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CHAPTER 8

Paramyxoviruses

Introduction

The paramyxoviruses are a heterogeneous group of viruses causing a variety of clinical diseases in humans, animals and birds as illustrated in Table 8.1. We shall examine in more detail below the structure and properties of the important human

| Virus type | Species pathogenicity under natural conditions | Main clinical syndrome |
|------------|-----------------------------------------------|------------------------|
| PIV-1      | +                                              | ARTDb                  |
| PIV-2      | +                                              | ARTD                   |
| PIV-3      | +                                              | ARTD                   |
| PIV-4      | +                                              | ARTD                   |
| PIV-5      | +                                              | Various                |
| Mumps (MuV)| +                                              | Mumps                  |
| Newcastle Disease Virus (NDV) | + | + | + | + | ARTD |
| Measles (MeV) | + | + | + | + | ARTD |
| Canine distemper (CDV) | + | + | + | + | ARTD |
| Rinder pest (RPV) | + | + | + | + | ARTD |
| Pneumonia (mouse) (PVM) | + | + | + | + | ARTD |
| Respiratory syncytial (RSV) | + | + | + | + | ARTD |

PIV, parainfluenza virus

* H, human; S, simian; E, equine; Rm, ruminant; P, porcine; C, carnivore; Rd, rodent; A, avian.

b ARTD, acute respiratory tract disease.
viruses in this group, namely measles, respiratory syncytial virus (RSV), mumps and parainfluenza viruses I–V. In brief, they are all enveloped, negative-stranded, riboviruses of helical symmetry (Fig. 8.1). The genome of the paramyxoviruses is a single negative strand of RNA of molecular weight approximately $5.4 \times 10^6$, containing the genes coding for the six known virus specific proteins. The HN (haemagglutinin-neuraminidase) F (fusion) and M (matrix) proteins are associated with the lipid membrane of the typical paramyxovirus. HN and F are glycoproteins (see below) constituting the surface spikes (see Fig. 8.1 for typical paramyxoviruses) and M is a non-glycosylated matrix protein, underlying the viral membrane. The F protein is formed by proteolytic cleavage of a larger precursor glycosylated polypeptide F$_0$. The three other proteins L, NP and 47K, together with the RNA, form the nucleoprotein core of the virion. The L and 47K proteins may constitute the virus RNA transcriptase enzyme, whose template is the genome RNA, complexed with NP. From the point of view of strategies of antivirals and vaccines, the RNA transcriptase enzyme and the antigenicity (Table 8.2) of the HN and F proteins respectively are important.

In infected cells, the paramyxovirus proteins are synthesized from mRNA species which are complementary to the genome, and which appear to be monocistronic. Two major size classes of mRNA have been detected – a 35S class containing the mRNA species for the L protein, and an 18S class containing at least five mRNA species (for a summary see Ball et al., 1978). The general strategy of replication is that of a typical negative stranded RNA virus (see Chapter 3), although whether precise details will differ between RSV, measles and mumps must await further studies.

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**TABLE 8.2.**
Antigenic composition and reactivity of paramyxoviruses (after Kelen and McLeod, 1977)

| Virus types | Core (S) antigen | Surface (V) antigens |
|-------------|-----------------|---------------------|
|             | Core (S) antigen | H     | N   | HL  | CFF |
| PIV-1–5     | +               | +     | +   | +   | +   |
| MuV         | +               | +     | +   | +   | +   |
| NDV         | +               | +     | +   | +   | +   |
| MeV         | +               | (+)a  | –   | (+)a| +   |
| CDV         | +               | –     | –   | –   | +   |
| RPV         | +               | –     | –   | –   | +   |
| PVM         | +               | (+)b  | –   | (+)b| +   |
| RSV         | +               | –     | –   | –   | +   |

a, with erythrocytes of simian origin only; b, with erythrocytes of rodent origin only.
8.1. Mumps virus

The catastrophe was dreadful: For the swelled testicles subsided suddenly the next
day, the patient was seized with a most frantic delirium, the nervous system was
shattered with strong convulsions, and he died raving mad the third day after.

Robert Hamilton.
An Account of a Distemper by the Common
People of England Vulgarly Called the
Mumps. paper to the
Royal Society of Edinburgh, 1790

The term mumps, probably from an old verb meaning 'to mope', is a good home-
spun word for a familiar illness (Christie, 1980). The disease, though, is of great
antiquity and was one of the first infections to be recognized and was described
by Hyppocrates in the 5th century BC.

8.1.1. The virus and mode of replication

The virus itself, like measles, belongs to the paramyxovirus group and is a roughly
spherical, enveloped RNA-containing virus, 150–200 nm in diameter (Fig. 8.1). The
internal nucleocapsid is surrounded by a lipid envelope (approx. 15 nm in thickness)
on which are situated the outer glycoproteins of the virion. The nucleocapsid is a
flexible helical structure with a "RNA backbone covered with protein subunits. The
RNA approximates to $7 \times 10^6$ M.W., and would have coding capacity for at least
8 proteins. A major protein of the virus is the NP and an additional polymerase
P protein is thought to be closely linked to the RNA and functioning in an RNA-
transcriptase complex. A membrane or M protein is another major constituent of
the virion and is assumed to contribute to the structural integrity of the virus, and
is located inside the lipid envelope. Two glycoprotein spikes are present, the larger
(HN) with HA and NA activities and a smaller glycoprotein F involved in haemo-
lytic and cell fusing activities (Heppertz et al., 1977, Naruse et al., 1981, Orvell,
1978, Herrler and Compans, 1982, Rima et al., 1980). The virus, therefore, has
some typical properties of the paramyxovirus group, but compared to other mem-
bers of the group rather little is known about the detailed biochemistry or molecular
biology of the virus.

In a recent study Merz et al. (1983) investigated intracellular mumps virus-specific
polypeptide synthesis by pulse- and pulse-chase-labelling with radioactive amino
acids and sugars. The major polypeptides seen on SDS-polyacrylamide gels were
NP (69 K M.W.), P (45K M.W.) and M (40K M.W.); a non-structural polypeptide
(22K M.W.) was also present in infected cell lysates. The HN (74K to 79K M.W.)
glycopolypeptide was detected in ($^3$H) glucosamine- and ($^3$H) mannose-labelled in-
fected cells. A 65K M.W. species that had incorporated these precursors was seen in pulse-labelled infected cell lysates, and this glycopolypeptide vanished during the chase interval, with the concomitant appearance of two glycopolypeptides (59K M.W. and 14K to 15K M.W.) which represented the F₁ and F₂ subunits of the F glycoprotein. Immunological data confirmed the relatedness of the 65K M.W. glycopolypeptide to the F glycoprotein and identified it as the precursor F₀. The F₀ precursor glycopolypeptide was seen in cells infected with both fusing and non-fusing strains of virus, and F₀ was processed completely to F glycoprotein for all infections.

Thus, mumps virus-infected cells contain seven virus-specified polypeptides, as has been described for other paramyxoviruses. The protein is the most abundant polypeptide in infected cells. Polypeptides corresponding to the nucleocapsid-associated polypeptide P and membrane (M) protein are also present. A polypeptide of M.W. 22K is found in infected cells which does not precipitate with anti-mumps virus antiserum and has a unique limited proteolysis peptide map; this is consistent with it being a non-structural (NS) polypeptide.

8.1.2. EPIDEMIOLOGY AND CLINICAL ASPECTS

Mumps is a common disease of childhood, although attacks in adult life are more frequent than of measles or chickenpox for example, suggesting a reduced infectivity of mumps compared with the latter 2 viruses. The virus spreads by aerosol and initially infects the upper respiratory tract. The incubation period varies from 7–23 days, but most commonly is 14–18 days. In experimentally infected children, virus was excreted in saliva for 2–6 days, before clinical signs of parotitis appeared and for 4 days or so after its onset. Approximately half of susceptible persons may never develop clinical signs of mumps infection, although they will commonly secrete virus and so be a source of infection for others. Clinically the disease often begins with malaise, fever and pain near the angle of the jaw. A typical swelling of the face develops within 14–18 hours and often distorts the features considerably. Often both parotid glands are affected, but there may be a delay between the onset in both glands. The patient may have difficulty in opening the mouth and there is often dryness of the throat, presumably because of reduced saliva flow from blocked salivary ducts. The temperature is often high for 2–3 days and the swelling lasts for 3 days but then subsides quickly. The most notorious complication of mumps is orchitis, developing 4–5 days after onset of the parotitis, although it may develop in the absence of preceding parotitis. The first indication is pain in the testicles, rapidly followed by swelling and tenderness. The pain may be very acute. There is often an accompanying sharp general reaction with high temperature and head-

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Fig. 8.1. Electron micrographs of typical paramoxyviruses. a, b, SV5 × 130 500; c, Sendai × 195 000 (courtesy of Dr. D. Hockley.)
ache. Symptoms tend to subside after 3–6 days. It has been estimated that the incidence of orchitis in adult mumps varies from 11.6% to 66% but an average incidence of 1 in 5 is usually accepted by clinicians. Some degree of testicular atrophy is common and may occur in up to half the patients and a further, more serious, complication is sterility. It is estimated that some degree of infertility may be expected in 10% of adult patients suffering from mumps orchitis, although this may be temporary. Christie (1980) believes that the fear of sterility following mumps has been greatly exaggerated.

A more important complication of mumps in children is meningitis (Kilham, 1949, McLean et al., 1961, Russell and Donald, 1958, Donohue et al., 1955) and to a much lesser extent encephalitis. However, the prognosis in aseptic meningitis is extremely good, with few reports of long term sequelae. Mumps encephalitis, on the other hand, can be a severe and sometimes fatal illness (Table 8.3). The essential lesion is demyelination, which occurs in foci distributed throughout the brain or spinal cord. Mumps virus may be found in the cerebrospinal fluid but not the brain tissue itself. Quite often the first indication is a convulsion in a young child or sudden coma in an older child or adult during the convalescent period after mumps, with a sharp rise in temperature. A less dramatic onset may include drowsiness and a change in behaviour. The extent of damage to the CNS varies considerably and some patients may recover, while others may develop paralysis. Mortality is estimated to be around 20%. The overall mortality from mumps is about 1.8 deaths per 10,000 reported cases (Table 8.3).

