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CHAPTER 4

Picornavirus infections

4.1. Picornaviruses

The family consists of a very large number of serologically distinct viruses (Table 4.1) with differing biological properties but which have, nevertheless, a very close relationship as regards morphology and physical and chemical properties (Table 4.2). Clinical illness caused by members of the group varies from asymptomatic infection, to common cold, pneumonia, aseptic meningitis, myopericarditis and paralysis (reviewed by Douglas, 1977 and Table 4.3).

The 'oldest' member of the group is polio virus which was recognized early by clinicians because of its characteristic paralytic disease and this virus will be examined in some detail below as regards virology, disease and prevention by vaccines and chemoprophylaxis. In the 1940s viruses with similar morphology and physico-

TABLE 4.1.
Picornaviruses of man

Picornaviruses	Serological types
Enteroviruses	
Poliovirus	1–3
Coxsackievirus group A	1–22, 24
Coxsackievirus group B	1–6
Echovirus	1–9, 11–27, 29–33
Enterovirus	68–70
Rhinovirus	1–150

Note that the enterovirus Hepatitis A is discussed in Chapter 16.

TABLE 4.2.
Morphological and physical properties of picornaviruses

Picornaviruses	Size (nm)	Morphology	Buoyant density (g/cm^3)	Heat stability (50°C, 30 min)	pH stability
Polioviruses	27–30	Icosahedron	1.32–1.34	No	Yes
Echoviruses	18–25	Icosahedron	1.32–1.34	No	Yes
Coxsackievirus A	18–25	Icosahedron	1.32–1.34	No	Yes
Coxsackievirus B	18–25	Icosahedron	1.32–1.34	No	Yes
Rhinoviruses	15–50	Icosahedron	1.38–1.43	Yes	No

chemical characteristics to polio were isolated, which paralysed infant mice, and they were divided into 2 groups dependent upon the different pathology in mice, namely Coxsackie A and B viruses. Thereafter, with the extensive use of tissue cultures in the 1950s, more enteroviruses were isolated which were not pathogenic for animals, or humans such as ECHO viruses (*e*nteric *c*ytopathogenic *h*uman *o*rphan) (reviewed by McLean, 1966, Artenstein et al., 1965, Karzon et al., 1956, Grist et al., 1975). Finally, in the late 1950s viruses were at last isolated from patients with common colds and these were classified as rhinoviruses (Tyrrell, 1965). Probably in excess of 150 viruses of the latter group circulate in man. Nowadays, all these viruses are designated as 'enterovirus' serotypes (Table 4.4).

4.2. Virus structure and replication of picornaviruses

Electron microscopy reveals the enteroviruses to be small spheres with a regular protein capsid structure 18–30 nm in diameter, although only a few members have been studied in any detail. The capsid is composed of 32 capsomeres in a pattern of cubic symmetry (Fig. 4.1) of an icosahedron with 180 polypeptide molecules per capsid. The molecular weight of the RNA approximates to $2–2.8 \times 10^6$ (7500 nucleotides) and sedimentation coefficients of the viruses range from 140 to 160 S. Most of the protein is encompassed in 4 polypeptides existing in equimolar amounts (VP1, 2, 3, 4) and an RNA capping peptide. In certain of the viruses, which have been studied in more detail, a single large protein VPO is synthesized and then the 4 capsid proteins are derived by a series of proteolytic cleavages (see below, and Cooper, 1977).

After infection and uncoating, the viral RNA is translated to form the giant polyprotein which is cleaved to yield capsid proteins and the viral RNA polymerase. This enzyme, which may exist in two forms – to synthesize plus- and minus-strands respectively – is unique to virus-infected cells, and is responsible for the synthesis of virus-specified RNA using an RNA template.

TABLE 4.3.
Picornaviruses associated with specific clinical syndromes

Syndrome	Virus group				
	Poliovirus	Coxsackievirus A	Coxsackievirus B	Echovirus	Rhinovirus
Paralytic poliomyelitis	Types 1–3	Types 2, 7, and 9	Types 3–5	Types 2 and 4	—
Aseptic meningitis	Types 1–3	Types 1, 2, 4, 5, 7, 9, 10, 14, 16, 22, and 24	Types 1–6	Types 1–9, 11, 12, 14–19, 21, 22, 25, 30, 31, and 33	—
Encephalitis	—	Types 9 and 16	Types 1–5	Types 2, 6, 9, 19, 11, and 25	—
Exanthemata	—	Types 2, 4, 9, 16, and 23	Types 1–5	Types 1–6, 9, 14, 16, 18, and 19	—
Herpangina	—	Types 1–6, 8, 10, 16, and 22	—	—	—
Hand, foot, and mouth disease	—	Types 5, 9, and 16	—	—	—
Acute lymphonodular pharyngitis	—	Type 10	—	—	—
Acute haemorrhagic conjunctivitis	—	Enterovirus 70	—	—	—
Epidemic pleurodynia	—	—	Types 1–5	—	—
Myocarditis/pericarditis	—	—	Types 1–5	—	—
Orchitis	—	—	Types 1–5	—	—
Gastroenteritis	—	—	—	Type 18	—
Common cold	All types	All types	All types	All types	All types
Acute febrile undifferentiated illness	All types	All types	All types	All types	—

(for additional details see Mirkovic et al. 1973, Carter 1933, and Ranzenhofer et al., 1958)

TABLE 4.4.
Antigenic differentiation of picornaviruses

Picornaviruses	Hemagglutination inhibition (HI)	Cross-reactions occur among indicated types
Poliovirus	None	1 and 2
		1 and 3
Coxsackievirus A	A20, A21, A24, A7	A3 and A8
		A11 and A15
		A13 and A18
Coxsackievirus B	B1, B3, B5, B6	12 and 29
	3, 6, 7, 11, 12, 13, 19, 20,	23 and 22
	21, 24, 25, 29, 30, 33	11 and 19
		6 and 30
		1 and 8
Rhinovirus	None	Many

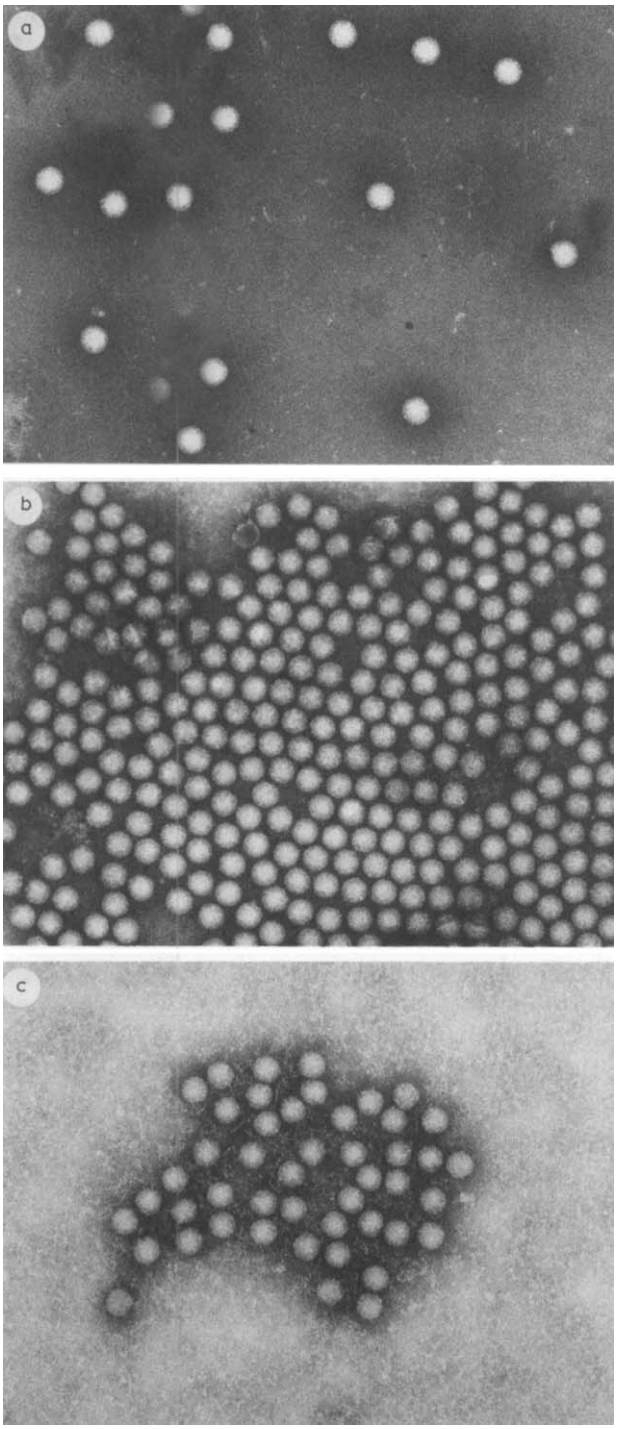
Note that more modern analytical techniques such as Western blotting may demonstrate hitherto unknown serological relationships (Thorpe et al., 1982)

The 3′ end of all picorna virus genomic RNA contains a stretch of adenosine residues (poly A) averaging 40–100 nucleotides. The function of poly A is not clear, although it is not important for the infectivity of genomic RNA. The 5′ end of the RNA is unique, uncapped and covalently linked to a protein (Lee et al., 1977, Wimmer, 1979). The protein, VPg (6–12 000 M.W. basic hydrophobic protein) and a comparable protein have been found on several picornavirus RNAs, including FMDV, EMC and rhinovirus. The significance of VPg is not known and genomic RNA without VPg is still infectious. It may act as a primer for the initiation of viral RNA synthesis and be covalently bound to the first nucleotide of the nascent strand, or may have a regulatory role.

A poliovirus specific Poly (A), oligo (U) dependent poly (U) polymerase has been purified from infected cell cytoplasm which can copy polio RNA to make complementary negative strands (Flanegan and Baltimore, 1977).

The initial transcription of input RNA produces a complementary (minus-strand) RNA whose only function is to act as template for plus-strand RNA synthesis, which may itself either be transcribed to form more complementary RNA, translated to form virus proteins or packaged to form completed virions. Much more plus- than minus-strand RNA must be produced, and the mechanism by which this is brought about is a major unsolved problem. Three RNA species have been isolated: virus RNA, a double-stranded RNA molecule known as replicative

Fig. 4.1. Electron micrograph of a typical picornavirus. a, poliovirus (× 195 000); b, poliovirus (× 170 000); c, poliovirus reacted with monoclonal antibodies (× 170 000). (Courtesy of Dr. D. Hockley, NBSC.)


a
b
c

form (RF) and a multi-stranded RNA species known as replicative intermediate (RI). There is evidence that the RI is an intermediate in virus RNA synthesis. It consists of an RNA molecule which serves as a template for the simultaneous semi-conservative synthesis of several RNA molecules (between six and seven for poliovirus) with nucleotide sequences complementary to that of the template. Two types of RI could exist – that using a minus-strand RNA to produce plus-strands (positive RI) and that using a plus-strand RNA to produce minus-strands (negative RI). Unlike RI, which is partially sensitive to the action of ribonuclease, RF is highly resistant to this enzyme, and since it is infective it must contain an intact plus-strand, which is hydrogen-bonded to a minus-strand.

RNA synthesis switches from exponential to linear synthesis early in infection, the time varying between 1 and 3.5 h after infection and depending upon input multiplicity. This switch implies that the concentration of positive RI (the structure responsible for the synthesis of the major product) is constant throughout the latter period of infection. This could most readily be explained if synthesis of the template of positive RI (the minus-strand) ceased at the switch point, or alternatively was produced at a rate equal to its degradation. It may be important that the switch occurs at the time when the virus RNA is beginning to be encapsidated to form progeny virus.

4.3. Polio – epidemiology and clinical aspects

To develop a sensible and effective strategy of immunization or chemotherapy for a particular disease, we need to know features of the virus epidemiology, how the virus is spread in the community, reservoirs, infectivity and how and where the virus replicates and spreads in the host.

The term 'poliomyelitis' comes from polios (grey) and muelos (marrow) and 'polio' has come to be used as a shortened version meaning paralysis. The virus exists as 3 serotypes originally called Brunhilde, Lansing and Leon but now known as types 1, 2 and 3 which are easily distinguished by neutralization tests, although some cross-immunity between them occurs (see Fig. 4.2 and Table 4.5). Second attacks of polio are rare (Bodian, 1951) and, as far as is known, vaccination with one serotype (e.g. type 1) will protect against all members of these type I serotypes, although individual viruses may differ in virulence (Boulger et al., 1979), biochemical properties (Romanova et al., 1981) and antigen composition (Nakano et al., 1963, Schild et al., 1980).

Polio is a highly infectious disease and infection rates may reach 100% in infant classes. Personal contact is the main method of spread. A seasonal incidence is marked in temperate countries but outbreaks may still occur in winter, whereas in tropical areas it occurs throughout the entire year (Paul, 1955).

Following initial infection of humans, virus is detected in the throat secretions

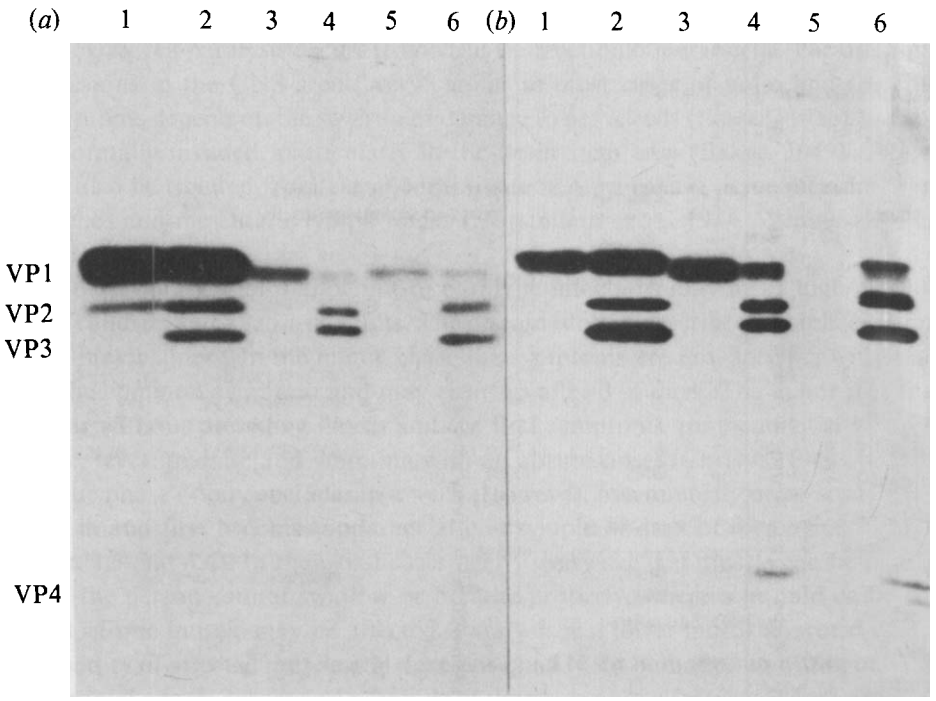


Fig. 4.2. Immunoblot experiments showing serological cross reactions between polioviruses (after Thorpe et al., 1982). (a) Immunoblot processed with rabbit antiserum 115B. Lanes 1, 3 and 5, antibody-labelled proteins; lanes 2, 4 and 6, biosynthetically labelled proteins. Lanes 1 and 2, poliovirus type 3; lanes 3 and 4, poliovirus type 2; lanes 5 and 6, poliovirus type 1. (b) Immunoblot processed with rabbit antiserum R51. Lanes 1, 3 and 5, antibody-labelled proteins; lanes 2, 4 and 6, biosynthetically labelled proteins. Lanes 1 and 2, poliovirus type 3; lanes 3 and 4, poliovirus type 2; lanes 5 and 6, poliovirus type 1. Antiserum R50 produced an identical blot (see also Table 4.5).

and faeces as virus replication continues in the tonsil and intestinal wall regions. Most patients excrete virus for 2–3 weeks and more rarely for 6 weeks. The virus then passes to the regional lymph nodes and probably then reaches the bloodstream. At this stage there is no clinical evidence of invasion of the central nervous system (CNS). There is little doubt that the virus travels along neural pathways from the periphery to the CNS and spread becomes neural once the CNS is reached. Certainly, if antibody is present in the blood the virus does not reach the CNS. A basis for a successful immunization is thus apparent already – induction of neutralizing antibody in the serum and local IgA antibody in the gut. The incubation period is as long as 2–3 weeks.

