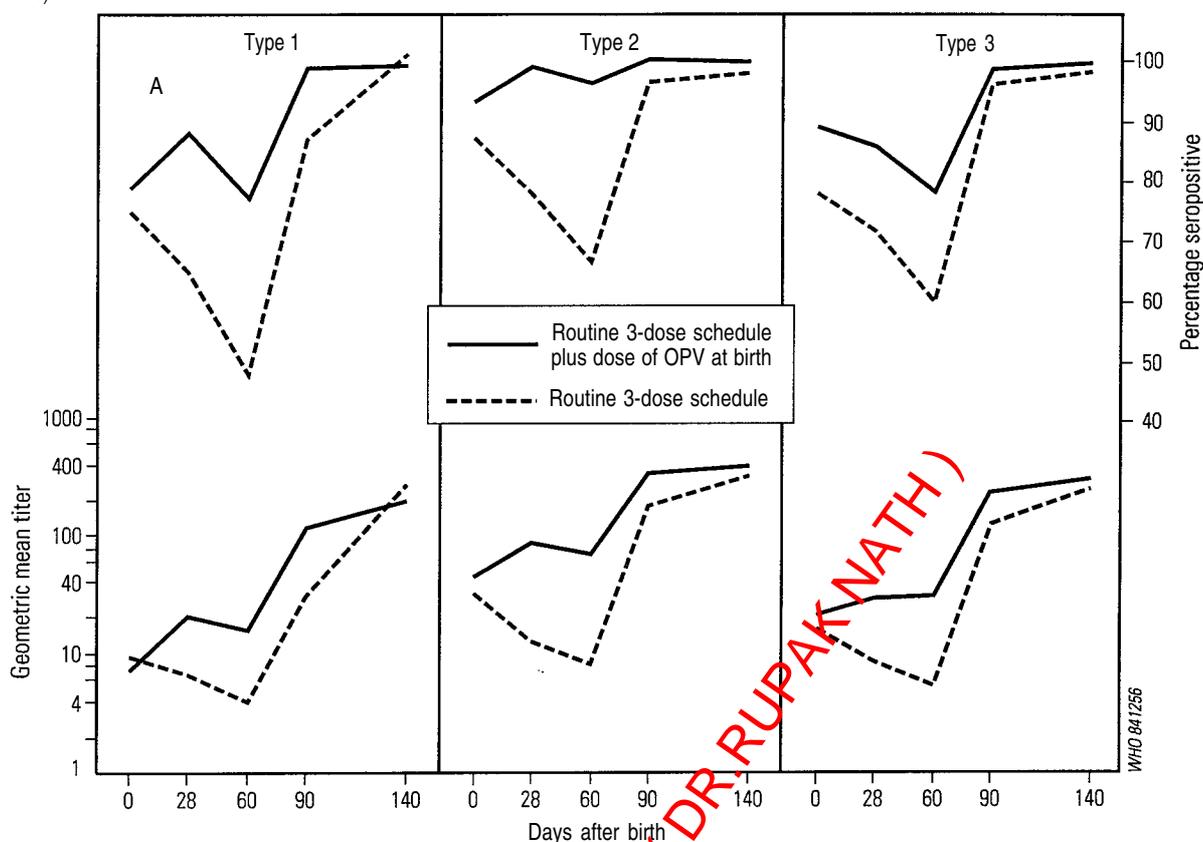
Figure 11. Comparison of seropositivity (A) and geometric mean titers of neutralizing antibody(B) against poliovirus types 1, 2, and 3 among children immunized at 3, 60, 90, and 120 days of life compared with children immunized at 60, 90, and 120 days of life, China *(De-xiang et al. 1986).*

early completion of the immunization series. An association between paralysis of a limb due to poliomyelitis and receipt of an injection of DPT vaccine in the limb during the preceding 30 days has been reported for many years *(McClosky 1950, Sutter et al. 1992)*. Cases of DPT-injection-associated paralytic polio are usually reported in children 6 months of age or older, reflecting the fact that most infants are protected from poliomyelitis during the first few months of life by maternal antibodies. As maternal antibody titers wane, susceptibility increases. Therefore it is desirable to complete a primary series of OPV/DPT immunization by 4 months of age, during which time the risk of post-injection poliomyelitis is extremely low.

If a dose of OPV cannot be given at birth or within the first two weeks of life, a fourth dose of OPV should be given at the same time as measles vaccine or at any contact with the health system that is four weeks after the third OPV dose.

9.3 Diarrhea

Since 1983, the EPI Global Advisory Group has recommended that diarrhea should not be considered a contraindication to OPV *(EPI 1984)*. However, to ensure full protection, a dose of OPV given to a child with diarrhea should not be counted as part of the series and the child should receive another dose at the first available opportunity.

9.4 HIV infection

In countries where HIV infection is considered a problem, individuals (including those with asymptomatic HIV infection) should be immunized with EPI antigens according to standard schedules. In countries where polio remains endemic, children with symptomatic AIDS may receive OPV according to the recommended EPI schedule *(Special Programme on AIDS and Expanded Programme on Immunization 1987)*. Live vaccines are not usually given to immunocompromised individuals; however, in areas where the risk of exposure to poliovirus is high, the benefits of immunization outweigh the apparently low risk of adverse effects from OPV, even in the presence of symptomatic HIV infection. On the basis of data available to the WHO in April 1993, no case of vaccine-associated poliomyelitis has been reported in an HIV-infected recipient or contact. However, vigilance should be maintained, and any case of poliomyelitis in an HIV-infected person should be reported to WHO *(Kim-Farley et al. 1993)*. IPV is an alternative to OPV for immunization of children with symptomatic HIV infection.

9.5 Supplemental immunization for polio eradication

In 1988 the World Health Assembly committed WHO to the global eradication of poliomyelitis *(Wright et al. 1991)*. This means the elimination of disease due to wild poliovirus, as well as the eradication of the wild virus itself. Available data suggest that poliovirus transmission in the Region of the Americas may have been interrupted or, at the very least, is approaching this point *(EPI 1992a)*.

Reaching a high level of coverage with OPV is the most important strategy to achieve polio eradication. Each year, cases of polio continue to occur needlessly in children who have not received polio vaccine. In 1992, 79% of children worldwide received at least 3 doses of OPV by their first birthday (based on data reported to WHO by April 1993). For the national immunization programme manager, coverage data need to be examined by district. Resources should be directed toward districts with coverage below 80%, with special attention to immunizing migrant populations, persons living in urban slums, and the poorest segments of the population.

In most developing countries routine immunization alone may not be sufficient to interrupt transmission of wild poliovirus. Supplementary immunization activities may be necessary. These strategies include:

- the use of OPV in national or subnational immunization days aiming at the administration of two doses of OPV one month apart to all children under 5 years of age, regardless of their previous immunization status;
- "mopping-up" immunization in selected high risk areas where wild poliovirus transmission persists ("mopping up" activities are similar to immunization days, but are they conducted on a house-to-house basis); and
- rapid and extensive outbreak immunization where suspected cases are detected.

In countries where the circulation of wild polioviruses has been greatly reduced or stopped, supplemental doses of OPV should be routinely given to maintain immunity against all three poliovirus types in all children below 1.5 years of age.

Acknowledgments

Abbreviations

DPT	diphtheria-pertussis-tetanus vaccine
eIPV	enhanced potency inactivated polio vaccine
ELISA	enzyme-linked immunosorbent assay
IPV	inactivated polio vaccine
OPV	oral polio vaccine
$TCID_{50}$	dose which infects 50% of tissue cultures

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