### TABLE 8.3
Cases, deaths, and case-fatality rates for mumps and mumps encephalitis, United States, 1966 to 1975 (after Hayden et al., 1978)

| Year | Mumps | | Mumps encephalitis |
|------|-------|---|------------------|
|      | Cases | Deaths | Deaths per 10,000 cases | Cases | Deaths | Deaths per 100 cases |
| 1966 | 128,295 | 43 | 3.4 | 628 | 10 | 1.6 |
| 1967 | 185,691 | 37 | 2.0 | 849 | 8 | 0.9 |
| 1968 | 152,209 | 25 | 1.6 | 408 | 2 | 0.5 |
| 1969 | 90,918 | 22 | 2.4 | 218 | 5 | 2.3 |
| 1970 | 104,953 | 16 | 1.5 | 288 | 5 | 1.7 |
| 1971 | 121,924 | 15 | 1.2 | 310 | 5 | 1.6 |
| 1972 | 74,215 | 16 | 2.2 | 163 | 0 | 0 |
| 1973 | 69,612 | 12 | 1.7 | 214 | 3 | 1.4 |
| 1974 | 59,128 | 6 | 1.0 | 149 | 2 | 1.3 |
| 1975 | 59,647 | 8 | 1.3 | 166 | 4 | 2.4 |
8.1.3. Prevention of Mumps by Vaccines

Because of the relatively high incidence of subclinical mumps (Henle et al., 1948) and because patients with parotitis may be infectious even before clinical signs appear, methods of prevention based on isolation of clinical cases or quarantining of contacts are not effective. Therefore work (after the pioneering studies of Enders et al., 1946) commenced in the 1950s to develop both inactivated mumps vaccine and live attenuated vaccine (Buynak and Hilleman, 1966, Ennis and Hopps, 1969, Henle, 1951, Kliachko and Maslennikova, 1957, Stokes et al., 1967). Because of the uncertain nature of the antigenic composition of the virus and because of the poor experience with inactivated RSV and measles vaccines (see pages 361 and 375) early experimental inactivated vaccines (although used quite successfully to immunize young army recruits) were soon superceded by live attenuated vaccines. A further negative aspect of inactivated mumps vaccine was the poor duration of immunity. A live attenuated mumps vaccine was licensed in the USA in 1968 and had been developed from a strain of mumps (Jeryl Lynn, Mumps vox) initially isolated in fertile hens eggs and which after several passages had been adapted to chick embryo cell cultures (earlier attenuated viruses had been passaged in eggs alone and proved either underattenuated causing mumps in recipients or overattenuated and not provoking antibody in recipients). Originally, mumps vaccine was used in a monovalent form but more recently, it has also been used in the USA in children particularly as a combined vaccine with live measles and rubella viruses (Weibel et al., 1973, 1978, Smorodintsev et al., 1970, Schwarz et al., 1975, Schell et al., 1975, Buynak et al., 1969). Clinical trials over the years have established that the protective efficacy ranged from 70–90% (MMWR Nov. 1982) and there was a good correlation with induction of virus neutralizing antibodies and clinical protection. The vaccine has proved to be safe in usage and no mumps-like syndrome could be attributed to the vaccine virus in controlled clinical trials in 500 or more patients including several hundred potentially susceptible adults. Similar vaccines can also be used in army recruits (Pentinnen et al., 1968). Since licensing of the vaccine in December 1967 in the USA more than 55 million vaccine doses have been used there. Other mumps vaccines have been developed since, particularly in Japan (Isomura et al., 1973, Hosai et al., 1970, Yamanishi et al., 1970).

Since 1968 and the introduction of the vaccine there has been a progressive decline in the number of reported cases of mumps in the USA. Thus, in 1976 a 42% decrease on the average annual total for the years 1971–75 was noted and the incidence of mumps in the USA has now reached an all time low. In 1981 there were only 4729 cases reported, showing a 97% decline from the 185 691 cases in 1967.

It is important that the immunity induced by mumps vaccine should last through adulthood and this feature of the vaccine has now been clearly established. In an early study of longevity of immunity (admittedly in a small number of patients) Weibel et al. (1978, 1980) reported no substantial decline of neutralizing antibody
titre for nearly 12 years. The initial level of neutralizing antibody reached after vacci-
nation was less than that following natural infection but even serum antibody
titres of 1:1 were associated with solid immunity to infection with the wild virus.

8.1.4. DEVELOPMENT AND EARLY CLINICAL TRIALS WITH A LIVE ATTENUATED MUMPS
VACCINE IN THE USA

An early example of an attenuated mumps vaccine and one still used today (alone
or in the form of a triple vaccine) was the Jeryl Lynn strain, named after a child
from whom the virus was originally isolated. The virus was attenuated empirically
by sequential passage in embryonated hens eggs and in chick embryo cell culture
and the first vaccines were prepared using virus grown in the latter cells (Hilleman
et al., 1967, 1968). In a large field trial, vaccine was given by subcutaneous injection
in a study involving a total of 1337 children. The children were assigned to family
or classroom groups or to a classroom-family group if one or more siblings in a
family were also entered into a classroom included in the study. The children in
classrooms were selected randomly to receive vaccine or to serve as unvaccinated
‘sentinels’ to enable a study to be made of any (unwanted) spread of vaccine virus.
482 children received vaccine and 855 acted as controls. The age range was from
eleven months to eleven years. Any clinical reactions were recorded for 28 days after
vaccination. Of the 402 children who were initially seronegative, 395 developed ant-
ibody after vaccination (98% seroconversion, Table 8.4). None of 407 contact con-
trols acquired mumps. Surprisingly recipients of vaccine who already had mumps
antibody often displayed an increase in antibody titre following vaccination. There
were no significant clinical reactions following vaccination. The occurrence of natu-
ral mumps in the community during the two years following immunization allowed
an assessment of the protective efficacy of the vaccine (Table 8.5). Among families,
attack rates were 6.9% in vaccinees and 84.7% in controls (protective efficacy

| Group of children | Children vaccinated | Children not vaccinated |
|------------------|---------------------|------------------------|
|                  | No. in whom antibody developed | Total | Percentage in whom antibody developed | No. in whom antibody developed | Total | Percentage in whom antibody developed |
| Classroom        | 224                  | 225 | 99.6 | 0 | 189 | 0 |
| Family           | 171                  | 177 | 96.6 | 0 | 218 | 0 |
| Total            | 395                  | 402 | 98.2 | 0 | 407 | 0 |
TABLE 8.5.
Evaluation of protective efficacy of mumps-virus vaccine among initially susceptible children (after Hilleman et al., 1968)

| Interval between vaccination and challenge | Study group | Vaccinated persons at risk | Unvaccinated controls at risk | Level of protective efficacy (%) |
|-------------------------------------------|-------------|-----------------------------|-----------------------------|--------------------------------|
|                                           |             | No. of cases | No. at risk | Rate (%) | No. of cases | No. at risk | Rate (%) |                          |
| month                                     |             |              |             |           |              |             |           |                          |
| 0-10                                      | Family      | 2            | 29          | 6.9       | 50           | 59          | 84.7      | 91.7                        |
|                                           | Classroom   | 2            | 114         | 1.8       | 49           | 113         | 43.4      | 95.9                        |
| 11-20                                     | Family      | 1            | 14          | 7.1       | 22           | 24          | 91.7      | 92.3                        |
|                                           | Classroom   | 1            | 28          | 3.6       | 24           | 40          | 60.0      | 94.1                        |
| Total period                              | All children| 5            | 174         | 2.9       | 133          | 224         | 59.4      | 95.1                        |

91.7%. Similarly, in the classroom the comparative rates were 1.8% and 43.4%, giving a vaccine efficacy of 95.9%. In this early study a few children were successfully immunized by jet gun and others with a combined measles–mumps formulation. Finally, the freeze-dried vaccine could be produced in batches of consistent quality, was very stable on storage at 4°C and was also stable after rehydration. A similar vaccine is now used very widely in the USA as a combined triple vaccine with live rubella and measles virus, and long term follow-up studies have indicated persistence of antibody over quite extended periods of time (Weibel et al., 1975, 1970).

8.1.5. A NEW LIVE ATTENUATED MUMPS VACCINE

A trial of a new mumps vaccine was reported recently by Ehrengut et al. (1983) and will be used to illustrate further general features of this type of vaccine (see also Isomura et al., 1973, Rossier et al., 1971, Smorodintsev et al., 1970).

The Urabe Am 9 strain was derived from a mumps virus isolated in primary human embryonic kidney cells from a child named Urabe with typical clinical signs and symptoms of mumps. The wild virus was attenuated by serial passages on several substrates by M. Takahashi, Osaka University, Japan. After isolation, it was passaged once more on human embryo kidney cells (HEK), then once in primary green monkey kidney cells six times in the amniotic membrane of specific-pathogen-free (SPF) hens' eggs, twice in SPF quail embryonic fibroblasts (both were cloning passages) and again four times in eggs. At this stage it was supplied to a vaccine manufacturer where it was passaged five times in the chorioallantoic membrane of SPF embryonated hens' eggs to produce the seed virus for vaccine production in chick embryo fibroblasts.