The most remarkable feature of the pathology of polio is the very high selectivity of the virus for nervous tissue, where it can cause rapid and widespread damage to the central nervous system. The primary changes are to the neurones which can be destroyed. As a response to this nerve cell damage an inflammatory reaction oc-

TABLE 4.5.
Characteristics of the antibodies to poliovirus 3 antigens used in the 'blotting' test (after Thorpe et al., 1982)

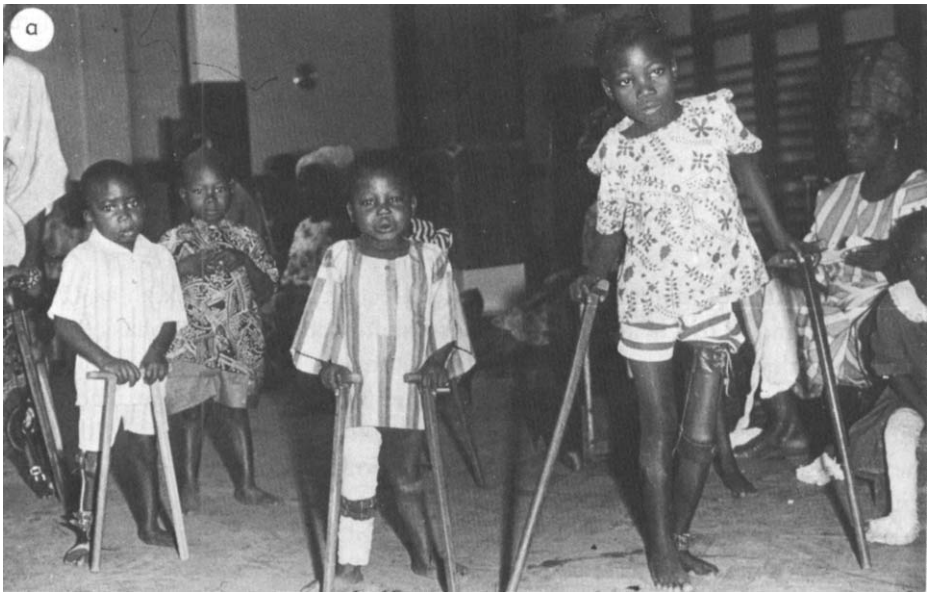
Antibody preparations		Virus[a] neutralization titre	Blocking antigen titre with poliovirus 3 antigens		Poliovirus proteins reacting with antibody on the blot								
					Type 1			Type 2			Type 3		
Designation	Specificity		D particles	C particles	VP1	VP2	VP3	VP1	VP2	VP3	VP1	VP2	VP3
115B	Polyclonal antiviral	100 000	30 000	30 000	++[b]	—	—	+++	—	—	++++	+++	+
GP35	Polyclonal anti-D	30 000	5000	20	++	—	—	+++	—	—	+++	+	—
R51, R50	Polyclonal anti-C	<2	3	3000	+	—	—	+++	—	—	+++	+	—
	Monoclonal anti-C	<2	1	50	—	—	—	—	—	—	+++	—	—
	Monoclonal anti-C	<2	1	50	—	—	—	—	—	—	+++	—	—
	Monoclonal anti-C	<2	1	50	—	—	—	—	—	—	—	—	—
	Monoclonal anti-D	<2	300	<10	—	—	—	—	—	—	—	—	—

[a] Against homologous virus.
[b] Autoradiographic bands were scored in order of increasing intensity from + (weak) to ++++ (very intense).
See also Fig. 4.2 for extra details of serological cross-reactivity.
See also Ferguson et al., 1982.

curs, with infiltration of surrounding tissue with polymorphonuclear cells and lymphocytes, but paralysis occurs from virus destruction of nerve cells. The distribution of lesions in the CNS seems very similar in most cases of polio and the clinical symptoms depend on the severity of damage to nerve cells (Lassen, 1956). The brain is normally invaded, particularly in the brain stem area (Baker, 1949). The virus can also be isolated from extraneural tissues e.g. lymphoid tissue including Peyers patches and mesenteric lymph nodes (Horstmann et al., 1954, Wenner and Rabe, 1951).

The proportion of inapparent to paralytic infections may be as high as 1000 to 1 in children and 75 to 1 in adults. The disease is often described as biphasic – minor and major illness. In the minor phase the symptoms are non-specific, with malaise as the common symptom and may clear up after 3–4 days. The major illness may occur without preceding illness and the first symptoms are meningitis, with headache, fever, malaise and vomiting with an abrupt onset (Christie, 1948). The meningitic phase often concludes in a week. However, in a minority of persons paralysis sets in and first becomes apparent after a couple of days of meningitis symptoms (Fig. 4.3 and 4.4). In the worst cases nearly every skeletal muscle can be paralysed and the person cannot swallow or breathe properly, whereas in mild cases only a part of one muscle may be affected. Paralysis is a lower motor neurone type with flaccidity of affected muscle and develops quickly. In bulbar poliomyelitis, paralysis of cranial nerves occurs and paralysis of the pharynx is a feature. In fact, the inability to swallow is the main clinical characteristic of bulbar poliomyelitis. Signs of other cranial nerve involvement may be present, facial weakness and paralysis of the flexor muscles of the neck being the commonest. Respiration may be deeply disordered and there may be pharyngeal paralysis (Baker, 1949).

In cases of paralytic polio, the full extent of paralysis is reached by 72 hours although not until 4 weeks have passed can the clinician make a reasonable prognosis, since by this time most reversible neuronal damage will have disappeared and residual irreversible damage can be assessed. Often great improvement takes place in these weeks but by the end of one month muscles still paralysed will probably remain paralysed. In bulbar polio the outlook is good if the patient is still alive by the 10th or so day since the pharyngeal muscles then begin to show signs of recovery. The basic principles for clinical care are outlined by Christie (1980) with his characteristic flair "to accept into ones care a patient stricken with a disease that may cut him off for all time from a return to normal life, but to ensure at every stage of his illness whether his life is at risk from respiratory failure or a limb is made useless by paralysis, that the exact nature of his disability is analysed as accurately as the medical sciences and clinical skill can measure it and, when that has been done, to bring to his aid all the sovereign art of modern medicine." Fortunately, the development of polio vaccines was given considerable priority in the late 1950s following the new developments of tissue culture technology (reviewed by Enders et al., 1980). Few clinicians, at least in Europe, USA, Japan, Australia and



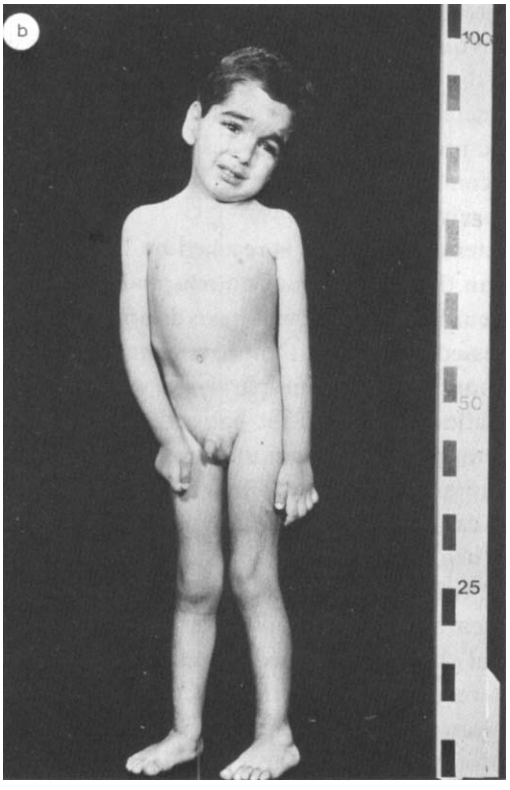


Fig. 4.3a and b. For legend see opposite page.

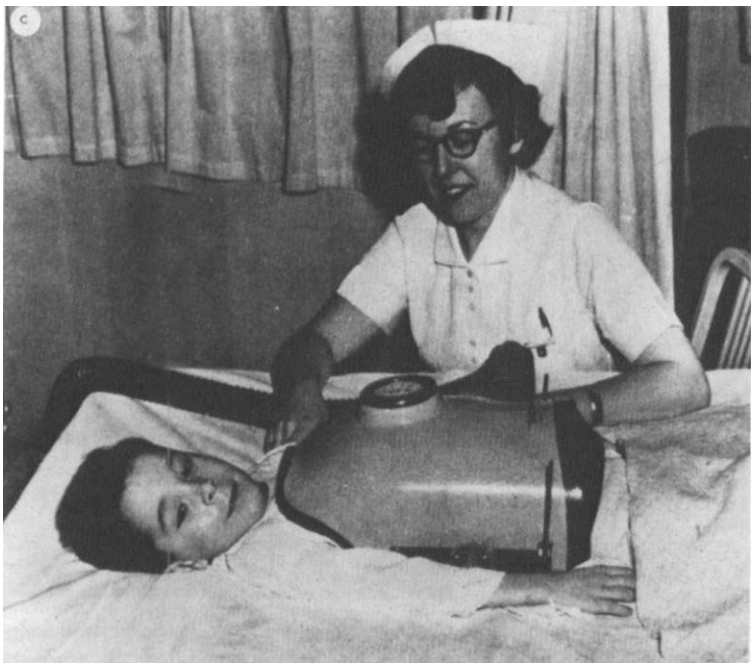


Fig. 4.3. Clinical poliomyelitis. a, children with polio (African continent); b, polio case in London (Courtesy of the late Dr. W.C. Marshall, Hospital for Sick Children, Great Ormond Street); c, artificial respiration.

the USSR have now to diagnose polio or to care for the ravages of the virus in children. The dramatic effects of vaccination on the incidence of polio is illustrated for European countries in Table 4.6.

Although the clincial effects of polio have been known since 1000 BC and a concise discussion of the disease was published by Underwood in 1789, surprisingly *epidemics* of polio have only been described in the last 100 years or so. It would seem in part that when sanitation improved, infection was encountered later in life and paralysis became more prominent. Polio vaccine (Melnick, 1954, Koprowski et al., 1956, Sabin, 1959, Salk, 1960, Salk and Salk, 1967) has been used so successfully in many countries that the virus and vaccine can be used as an example of a situation where the remote risks of vaccination itself begin to assume major importance (see below). In the US before the introduction of vaccine approximately 20 000 cases of polio-caused paralysis were recorded annually and now this number approximates to 10.

4.4 Polio vaccines – general comments

Polio has been controlled successfully by vaccines in many temperate countries of

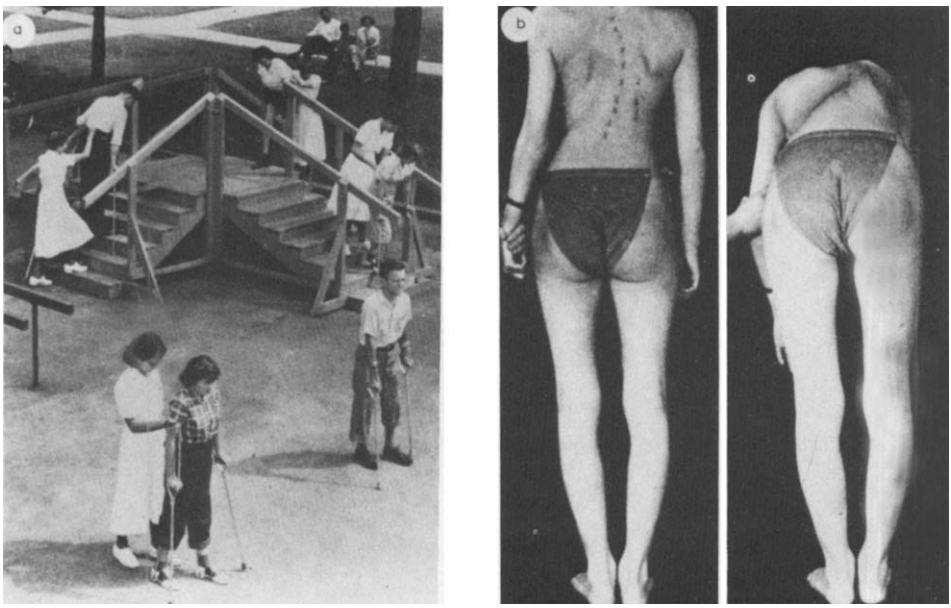


Fig. 4.4. Common residual clinical problems after recovery from acute poliomyelitis. a, physiotherapy for paralysed limbs (Third International Poliomyelitis Conference, 1958); b, scoliosis (from Ottolenghi 1958).

the world (Böttiger et al, 1966, Nathanson, 1982) but not in many tropical, third world countries (Sabin, 1982, John and Christopher, 1975, Swartz et al., 1972).

There is little precise information concerning the antigenic determinants of polioviruses and their involvement in virus structure and function. Polioviruses have been classified into three serotypes (1, 2 and 3) according to the ability of type-specific antisera to neutralize infectivity. The virus exists in two different antigenic forms: the D antigen, which is associated with the infectious particles, and the C antigen, which is associated with non-infectious particles (Mayer et al., 1957, le Bouvier et al., 1957, Roizman et al., 1959, Minor et al., 1980). As infectivity is associated with D antigen particles, it has been assumed that this immunogen is largely responsible for the production of neutralizing antibodies. Most immunochemical work with polioviruses, as well as with other picornaviruses, has been carried out with intact virus particles in which the capsid proteins are arranged in complex structural configurations, so that the immunological and antigenic properties of individual virus proteins could not be shown. In contrast, for foot-and-mouth disease virus it has been possible to establish that immunization with the trypsin-sensitive protein (VP1) induces neutralizing antibodies (Meloen et al., 1979). (see section 4.12 on immunogenic peptides of polio VP1.)

Relevant to vaccines, no single common antigen has yet been described for picornaviruses, but, as stated above, we should remember that very little detailed bio-

TABLE 4.6.
Poliomyelitis in European countries in 1960 and 1969 (i.e. before and after extensive immunization campaigns)

Country	No. of poliomyelitis cases	
	1960	1969
Austria	404	—
Belgium	300	2
Denmark	22	1
Finland	273	—
France	1663	69
West Germany	4139	25
Hungary	38	2
Ireland	183	7
Italy	3555	56
Netherlands	29	15
Norway	59	1
Poland	301	6
Portugal	244	1
Spain	1632	387
Sweden	18	—
Switzerland	139	1
United Kingdom	378	15
Yugoslavia	1680	24
Total	15057	612

chemistry has been carried out with many of these viruses. Indeed RNA hybridization shows a high degree of homology between certain members and so common amino acid sequences with corresponding antigenic relationships may exist. Certainly, antigenic cross-reactions have been noted among certain serotypes such as coxsackie A3 and A8 for example, between A9 and 11 coxsackie B viruses. Again with the ECHO viruses, heterotypic cross reactions occur between some viruses, e.g. types 1 and 8, and between 12, 29, 23 and 22. Thorpe et al. (1982) using the sensitive immunoblot technique have described antigenic relationships between VP1 of polio types 1, 2 and 3. Furthermore, certain monoclonal antibodies directed towards the C antigen of polio type 3 reacted with VP1 of this virus. VP1 seems to be immunodominant regardless of the form of the immunizing antigen. A recent report (Blondel et al., 1982) suggests that immunization with VP1 causes the production of neutralizing antibodies and in this respect the immune response to poliovirus VP1 may be similar to that to VP1 of foot-and-mouth disease virus and to VP2 of coxsackie virus (Meloen et al., 1979, Beatrice et al., 1980). Although homotypic antibodies binding to VP2 were detected with hyperimmune sera, no heterotypic cross-reactivity was observed for this protein.

To some extent views about the use of either killed or live polio vaccines are in a state of flux at present (Melnick, 1978, Sabin, 1982). Perhaps not unexpectedly the virologists who hold dominating and contrasting views are Drs. Salk and Sabin (originators of inactivated and attenuated polio virus strains respectively). Also, perhaps not unexpectedly Sabin enthusiastically supports the widespread use of live vaccine whilst Salk confines his interest to killed polio vaccine. Both vaccines have their merits and weaknesses and it may not be possible to decide between them (Tables 4.7 and 4.8). To some extent a judgement on their relative efficacy and usefulness may depend on the purpose of the vaccination campaign and where in the world it is being conducted. If our goal is total eradication of virulent polio virus from the world community (which should be possible, there being no animal reservoir of the virus, and the virus exists as a limited number of 3 serotypes) then inacti-

TABLE 4.7.
Killed poliovaccine: advantages and disadvantages (after Melnick, 1980)

Advantages
Confers humoral immunity in satisfactory proportion of vaccinees if sufficient numbers of doses are given
Can be incorporated into regular paediatric immunization, with other vaccines (DPT)
Absence of living virus excludes potential for mutation and reversion to virulence
Absence of living virus permits use in immunodeficient or immunosuppressed individuals and their households
Appears to have greatly reduced the spread of polioviruses in small countries where it has been properly used (wide and frequent coverage)
May prove especially useful in certain tropical areas where live vaccine has failed to 'take' in young infants
Disadvantages
Several studies have indicated a disappointing record in percentage of vaccinees developing antibody after three doses[a]
Generally, repeated boosters have been required to maintain detectable antibody levels[a]
Does not induce local (intestinal) immunity in the vaccinee; hence vaccinees do not serve as a block to infection with wild polioviruses
More costly than live vaccine, in single-dose cost, administration expense, and total amount required, including boosters
Subject to problems from present and growing scarcity of monkeys (but could be resolved if high-titer virus could be grown in human diploid cells and shown, in field tests with adequate numbers of persons, to be free of any problems resulting from *injection* of virus grown in human cells
Use of antigenically potent but virulent polioviruses as vaccine seed creates potential for tragedy if a single failure in virus inactivation were to occur in a batch of released vaccine

[a] Some of the disappointing results in the decade after killed vaccine was introduced may have been due in part to problems that may now have been corrected. Present antigens are stronger than the older antigens. (See also Marsden et al., 1980 and Boulger et al., 1979 for a further discussion of assessment of neurovirulence of poliovirus vaccine.)

TABLE 4.8.
Live poliovaccine: advantages and disadvantages (after Melnick, 1980)

Advantages
Confers both humoral and intestinal immunity, like natural infection
Immunity induced may be lifelong
Induces antibody very quickly in a large proportion of vaccinees
Oral administration is more acceptable to vaccinees than injection, and is easier to accomplish
Administration does not require use of highly trained personnel
When stabilized, can retain potency under difficult field conditions with little refrigeration and no freezers[b]
Under epidemic conditions, not only induces antibody quickly but also rapidly infects the alimentary tract, blocking spread of the epidemic virus
Is relatively inexpensive, both to produce the vaccine itself and to administer it, and does not require continued booster doses
Can be prepared in human cells, thus is not dependent on continuing large supplies of scarce monkeys (this also eliminates theoretical risk of including monkey virus contaminants in the vaccine)
Disadvantages
Being living viruses, the vaccine viruses do mutate, and in rare instances have reverted toward neurovirulence sufficiently to cause paralytic polio in recipients or their contacts
Vaccine progeny virus spreads to household contacts[a]
Vaccine progeny virus also spreads to persons in the community who have not agreed to be vaccinated[a]
In certain warm-climate countries, induction of antibodies in a satisfactorily high proportion of vaccinees has been difficult to accomplish unless repeated doses are administered. In some areas, even repeated administration has not been effective.
Contraindicated in those with immunodeficiency diseases, and in their household associates, as well as in persons undergoing immunosuppressive therapy

[a] Some people consider this spread into the community to be an advantage, but the progeny virus excreted and spread by vaccinees often is a mutated virus. Obviously it cannot be a safety-tested vaccine, licensed for use in the general population.
[b] see Magrath, 1976.

vated vaccine must be used in the final thrust, although much earlier work could be carried out with live vaccine or a combination of live and killed vaccine. This is because attenuated viruses may (and do) revert to virulence on occasion (Nathanson, 1982). As mentioned above the genetic determinants of virulence or attenuation for polio virus are not known and it will require extensive biochemical analysis of the genome of a number of attenuated and virulent viruses, mutants and recombinants to establish the genetic basis of virulence. At this stage one could envisage that an attenuated and *stable* variant could be constructed by using DNA technology (see Chapter 2). On the other hand, it may not be practicable to produce large enough quantities of inactivated vaccine cheaply enough for a total eradication campaign and moreover, if the manufacture of vaccine is extended more widely

then escapes of virulent virus in incompletely inactivated vaccine may occur. An early example of such an accident was the Cutter affair in the USA where 92 children contracted paralytic poliomyelitis following immunization with insufficiently inactivated vaccine (Nathanson and Langmuir, 1963). It could be added however that nowadays, should large scale manufacture of inactivated polio vaccine be reinitiated then viruses would be chosen with less than full virulence to avoid potential problems of incomplete inactivation (Salk et al., 1981). Genetic engineering techniques could now be explored to produce immunogenic polio proteins and synthetic peptides which would side step any 'failure of inactivation' problems although these synthetic techniques are likely to produce problems of their own (see Chapter 2).

We can conclude that both vaccines have had their successes and failures, both vaccines are not in evolutionary blind alleys and can be improved in the near future, and both vaccines probably have a long and useful life and function ahead of them.

4.5. Early history of laboratory studies with polio virus

Until the 1930s polio virus could only be identified by infection of monkeys and subsequent production of paralytic disease. This property of neurotropism of polio in vivo and early studies in cell culture which were wrongly interpreted as indicating that polio could only replicate in neural tissues delayed work with the virus and, moreover, erroneously led to deductions that droplet infection and subsequent invasion of the CNS via the olfactory nerve endings was the major point of entry of the virus in humans. In fact it could be said that the early studies of 'chemotherapy' were initiated by these observations and sodium alum, zinc sulphate and picric acid were all used unsuccessfully to prevent paralysis (Armstrong and Harrison, 1935, Sabin et al., 1936, Olitsky and Sabin, 1937, Schultz and Gebhardt, 1937, Armstrong, 1937). Sabin and Ward (1941) were among the first to appreciate that the alimentary tract was a major site for polio virus replication in man. Studies in the 1940s and 1950s established that although polio virus could be isolated in flies and cockroaches, and antibodies detected in cattle and chimpanzees (Sabin and Ward, 1942, Melnick and Penner, 1952, Sabin and Fieldsteel, 1952), nevertheless the viruses are maintained in nature by human to human transmission. Epidemiological studies in Eskimoes demonstrated that antibodies to polio persisted for at least 40 years in the absence of re-infection and that natural infection gave rise to complete or partial resistance in the intestine. The scientific ground work was therefore laid for the production and application of polio vaccines. Theiler (1941) reported laboratory modification of the neurotropic property of polio and Koprowski et al. (1952) described for the first time that an immune response could be produced in humans following infection with an attenuated (mouse adapted) type 2 polio virus which had reduced intra-cerebral virulence for monkeys. Finally, the clear demonstration by Enders et al. (1952) that all three polio virus types could replicate in

TABLE 4.9.
Results of titrations in monkeys and in tissue cultures of materials from a tissue culture line of Type 1 virus (Brunhilde strain). (after Enders et al., 1980)

Material titrated	Monkey infectivity (PD_{50}/1 ml)	Tissue culture infectivity (ID_{50}/1 ml)	Ratio: TC infectivity / Monkey infectivity
Monkey cord	10^5	10^5	(1)
Susp. cell cult. pass. 2	10^3	10^5	100
Susp. cell cult. pass. 10	19^1	10^5	1000
Roller cult. pass. 1	<1	10^4	>10 000
Roller cult. pass. 2	<1	10^5	>100 000

cell cultures of non-nervous tissue (see below) and simultaneously lose monkey virulence (Table 4.9.) opened the way to the extensive laboratory studies leading to the development of successful formalin inactivated and also live attenuated polio vaccines.

4.6. Early development of inactivated polio vaccine

Salk commenced work on producing an inactivated polio vaccine with the premise that circulating serum IgG antibody provided protection against the clinical signs of polio and therefore as long as a killed vaccine induced such antibody in sufficient quantity it would be effective. (Earlier work in his laboratory had been orientated towards developing live attenuated viruses, but this ceased as soon as it was apparent that tissue culture cultivated formalin-inactivated virus was immunogenic.) His group demonstrated that virus concentration (and hence antigen concentration) was the single most important variable factor in vaccine production, rather than the inactivation process per se. Inactivation with 1/4000 formalin at 35°C at pH 7.0 resulted in a constant rate decline of virus infectivity for the first $2\frac{1}{2}$ days. However, even at this early stage inactivation of lots of vaccine varying from 30 to 50 litres was showing some variation between manufacturers, with satisfactory 'base line' virus inactivation in one manufacturer detected at 6–9 days but 13 days at another production plant (12 days was later chosen as safer after the Cutter incident). If inactivation proceeded for too lengthy a period then immunogenicity decreased, and hence the time period was critical.

Early experiments in monkeys established that vaccine given in three divided doses was more immunogenic than a single large dose. Table 4.10 summarizes the data from some of the first trials in children who had no antibody prior to immunization with polio vaccine and the effect of boosting at 7 months and at 20 months (Table 4.11). Quite clearly the era of successful immunization against polio had commenc-

TABLE 4.10.
Antibody levels before and 7 months after primary vaccination and after 'booster' injection in persons inoculated with an aqueous poliomyelitis vaccine (inactivated). (after Salk, 1955)

Subject no.	Age in years	Type 1			Type 2			Type 3		
		Pre-vacc.	7 mos. later	12 days after 'booster'	Pre-vacc.	7 mos. later	12 days after 'booster'	Pre-vacc.	7 mos. later	12 days after 'booster'
Group A										
F-332	3	0	8	256	0	128	1024	0	0	512
F-4	6	0	64	1024	0	4	1024	0	4	512
F-2	9	0	32	512	0	8	128	0	4	256
F-45	9	0	32	4096	0	32	512	0	16	1024
F-47	10	0	16	1024	0	4	256	0	8	1024
F-328	10	0	64	4096	0	4	256	0	4	256
F-335	11	0	32	2048	0	0	128	0	8	1024
Group B										
F-80	6	0	4	512	0	0	256	0	4	512
F-77	7	0	4	512	0	4	128	0	8	512
F-79	10	0	4	128	0	4	256	0	4	128
F-336	12	0	16	1024	0	16	256	0	16	512

Primary vaccination – 3 doses, 1 week apart.
Group A – 1 ml of trivalent vaccine, intramuscularly.
Group B – 0.1 ml of each type of monovalent vaccine, intradermally.

ed, although problems of incomplete and variable inactivation of virus were to be noted immediately the vaccine process was scaled up to produce the tens of millions of doses required for mass immunization (Nathanson and Langmuir, 1963).

As regards production of polio vaccine nowadays, human diploid cells are used by some manufacturers and still other cell substrates are being considered for polio vaccine production mainly because of the immediate shortage of monkeys and safety considerations but additional advantages might be increased yields of virus (Van Wezel et al., 1978, Marel et al., 1981). Possible new cell substrates are:

1: subcultured monkey cells rather than primary cells. In addition, newly developed techniques of perfusion with trypsin gives increased yields of cells.
2: primate embryo diploid cells (in addition to human diploid cells).
3: continuous cell lines e.g. Vero (these cells have been suggested because of evidence of absence of tumourigenicity).
4: 'suspension' cultures of continuous cell lines using Sephadex beads.

Captive bred monkeys have fewer adventitious 'foamy' viruses and so are more suitable as a source of kidney cells than captured animals. Good cell growth of MK

TABLE 4.11.
2-Year follow-up in first group of subjects in whom polio vaccination studies were undertaken (after Salk, 1955)

Age at time of first vacc. (years)	Identification no.	Type 2 antibody titre vs. 100 ID_{50} virus			
		Before vacc.[a]	2 months	Booster at 20 months[b]	22 months
2	W-44	0	32	8	2048
4	W-31	0	64	8	1024
5	W-18	0	16	8	512
6	W-27	0	8	2	512
8	W-20	0	128	64	256
9	W-33	0	8	0	256
10	W-8	0	4	0	512
10	W-26	0	16	8	256
11	W-74	0	32	4	512
14	W-34	0	16	8	256
17	W-1	0	32	16	128
17	W-32	0	32	16	N.T.
31	W-3	0	16	8	256

[a] 2 doses I.D. of 0.1 ml each 6 weeks apart monovalent vaccine.
[b] 1 dose I.M. of 1 ml trivalent vaccine.
N.T., not tested.

cells is obtained in monolayers and in microcarrier cultures for up to 3 passages (approx. 12 cell generations) although the cells stop growing after 7–8 passages. 15–20 monkeys per year will then be required to produce $3–4 \times 10^6$ doses of vaccine. Although diploid cells have many favourable properties (absence of viruses, strict control on karyotype (Chapter 2)) they require high quality serum for growth and are less suitable for large scale cultivation. Finally, Vero cells may be suitable particularly since polio virus will be inactivated with formalin at the final stages (so inactivating any adventitious or unknown virus) and these cells can be cultivated in microcarrier systems.