None of 197 subjects given the vaccine in two clinical trials had local reactions
TABLE 8.6.
The incidence of local and systemic reactions related to the dose of vaccine administered and the serological status of the vaccinees (after Ehrengut et al., 1983)

| Study number | Vaccine dose (TCID<sub>50</sub>/dose) | Number of subjects | Average age and range | Serological status | Number with reactions |
|--------------|-------------------------------------|--------------------|-----------------------|--------------------|----------------------|
|              |                                     |                    |                       | Neg | Pos | Local Neg | Pos | Systemic Neg | Pos |
| MUM-018      | 10<sup>4.7</sup>                    | 34                 | 5.0 (1-9 years)       | 15  | 19  | 0         | 0   | 0           | 0   |
|              | 10<sup>3.7</sup>                    | 36                 | 4.6 (8 months-7 years)| 19  | 17  | 0         | 0   | 1           | 1   |
| MUM-021      | 10<sup>2.8</sup>                    | 32                 | 5.4 (3-8 years)       | 15  | 17  | 0         | 0   | 1           | 1   |
|              | 10<sup>3.7</sup>                    | 95                 | 5.3 (7 months-8 years)| 50  | 45  | 0         | 0   | 4           | 7   |

although a low number developed systemic signs (Table 8.6). Sero conversion was noted in 89-100% of persons in four separate groups. Twenty-seven subjects retested for antibody nearly 3 years later still had EHI antibodies although levels had dropped somewhat. Thus, the Urabe Am 9 mumps vaccine strain in common with other strains used widely (e.g. Jeryl Lynn) is relatively well tolerated and immunogenic, although not completely free from side reactions.

8.1.6. GENERAL RECOMMENDATIONS FOR MUMPS IMMUNIZATION (USA)

It is suggested that susceptible children, adolescents and adults should be vaccinated against mumps, unless vaccination is contraindicated. Mumps vaccine can be of particular value to children approaching puberty and for adolescents and adults, especially males who have not had mumps in childhood. Live mumps vaccine is recommended for all children at any age after 12 months, although it is not recommended for younger infants because of persisting maternal antibodies which would interfere with virus take. Contraindications include pregnancy, since although there is no good evidence of mumps causing congenital abnormalities in humans, nevertheless, the wild virus can infect the foetus and placenta. Mumps vaccine virus has also been isolated from the placenta, although not from the foetus. Since the virus is grown in chick embryo cell culture, persons allergic to egg protein should be vaccinated with extreme caution. Persons with immune deficiency diseases or suppressed immune responses that occur with leukaemia, lymphoma, generalized malignancy or during therapy with corticosteroids, alkylating drugs or radiation should not be vaccinated. This is because the replication of mumps vaccine virus may be potentiated in patients with immune deficiency diseases.

The principle strategy for removing the burden of mumps disease in the USA is through achieving or maintaining high immunization levels and CDC constantly
encourage private physicians and public health clinics to carry out routine vaccination as part of the health care programme. As an example of what can be achieved, the state of Massachusetts initiated a mumps control programme and offered vaccine to 5–14 year olds beginning in 1969 and 1–4 year olds additionally by 1971. A saving of over $300 000 is achieved when the cost of the vaccine programme is compared to the costs of avoided medical care. It seems very likely that the future of mumps vaccine will be directed by the emphasis put on rubella and measles vaccine and combined virus vaccines which, in the USA at least have proven to be efficacious, easy to apply and without serious side effects (Tables 8.7 and 8).

**TABLE 8.7.**
Occurrence of fever among triple-seronegative children who received combined measles–mumps–rubella vaccine (Moraten measles, Jeryl Lynn mumps, HPV-77 duck rubella) (after Hilleman et al., 1973)

| Maximum oral temperature (°F) | Vaccinated children (228): days after vaccination | Unvaccinated children (106): days observed |
|-------------------------------|-----------------------------------------------|------------------------------------------|
|                               | 5–12 | 13–18 | 5–12 | 13–18 |
| <99                           | 105 (47%) | 140 (64%) | 57 (59%) | 64 (66%) |
| 99–100.9                      | 86 (39%) | 69 (32%) | 36 (37%) | 25 (26%) |
| 101–102.9                     | 26 (12%) | 7 (3%) | 3 (3%) | 8 (8%) |
| 103–104.9                     | 6 (3%) | 2 (1%) | 1 (1%) | — |
| Not taken                     | 5 | 10 | 9 | 9 |

**TABLE 8.8.**
Rates of symptoms among vaccinees and unvaccinated controls following measles–mumps–rubella vaccination, University of Lowell, Massachusetts, 1980 (from CDC Measles Surveillance Report, 1982)

| Symptom       | Percentage of vaccinees | Percentage of controls | P value |
|---------------|------------------------|------------------------|---------|
|               | n=388                  | n=175                  |         |
| Fever         | 12                     | 8                      | N.S.    |
| Rash          | 4                      | 4                      | N.S.    |
| Sore throat   | 27                     | 22                     | N.S.    |
| Cough         | 23                     | 18                     | N.S.    |
| Headache      | 30                     | 20                     | <0.02   |
| Photophobia   | 12                     | 14                     | N.S.    |
| Arthralgia    | 15                     | 7                      | <0.01   |

N.S., not significant
8.2. Respiratory syncytial virus

8.2.1. Respiratory syncytial virus-structure and replication

Electron microscopy shows the virus to be 120 to 200 nm in diameter, with an envelope containing surface projections (Fig. 8.2) (Bloth et al., 1963). Attempts to identify and characterize the proteins of RSV have been hampered by several factors. Loss of infectivity during purification procedures and the fact that 80% of sucrose band-purified material is nonviral make the study of purified viral proteins difficult (Fernie and Gerin, 1980) and may account for some of the discrepancies among

Fig. 8.2. Electron micrographs of respiratory syncytial virus. a, b: negative staining of virions; c, d: sectioned cells with budding RSV (courtesy of Dr. T. Bachi, University of Zurich.)
different laboratories. Between 7 and 10 presumed viral polypeptides ranging in molecular weight from 200,000 (200K) to 10K (Table 8.9 and Fig. 8.3) have been identified by polyacrylamide gel electrophoresis of radiolabeled RSV protein (Fernie and Gerin, 1982, Bernstein and Hruska, 1981). A 90K glycoprotein and a 48K glycoprotein have been identified and suggested as surface glycoproteins based upon their removal by trypsin and detergent treatment of purified RSV. Recently, the 48K glycoprotein has been shown to be connected to a 20K protein by disulfide bonds, forming a 70K protein. The functional designation of the 90K and 70K glycoproteins has not been made. A 41K protein is considered to be the nucleocapsid (N) protein, and a 27K protein is considered to be the matrix protein (Peebles and Levine, 1979).

Although related to paramyxoviruses, RSV does not possess similar biochemical markers such as a haemagglutinin or neuraminidase activity. Like the paramyxoviruses, however, RSV promotes cell-cell fusion, accounting for the characteristic giant cell syncytia formation that is seen when the virus is grown in permissive cell lines. The ability to form syncytia permits the spread of virus by fusion of infected cells to adjacent noninfected cells. It is presumed that a surface protein of the virion is responsible for the fusion process, as has been demonstrated with other paramyxoviruses. In an attempt to define the so-called fusion protein of RSV, Walsh and Hruska (1983) produced six monoclonal antibodies directed against respiratory syncytial virus proteins. Each was characterized by immunoprecipitation and indirect immunofluorescence. One was directed against the nucleocapsid protein, NP 44, two were directed against a 37K protein, two were directed against the 70K envelope protein, VP70 (Table 8.10). Indirect immunofluorescence stain patterns of infected HEp-2 cells defined GP 90 and VP 70 as viral proteins expressed on the cell surface, whereas NP 44 and the 37K protein were detected as intracytoplasmic inclusions. One of the anti-GP 90 antibodies neutralized virus only in the presence

| New nomenclature | Previous designation | Evidence for virus specificity |
|------------------|----------------------|-------------------------------|
| Detroit          | Glasgow              |                               |
| VP200            | VP0                  | VP200                         | ?                             |
| GP1              | VP1                  | ?                             |
| VGP48            | VP2                  | VP48                          | Immunoprecipitation           |
| VPN41            | VP3                  | VP41                          | Immunoprecipitation           |
| VPP32            | VP4                  | VP32                          | Mutation                      |
| VPM27            | VP5                  | VP27                          | Immunoprecipitation           |
| VP25             | VP6                  | VP25                          | Immunoprecipitation           |
| VP10             | —                    | VP10                          | —                             |

See also Peeples and Levine, 1979; Veba, 1980; Cash et al., 1979; Belshe et al., 1977.
TABLE 8.10.
Monoclonal antibodies to RSV proteins (after Walsh and Hruska, 1983)

| Immunoglobulin class | RSV protein specificity (mol wt) | Virus neutralization titre |
|----------------------|----------------------------------|---------------------------|
|                      |                                  | Without complement | With complement |
| IgG2a                | NP 44                            | <1:4                     | <1:4             |
| IgG1                 | VP 37                            | <1:4                     | <1:4             |
| IgG1                 | VP 37                            | <1:4                     | <1:4             |
| IgG2b                | VP 70                            | 1:512                    | >1:2.048         |
| IgG2a                | GP 90                            | <1:4                     | 1:2.048          |
| IgG2a                | GP 90                            | <1:4                     | <1:4             |

of complement, but did not inhibit cell-cell fusion. The anti-VP 70 antibody neutralized virus without complement and inhibited cell-cell fusion of previously infected HEp-2 cells, thus identifying VP 70 as the fusion protein.

Based on the molecular weight and surface location of GP 90 of RSV, Fernie and Gerin (1980) suggested that it may be analogous to the haemagglutinin of the paramyxoviruses which functions as the viral glycoprotein responsible for adsorption of virus to cells. Although GP 90 of RSV does not possess haemagglutinating or neuraminidase activity, it is reasonable to suspect that it also might function in cell attachment. The inability to neutralize RSV with two anti-GP 90 monoclonal antibodies does not negate this possibility (Walsh and Hruska, 1983). These monoclonal antibodies may be directed toward an epitope on GP 90 distinct from the one responsible for virus-host cell adsorption. That one of the anti-GP 90 antibodies was capable of neutralizing virus in the presence of complement suggests that neutralization is mediated by viral membrane lysis and that antibodies to GP 90 might be important in immunity to infection. Although able to neutralize RSV in the presence of complement, this anti-GP 90 antibody was unable to prevent the spread of infection by cell-cell fusion.