Criteria for seed polio viruses for inactivated vaccine include stability, immunogenicity, and replication rate but the most important factor is immunogenicity. Type 2 strains (MEF1) with type 3 (Saukett) induce much higher antibody levels than a comparable antigenic mass of type 1 (G. Mahoney) polio. It has also been recognized that antigenic differences exist within polio types and so the antigenic structure of the vaccine strain should be representative of field strains. Type I virus still gives problems of antigenic instability after formalin inactivation. For final vaccine production, cell debris are removed by filtration and the virus suspension concentrated by ultrafiltration and standardized using D antigen concentration, or by single radial diffusion techniques. Gel filtration on Sepharose (to remove remaining

serum proteins), followed by ion exchange on DEAE Sephadex is used to remove further impurities. The original inactivation techniques developed by Salk (1955) using formaldehyde at elevated temperatures has proved successful also for purified polio virus suspensions (Van Wezel et al., 1978). It could be added that attenuated polio viruses could be used nowadays for production of inactivated polio vaccine thus avoiding potential problems of virus escaping inactivation and infecting and paralysing recipients.

4.7. Early development of attenuated polio viruses

During 1953–1954 Sabin and co-workers attempted to attenuate highly virulent polio virus (Mahoney, Y-SK and Leon) by cultivation in cynomolgus kidney tissue cultures. However, it should be realised that several other groups were actively working at the same problem and several potential 'candidate' vaccine strains had been selected (Koprowski et al., 1952, Enders et al., 1952, Melnick, 1954). They soon found that mere passage had no attenuating effect but, significantly, use of *high* inocula together with *rapid* passage favoured the overgrowth of virions able to replicate well in non-nervous tissue and the harvests had diminished virulence for monkeys (Table 4.12). This suggested a mixed population originally and therefore terminal cloning was carried out and non-virulent variants of all three polio types obtained (Sabin, 1955). A marked diminution in intramuscular and oral infective capacity for cynomolgus monkeys was noted for these strains (Table 4.13) and these monkeys produced antibodies in response to infection. Sabin had selected a variant of the mouse passaged Mahoney strain (type 1) which was potentially useful

TABLE 4.12.
Intracerebral virulence in cynomolgus monkeys of type 1 poliomyelitis virus (Mahoney strain) propagated in different ways in cynomolgus kidney tissue cultures (after Sabin, 1955)

Kidney passage 10 Serial passages with minimal inocula		Kidney passage 33 Rapid passages with large inocula followed by purification by terminal dilution technique		
No. of TCD_{50} inoculated	No. of monkeys paralyzed	No. of TCD_{50} inoculated	No. of monkeys paralyzed	No. showing CNS lesions
		16 000 000	0/4	0/4
		1 600 000	0/4	0/4
500 000	5/5	160 000	0/4	0/4
50 000	5/5	16 000	0/4	0/4
5000	5/5	1600	0/4	0/4
500	5/5	160	0/4	0/4
50	3/5	16	0/4	0/4
0.5	1/5			

TABLE 4.13.
Intracerebral activity in cynomolgus monkeys of optimum single-plaque strains of each type of polio virus grown in monkey kidney tissue culture (after Sabin, 1955)

Type	Strain	PFU virus per ml	Paralytogenic effect of 1 ml of indicated dilution	
			Undiluted	10^{-1}
1	LSc, 2 ab	4.2×10^{7}	0/10	0/5
2	P-712, Ch, 2 ab	3.6×10^{7}	0/10	0/5
3	Leon, 12 a_1b	4.3×10^{7}	0/10	0/5

as a vaccine strain, and early tentative trials in volunteers confirmed attenuation of these viruses (Table 4.14).

4.8. Early large scale immunization trials with inactivated and attenuated polio vaccines

The WHO Expert Committee on Poliomyelitis in 1957 recommended large scale trials of the newly developed formalin-inactivated vaccine and particularly well controlled trials in the USA (Francis et al., 1957) demonstrated efficacy and safety of the new vaccine. However, Sabin (1955) had by now developed several live attenuated candidate vaccine viruses and since he did not want to interfere with the still progressing trials of Salk vaccine in the USA, he initiated large scale trials in Eur-

TABLE 4.14.
Resistance of alimentary tract to infection with attenuated strains in nonimmune and naturally immune adults as well as in adults who received single feedings of attenuated virus or 2 doses of Salk vaccine intramuscularly (after Sabin, 1958)

Category	Type 1 LSc	Type 2 P-712	Type 3 Leon
No homotypic low-avidity or high-avidity antibody	17/19[a]	15/15	17/17
Naturally acquired low-avidity antibody *only* (pH test) (with few exceptions may be result of heterotypic infection)	15/19	2/3	7/7
Naturally acquired low-avidity *and* high-avidity antibody present	1/11	3/11	6/12
Ingested homotypic attenuated virus 8 to 15 months before test. No homotypic low- or high-avidity antibody before Salk vaccine.	1/10	1/7	1/6
Received 2 doses Salk vaccine – tested 2 weeks to $3\frac{1}{2}$ months after 2nd dose	9/9	6/7	9/9

[a] Number of infections detected.

ope. By this stage the genetic basis of virulence of these new polio strains had been established, at least tentatively, and several phenotypic virological markers which co-segregated with virulence had been observed. Thus, attenuated polio strains often possessed *ts* or *ca* properties. In fact it appeared, in retrospect, that since the early tissue culture passage experiments to 'attenuate' these viruses had been carried out at low temperatures of 35°–36°C this may have also increased the selection pressure for *ts* attenuated virus subpopulations (Sabin, 1958).

Large scale polio immunization was carried out in Europe in collaboration with the large and active virology group in Leningrad and these represent, in retrospect, a classic series of experiments both scientifically and for the benefit of mankind. Initially small groups of children were immunized, but as increasing clinical and laboratory experience showed no adverse effects the campaign was widened. Thus, some of the early clinical trials were carried out with Sabin live vaccine virus in the baltic republics of Latvia, Lithuania and Esthonia (Chumakov et al., 1959). Twenty seven thousand individuals were immunized with vaccine provided by Sabin but a further 10 million people were fed with first passage material grown in the USSR. The first cycle of immunizations was carried out in January–March 1959 (Table 4.15 and 4.16). Almost half the susceptible population in Lithuania received live virus vaccine and convincing proof of the highly immunogenic characteristics of the virus was obtained. Trivalent mixtures were also used effectively with children developing antibody to all three types.

In separate studies, Smorodintsev et al. (1959) also used a cautious approach and between April 1957 and April 1958 observed 150 vaccinated children and contacts before vaccinating a further 822 children in May 1958 and 12 000 children in January 1959. By March 1959 a further 1.8×10^6 children were fed vaccine virus. Table 4.16 shows illnesses in 10 000 immunized children and non-immunized controls in Riga. No clinical polio was detected in the vaccinated group or the internal control group.

Early experiments by Smorodintsev in Leningrad also established that no marked increase in virulence occurred on human–human passage of these attenuated virus-

TABLE 4.15.
Scope of vaccination with Sabin's polio vaccine of the population in Lithuanian SSR, by age groups (up to May 10, 1959) (after Chumakov et al., 1959)

Age group	Population (in thousands)	No. vaccinated (in thousands)	% vaccinated
0–7	390	164	42
7–15	450	256	57
15–19	270	147	54
Total	1110	547	49.7

TABLE 4.16.
Analysis of the reaction-causing properties of live poliomyelitis vaccine according to data concerning vaccinations in schools in Riga (Latvian SSR) (after Smorodintsev et al., 1959)

Recorded diseases	Rate of sickness among the groups mentioned (per 10000 persons for 4 months)		
	Live vaccine Schoolchildren 8311 Children of pre-school age 1346	Internal control Schoolchildren 4188 Children of pre-school age 768	External control Schoolchildren 3300 Children of pre-school age 1062
I. Poliomyelitis and similar diseases	—	—	9.3
	—	—	—
II. Acute infectious diseases	3697.6	3662.3	3506.0
	2526.0	2556.4	2824.8
III. Diseases of the respiratory organs and ear, nose and throat	474.5	496.0	472.7
	1315.0	1119.8	1468.1
IV. Diseases of the digestive organs	21.5	23.4	18.1
	74.3	78.4	75.3
V. Other diseases (pyelitis, nephritis, bronchial asthma and lymph-adenitis)	10.3	22.3	21.1
	29.7	26.0	28.2
VI. Injuries	2.4	2.5	12.1
	—	—	—

es (Smorodintsev et al., 1959) and the vaccines began to be widely used (Table 4.17 and 18). However, in these early studies it soon became apparent that problems of vaccine 'take' could occur in countries with poor hygiene and where many other enteroviruses were present in the gut which could interfere with the replication of the polio vaccine virus (Sabin, 1959, 1963).

4.9. National campaigns to eradicate polio

Several developed countries have now virtually eradicated polio and these include, for example, the USA, USSR, UK, Scandinavia (Böttiger, 1969) (Fig. 4.5) and certain other Western and Eastern European countries. However, polio is still com-

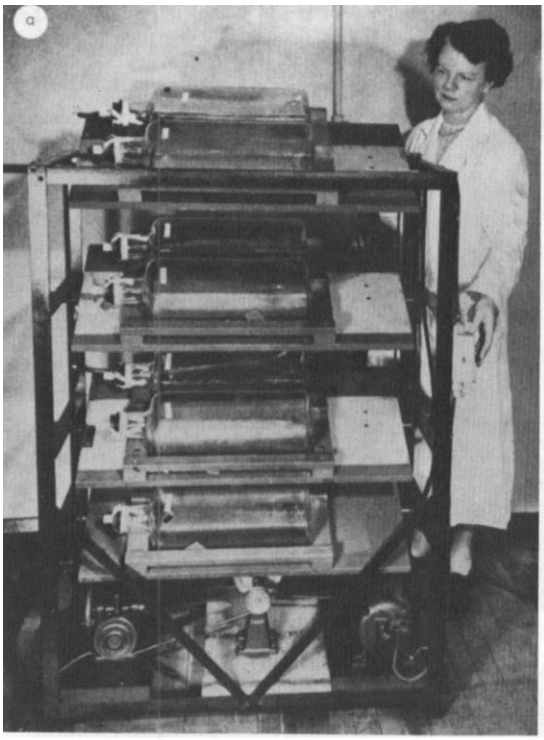
TABLE 4.17.
Distribution of live poliovirus vaccine in the USSR up to December 31, 1960 (after Weissfeiler, 1961)

Republic	Distributed doses of live vaccine	Estimated number of the vaccinated persons
RSFSR	166 590 800	42 604 150
Ukraine	47 752 300	14 152 778
Kazakh	6 438 700	3 385 184
Uzbek	8 030 000	3 872 000
Byelorussia	12 495 600	2 888 000
Georgia	3 199 200	1 320 000
Azerbaijan	2 991 000	1 261 920
Moldavia	1 545 000	1 007 600
Lithuania	2 750 000	1 760 000
Latvia	2 608 000	880 000
Kirghiz	1 902 000	968 000
Tajik	1 852 200	968 000
Armenia	1 702 000	792 000
Turkmenia	1 478 000	792 000
Estonia	1 948 000	827 240
Total	263 282 800	77 478 872

mon in underdeveloped countries and a quandary for eradication here is that live polio vaccine often works erratically and so is less than satisfactory in these countries (Chandra, 1975, Swartz et al., 1972, Hale et al., 1959, Hale et al., 1961). Also inactivated polio vaccine may not produce herd immunity which is assumed to be

TABLE 4.18.
Data on the use outside the USSR of live vaccine prepared in the USSR from Sabin's attenuated strains (after Weissfeiler, 1961)

Country	Amount of vaccine doses	Calculated number of vaccinees
Albania	803 000	450 000
Bulgaria	6 850 000	2 000 000
People's Republic of China	9000	3000
Czechoslovakia	3 500 000 (types 2 and 3)	2 000 000
German Democratic Republic	18 100 000	5 000 000
Hungary	7 700 000	2 400 000
Japan	600	
Korean People's Democratic Republic	750 000	350 000
People's Republic of Viet Nam	4 500 000	950 000
Total	42 712 600	13 153 000



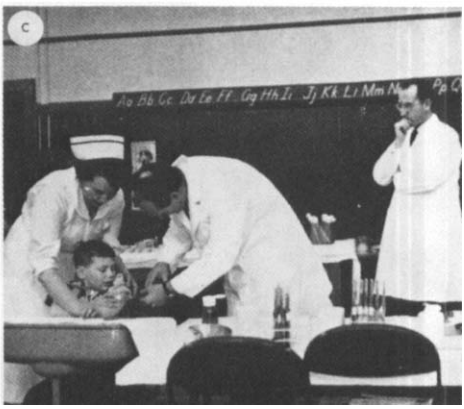


Fig. 4.5a, b and c. Illustrations of early studies with polio vaccine production, control and usage. a, polio vaccine production in roller bottles containing monkey kidney cell; b and c, children in the first 3 school grades took part in the nation-wide vaccine trials in the USA. In some areas, only second-grade children received the vaccine, with first- and third-grade children serving as uninjected controls. In others, half the children in all 3 grades received vaccine and the other half medium No. 199 without virus. (illustrations from Third International Poliomyelitis Conference, 1955).

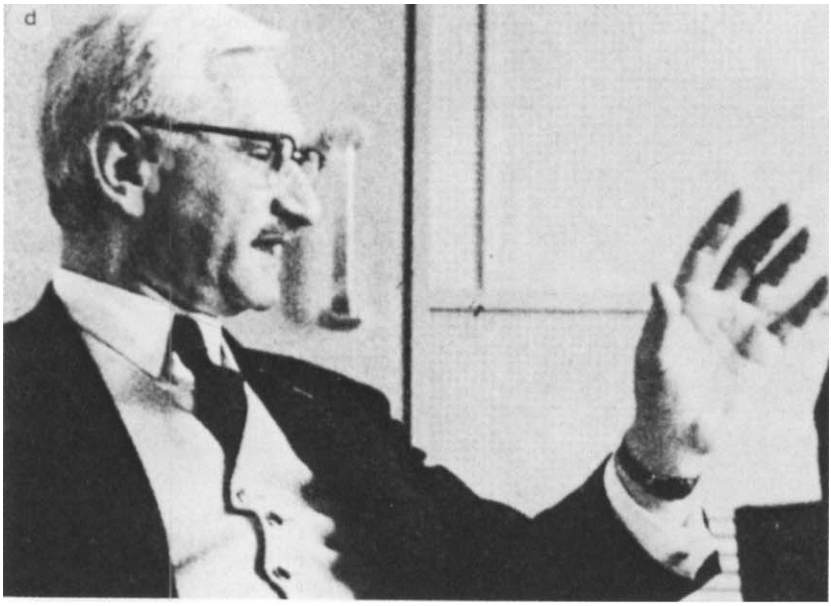


Fig. 4.5d, e: The scientists, Dr. A. Sabin and Dr. J. Salk, who initially developed live and inactivated polio vaccines.

so important, since in these countries, the faecal-oral route of transmission is most common and inactivated polio may only reduce transmission via the pharyngeal route (Gelfand et al., 1959, Chin et al., 1961). Therefore, further research is clearly needed before a decision is made on the strategy for the control and eradication of poliomyelitis in developing countries. Meanwhile, however, we might learn from the very useful data on the experience of eradication of polio in certain countries like the USA using live vaccine, or in Scandinavia using inactivated vaccine.

From 1951 to 1977 the incidence of polio in the USA dropped steeply from a level of 10 000–20 000 cases a year to 10 cases a year. Similar figures were obtained in the UK and Scandinavia (Fig. 4.6). Naturally occurring poliomyelitis due to indigenous wild poliovirus essentially disappeared from the USA after 1972 (Nathanson, 1982). Nevertheless, 5–10% of children under 16 years of age in the USA are considered to remain susceptible to polio because vaccine coverage is not 100% (Table 4.19). This success in eradication of polio in the USA was achieved mainly by the use of attenuated vaccine (Table 4.20), but the continuing problem with this vaccine is the, albeit small, number of vaccine-associated cases of poliomyelitis (Schonberger et al., 1976) which approximate to 2 paralytic cases per million vaccinees. In the USA with 3.5 million children being immunized yearly 7 paralytic cases are expected, caused by the vaccine virus, of which one third occur in recipients of vaccine and two thirds in contacts, who are mainly adults.

At present it is estimated that 2–5 million children in the USA are still susceptible to polio type I, providing a substantial pool of susceptibles for epidemics to occur.

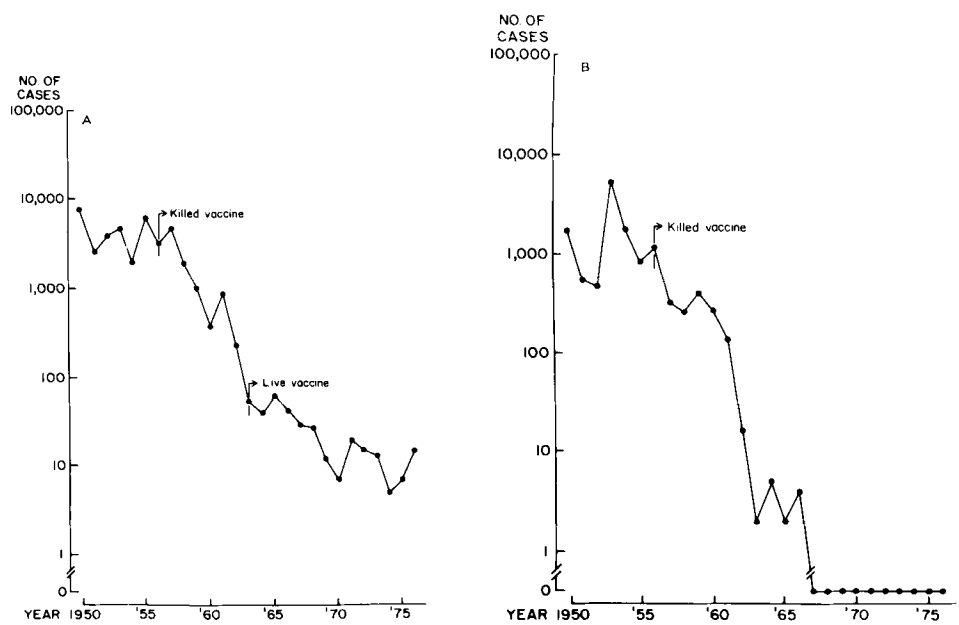


Fig. 4.6. Total reported cases of poliomyelitis – UK (1950–76), Sweden and Finland (1950–1976).

TABLE 4.19.
Poliomyelitis vaccination status by age group in the United States, 1965–1981 (after CDC Poliomyelitis Surveillance, 1982)

Year	Percentage of children who received 3 doses of OPV		
	Age group		
	1–4	5–9	10–14
1965	73.9	89.9	92.1
1966	70.2	88.2	90.0
1967	70.9	88.3	89.7
1968	68.3	84.9	87.8
1969	67.7	83.6	85.7
1970	65.9	82.3	85.3
1971	67.3	81.2	83.9
1972	62.9	78.9	81.8
1973	60.4	71.4	69.3
1974	63.1	73.5	69.8
1975	64.8	76.7	71.5
1976	61.6	71.8	65.2
1977	60.1	69.9	62.8
1978	61.4	74.2	67.7
1979	59.1 (76.0)	71.8 (88.5)	67.2 (84.5)
1980	58.8 (77.7)	71.5 (89.8)	68.7 (87.6)
1981	60.0 (78.3)	73.1 (89.5)	70.2 (87.5)

An example of the problem was the outbreak of polio in an unimmunized pocket of Amish fundamentalist protestants, where 13 cases of polio occurred (Chapter 17). In the post-eradication era in the USA there is a constant risk of introduction of wild type virus. It would seem unnecessary to change vaccine at present in the USA, but inactivated polio vaccines could be used with benefit in immunocompromised individuals (Davis et al., 1977), for adult contacts of children immunized with live vaccine, or for boosting immunity in adults travelling abroad.

An important, and as yet incompletely answered question, is whether inactivated polio vaccine could reduce the spread of virus in the alimentary tract or circulation of virus in the community. Early experience with inactivated polio vaccines in the USA in 1960 would suggest that inactivated polio vaccine *did* inhibit virus circulation (Marine et al., 1962). Thus, studies of polio outbreaks in single towns with high and low socio-economic groups showed clustering in the latter groups and few cases in the former groups. Also polio virus isolations from sewage were infrequent in the upper class areas but were frequent in the lower class areas. In an outbreak in 1960 on Rhode Island polio clustered in two lower class areas in Pawtucket (a high attack rate of 12.8%), whereas other areas remained free from polio. Sewage from

TABLE 4.20.
Doses (millions) of poliomyelitis vaccines distributed by year, United States, 1962–1981 (CDC Poliomyelitis Surveillance, 1982)

Poliomyelitis vaccine	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981
Inactivated (IPV)	15.3	19.0	8.8	7.5	5.5	4.0	2.7	—	—	—	—	—	—	—	—	—	—	0.04	0.03	0.02
Live, oral (OPV)																				
Monovalent (MOPV)																				
Type 1	33.1	38.7	24.9	4.7	1.4	1.3	0.5	0.4	0.3	0.2										
Type 2	37.0	34.2	29.8	3.4	1.3	0.9	0.5	0.4	0.2	0.1										
Type 3	13.7	54.2	28.4	3.7	1.4	1.0	0.6	0.4	0.3	0.2										
Trivalent (OPV)	—	4.2	24.0	17.4	24.0	18.0	23.9	22.5	25.8	25.5	24.7	24.9	25.2	24.8	19.4	23.2	24.6	24.5	23.7	22.8
Total	99.1	150.3	115.9	36.7	33.6	25.2	28.2	23.7	26.6	26.0	24.7	24.9	25.2	24.8	19.4	23.2	24.6	24.5	23.7	22.8

the lower class area contained type I polio. The most likely interpretation is that the better vaccinated affluent neighbourhoods were not subject to the silent ecological spread of virus at a time when there was an intense epidemic in a low class area. More recently there has been some evidence from the Netherlands polio outbreak that even without 100% immunization a barrier is provided to the transmission of polio by inactivated vaccine. Although there was some evidence of infection in unimmunized classmates of cases, widespread transmission of virus did not occur.

As mentioned above, it seems quite possible that inactivated polio vaccine has an inhibitory effect on pharyngeal excretion of polio virus. Gelfand et al. (1959) followed families who had received inactivated polio vaccine. When wild polio was introduced into the household previous immunization of children had no influence on virus spread and faecal excretion of virus was the same in vaccinated and unvaccinated persons. In contrast, spread of polio *was* limited in upper economic households and a possible explanation is that inactivated vaccine reduced excretion of *pharyngeal* virus, whilst the level of hygiene in these homes was high enough to limit spread of virus by the faecal oral routes.

4.10. Future control of polio in developed and underdeveloped countries

Paralytic polio still occurs in all countries of the world but the incidence rates vary from a minute 2.6 per 100 million persons per year in the USA to over 200 cases of persisting paralysis per million persons per year in certain African countries. Recent 'Lameness surveys', a technique pioneered by WHO in the Expanded Programme on Immunization (Chapter 2) have changed previous ideas about poliomyelitis in tropical areas. It is now clear that residual paralytic polio in Burma, Egypt, Thailand, Indonesia, Ivory Coast and Brazil, India, Yemen and Niger is higher now than it was during the prevaccine era in the USA and Sweden. The old dogma that in the tropics the infection rate with polio is high but the paralysis rate is low, and that an increased incidence of the paralytic disease was related to emerging epidemics and an improvement in the standard of living reflected in diminishing infant mortality rates, may no longer hold (Sabin, 1982). Between 50% and 75% of lameness in certain developing countries has been found to be caused by poliomyelitis virus. In the absence of immunization programmes some 4 out of 1000 school age children will be disabled by polio. This estimate does not take into account those who have been afflicted and died, and those who have recovered (Tarantola, 1982). Obviously, on a world scale there is a serious imbalance as regards incidence of polio which requires correction as a high priority. The complete list of reasons for the calamatous differences between countries are not absolutely clear and disagreement exists in the current scientific literature. But, an important reason is the absence of a modern medical and social infrastructure in certain developing countries.

Even although the scientific debate may and will continue about the merits and drawbacks in underdeveloped countries of inactivated versus attenuated polio vaccines it is, nevertheless, clear from the studies of Sabin and others that a vigorous and concerted *rapid* immunization campaign (Sabin, 1982) using oral attenuated polio vaccine will stop polio in tropical and semi-tropical underdeveloped countries. As early as the 1960s Cuba showed the way with annual mass vaccination campaigns on two Sundays of the year for *all* children under five years of age regardless of the number of doses they might have had previously. Only such annual mass vaccinations make it possible to reach the extremely high proportions of children necessary to break the chain of infection. When vaccination is more spread out in time, wild polio and other enteroviruses remain dominant in the community. As another example, Brazil in 1980 and 1981, organized an army of volunteers to vaccinate almost all children (18 million) under 5 years of age twice a year on a single day (June and August in 1980 and August and October in 1981). A precipitous drop in reported cases followed (Sabin, 1982). An alternative option is to use more doses of vaccine. John (1976) found that multiple doses of conventional oral vaccine containing 10^5 and $10^{5.5}$ $TCID_{50}$ for types 1, 2 and 3 (five or more doses) were required to give more than 90% seroconversion, but such an approach requires good organization and mass campaigns.

Fang-chou et al. (1982) have described how polio has been controlled recently in sub-tropical areas in China using oral attenuated vaccine. In Guangxi autonomous region, with a population of 20 million persons, apparently healthy children were found to be heavily infected with non-polio enteroviruses. Even as late as 1971 several polio epidemics were detected since the polio vaccination rates were still low, especially in rural areas. An intensive campaign was initiated in 1973 using 'barefoot doctors' and special immunization teams in rural areas and the incidence of polio has since declined from 0.41 (1973) to 0.05 (1977) and 0.02 (1979) per 100 000 persons. Fang-chou et al. (1982) conclude "the key to elimination of paralytic poliomyelitis from a community is to have a powerful leadership and authority in public health that can provide effective administrational guidance, and measures to train and organize health personnel."

The alternative approach is to use killed polio vaccine. In an early study Beale (1969) showed that vaccine given at 3, 4 and 5 months with a booster at one year gave a high seroconversion rate. The polio was mixed with diphtheria, tetanus and pertussis vaccine, thus protecting against 4 diseases in one immunization course. Immunization in Mali or Indonesia with 40 D units of currently used polio vaccine can result in essentially 100% seroconversion and it is probable that a single such dose at 6 months will provide immunological memory; subsequent contact with natural virus would boost immunity (reviewed by Beale, 1982 and see also Table 4.21). Krishnan et al. (1983) evaluated the immunogenic efficacy of inactivated polio vaccine in India in infants given a quadruple vaccine incorporating polio with diptheria-pertussis-tetanus. A total of 150 babies aged 6–45 weeks were immunized with

three doses of vaccine containing 20, 2 and 3.5 D antigen units of polio types 1, 2 and 3 respectively at intervals of 4–8 weeks and the overall sero-conversion rates to poliovirus types 1, 2 and 3 were 99%, 89% and 91% respectively. An otherwise adverse effect of maternal antibody on the immune response is reduced by increasing the intervals between doses from 4 to 8 weeks.

4.11. Characterization of polio viruses isolated in the USA between 1969–1981: vaccine associated cases

A total of 203 cases of paralytic polio (most with pure spinal paralysis and 39 cases of spinal involvement although in 93% of cases one or both lower limbs were paralysed) were reported in the USA during this period and this represents a dramatic decrease from the 10 000 cases or so reported each year in the 1950s. Therefore the attack rate of paralytic polio has declined from a high of 13.7 cases per 100 000 population (1952) to 0.003 cases per 100 000 population in 1981. Of these 203 cases, 40 were associated with epidemics, 127 were endemic, 22 were imported and 14 were in immune deficient persons. Of particular interest is the group of 86 vaccine-associated cases which were examined: 26 had occurred in recipients of oral polio vaccine, while 45 occurred in household contacts of vaccinees and 15 in non-household contacts. Perhaps, as to be expected, there was no seasonal distribution of cases and most (99 cases) were under 5 years of age. Compared to the 1960s more adult cases were being observed. Analysis of polio virus isolates by oligonucleotide mapping showed that most isolates from vaccine cases were vaccine-like in oligonucleotide spot pattern (see Chapter 17 for more details).

Of the 167 polio cases analyzed carefully, 77 involved polio type 1, 45 type 2 and 45 type 3. Type I was responsible for all 38 epidemic associated cases but was responsible for only 4 vaccine associated cases, which were more often associated with

TABLE 4.21.
Antibody response to inactivated polio vaccine (IPV) in India (after Jacob John, 1982)

Maternal antibody	Interval between doses	Number of infants showing seroconversion to poliovirus		
		Type 1	Type 2	Type 3
Absent	4 weeks	44/44 (100)[a]	47/52 (90)	57/63 (90)
Absent	8 weeks	29/29 (100)	26/26 (100)	41/43 (95)
Present	4 weeks	25/25 (100)	7/17 (41)	4/6 (67)
Present	8 weeks	21/21 (100)	23/24 (96)	6/7 (86)
Both groups	4 weeks	69/69 (100)	54/69 (78)	61/69 (88)
	8 weeks	50/50 (100)	49/50 (98)	47/50 (94)
Both groups	4 and 8 wks	119/119 (100)	103/119 (86)	108/119 (91)

[a] No. responding/no. tested, with seroconversion rate as percent within brackets.

type 3 or type 2 viruses. A more recent analysis of the epidemiological characteristics of paralytic polio cases in the USA is illustrated in Table 4.22.

We have left to last in this chapter any indication of potential new developments in polio vaccines, either as regards live or inactivated vaccine. It has taken many years to establish the safety of existing polio vaccines and therefore it is unlikely that any new technical developments will be introduced quickly, unless (and this is unlikely) they represent a striking scientific breakthrough in, for example, immunogenicity (i.e. producing cross-protection against a variety of enteroviruses) or a marked reduction in cost (it should be remembered that live polio vaccines are very cheap). A more extensive discussion, with new data, is presented in Chapter 2 and we shall content ourselves here with a brief review of typical recent data with polio immunogenic peptides since this is an area of intense investigation at present.