The fusion protein may be involved in the cell penetration step of some enveloped viruses by fusing the viral envelope to the host cell membrane, resulting in delivery of the ribonucleoprotein into the cell cytoplasm. Neutralization of RSV by anti-VP 70 antibody might be mediated by inhibition of virus-cell fusion. The anti-VP 70 monoclonal antibody described by Cote et al. (1981) was able to neutralize virus, but did not inhibit cell-cell fusion. This suggests that either the fusion protein facilitates cell adsorption or that neutralization of virus occurs by a mechanism other than inhibition of cell attachment. It is also possible that virus-cell fusion and cell-cell fusion may be mediated by different regions of the fusion protein.

Reinfecction with RSV in the presence of circulating neutralizing antibodies is common in both infants and adults (Hall and Douglas, 1982). However, the protein
specificity of these neutralizing antibodies has not been fully explored. Because of the cell-cell spread of RSV, neutralizing antibody to GP 90 may not be as effective in preventing infection as neutralizing antibody that is directed to the fusion protein and can also limit cell-cell spread of virus. Of course, these studies have particular relevance as regards new RSV vaccines and particularly vaccines containing proteins from cloned genes.

The analysis of the RS virion RNA and the viral-specific messenger mRNAs synthesized in infected cells, has been initiated but is not complete. Seven genetic complementation groups exist, but there is no firm biochemical data describing the number of genes on the viral genome or the number and identification of the gene products specified. Huang and Wentz, 1982 have presented data which suggest that RS virus is a negative-strand RNA virus. One of the characteristics of a negative strand virus is that the virion contains an RNA dependent RNA polymerase that is capable of synthesizing messenger RNA in the presence of inhibitors of protein synthesis. Huang and Wertz (1982, 1983) have established polypeptide coding assignments for six RSV mRNAs by translation in vitro. RNA band 1 is complex, can be separated into at least two components, and codes for three polypeptides of 9.5K, 11K and 14K. RNA2 codes for the 32K polypeptide, RNA3 for the 27K polypeptide, RNA4 for the 41K polypeptide and RNA5 for a 59K polypeptide (which may be a post-translation modified protein).

8.2.2. CLINICAL ASPECTS

This virus is the most important cause of severe respiratory infections in young infants under the age of 18 months (Chanock et al., 1961, Parrott et al., 1961, Glezen and Denny, 1973, Holzel et al., 1968, Gardner, 1973, Report to the MRC, 1978, Loda et al., 1968, McDonald et al., 1982, Kim et al., 1973) and may also infect (Johnson et al, 1961) and cause exacerbation of bronchitis in adults (Kravetz et al., 1961, Johnson et al., 1961). There has been a close correlation between recovery of RSV and cot deaths. The virus is ubiquitous in both temperate and tropical climates and may cause epidemics of bronchiolitis in infants for several weeks during the winter. Infants tend to shed virus for up to 3 weeks and so may be an important focus of infection even after leaving hospital. RSV can cause a mild upper respiratory tract infection or a severe attack of acute bronchiolitis. Often the attack commences like a common cold but within 24 hours the child may be acutely ill with dyspnoea, being distressed and cyanosed. In other infants the clinical picture may resemble pneumonia (Christie, 1980). The pathology is that of a necrotizing bronchiolitis in which partial blocking of the bronchioles leads to their collapse. Peribronchial infiltration may spread out into the lungs to give widespread interstitial pneumonia. Most adults have detectable levels of neutralizing antibody to RSV but the actual titre of antibody may have no direct relationship with ability to resist infection (reviewed by Jackson and Muldoon, 1975). Young children may have
Fig. 8.3. Polypeptides of the Long strain of RSV. Radioimmunoprecipitation analysis and SDS-PAGE of RS virus polypeptides from extracts of infected HEp-2 cells using 1 µl of a human RS virus pneumonia hyperimmune serum. Left: cells labelled with \(^{35}\text{S}\) methionine plus \(^{35}\text{S}\) cysteine; right: cells labelled with \(^{3}\text{H}\) glucosamine. Lanes V, RS virus-infected cells; Lanes C, mock-infected control cells. Bands arrowed represent polypeptides which were consistently revealed during repeat analyses. (after Ward et al., 1983.)

Several infections with RSV over a period of only a few years. Rather, resistance to infection appears to correlate more closely with levels of nasal IgA antibody. Repeated natural infections do result in a cumulative acquisition of resistance to illness (Tyeryar, 1983).
8.2.3. Failure of inactivated vaccines against RSV

Soon after the isolation of RSV and delineation of its clinical effects in young children (Chanock et al., 1961) it was realized, that protection against disease would be difficult to achieve by immunization, because moderate levels of serum neutralizing antibody from previous natural infection did not protect fully against RSV caused low respiratory tract illness (Kapikian et al., 1969). However, more recent data, summarized by Tyeryar, 1983, indicates that natural infection induces a state of partial immunity to re-infection detectable for at least one year. Also RSV-caused bronchiolitis and pneumonia occurred more often in the first four months of life when maternally transmitted antibody was at high levels. Nevertheless, following preliminary studies of a formalin inactivated RSV vaccine in adults, the vaccine was used to immunize children in a local authority home. The vaccine utilized the Bernett strain of RSV isolated originally in HEK (human embryo kidney) cells and passed 3 times in HEK cells and 10 times in vervet MK cells. MK cell harvests were clarified by filtration, inactivated with formalin, and safety tested in animals and tissue cultures. The vaccinated and control groups were comparable in age, sex and race and there were no significant side effects following immunization. The vaccine was immunogenic and 96% of children who lacked CF antibody subsequently developed antibody after 1-3 injections. (In the 7 children tested, relatively high levels of neutralizing antibody also developed.) Approximately 9 months later a sharp outbreak of RSV occurred in the home. Virus was recovered from 41% of 146 residents and serological evidence of infection was detected in 92% of 40 seronegative residents. The vaccine not only failed to induce protection but induced an exaggerated and altered clinical response to RSV in the younger vaccinees. Nine of 13 vaccinated children (6-23 months of age) developed pneumonia compared to 4 of 47 non-vaccinated children (Table 8.1). Five of these 13 vaccinated children developed pneumonia serious enough to require hospitalization, whereas none of the 47 non-vaccinated children were hospitalized. This paradoxical effect of vaccination was not observed in Arthur Cottage which housed older children (24-65 months of age), but lack of protection against illness was observed. Kapikian et al. (1969) considered that the failure to produce protection in the older children may have been due to poor induction of locally produced IgA antibody as observed previously with parainfluenza type 1 virus infections, whereas the enhanced clinical effect noted in younger infected children may have resulted from interaction of serum antibody and RSV antigen in the lungs of children who lacked respiratory tract IgA antibody. The authors also concluded that administration of gamma globulin to infants should be approached with caution during periods of RSV prevalence.

Essentially identical results of a paradoxical response to RSV immunization were also reported by Kim et al. (1969). In response to three injections of alum precipitated, 100 × concentrated, formalin inactivated RSV vaccine, 43% of infant vaccinees displayed a 4-fold or greater rise in serum neutralizing antibody and 91% displayed
TABLE 8.11.
Incidence of pneumonia and RS virus isolations according to age and vaccine status in residents of Harrison and Arthur Cottages (after Kapikian et al., 1969)

| Age (months) | Vaccinated group | Nonvaccinated group |
|--------------|------------------|---------------------|
|              | No. in group     | Pneumonia no. | RS isolated no. | No. in group | Pneumonia no. | RS isolated no. |
| Harrison     |                  |                |                |              |                |                |
| 6-11         | 2                | 2 (69%)        | 1 (69%)        | 7            | 1 (9%)         | 4 (49%)        |
| 12-23        | 11               | 7              | 8 (69%)        | 40           | 3 (9%)         | 19 (49%)       |
| 24-35        | 2                | 0              | 0              | 10           | 0              | 6              |
| 36-58        | 0                | 0              | 0              | 22           | 0              | 3              |
| Total        | 15               | 9 (60%)        | 9 (60%)        | 79           | 4 (5%)         | 32 (41%)       |
| Arthur       |                  |                |                |              |                |                |
| 6-11         | 0                | 0              | 0              | 0            | 0              | 0              |
| 12-23        | 2                | 0              | 1 (50%)        | 1            | 0              | 0              |
| 24-35        | 9                | 4 (44%)        | 3 (33%)        | 20           | 6 (30%)        | 9 (45%)        |
| 36-65        | 11               | 0              | 5 (45%)        | 9            | 1 (11%)        | 1 (11%)        |
| Total        | 22               | 4 (18%)        | 9 (41%)        | 30           | 7 (23%)        | 10 (33%)       |

*a Percentage significantly different ($P < 0.0001$).