TABLE 4.22.
Paralytic poliomyelitis cases by epidemiological characteristics, United States, 1980–1981 (CDC Poliomyelitis Surveillance, 1982)

Year	Epidemiologic classification	State	Age	Sex	Month of onset	Prior polio vaccination	Poliovirus type implicated[c]
1980	Endemic, not VA[a] (IIA)	CA	11 months	M	June	None	2
	Recipient, VA (IIB)	NJ	3 months	M	February	None[b]	3
	Contact, VA (IIC1)	LA	6 months	F	January	1 TOPV	1, 2, 3
	Contact, VA (IIC1)	WA	25	F	May	1 TOPV, 3 IPV	?
	Contact, VA (IIC2)	MI	28	M	October	None	2
	Imported (III)	OR	67	F	February	None	3
	Imported (III)	CA	4 months	F	January	None	1
	Immune deficient (IV)	WY	2 months	M	March	1 TOPV	2
	Immune deficient (IV)	NJ	1	F	December	4 TOPV	2
1981	Recipient, VA (IIB)	CA	3 months	F	October	None[b]	?
	Contact, VA (IIC1)	NB	41	M	August	None	2
	Contact, VA (IIC1)	MN	29	M	August	None	2
	Contact, VA (IIC1)	WV	8 months	F	April	None	2
	Contact, VA (IIC2)	MD	14	F	January	1 TOPV	2
	Contact, VA (IIC2)	WA	27	M	June	None	2
	Immune deficient (IV)	MO	17	M	July	3 TOPV, 4 IPV	1

[a] VA = vaccine associated
[b] Excludes OPV administered within 30 days prior to onset of illness
[c] By virus isolation and/or fourfold rise in antibody titer

4.12. Immunogenic synthetic peptides corresponding to antigenic areas of polio VP1 protein

Recently Emini et al. (1983) have synthesized five peptides containing amino acid

sequences from type 1 poliovirus structural protein VP1. Each of the peptides was able to prime the immune system of rabbits, which on subsequent challenge with a single inoculation of whole polio virus produced a long lasting virus neutralizing IgG antibody response. The essential data is summarized in Table 4.23 and 4.24. The sequences were chosen after a hydrophilicity analysis of protein sequence and a sequence variation analysis obtained by comparing the amino acid sequence of VP1 of the three polio serotypes. It was assumed that hydrophilic regions of VP1 were more likely to be exposed at the virus surface. Four of the five peptides were recognized by neutralizing monoclonal antibodies raised to the intact type I poliovirus. A single peptide (No. 3) elicited virus neutralizing antibodies when coupled to a carrier protein. Finally, the peptides were able to prime animals who subsequently reacted rapidly when immunized with whole polio virus. The priming was specific in the sense that priming only occurred for the homologous type I virus. It is just possible that priming alone might give sufficient protection against poliovirus infection. A major study of poliovirus antigenic sites has been reported by Minor et al. (1983).

TABLE 4.23.
Priming of anti-poliovirus immune response by peptides 2, 3, 4 and 5 (after Emini et al., 1983)

Peptide	Rabbit	Inoculated poliovirus type	Weeks after virus inoculation	Plaque titre reduction (log_{10} PFU ml^{-1})			Antiserum		
				Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
2	A	1	1	>8.5	3.6	3.3	+	–	–
	B	1, 2, 3	1	5.5	3.2	1.4	+	+	–
			5	6.3	2.7	<1.0	+	+	–
3	B	1, 2, 3	1	7.8	1.9	<1.0	+	–	–
			5	8.0	<1.0	<1.0	+	–	–
4	A	1	1	6.7	<1.0	<1.0	+	–	–
			5	6.2	<1.0	<1.0	+	–	–
	B	1, 2, 3	1	5.8	1.3	<1.0	+	–	–
			5	6.4	1.1	<1.0	+	–	–
5	A	1	1	>8.5	1.7	<1.0	+	–	–
			5	>8.5	<1.0	<1.0	+	–	–
	B	1, 2, 3	1	6.7	1.8	<1.0	+	–	–
			5	6.5	<1.0	<1.0	+	–	–

All rabbits were inoculated with 1.0 mg per inoculation of carrier protein-linked peptide (diluted 1:1 with CFA) by the i.d., s.c. and i.m. routes at 0, 4 and 5 weeks, respectively. Approximately 5.0 log_{10} PFU of the appropriate poliovirus type(s) (1:1 with CFA) was inoculated, i.m., 10 days following the final peptide inoculation.

TABLE 4.24.
Sequences of immunologic peptides of Polio type I (after Emini et al., 1983)

Peptide 1	H_2N-TYR-GLY-GLY-ARG-SER-ARG-SER-GLU-SER-GLY-COOH
Peptide 4	H_2N-CYS-GLY-GLY-ARG-SER-ARG-SER-GLU-SER-SER-ILE-GLU-SER-PHE-COOH
Peptide 2	H_2N-TYR-GLY-GLY-SER-THR-THR-ASN-LYS-ASP-LYS-GLY-COOH
Peptide 3	H_2N-TYR-GLY-GLY-ASP-ASN-PRO-ALA-SER-THR-THR-ASN-LYS-ASP-LYS-COOH
Peptide 5	H_2N-TYR-GLY-GLY-ASP-ASN-THR-VAL-ARG-GLU-THR-GLY-COOH

4.13. Specific inhibitors of polio

Since the vaccine has been so successful in preventing the disease of polio, rather little new work has been initiated in recent years to study specific inhibitors of polio virus replication, although important studies were carried out earlier with guanidine and hydroxybenzimidazole compounds (see below). In contrast, inhibitors against rhinoviruses have been sought extensively, although without success until recently (reviewed by Tyrrell et al., 1983, Reed, 1980). Little work has been carried out with rhinovirus vaccines, mainly because of the awesome problems of multiplicity of serotypes.

4.13.1. Benzimidazoles

Early work by Hollinshead and Smith (1958) described the inhibitory effects of 2 (α - hydroxybenzyl)benzimidazole (HBB) (Fig. 4.7) on polio type 2 replication in mice: death of the animals was prevented. Early tissue culture experiments established that the compound exerted an antiviral effect in vitro, was not simply virucidal and had no toxic effect on uninfected tissue culture cells (Tamm and Nemes, 1959). This compound was one of the first antiviral molecules to be discovered. However, HBB showed some unwanted characteristics which were to become familiar to specialists working with later antivirals – drug resistant virus mutants could be isolated with relative ease, and the compound had a very narrow antiviral spectrum more or less limited to enteroviruses (Table 4.25).

HBB acts during the first 2–5 hours after polio virus infection of cells, although the precise point of action is not known. Virus adsorption, penetration and uncoating, however, are not affected and virus RNA and protein synthesis are not inhibited. Virus macromolecular synthesis is thought to be the target site for inhibition, and cessation of virus RNA synthesis eventually stops viral protein synthesis. An interpretation of the rather conflicting data is that HBB inhibits the synthesis of a virus directed RNA polymerase, which in turn halts replication of virus RNA leading to inhibition of synthesis of viral capsid polypeptides. Alternatively, other experiments suggest that HBB inhibits viral RNA synthesis, thereby inhibiting replication of infectious virus.

HBB significantly delays the development of enterovirus CPE in MK cells, although the cells ultimately succumb to virus induced CPE, presumably because of

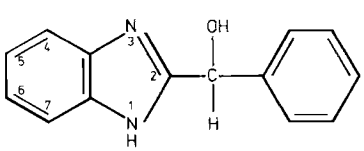


Fig. 4.7. Molecular structure of 2-(α-hydroxy-benzyl)benzimidazole.

TABLE 4.25.
Antiviral spectrum of HBB

Viruses inhibited in vitro	Viruses not inhibited in vitro
Polio 1–3	Echovirus 22, 23
Coxsackie B 1–6	
Coxsackie A 9	Other Coxsackie viruses
Echovirus 9, 11–21, 24–27	Rhinoviruses
Porcine and bovine enteroviruses	FMDV
Arenavirus (e.g. LCM)	

virus induced cell protein and RNA shut off. Production of infectious virus is very significantly inhibited by HBB in vitro with >8.0 $\log_{10}$ LD_{50}/ml inhibition of certain enteroviruses. However, as with many antivirals much less antiviral effect is noted in vivo.

A large number of derivatives of HBB have been investigated for virus inhibitory effects (Tamm et al., 1969) and it has been found that the hydroxybenzyl group at position 2 of the benzimidazole ring is of critical importance. Also, replacement of the benzimidazole nucleus of HBB by an imidazole ring results in complete loss of antiviral activity. Certain substituted derivatives such as 1-propyl, 1-β-methyl-propyl and 1-phenyl-HBB are more active and selective in vitro than HBB itself. Thus, the selective virus inhibitory activity of HBB is dependent upon the overall geometry of the molecule and most structural modifications lead to a loss of viral inhibitory activity and selectivity.

4.13.2. Guanidine

As early as 1961 Rightsel et al. published details of the inhibition of polio virus by guanidine and soon afterwards the by now familiar drug resistant or even drug dependent mutants were detected (Loddo et al., 1963).

Many enteroviruses are inhibited in vitro by guanidine (see Table 4.26 and Fig. 4.8) but nevertheless the antiviral spectrum of the compound is severely restricted to RNA viruses.

Guanidine has no direct virucidal effect and enterovirus attachment and penetration are not inhibited. (Crowther and Melnick, 1961). Inhibiting effects begin midway through the virus latent phase and continue through the reproduction cycle. The principal site of guanidine action appears to be the synthesis of viral RNA and more than one step may be affected (reviewed by Tershak et al., 1982, Caliguiri and Tamm, 1968). Polio RNA polymerase is not inhibited in vitro, presumably because initiation of viral RNA synthesis does not occur in cell free extracts but only results in completion of RNA chains in the process of synthesis during extraction of the

TABLE 4.26.
Antiviral spectrum of guanidine (after Tershak et al., 1982)

Viruses inhibited in vitro	Viruses not inhibited in vitro
Polio	Orthomyxoviruses
Coxsackieviruses	Coxsackie B6
Echoviruses	Echo 6, 7, 8, 12
Rhinoviruses	Paramyxoviruses
FMDV	Reoviruses
Togavirus (Sindbis and SVF)	Togaviruses
Tobacco necrosis	Herpes
Tobacco mosaic	Vaccinia
	Adenovirus

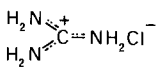


Fig. 4.8. Molecular structure of guanidine.

crude enzyme (although more recently pure preparations of picornavirus polymerase that utilize exogenous viral RNA have been obtained and could now be tested). Inititation of synthesis of viral RNA seems to be depressed by guanidine, and release of completed RNA chains is restricted. Current data would point to the initiation step of viral RNA synthesis as the site of action of guanidine (Tershak et al., 1982). In addition, guanidine exerts mild conformational changes in viral proteins and specifity could be due to a specific amino acid sequence in picornaviruses compared to cell proteins or proteins of other viruses. It is possible that synthesis and encapsidation of viral RNA both require capsid subunits and Vpg_1 and any interaction with guanidine would cause multiple aberrations.

Guanidine, in combination with HBB has been used to successfully treat echovirus type 9 and coxsackie A9 infections in newborn mice, although neither compound alone was effective (Eggers, 1982). Theoretical levels of inhibitor were 200 μM HBB and 2mM guanidine.

4.13.3. ARILDONE

Several acyclic β-diketones were shown to inhibit the replication of equine picornaviruses (Diana et al., 1977). Arildone, 4-[(2-chloro-4-methoxy)phenoxy]hexyl-3,5-heptadione, emerged as a promising candidate antiviral, having broad spectrum in vitro antiviral activity against equine rhinovirus and interestingly HSV (Table 4.27, 28). The compound arildone is composed of a β-diketone separated from the substituted benzene ring by an alkyl chain of 6 carbons. It is stable to heat but virtually

insoluble in water, which is a drawback. Substituents of the benzene ring contributed lipophilicity and substitution and addition of more hydrophobic substituents decreased the antiviral activity of the compound. Shorter and longer alkyl chains decreased antiviral activity (reviewed by McSharry and Pancic, 1982).

The compound is thought to inhibit polio replication by preventing intra-cellular uncoating of the virion (McSharry et al., 1979) perhaps by stabilizing capsid proteins so that they cannot undergo conformational changes required for uncoating and release of virion RNA. The isolation of drug resistant mutants of polio also suggests a direct antiviral effect on the virus. Certainly arildone appears to stabilize polio in the presence of heat and alkali (Table 4.29).

Arildone was assessed for its ability to prevent paralysis and death in mice infected intracerebrally with a lethal dose of human poliovirus type 2 (McKinlay et al., 1982). Intraperitoneal administration of arildone suspended in gum tragacanth prevented paralysis and death in a dose-dependent manner (minimal inhibitory dose = 32 mg/kg, twice daily) and protected animals from virus challenges in excess of twenty 50% lethal doses (Table 4.30). Oral medication with arildone solubilized in corn oil was similarly effective in preventing poliovirus-induced paralysis and death. Arildone was therapeutically effective even when intraperitoneal medication

TABLE 4.27.
Effect of arildone on virus infectivity

Virus	MIC (μM)[a]	
	CPE	Plaque reduction
Poliovirus 2	0.8	< 0.27
Murine cytomegalovirus		10.8
Herpes simplex virus 1		
Sheely strain	16.2	< 1.35
Robinson strain		< 5.4
McKrae strain		< 5.4
Herpes simplex virus 2		
Curtis strain	16.2	< 5.4
75–1000 strain		< 5.4
Varicella zoster virus		< 2.7
Corona virus A 59		>27
Vesicular stomatitis virus		
Indiana serotype	1.9	>27
Influenza A_0/WSN/(H_0N_1) virus		>27
Vaccinia virus	8.1	>13.5
Adeno virus		>27
Sindbis virus		>27

[a] Minimal inhibitory concentration expressed as μM arildone required to reduce CPE or plaque formation by 50%

TABLE 4.28.
Effect of arildone on virus replication

Virus	MIC (μM)
Murine cytomegalovirus	<8.1
Semliki forest virus	<8.1
Vesicular stomatitis virus	2.7
Poliovirus 2	<2.7
Herpes simplex virus 1	2.7
Herpes simplex virus 2	<2.7
Coxsackievirus A 9	<5.4

was delayed for 48 h postinfection. Analysis of the virus titres in the central nervous system tissues of animals infected with 200 50% lethal doses demonstrated that arildone reduced titres in the brain and spine by approximately 3 and 4 $\log_{10}$ PFU per g of tissue, respectively, implying that direct inhibition of virus replication was responsible for host survival. However, this animal model depends on direct inoculation of polio into the CNS and therefore the relevance of the study to human polio must await further study. It should be noted that the acute oral LD_{50} of arildone in mice is in excess of 8 g/kg and monkeys and rats medicated daily for 5 weeks with 1000 mg/kg showed no toxic reactions.

In summary, these compounds excite little interest with clinicians because of the success of polio vaccines and relative lack of interest in other enteroviruses. Their main use at present appears to be in laboratory studies of enterovirus replication, including genetic markers.

TABLE 4.29.
Effect of arildone on thermal inactivation of poliovirus

Time (min)[a]	Poliovirus titre (PFU/ml)		
	Eagle's MEM	Eagle's MEM +0.01% DMSO	Eagle's MEM +2.7 μM arildone +0.01% DMSO
0	3.4×10^9	4.3×10^9	4.0×10^9
2.5	2.5×10^9	2.3×10^9	4.5×10^9
5	2.2×10^8	2.4×10^8	2.3×10^9
10	2.3×10^7	6.1×10^7	2.5×10^9
20	1.3×10^6	3.4×10^6	2.2×10^9

[a] Time of incubation of poliovirus 2 at 47°C.