A 4-fold or greater rise in serum CF antibody. When RSV became prevalent in the community, the rate of RSV infection in infants who received this vaccine was not different from that in control infants who received parainfluenza vaccines. However, a high proportion of RSV vaccinees required hospitalization at the time of RSV infection, whereas only 5% of such infections among parainfluenza vaccinees (control group) resulted in admission to the hospital (see also Table 8.12). Illnesses among the RSV vaccinees who underwent natural infection included pneumonia, bronchiolitis, and bronchitis, with pneumonia in a majority and rhinitis, pharyngitis and bronchitis in a few. Thus, infants who received this vaccine were not protected against natural infection and also, when they became naturally infected their illness was more severe than that seen in cohorts who received a similar parainfluenza type 1 vaccine. These findings, similarly to those of Kapikian et al. (1969), indicated that vaccine-induced RSV serum antibody alone did not protect against illness and suggested that serum antibody (particularly against certain of the viral proteins) without local respiratory antibody might actually play a part in the production of disease. The highest incidence of serious RSV illness occurring naturally was under six months of age, when maternally derived serum antibody was present. These findings together with those of Kapikian et al. (1969) suggest that RSV illness in infants is, at least in part, an immunologic phenomenon, wherein the virus and serum antibody interact to produce severe illness.
TABLE 8.12.
RS virus infection and illness in groups of infants after receiving 1 or more injections of inactivated respiratory syncytial and parainfluenza vaccines (after Kim et al., 1969)

| Vaccine received | No. vaccinees | No. vaccinees with later RS infection as indicated by | No. vaccinees with designated illness at time of RS infection | No. requiring hospitalization at time of RS infection |
|------------------|---------------|------------------------------------------------------|----------------------------------------------------------|------------------------------------------------------|
|                  |               | Virus CF recovery | CF antibody rise | Virus recovery and/or CF rise | P B or B-P | BR-PH | URI |
| RS               | 31            | 18                | 20              | 23              | 6 13 0 | 4 18 |
| Para 1           | 20            | 7                 | 12              | 12              | 2 0 0 | 10 1 |
| Trivalent parainfluenza | 20 | 7 | 9 | 9 | 0 2 2 | 5 0 |
| Total parainfluenza | 40 | 14 | 21 | 21 | 2 2 2 | 15 1 |

P, pneumonia; B, bronchiolitis; BR-PH, severe bronchitis-pharyngitis; URI, mild rhinitis, pharyngitis and/or bronchitis.

8.2.4. EXPERIMENTAL LIVE ATTENUATED RSV VACCINES

Since anti-RSV serum IgG antibody of maternal origin, or antibody induced by inactivated RSV vaccine failed to produce protection against natural disease (Kapikian et al., 1969) efforts commenced to make a cold adapted (ca) or ts virus, which could be used as a candidate strain for a live vaccine (Gharpure et al., 1969, Belshe et al., 1978). Such a vaccine would presumably induce local IgA antibody in the respiratory tract and so more closely mimic natural infection with RSV. Initial experiments with a ca virus (Kim et al., 1971), adapted to 26°C failed to produce a completely attenuated virus because, for example, a child given the virus developed mild lower respiratory tract disease. The same group used the standard mutagenesis technique with 5-fluorouridine (Wright et al., 1970) to produce a ts mutant designated RS-A2 ts-1. The mutant, after propagation at 30°C in primary bovine embryo kidney cells did not infect cells at or above 37°C. In the in vivo hamster model the ts mutant only infected the upper respiratory tract and, moreover, there was no laboratory evidence of phenotypic reversion, which is the most important problem with ts viruses. Initial experiments in adults, who were infected intranasally, indicated that no disease was produced and the vaccinees resisted subsequent challenge with virulent wild type virus (Wright et al., 1971). The next step was to infect infants and children with virus using a coarse spray. The ts-1 mutant produced infection in 27 of 32 infants as shown by recovery of virus (Table 8.13) and increases in antibody titre (up to 48-fold) (Table 8.14). Children excreted vaccine virus for a mean of 6.1 days (Kim et al., 1973).

Another candidate live attenuated RSV vaccine strain, ts-2, has been found un-
TABLE 8.13. Evidence of infection in infants and children who received the ts-1 mutant respiratory syncytial virus (RSV) (after Kim et al., 1973)

| Previous RSV infection | No. in group | No. with indicated evidence of infection |
|------------------------|--------------|----------------------------------------|
|                        |              | Virus recovery | Rise in neutralizing antibody | Virus and/or an immunological response |
|                        |              | Serum | Nasal secretion |
| No                     | 7            | 7     | 7   | 7   | 7   |
| Yes                    | 25           | 20    | 7   | 19  | 25  |
| Total                  | 32           | 27    | 14  | 26  | 32  |

a Based upon presence or absence of detectable plaque reduction antibody in serum.

TABLE 8.14. Serum neutralizing antibody response to ts-mutant RSV (after Kim et al., 1973)

| Previous RSV infection | No. in group | Virus shed | Time (weeks) | Mean serum antibody titre (reciprocal) | Fold antibody rise |
|------------------------|--------------|------------|--------------|----------------------------------------|-------------------|
|                        |              | Yes or no  | Number       |                                        |                   |
| No                     | 7            | Yes        | 7            | 0                                      | <20               |
|                        |              |            |              | 2                                      | 117               | 5.8×               |
|                        |              |            |              | 3                                      | 407               | 26×                 |
|                        |              |            |              | 7                                      | 683               | 46×                 |
| Yes                    | 25           | Yes        | 20           | 0                                      | 350               |
|                        |              |            |              | 2                                      | 711               | 2×                  |
|                        |              |            |              | 3                                      | 841               | 2.4×                |
|                        |              |            |              | 7                                      | 736               | 2.1×                |
| No                     | 5            | No         | 0            |                                        | 518               |
|                        |              |            |              | 2                                      | 525               |
|                        |              |            |              | 3                                      | 477               |
|                        |              |            |              | 7                                      | 537               |

suitable for further work because of over- attenuation (reviewed by Tyeryar, 1983). When tested in adult volunteers there was only slight evidence of infection and none of the 14 volunteers shed virus, and only 2 had an antibody response. Similarly, only two of seven seronegative children were infected.

In another approach, a live virus vaccine to be administered parenterally has been tested. The vaccine was prepared by passaging a clinical isolate of RSV in WI38 cells. In a randomized, double-blind, placebo-controlled trial encompassing 510 children, 233 received the vaccine and 277 received placebo. Children with low levels of pre-existing neutralizing antibodies did not respond to the vaccine, whereas
seronegative children did produce antibodies, but to titres lower than those observed in children after natural infection. RSV infection occurred in the various groups at similar rates. Of the 112 children for which pre-epidemic/post-epidemic serum pairs were available 48.2% (27 of 56) placebo recipients and 50% (28 of 56) of vaccine recipients had serological evidence of RSV infection. Further, 25% (25 of 100) placebo recipients and 17.3% (19 of 98) vaccinees developed RSV illness, as confirmed by viral isolations. Thus, parenterally administered live RSV vaccine did not protect from subsequent RSV infection or illness and this simply repeats the negative results of earlier trials with killed vaccine documented above. Among the 510 study children there were 23 illnesses requiring hospitalization for respiratory tract diseases during the study. Five of the illnesses were caused by RSV infection, as determined by isolation of virus, fourfold increases in antibody titre, or both. Three RSV illnesses were in the vaccine group, and two were in the placebo group. Fortunately, none of the illnesses was particularly severe, and none was life-threatening. No untoward clinical reactions were noted in most infants (Table 8.15), although in 7 infants a mild non-febrile rhinitis was noted. However, two worrying features of the ts-1 vaccine strain were spread to non-vaccinees and laboratory evidence of reversion to wild-type phenotype. Thus, of a total of 139 viruses recovered from vaccinees, 34 showed evidence of genetic alteration from the ts phenotype, and 8 viruses completely lost the ts property. Although these revertants represent less than 0.01% of virus produced in the respiratory tract at the time of peak virus replication, they nevertheless made the vaccine unsuitable for more widespread use. Experiments with alternative ts mutants are still in progress (Richardson et al., 1978).

8.2.5. APPLICATION OF RECOMBINANT DNA TECHNOLOGY FOR RSV

As alluded to above, the poor growth of RSV in tissue culture systems has hampered biochemical work with the virus, and its polypeptide and RNA composition have only recently been described (Huang and Wertz, 1983, Pringle et al., 1981). No satisfactory vaccines have been developed using the conventional biological methods (Hall, 1980) employed so successfully for many other viruses and therefore a remaining possibility is to apply recombinant DNA techniques to isolate gene products and also to analyze the structure and nature of expression of the RSV

| Previous RSV infection | No. in group | No. infected | No. with indicated response |
|------------------------|--------------|--------------|----------------------------|
|                        |              |              | Rhinorrhea | Fever | Otitis media |
| No                     | 7            | 7            | 7          | 1     | 1            |
| Yes                    | 25           | 25           | 6          | 0     | 0            |

TABLE 8.15.
Clinical response to infection with ts-1 mutant RSV (after Kim et al., 1973)
Complementary cDNA clones were constructed containing RSV N, P, M and NS gene sequences. (The authors assume that the gene order in RSV is similar to that of paramyxoviruses, namely 3' NFo .M PH N L 5'.) In this case Venkatesan et al. (1983) have clones containing sequences adjacent and 3' to Fo and the HN equivalent and so it should be possible to obtain cDNAs coding Fo and HN equivalents in RSV. The cDNA clones could also be transferred to eukaryotic expression vectors. Both the fusion and major surface glycoprotein antigen would be required for any new inactivated vaccine.

8.2.6. CHEMOPROPHYLAXIS OF RSV

Dubovi et al. (1980, 1981) have described the inhibition of RSV induced cell fusion in cells by an aromatic diamidine, bis(5-amidino-2-benzimidazolyl)methane (BABIM) which is active in vitro at 1 µM. The effect was RS virus specific, and did not extend to parainfluenza type III or SV strains, for example. The compound appeared to retard virus penetration and reduced the yields of virus under multiple growth cycle conditions (Table 8.16). It is postulated that 'fusion from within' is inhibited and the compound is known to be a potent reversible inhibitor of trypsin.

8.2.6.1. Inhibition of RSV by ribavirin

The compound inhibited plaque formation by the Long strain of RSV by 50% at concentrations of 3.2 µg/ml in cells (Table 8.17). Similarly 'wild' strains of virus were inhibited in vitro, although they were rather less susceptible than laboratory viruses (Hruska et al., 1980). More than 100-fold reduction in released RSV was detected and the virus induced cytopathic effect was reduced by ribavirin. The compound, therefore, appeared to inhibit RSV to the same extent as influenza A and B viruses (see page 313).

In further experiments Hruska et al. (1982) demonstrated mild in vivo inhibition of RSV replicating in the nasal turbinates and lung tissues of infected cotton rats.

| m.o.i.    | Control (TCID50/ml) | 50 µM BABIM |
|----------|---------------------|-------------|
| 0.1      | 5 x 10^7            | 6.3 x 10^6  |
| 0.01     | 3.2 x 10^7          | 1.2 x 10^6  |
| 0.001    | 2.8 x 10^4          | 1.1 x 10^4  |

*RS virus at various m.o.i. was adsorbed to Hep-2 monolayers for 2 hr at 36°. Monolayers were rinsed three times and overlaid with MEM containing 2% FBS and 1% DMSO.
An antiviral effect was detected when ribavirin was administered by aerosol intranasally and also intraperitoneally (Table 8.18).

8.2.6.2. Ribavirin treatment of experimental RSV infection in adult volunteers

Hall et al. (1983) infected 16 young adults intranasally with RSV (5 log_{10} TCID_{50}) and treated them by ribavirin aerosol commencing on day 3, for 12 hours per day for 3 days (virus shedding and the first clinical signs normally commence on day 3). The aerosol was given during 3 periods in 24 h using a face mask. It was estimated that 12 h of treatment would provide a deposited dose of 660 mg ribavirin per patient.

The average onset of virus shedding of the placebo-treated group on day 4.1 was not significantly different from that of the ribavirin-treated group on day 3.5. The

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### TABLE 8.18.
Titres of RSV in tissues of cotton rats 4 days postinfection after continuous aerosol treatment with ribavirin (after Hruska et al., 1982)

| Treatment | Titre at concentration of ribavirin in reservoir (mg/ml) |
|-----------|--------------------------------------------------------|
|           | 0 (n=6) | 1 (n=6) | 2 (n=5) | 4 (n=10) |
|           | Lungs    | Turbinate | Lungs    | Turbinate | Lungs    | Turbinate | Lungs    | Turbinate |
| Controls  | 5.0±0.9  | 5.4±0.7   | 5.1±0.7  | 4.9±0.4  | 5.3±0.5  | 5.75±0.3 | 4.4±0.6  | 4.35±0.6  |
| Ribavirin | 4.5±0.5  | 5.3±0.3   | 4.4±0.3  | 4.0±0.7  | 4.3±0.5  | 4.8 ± 0.6 | 3.6±0.1  | 3.6±0.3   |
| P         | <0.2     | <0.35     | <0.1     | <0.025   | <0.01    | <0.01    | <0.025   | <0.025    |

* Controls were not given aerosol treatment.
peak amount of virus shed on each day varied from 0.7 to 5.45 log_{10} TCID_{50}/ml in the placebo-treated group and from 0.95 to 4.95 log_{10} TCID_{50}/ml in the ribavirin-treated group. If the total amount of virus shed by each subject (i.e., the sum of the average shedding of each day) was compared for the two groups, the average amount of total shedding was greater in the placebo group (1.1 \times 10^4 TCID_{50}), compared with the ribavirin-treated subjects (0.6 \times 10^3 TCID_{50}). This difference, however, was not statistically significant.

During the first three days of shedding (days 3, 4 and 5 of the study), the proportion of subjects shedding the virus was not significantly different between the two groups. In the latter part of the study, days 6 through 9, however, after the 36 hours of ribavirin treatment, the proportion of infected subjects still shedding the virus was significantly less in the ribavirin-treated group ($\chi^2 = 3.99, P < 0.5$).

Twelve of the 13 infected subjects manifested signs or symptoms of upper respiratory tract illness, and one subject in the ribavirin-treated group remained asymptomatic. The frequency of occurrence of minor upper respiratory tract symptoms did not differ significantly between the two groups. The frequency, however, of the more major complaints of systemic symptoms, cough and fever, occurred significantly more often in the placebo-treated group (Table 8.19). Nine (13%) of 70 recorded oral temperatures in the placebo-treated subjects were greater than 37.5°C, compared with one (1.6%) of the recorded temperatures in the ribavirin-treated group ($P < 0.002$). Similarly, the average symptom score for minor symptoms did not significantly differ between the two groups, but the score obtained from systemic symptoms and fever was significantly greater in the placebo-treated group (Table 8.20).

It should be noted that the infection was mild and even so ribavirin only ameliorated systemic symptoms of cough and temperature. However, no toxic signs of the drug were noted suggesting that treatment could be extended.

### 8.2.6.3. Treatment of infants with RSV with ribavirin

Hall et al. (1983) have also described treatment of infants hospitalized with RSV

### TABLE 8.19.

Average symptom score of RSV infected subjects treated with placebo versus subjects treated with ribavirin (after Hall et al., 1983)

| Symptom          | Placebo-treated group ($n=7$) | Ribavirin-treated group ($n=6$) | $P^*$  |
|------------------|------------------------------|---------------------------------|-------|
| Minor symptoms   | 6.2                          | 9.6                             | N.S.  |
| Systemic symptoms| 2.9                          | 0.5                             | <0.01 |
| Fever            | 4.4                          | 0.8                             | <0.01 |

*Wilcoxon's rank test.*
infection with an aerosol of ribavirin. The compound was administered continuously, except during a period of 1–3 hours each morning, for a minimum of 3 days giving (in adults) approximately 0.82 mg per kg drug per hour. Thirty-three infants were studied, all with RSV pneumonia. On admission to hospital the severity of illness was the same in placebo and treated groups, but after 1 day of therapy a difference in clinical improvement between the 2 groups in favour of ribavirin was detected. The change in degree of improvement in lower respiratory tract signs, except for wheezing, was significantly greater in the drug treated group, whereas the change in temperature and upper respiratory tract signs was not. By the end of treatment the quantity of virus in nasal washes from the ribavirin group was significantly less (Table 8.21). Moreover, the average number of days of RSV shedding

| Group (no.)   | Titre (log$_{10}$ TCID$_{50}$/ml) |
|--------------|-----------------------------------|
|              | Before treatment | At day 3 | At end of treatment |
| Ribavirin (12) | 7                   | 2.1*     | 1.2*               |
|               | 0.4–5.7            | 0–4.2    | 0–1.7             |
| Placebo (13)  | 3.0                 | 2.1      | 1.3                |
|               | 0.4–5.9            | 0–4.2    | 0–4.2             |

*Not significantly different from value for placebo group.
*Significantly different from value for placebo group.

**TABLE 8.20.**
Mean severity score for RSV induced signs or symptoms at start and end of treatment with ribavirin (after Hall et al., 1983)

|                     | Ribavirin group | Placebo group | P value* for change in score |
|---------------------|-----------------|---------------|-----------------------------|
|                     | Start | End | Start | End |                      |
| Temperature (°C)    | 37.9  | 37.2 | 37.9  | 37.4 | N.S.                 |
| Nasal congestion and discharge | 1.8 | 0.6 | 2.2 | 1.0 | N.S. |
| Cough               | 2.3   | 0.9 | 1.8 | 1.6 | <0.01 |
| Rales               | 2.2   | 0.5 | 1.6 | 1.4 | <0.01 |
| Wheezing            | 1.1   | 0.2 | 1.3 | 0.8 | N.S.                 |
| Retractions         | 2.2   | 0.2 | 1.5 | 1.0 | <0.01 |
| Lethargy            | 2.3   | 0.2 | 2.0 | 1.2 | <0.01 |

*P value for unit change in score from start to end of therapy for placebo group versus ribavirin group (Mann-Whitney U test and non-paired t-test). N.S. denotes not significant.

**TABLE 8.21.**
Titres of respiratory syncytial virus in nasal-wash isolates (after Hall et al., 1983)
from the start of therapy was 4.3 days in the placebo group and 2.9 days in the ribavirin group. Finally, no toxic side effects of aerosol therapy were detected in these infants.

Since ribavirin therapy is administered by aerosol over long periods it is appropriate at this time only for hospitalized infants. The number of infants requiring hospitalization for RSV infection is nevertheless appreciable. In long-term studies in Washington, D.C., infection with this virus accounted for 23% of all hospital cases of acute respiratory-tract disease, and one of every 100 primary RSV infections resulted in a hospital admission (Parrott et al., 1974). In the UK, the rate of hospitalization for RSV infections is as high as 1 in every 50 infants in the first year of life. Ribavirin therapy may be particularly beneficial for children at risk for severe and often fatal RSV infection, such as infants with congenital heart disease (MacDonald et al., 1982).

8.3. Measles

8.3.1. Measles (Morbillus, a diminutive of morbus – a small or childhood disease)

Measles is an Anglo-Saxon word (Middle English: ‘maseles’) and true to form the disease is still regarded here, as in those earlier times, as an almost normal incident of Anglo-Saxon childhood! This is certainly not so in other countries and, for example, the US may soon rid itself of indigenous measles and may therefore become an island to itself. Other countries with vigorous immunization policies are the USSR, Sweden and the DDR. Measles has also been implicated in at least one neurological disease, resulting from persistent viral infection, namely subacute sclerosing panencephalitis (SSPE) (Hall and Ter Meulen, 1976, Modlin et al., 1977), and also a fatal encephalopathy during immunosuppression (Olding-Stenkvist et al., 1982).

8.3.2. Structure of measles virus and mode of replication

A M.W. of $4.5 \times 10^6$ for measles virus genomic RNA has been obtained, which is intermediate between values for other negative strand viruses of $3.8 \times 10^6$ for VSV and $5 \times 10^6$ for RSV. This is in agreement with the estimated number of virus coded proteins, namely 5 for VSV, 6 for measles and 7 for RSV.

Most studies on virus proteins have been carried out in the last 5 years and as with other paramyxoviruses formidable difficulties have had to be overcome in virus purification. In appearance the virus is a typical paramyxovirus (Fig. 8.4). The virions appear to have six proteins, similar to those of typical paramyxoviruses, namely L, H, P, N, F and M. The precise role of many of these proteins is still
speculative and even the question as to whether the H and F proteins are present in a single complex spike on the virus is unknown.

Nucleocapsid (N) protein (60K) is directly associated with the RNA genome and is a major protein of all paramyxoviruses in both the virion and the virus infected cell. The electrophoretic mobility of N varies among measles virus strains (Rima, 1983) and some changes could be caused by phosphorylation of the protein. The N protein is a major cross-reacting protein of the morbilliviruses, although monoclonal antibodies may be used to distinguish different members of the group (Birrer et al., 1981a, b).