TABLE 4.30.
In vivo activity of arildone (after McKinlay et al., 1982)

Expt. no. (size of inoculum)	*Arildone treatment regimen*			No. of expts × no. of mice challenged per expt	% Survivors[a]		
	Route of administration/ vehicle	Treatment schedule	Individual dose (mg/kg)		Day after challenge		
					5	10	20
1 (2 LD_{50})	i.p./GT[b]	Doses delivered 4 h before challenge, 2 h postchallenge, and twice daily for 13 days thereafter	0	5×10	98± 2.0	46± 5.1	26± 5.1
			32	5×10	100± 0	88± 3.8	42± 2.5
			63	5×10	95± 2.0	90± 3.2	78± 3.8
			125	5×10	100± 0	100± 0	94± 2.4
			250	5×10	100± 0	100± 0	100± 0
2 (20 LD_{50})	i.p./GT	Doses delivered 4 h before challenge, 2 h postchallenge, and twice daily for 13 days thereafter	0	3×10	97± 3.3	23±12.0	3± 3.3
			250	3×10	100± 0	100± 0	100± 0
3 (2 LD_{50})	i.p./GT	Doses delivered 4 h before challenge, 2 h postchallenge, and twice daily for 5 days thereafter	0	3×10	100± 0	43± 3.3	20± 1.0
			250	3×10	100± 0	100± 0	93± 6.6
4 (2 LD_{50})	i.p./GT	Doses delivered 48 h postchallenge and twice daily for 14 days thereafter	0	3×10	97± 3.3	50± 5.8	30±10.0
			250	3×10	100± 0	97± 3.3	87± 3.3
5 (2 LD_{50})	Oral/GT	Doses delivered 4 h before challenge, 2 h postchallenge, and twice daily for 13 days thereafter	0	4×10	92± 4.8	52± 4.8	25± 9.5
			250	4×10	100± 0	87± 6.3	59± 6.3
6 (2 LD_{50})	Oral/corn oil	Doses delivered 4 h before challenge, 2 h postchallenge, and twice daily for 13 days thereafter	0	3×10	90±10.0	40± 5.8	30±10.0
			250	3×10	100± 0	100± 0	93± 3.3

[a] Geometric mean ± standard error of the mean.

[b] Arildone suspended in 1% gum tragacanth plus 2% Tween 80.

4.14. Rhinoviruses

Minor respiratory infection (common cold) is one of the commonest viral diseases in the UK and other temperate and even sub-tropical countries. In addition, they cause exacerbation of chronic conditions such as asthma, chronic bronchitis or cardiac failure (reviewed by Andrewes, 1962, Hope-Simpson, 1958). We have mentioned above that, at present, few cross-reacting antigenic determinants have been detected among this group of 150 viruses and hence development of effective vaccines would be difficult. Since the viruses are all closely related as regards physico-chemical properties and presumably biochemistry receptor sites etc., it would appear more logical to place an emphasis on development of antiviral drugs or interferon. Most pharmaceutical groups searching for new antivirals include typical rhinoviruses in their antiviral screening programme and most of the latter work is done in tissue culture systems because of the absence of an animal model of human rhinoviruses. Suitable compounds are tested in volunteers for pharmacological properties and any nontoxic compounds are then tested at, for example, the Common Cold Research Unit at Harvard Hospital, Salisbury, or in comparable units in Leningrad and Houston. Volunteers in the UK unit are aged from 18–50 years and on arrival at the unit are given a medical examination and a preliminary serum sample is taken and subsequently used to estimate the level of any anti-virus antibody (Beare and Reed, 1977). During the experimental period volunteers are isolated in pairs in their own self-contained apartments and no physical contact is allowed with other persons (Fig. 4.9). Subjective symptoms and objective criteria are used to grade reactions induced by experimental viruses ranging from very mild (trivial reactions), mild (local symptoms), moderate (both local and constitutional symptoms) and severe (febrile and other unpleasant reactions necessitating bed rest). The number of paper handkerchiefs used each day is counted and may often increase from a baseline of 3 per day to 35 per day during the symptoms of a cold. After 3 days quarantine, antiviral medication is started and the volunteers are then infected with rhinovirus intranasally (Fig. 4.10). Nasal washings are taken for several days thereafter for virus reisolation studies. Drugs may be administered locally by spray or orally. In the latter case there must be evidence that enough compound reaches the secretions of the upper respiratory tract. On the other hand, mucociliary clearance mechanisms are efficient and may rapidly reduce the local concentration of a nasally administered drug.

Reed (1980) has summarized earlier trials in volunteers with both specific antivirals and interferon (Tables 4.31 and 4.32) (see also Chapter 3). In general, guanidine-type molecules have given disappointing results in volunteers but more recently a benzimidazole has been shown to have mild antiviral effects (enviroxime or 2-amino-1-(isopropyl sulphonyl)-6-benzimidazole phenyl ketone oxime (Table 4.33, Fig. 4.11). The compound completely suppressed plaque formation in vitro by rhinoviruses types 3, 9, 14 and 31 (Table 4.33). Moreover, enviroxime inhibited a fur-

Fig. 4.9. Accommodation at the Salisbury Common Cold Unit (after Beare and Reed, 1977).

ther 12 rhinovirus serotypes by at least 3.0 $\log_{10}$ units at 0.5 μg/ml. Enviroxime was equally effective in human embryo fibroblasts and HeLa cells and also in human embryo nasal trachea organ culture at concentrations of 0.2 μg/ml. In clinical trials (Table 4.34) both intranasal and oral administration of the compound was used, and the severity of colds was reduced (Fig. 4.12) although at the borderline of statistical significance. We should remember also that volunteers were given a minimum virus challenge in the presence of a maximum (near toxic) level of drug. Even under these conditions the antiviral effect was not striking, but it nevertheless represents the first synthesized antiviral coumpound to have detectable anti-rhinovirus effects in man.

However, trials of enviroxime have also been carried out by Hayden and Gwaltney (1982), who were unable to detect any antiviral effect (Table 4.35). Intranasal administration of enviroxime by aerosol spray was associated with drug levels in nasal secretions that 1 h later averaged 750-fold higher than those inhibitory for rhinoviruses in vitro (0.2 μg/ml). However, administration of intranasal enviroxime (one spray per nostril, five times per day) to susceptible volunteers, beginning 1 day before and continuing for 4 days after virus exposure, did not significantly reduce infection or illness due to experimentally induced rhinovirus type 39 infection. The combined results of two separate trials yielded an infection rate of 100% for 21 pla-

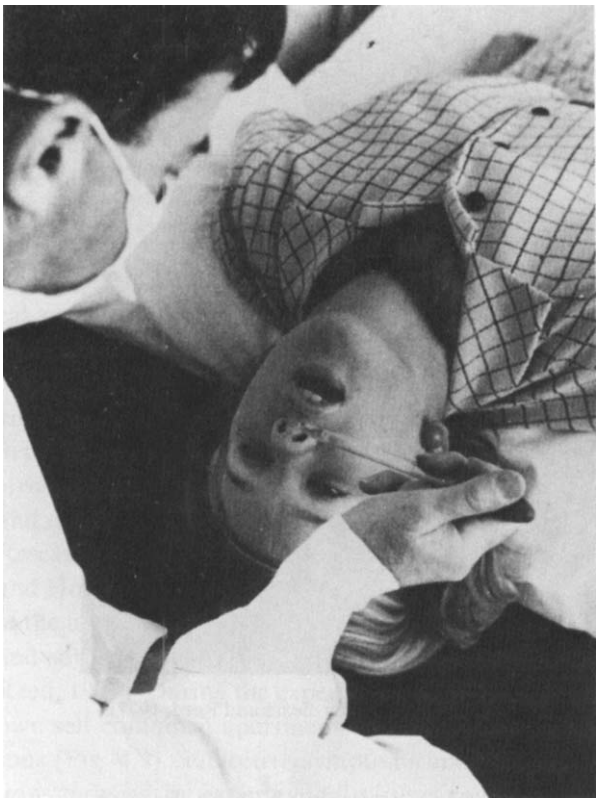


Fig. 4.10. Method of administration of virus inoculum in the form of nasal drops (after Beare and Reed, 1977).

cebo-treated and 89% for 19 enviroxime-treated subjects. Approximately one-half of the volunteers in each group had seroconversion to the challenge virus. Overall, 52% of the placebo-treated and 53% of the enviroxime-treated subjects developed colds. No significant differences in symptom scores, nasal mucus weights, or numbers of nasal tissues used were observed between the two groups. Two-thirds of the enviroxime-treated volunteers noted intranasal irritation immediately after sprays, as compared with only one-third of the placebo-treated subjects.

Another recently investigated compound, dichloroflavan, (Fig. 4.13) was shown to have no detectable anti-rhinovirus effects in volunteers (Fig. 4.14, Table 4.36) although the compound has anti-rhinovirus activity in vitro (Table 4.37), but this could be attributed to pharmacology problems with perhaps only low concentrations reaching the nasal mucosa.

4.15. Some recently discovered inhibitors of picornaviruses

A nitrobenzene derivative, MDL-860, was found to inhibit plaque formation, cyto-

TABLE 4.31.
Broad spectrum antiviral agents tested in humans experimentally infected with rhinoviruses

Preparation	Dosage	Virus	Effect[a] of medication on:		
			Symptoms	Virus shedding	Antibody response
Human leukocyte interferon	1.4×10^7 U spread over 4 days 9–12 doses/day[b]	Rhinovirus type 4	+	+	±
Human fibroblast interferon	Between 6×10^5 and 4×10^7 U, spread over 4 days 3 doses/day[b]	Rhinovirus type 4	–	–	–
Polyriboinosinic polyribocytidylic acid	7.0 mg/day for 1 day, 3.5 mg/day for 6 days, given as 4 or 7 doses/day[b]	Rhinovirus type 13	+	+	–
Polynucleotide from fungal virus (BRL 5907)	5.0 mg/day for 3 days 5 doses/day[b]	Rhinovirus type 4	±	±	–
Synthetic low M.Wt. inducers CP 20 961 and CP 20 888	In most studies, 5 doses of 50 mg, 3 before virus challenge and 2 after it[b]	Rhinovirus types 13, 14 and 21	(Significant effects on symptoms and virus shedding in some but not all studies.)		
Isoprinosine	4–6 g/day[c]	9, 31, 44, 32 and 21	(Significant effects, mainly on symptoms, in some but not all studies.)		

[a] + indicates a definitely favourable effect, usually statistically significant; ± indicates minor beneficial effects, not statistically significant
[b] Intranasal medication
[c] Oral medication

pathic effect, or both in 11 of 12 picornaviruses (Table 4.38) at concentrations which did not affect cell growth (Torney et al., 1982). The compound did not directly inactivate the virus and inhibited actinomycin D-resistant ^{3}H-uridine uptake in cells infected with coxsackie virus A_{21} or rhinovirus 1-A, whereas incorporation into uninfected cells was not inhibited. With three picornaviruses (echovirus type 12, poliovirus type 2, and rhinovirus type 1-A) made photosensitive with neutral red, MDL-860 did not appear to cause a significant reduction in their loss of photosensitivity (uncoating) during the first 3 h of infection. The authors concluded that MDL-860 appeared to inhibit some early event in virus replication, after uncoating, which is required for synthesis of viral RNA.

In multiple growth cycle experiments, 1 μg of MDL-860 per ml caused a reduction in virus yield of at least 1.0 $\log_{10}$ 50% tissue culture infectious doses per 0.2

TABLE 4.32.
Some synthetic antiviral compounds tested against rhinovirus infection in vivo. Experiments were done in humans except where indicated (after Reed, 1980)

Preparation	Route	Effect[a] of medication on:			
		Rhinovirus	Symptoms	Virus shedding	Antibody response
SKF 21687 (triazinoindole)	Intranasal	44	–	–	–
SKF 40491[b] (triazinoindole)	Intranasal	2	ND	+	+
As above[b]	Oral	1A	ND	–	–
As above	Intranasal	3	–	±	–
19326 RP (imidazo-thiazole)	Intranasal	9	±	+	–
Abbott 36683[c] (bis-benzimidazole)	Oral	30, 49 or 44	Side effect at high dose	+ at high dose only	+ at high dose
ICI 73602 (ureidoguanidine)	Intranasal	3	–	–	–
CL 88, 277 (substituted guanidine) (-phenyl-3-(4-phenyl-2-thiazolyl)guanidine	Intranasal	44	–	±	±

[a] +indicates a definitely favourable effect, usually statistically significant, ± indicates minor beneficial effects, not statistically significant
[b] In gibbons
[c] In chimpanzees

TABLE 4.33.
Effect of incorporation of antiviral compounds in the overlay medium of HeLa cells on the titre of four preparations of rhinovirus (after Reed, 1980)

Compound	Concentration (μg/ml)	Reduction in titre ($\log_{10}$ PFU) using rhinovirus serotypes			
		3	9	14	31
SKF 40491	2.0	2.0	1.4	2.0	3.0
RP 19326	2.0	0.7	2.0	1.0	1.0
CP-196J	0.5	>4.7	>3.3	>4.0	>4.4
Enviroxime	0.6	>5.3	>6.6	>4.5	>5.9

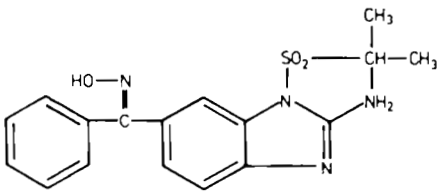


Fig. 4.11. Molecular structure of enviroxime.

TABLE 4.34.
Prophylactic effect of enviroxime versus rhinovirus type 9 (after Phillpotts et al., 1981)

Treatment	Clinical colds				Virus shedding
	Total	Severe or moderate	Mild	None or insignificant	
Enviroxime	18	0	4	14	10
Placebo	18	3	3	12	15

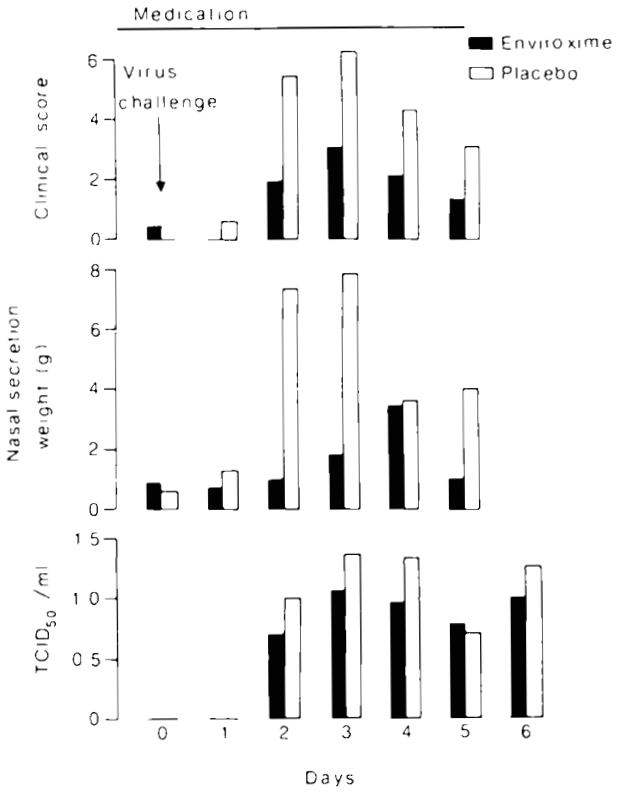
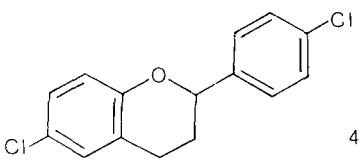


Fig. 4.12. Clinical antiviral effects of enviroxime. (after Tyrell et al., 1983)

TABLE 4.35.
Frequency and severity of illness in enviroxime- and placebo-treated volunteers with experimental rhinovirus type 39 infection (after Hayden and Gwattney, 1982)

Trial	Treatment	No. of colds/ no. of volunteers (%)	Total symptom score	Wt of nasal secretions (g)	No. of tissues used
First	Placebo	3/7 (43)	6.0±2.4	11.9±6.3	32±12
	Enviroxime	4/7 (57)	6.1±2.1	12.9±4.2	39±11
Second	Placebo	8/14 (57)	8.1±1.4	21.2±5.4	42± 9
	Enviroxime	6/12 (50)	10.8±3.1	17.1±4.5	37± 9
Combined	Placebo	11/21 (52)	7.4±1.3	18.1±4.2	39± 7
	Enviroxime	10/19 (53)	9.3±2.5	15.5±3.2	38± 7



4,6, Dichloroflavan

Fig. 4.13. Molecular structure of 4,6 dichloroflavan.