P protein (formerly called P2) is the second largest structural protein and is designated P because of association with the RNA polymerases. It has a molecular weight of 70K and is phosphorylated (Hall et al., 1980) which may contribute to the observed electrophoretic variations in measles virus strains. The P protein is very susceptible to proteolysis and, for example, in measles virus infected cells a 37K protein derived from P has been described (Rima et al., 1981).

The L protein is the largest protein, is not glycosylated and has a molecular weight between 100 and 200K. It is present in purified virus (Vainionpaa, 1979) and infected cells. The L protein does not appear to be phosphorylated and has been identified as virus specific using monoclonal antibodies (Giraudon and Wild, 1981). The L protein is a very minor component of the virion.
The HA protein (H or G protein) is the major glycoprotein and can be detected in the virion, with a molecular weight of 79–80K. In infected cells and in the virion the H protein is present as 150K–160K dimers. Variation in electrophoretic mobility of H proteins has been observed among strains of measles virus. Monospecific antisera to the H protein of measles virus have neutralizing and HI activity, while some monoclonal antibodies have haemolysis-inhibiting antibody. Antigenic variation among H proteins of measles viruses is also detectable with monoclonal antibodies (Birrer et al., 1981b).

The fusion glycoprotein (F₀) is converted to the biologically active form (F) by proteolysis, and the resulting polypeptides are held together by disulphide bonds. In the morbilliviruses F₁ (40K) is not glycosylated. The F₂ portion of the F protein is glycosylated and is detected as a 20–25K glycopolypeptide. The proteolytic activation of the F₀ protein appears to depend on the cell line in which the virus replicates. The membrane or matrix protein (M) is the smallest but most abundant protein of the virus with a molecular weight of 36–37K (Mountcastle and Choppin, 1977). The M protein is detectable in measles virus infected cells and under certain salt conditions is associated with the purified nucleocapsid complex. The protein is not glycosylated and can be purified by extraction in 1 M KCl. The M proteins of measles and SSPE viruses can be distinguished by fingerprinting techniques (Hall et al., 1980). The M protein is thought to have a role in virus assembly and budding and may have a function in virus persistence since both the antigenic composition and electrophoretic characteristics of M differs in persistent virus compared to acute infection virus (Ter Meulen and Carter, 1982). Sera from SSPE patients have reduced antibody frequency and titres to the measles virus M protein (Hall et al., 1979b, Machamer et al., 1980) and a number of SSPE strains have been described which produce reduced amounts of M (or no M) in infected cells (Machamer et al., 1981) but other SSPE strains also produce reduced amounts of H or F proteins. It is possible that certain SSPE virus proteins may be rapidly degraded or, alternatively, there may be defects in the transcription or translation of the various RNAs. Precise definition of the non-structural proteins must await further study but 'candidate' NS proteins are detectable in measles infected cells with molecular weights of 65K, 38K and 18K (reviewed by Rima, 1983).

8.3.3. CLINICAL DISEASE AND EPIDEMIOLOGY

Measles virus enters the body and infects via droplet spread, and perhaps also via the conjunctiva. During the incubation period of 8–11 days, virus spreads to the nearest lymph nodes, replicates and is soon detected in the bloodstream, spleen, liver, bone marrow and other organs (Fenner, 1948). Certain symptoms, such as the virus induced lesions or rash (Fig. 8.5) are probably due to inflammatory reactions and to the release of abnormal cell products by virus infected cells. Giant cells containing up to 100 nuclei have been described in the pharynx and tonsils,
skin, respiratory epithelium, lymph nodes and Peyers patches and the brain. The virus is widespread in the skin but disappears quickly. By the time the skin has reacted and the rash is apparent virus content in the internal organs has dropped, and within 1 to 2 days little virus is excreted. The most infectious stage of the disease is for several days before the rash.

There is little doubt that measles is one of the most ubiquitous of human viruses, with a world wide distribution, and can cause disease in any climate or environment (Assaad, 1983, Hethcote, 1983). In countries with a high standard of living, epidemics occur in cycles every 2–3 years, attacking predominantly 3–5 year-olds in a relatively mild way (Table 8.22). In third world countries, on the other hand, measles has a high incidence in the under 2 year olds and is a severe disease with unusual clinical features such as blindness and a high fatality rate (Table 8.23, Morley, 1969, Smith and Foster, 1970, Wakeham, 1978). The state of nutrition may affect the virus epidemiology and malnutrition may reduce the immune response (Chen et al., 1980, Dossetor and Whittle, 1975). The measles then accentuates the effects of malnutrition and diarrhoea, for example, may persist after measles, leading further to protein loss. Death rates of 42%, 17% and 7% have been recorded in Afghanistan, for example, in children aged 0–4, 5–9 and 10–14 years respectively. Certain of the clinical features of measles in third world countries today resemble descriptions of measles in Europe 100 years ago (Christie, 1980, Heymann et al., 1983).

Complications of measles in children in Europe are croup (4%), otitis media, eye complications and central nervous system changes (0.4%). Croup is often a complication of the prodromal phase and nearly every child with a sharp attack of measles has symptoms of bronchitis and X-ray signs of pneumonitis because of virus inva-

| Country                  | Age (years) |
|--------------------------|-------------|
| Nigeria (urban)          | 1–2         |
| Kenya                    | 1–2         |
| Ghana, Zambia, Rhodesia  | 2–3         |
| Cameroon (urban)         | <1          |
| Indonesia                | 1–4         |
| India                    | 2–4         |
| India (rural)            | 1–2         |
| Guatemala                | 1–2         |
| United Kingdom           | 5–9         |
| United States (prevaccination) | 4–5    |
|                          | 10–14       |
| Denmark                  | 6           |
TABLE 8.23.
Case fatality rates for measles from community studies around the world

| Country                     | Year | Case fatality rate (%) |
|-----------------------------|------|------------------------|
| India (Punjab)              | 1959 | 1-2                    |
| Chile                       | 1960 | 4                      |
| The Gambia                  | 1961 | 14                     |
| Guatemala                   | 1963 | 4.5                    |
| Nigeria                     | 1963 | 7                      |
| Nigeria (war areas)         | 1968 | 15                     |
| India (Madras)              | 1969 | 1.5                    |
| India (Aurangabad)          | 1972 | 2.2                    |
| Kenya                       | 1976 | 6.5                    |
| Bangladesh                  | 1976 | 3.7                    |
| Ghana                       | 1978 | 3                      |
| Indonesia                   | 1978 | 25.5                   |
| United States (prevaccination) | 1961 | <0.02                  |
| United States (vaccine era) | 1975 | 0.01                   |
| United States (epidemic on Indian reservation) | 1974 | 4                      |
| United Kingdom              | 1963 | 0.2                    |

sion of the respiratory tract (Miller, 1964, Christie, 1980). In underdeveloped countries bacterial pneumonia is a common and often fatal complication of measles including pneumococci, haemolytic streptococci and *H. influenza*. Virus pneumonia caused by measles itself has been reported in children in North Africa and the fatality rate may approach 50%.

Otitis media used to be a common complication of measles but nowadays is much reduced, possibly because of a slow change in the disease itself rather than the use of antibiotics. Convulsions are commonest when the rash is appearing and often are classical febrile convulsion. The incidence of measles encephalitis approximates to 1 in 1000 cases but drowsiness and irritability are very common in measles and so the actual incidence may be much higher. The mortality in patients with coma is around 30-40%. Sub-acute sclerosing panencephalitis (SSPE) occurs at a rate of approximately 1 per 100,000 measles cases.

### 8.3.4. Prevention by vaccination

Since patients are most infective in the prodromal period and to isolate them when the rash is out serves little purpose, we must orientate particularly towards vaccines or specific antivirals to prevent measles as an epidemic disease. Immunoglobulin given intramuscularly (250 mg to infants, 500 mg to over 1 year olds) to contacts within 6 days or so of exposure to measles either prevents or modifies the disease. Immunoglobulin prophylaxis is considered to be a valuable measure in persons at
special risk and who have not been vaccinated but, of course, could not be considered for use at present on a wider scale.

8.3.5. EARLY STUDIES WITH INACTIVATED AND UNDER-ATTENUATED LIVE MEASLES VACCINES: EVIDENCE OF SIDE-REACTIONS

Since some of the first produced live attenuated measles vaccines (e.g. 'Enders-Edmonston', isolated from a boy of that name and passaged 24 times in kidney cells, 28 times in primary amnion cells and 6 times in chick embryos) produced unacceptable side reactions in children (Enders et al., 1960), it was initially recommended that either the vaccine be given with gamma globulin or that killed measles vaccine be administered first, followed after several months by a booster of live attenuated virus (MRC report, 1965). This practice is now discontinued because of the development of more suitable attenuated viruses (e.g. Schwartz strain, produced by 77 additional passages of Edmonston virus in chick embryo fibroblast cells at 32°C (Krugman, 1983)) and also because of untoward side reactions noted in some children given the above combination. This again emphasizes the caution with which vaccination trials have to proceed, particularly with viruses where the virus structure is unknown.

In this regard, McNair-Scott and Bonanno (1967) described febrile (104°–105°F) illness in 16 patients who were given the inactivated measles vaccine course followed by a challenge with live measles virus. Redness and swelling at the site of injection of live virus vaccine was noted and blisters developed in 4 and cysts in 3 patients (Table 8.24). The clinical appearance was that of an Arthus type immunological reaction in a sensitised person. Moreover, Norrby (1966) described the occurrence of severe pneumonia after infection with natural measles virus in 5 of 125 children previously immunized with killed measles vaccine, Rauh and Schmidt (1965) described 54 patients infected with natural measles two years after immunization with killed vaccine of whom 9 showed an atypical infection characterized by urticarial.