TABLE 4.36.
Results of Rhinovirus type 9 challenge – dichloroflavan trial (after Tyrrell et al., 1983)

Treatment	Clinical colds				Virus shedding
	Total	Severe or moderate	Mild	None or insignificant	
Dichloroflavan	26	5	4	17	22
Placebo	23	5	5	13	17

ml for 8 of 10 enteroviruses and 72 of 90 rhinovirus serotypes (Table 4.39). This antiviral activity was dependent on both compound concentration and virus inoculum size. At concentrations that had no toxic effects on cell cultures, MDL-860 did not inhibit cytopathic effect or hemadsorption activity due to coronavirus 229-E, vesicular stomatitis virus, herpes simplex virus type 1, adenovirus, influenza virus A, or parainfluenza virus 1. Compound concentrations up to 25 μg/ml did not cause cytopathic effect in short-term incubated cultures of rhesus monkey, WI-38, or HeLa cells and 10 μg/ml did not inhibit the replication of HeLa cells (Powers et al., 1982).

TABLE 4.37.
Sensitivity of rhinoviruses to dichloroflavan (after Bauer et al., 1981)

Serotype	IC_{50} (μM)	Serotype	IC_{50} (μM)
1A	0.013	13	0.66
1B	0.007	14	Inactive
2	0.04	15	0.17
3	10.10	16	0.02
4	Inactive	18	0.29
5	Inactive	19	0.81
8	8.00	21	1.70
9	0.011	29	0.008
12	0.15	30	52.00
		31	0.013

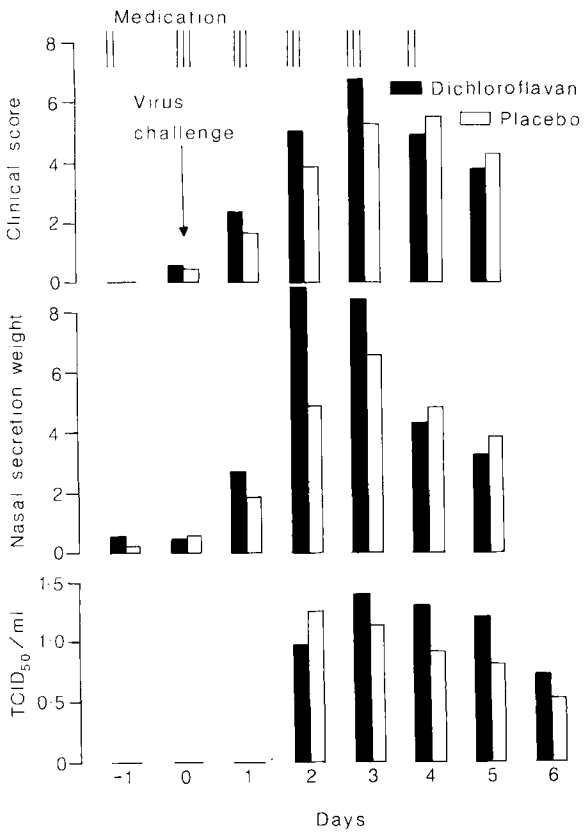


Fig. 4.14. Dichloroflavan has no prophylactic effect against rhinovirus type 9 in volunteers (after Tyrrell et al., 1983).

TABLE 4.38.
Effect of MDL-860 on virus plaque formation (after Torney et al., 1982)

MDL-860[a] (μg/ml)	No. of PFU		
	RV Hank	Echo 12	Coxsackie B_4
0	38	37	44
0.06	37	33	45
0.125	16	21	40
0.25	9	13	30
0.5	6	2	17
1.0	0	0	3
2.0	0	0	3

[a] MDL-860 was present only in the overlay medium

Ro 09-0179 (4′,5-dihydroxy-3,3′,7-trimethoxyflavone), isolated from a Chinese medicinal herb, was found to have potent antiviral activity by Ishitsuka et al. (1982). It selectively inhibited the replication of human picornaviruses, such as rhinoviruses and Coxsackie viruses in tissue culture (Table 4.40), but not other DNA and RNA viruses. A further compound, Ro 09-0179, prevented coxsackie virus (B1) infection in mice (Table 4.41). The critical time for the inhibition of rhinovirus replication by the compound was 2 to 4 h after virus adsorption, i.e., in the early stages of virus replication. It markedly inhibited coxsackie virus and rhinovirus RNA synthesis in infected HeLa cells, but not in a cell-free system using the RNA polymerase complex isolated from the infected cells. In the infected cells, the RNA polymerase complex was not formed in the presence of the drug and therefore, it was suggested that Ro 09-0179 interferes with some process of viral replication which occurs between viral uncoating and the initiation of viral RNA synthesis.

Furthermore, studies of various analogs related to 4′,5-dihydroxy-3,3′,7-trimethoxyflavone led to the identification of 4′-ethoxy-2′hydroxy-4,6′-dimethoxychalcone

TABLE 4.39.
Inhibitory effect of MDL-860 (1 μg/ml) on picornavirus replication in vitro (after Powers et al., 1982)

Virus (no. of strains)	No. (%) of strains with mean decrease in yield ($log_{10}TCID_{50}$/0.2 ml) of:		
	≥1.0	0.5–0.9	<0.5
Rhinovirus (90)	72 (80)	12 (13)	6 (7)
Enterovirus (10)	8 (80)	1 (10)	1 (10)

(Ro 09-0410), a new and different type of antiviral agent (Ishitsuka et al., 1982). Ro 09-0410 had a high activity against rhinoviruses but no activity against other picornaviruses. Of 53 rhinovirus serotypes tested, 46 were susceptible to the compound in HeLa cell cultures. The concentration of Ro 09-0410 inhibiting 50% of the types of rhinovirus was about 0.03 μg/ml, whereas the 50% cytotoxic concentration was 30 μg/ml. Ro 09-0410 inactivated rhinoviruses in direct dose-, time-, and temperature-dependent fashion. We can conclude that the inactivation may be associated with the binding of the agent to some specific site of the rhinovirus capsid.

Finally, we end up near the beginning of attempts at chemotherapy with rhinoviruses, namely with interferon. Merigan et al. (1973) showed that 14×10^6 units (50 μg approximately) of partially purified human leukocyte interferon could protect volunteers against infection with rhinovirus type 4. At the time this experiment used up a great proportion of the total world supply of interferon and the pretreatment and treatment was prolonged! This classic experiment has now been repeated but

TABLE 4.40.
Reduction of yield of echovirus from Ro 09-0179-treated cells in culture (after Ishitsuka et al., 1982)

Ro 09-0179 added (μg/ml)	Reduction of yield (log_{10}) of echovirus type:			
	7	11	12	19
0.5	2.8	2.5	>3.6	3.1
2.0	3.7	3.3	>3.6	3.6

TABLE 4.41.
Inhibition of viraemia caused by Coxsackievirus B1[a] (after Ishitsuka et al., 1982)

Treatment	Virus titre (log_{10} PFU/ml of blood) at day after infection:		
	1	2	3
None	5.7	6.5	7.0
	6.2	6.7	6.3
	6.4	7.3	6.7
Ro 09-0298 (10 mg/kg)	<4	<4	<4
	<4	<4	6
	<4	<4	6.7
Ro 09-0298 (20 mg/kg)	<4	<4	<4
	<4	<4	<4
	<4	<4	5.7

[a] Groups of three mice were infected intraperitoneally with about 10 LD_{50} of coxsackievirus B1 and were treated orally four times with Ro 09-0298 at 1, 2, 5, and 19h after infection

using highly purified and ten-fold more interferon, and using rhinovirus type 9 (Scott et al., 1982). The data is summarized in Table 4.42. Symptoms were markedly reduced but virus infection and seroconversion were not prevented and it was possible that interferon only *delayed* the onset of the cold. There is still a long way to go before the common cold is vanquished!

4.16. Acute haemorrhagic conjunctivitis (AHC or Apollo 11 disease)

This infection caused by enterovirus 70 now has a unique position in virology as one of the two human viruses which are pandemic in their epidemiology (with influenza still heading the 'record' as the classic example). The virus infection was first described by Chatterjee et al. (1970) in Ghana as a disease with an incubation period as short as 24 h and was called Apollo 11 disease in the locality because the outbreak coincided with the time of Apollo 11 landings on the moon. Within 3 years outbreaks had occurred in Japan (Kono, 1975) and a new picornavirus was isolated from the conjunctival scrapings. Enterovirus 70 has now spread almost around the world. (Hierholzer et al., 1975, Likar et al., 1975, Mirkovic et al., 1973).

Although Apollo 11 disease is most usually rather benign with complete recovery within 2 weeks, some neurological complications have nevertheless been described affecting about 1 in 10 000 cases. Most of the paralysed cases are men and typically the neurological phase begins 2–3 weeks after the onset of conjunctivitis with an acute hypotonic asymmetrical proximal paralysis of the lower limbs with fever, malaise and nerve root pains. Cranial nerve paralysis is often noted. Paralysis may be

TABLE 4.42.
Clinical grades of colds, rhinovirus 9 shedding on any day, and seroconversion (rise in serum neutralizing antibody of over fourfold) in volunteers treated with interferon or placebo and challenged with rhinovirus 9 (after Scott et al., 1982)

Grade of cold	No. of volunteers receiving:					
	Interferon			Placebo		
	Total	Virus secretion	Sero-conversion	Total	Virus secretion	Sero-conversion
Nil	5	3	2	1	1	0
Doubtful or very mild	3	2	1	2	1	1
Definite colds:						
Mild	0	0	0	1	1	1
Moderate	0	0	0	7	7	4
Total	8	5	3	11	10	6

permanent in up to 50% of those affected and hence in the near future the virus may be an important candidate for vaccine development or chemoprophylaxis. Epidemics have reached enormous proportions (Table 4.43) with over a million cases, for example, in Calcutta in 1971. The disease is most frequently seen in young adults and is not common in children, although serological evidence shows that infection occurs no less frequently in children (Table 4.44). Under conditions of poor hygiene and overcrowding rapid and extensive spread can occur.

Enterovirus 70 is seldom found in the faeces of patients. The viruses are rather fastidious from a cultivation point of view but may be isolated in human diploid fibroblasts and organ cultures of human embryonic conjunctiva. They are not pathogenic for suckling mice. Sensitive kinetic neutralization tests have shown that serological differences occur among isolates of enterovirus 70 (Higgins, 1982).

Apollo 11 disease is an excellent example of how a new disease can occur in man not only as a result of a previously unknown virus (e.g. Marburg virus) but also when members of a group of viruses behave in a previously undescribed fashion, with coxsackie A24 and enterovirus 70 causing disease not previously recognized as characteristic of the enteroviruses.

Prevention

At present no vaccine or specific treatment exists. In fact even if a live or inactivated vaccine were to be developed it is not clear which route of administration, for exam-

TABLE 4.43.
Number of patients with AHC reported or treated and estimates of actual number of cases in different parts of the world (after Kono, 1975)

Country or city	Period	No. of cases	
		Reported or treated	Estimates of the whole
Africa			
Accra, Ghana	Jun–Oct 1969	13664	
Lagos, Nigeria	Sep–Dec 1969	12799	over 100000
Morocco	Dec 1970–May 1971	137991	
Tunisia	Feb 1971		over 50000
Sana, Yemen	Feb 1972		30000
Asia			
Singapore	Sep–Oct 1970	60180	
Singapore	Jun–Dec 1971	38150	
Hong Kong	Aug 1971	6420	
Bangkok, Thailand	Jun–Nov 1971	2105	
Japan	Aug 1971–Feb 1972		30000
Bombay, India	Mar–Sep 1971		500000
Calcutta, India	May–Jul 1971		1000000

TABLE 4.44.
Age distribution of patients with AHC under treatment at ophthalmologic clinics (after Kono, 1975)

Age groups	Singapore	Bangkok	India	Nairobi	Age groups	Tokyo
10 or under	2.5	13.1	6.4	8	0–9	4.0
11–20	33	30.5	21.8	9.3	10–19	9.5
21–80	20	24.4	38.1	40	20–29	21.0
31–40	20	14.2	19.8	16	30–39	18.5
41–50	12.5	9.3	8.5	16	40–49	18.5
51–60	10.6	4.5	3.7	4	50–59	9.5
61–70	1.2	2.9	1.0	5.3	60–69	10.5
71 or over		1.0	1.0	1.3	70 or over	8.5

ple, would be most effective. Specific antivirals could prevent the secondary paralysis syndrome, especially when the rather long interval between conjunctivitis and paralysis is considered, but a large number of persons would have to be treated to prevent a single case of paralysis. As with certain arboviruses (see Chapter 5) resources might be more usefully diverted to altering and improving hygiene and living conditions in developing countries where the highest attack rates have been recorded.

4.17. Summary

Polio has been well controlled in most developed countries using live or (in a few countries) inactivated vaccines. Research work has intensified using genetic engineering techniques to produce live attenuated viruses with defined and stable mutations so as to prevent reversion to virulence, and also to produce immunogenic oligopeptides or proteins for a new generation of inactivated polio vaccines. Chemotherapy is therefore not required for polio infections.

In contrast, no vaccines have been developed against rhinovirus infections and nor are vaccines thought to have a use, unless broadly reacting antigenic determinants can be located. Several interesting but only weakly effective antiviral compounds have been selected against rhinoviruses and this is a major research area at present. Studies continue also with interferon, but because of toxicity problems these look less interesting at present.

Sequence and biochemical data is now available for several additional enterovirus strains (including the pandemic enterovirus type 70 and hepatitis A) and this could open new possibilities both with antivirals or vaccines (e.g. synthetic peptides) in the near future. At present little attention has been paid to preventing the spread of many important enteroviruses of the coxsackie and ECHO virus group, although

some of these viruses can result in serious neurological sequelae, resembling polio viruses in this regard.

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