### TABLE 8.24.
Reactions at inoculation site of live measles virus after 2 doses of killed measles virus vaccine more than three months previously (after McNair Scott and Bonanno, 1967)

| Type of reaction          | No. of children | Stated day of onset | No. of children in whom day of onset was known |
|---------------------------|-----------------|---------------------|-----------------------------------------------|
| Fever                     | 16              | 1st                 | 2                                             |
| Local swelling and erythema| 13              | 3rd                 | 1                                             |
| Local swelling and blisters| 4              | 4th                 | 3                                             |
| Local swelling and cysts  | 3               | 5th                 | 1                                             |
| Generalized rash          | 2               | 6th                 | 3                                             |

Average stated duration, before therapy, 3–7 days in 5 patients.
vesicular and petechial rashes, swollen hands and feet, pneumonia (4 cases) and erythema multiforme.

In a further report Fulginiti et al. (1967) described atypical measles illness in 10 children who had received inactivated measles vaccine 5–6 years earlier (Table 8.25). The illness was characterized by a 2–3 day prodrome of fever and pain, followed by a maculo-papular rash with petechial vesicular and urticarial components. Peripheral oedema and pneumonia occurred in almost all the children. All the children were hospitalized and 9 were judged as seriously ill (in fact Rocky Mountain spotted fever was misdiagnosed in one case). The authors concluded that these cases represented only a fraction of the total in the community and recommended that the inactivated measles vaccine should be discontinued.

Nowadays only live ‘further’ attenuated measles vaccines (MRC report, 1977, Miller, 1982, Krugman, 1983) are used and these are highly effective vaccines (Table 8.26). The vaccine is given currently to children no earlier than 12 months (because of maternal antibodies) and preferably at 15 months of age. Vaccine is not administered to children with acute leukaemia or under immunosuppression (Mitus et al., 1962, Aicardi et al., 1977).

In a detailed follow up study of 5000 children given a single dose of live attenuated measles vaccine (Schwartz strain) when 10 months to 2 years of age, a high level of protection was noted (MRC report, 1977, Table 8.26). Moreover this immunity lasted over a 10 year period (Table 8.27). Throughout the trial when measles occurred in vaccinated children more of the cases were mild than in unvaccinated

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**TABLE 8.25.**

Clinical findings in patients with atypical measles who had been immunized previously with inactivated measles vaccine (after Fulginiti et al., 1967)

| Case no. | Age (years) | Sex | Temperature | Rash$^b$ | Oedema | Pneumonia$^c$ |
|----------|-------------|-----|-------------|----------|--------|---------------|
|          |             |     | Maximum $^\circ F$ $^\circ C$ Duration (days) |          |        |               |
| 1        | 6           | F   | 105 (40.6)  | 7        | M, V, P | +             | RML          |
| 2        | 6           | M   | 103 (39.4)  | 7        | M, P    | +             | ?            |
| 3$^a$    | 7           | F   | 104 (40.0)  | 7        | M, V, P | +             | B+E          |
| 4        | 7           | F   | 105 (40.8)  | 7        | M, P    | +             | RLL+E        |
| 5        | 6           | M   | 104 (40.0)  | 5        | M, P    | 0             | B            |
| 6$^a$    | 6           | F   | 104 (40.0)  | 5        | M, V, P | +             | RML          |
| 7        | 8           | F   | 105 (40.6)  | 4        | M       | 0             | RML          |
| 8        | 6           | M   | 105 (40.6)  | 6        | M, P    | 0             | B+E          |
| 9        | 7           | F   | 103 (39.4)  | 4        | M, V    | +             | RLL          |
| 10       | 8           | F   | 105 (40.6)  | 5        | M       | +             | RLL          |

$^a$ Siblings.

$^b$ M signifies maculopapular; V, vesicular; P, petechial.

$^c$ RML signifies middle lobe of the right; RLL, lower lobe of the right; B, bilateral; E, effusion.
TABLE 8.26. Confirmed cases of measles in four periods according to vaccine group (after MRC report, 1977)

| Period          | Group            | No. of cases | Doctor’s assessment |
|-----------------|------------------|--------------|---------------------|
|                 |                  |              | Mild No. (%)       | Moderate No. (%) | Severe No. (%) |
| 1964/65 (9 months) | Killed/live vaccine | 174          | 133 (76)            | 37 (21)         | 4 (2)          |
|                 | Live vaccine     | 183          | 137 (75)            | 42 (23)         | 4 (2)          |
|                 | Unvaccinated     | 1993         | 938 (47)            | 969 (49)        | 86 (4)         |
| October 1965–September 1969 | Killed/live vaccine | 327          | 245 (75)            | 77 (24)         | 5 (2)          |
|                 | Live vaccine     | 169          | 126 (75)            | 39 (23)         | 4 (2)          |
|                 | Unvaccinated     | 1501         | 631 (42)            | 781 (52)        | 89 (6)         |
| October 1969–September 1972 | Killed/live vaccine | 119          | 85 (71)             | 32 (27)         | 2 (2)          |
|                 | Live vaccine     | 42           | 28 (67)             | 14 (33)         | 0 (0)          |
|                 | Unvaccinated     | 203          | 74 (36)             | 124 (61)        | 5 (2)          |
| October 1972–September 1976 | Killed/live vaccine | 38           | 20 (51)             | 17 (45)         | 1 (3)          |
|                 | Live vaccine     | 32           | 18 (56)             | 14 (44)         | 0 (0)          |
|                 | Unvaccinated     | 47           | 16 (34)             | 30 (64)         | 1 (2)          |

For example, no severe cases of atypical measles as noted in the USA after multiple doses of inactivated measles vaccine (Fulginiti et al., 1967) were noted. The persistence of clinical protection was also confirmed by the serological investigations which showed that only one child in the sample investigated (Table 8.27) had no detectable antibody after 10 years.

Common reactions associated with the ‘more attenuated’ measles vaccine strains used now (Norrby, 1978) are fever and rash occurring in 5–30% of recipients, generally during the second week after immunization. Febrile convulsions are rare. In a recently published study in the UK involving 10 035 children vaccinated with the Schwartz vaccine virus, 20% had mild symptoms, 8% moderate symptoms and 1% severe symptoms in the two weeks after vaccination. In all, 5% of children were seen by the doctor for possible serious side effects and 12 (0.1%) were admitted to hospita-

TABLE 8.27. H.I. antibody levels after 10 years according to vaccine group (after MRC report, 1977)

| Vaccine group   | No. of children tested | No. and percentage according to H.I. titrea |
|-----------------|------------------------|---------------------------------------------|
|                 |                        | <4  | 4   | 8   | 16  | 32  | 64  | 128 | 256 and over |
|                 |                        | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | No. (%)   |
| Killed/live vaccine | 47                   | 2 (4) | 1 (2) | 1 (2) | 3 (6) | 19 (40) | 9 (19) | 9 (19) | 3 (6)    |
| Live vaccine    | 71                    | 1 (1) | 1 (1) | 2 (3) | 16 (23) | 17 (24) | 19 (27) | 12 (17) | 3 (4)    |
| Unvaccinated    | 37                    | 12 (32) | — (0) | — (0) | 3 (8) | 5 (14) | 7 (19) | 7 (19) | 3 (8)    |

aA titre of 16 is equivalent to 1 international unit.
tal, nine with convulsions. All of the latter group were febrile. The incidence of febrile convulsions during this two week period was three times that expected for children aged 10–18 months. We should remember that convulsions due to natural measles at this age are 7–10 times more frequent than noted above during vaccination (Miller, 1982). Furthermore, intensive surveillance following the use of $90 \times 10^6$ doses of live measles vaccine in the USA has established an encephalitis incidence as low as 1 per million doses of vaccine, as compared to an incidence of 1 per 1000 due to natural measles in a comparable situation.

The Centre for Disease Control in the USA has established a system to monitor adverse effects of measles vaccines and all illnesses occurring within 4 weeks of vaccination in the immunization projects have to be reported (Table 8.28). Unfortunately reports from the private sector in the USA are much less reliable and hence a complete assessment of risk is not possible. However, during 1979–80 there were 19 reported cases of neurological events (excluding cases of febrile seizures) within 30 days of immunization with live measles virus, and one patient died. Three of the cases had a diagnosis of encephalitis or encephalopathy including Reye syndrome and the persons were less than 10 years of age (approximating to 0.64 cases per $10^6$ doses of vaccine). Of course it is possible that some of the cases were only

| Types of adverse event                  | Number of adverse events by vaccine |
|----------------------------------------|-----------------------------------|
|                                        | MMR$^a$ | MR$^b$ | Measles | Total |
|                                        | '79 '80 | '79 '80 | '79 '80 | '79 '80 |
| Allergic reactions                     | 12 11   | 1 1    | 2 4     | 15 16 |
| Anaphylaxis                            | 2 1     | 0 0    | 0 0     | 2 1   |
| Arthritis and/or arthralgia            | 7 8     | 2 2    | 0 3     | 9 13  |
| Convulsions–febrile                    | 22 30   | 2 0    | 1 0     | 25 30 |
| Convulsions–non-febrile                | 3 1     | 2 0    | 1 1     | 6 2   |
| Encephalitis and/or encephalopathy     | 1 1     | 1 0    | 1 0     | 3 1   |
| Reye syndrome                          | 0 0     | 1 0    | 1 0     | 2 0   |
| Guillain–Barré syndrome (GBS)          | 2 1     | 1 0    | 0 0     | 3 1   |
| Paralysis–non-GBS                      | 0 1     | 0 0    | 1 0     | 1 1   |
| Sudden infant death syndrome           | 0 0     | 1 0    | 0 0     | 1 0   |
| Deaths from all causes                 | 1 2     | 1 0    | 2 0     | 4 2   |

$^a$ Measles-mumps-rubella vaccine.

$^b$ Measles-rubella vaccine.
coincidentally related to measles vaccination and may have been caused by other agents.

8.3.6. **Global eradication of measles**

From a technical point of view both measles and polio could follow smallpox and be eradicated world-wide by mass immunization campaigns. We have noted above the serious morbidity and also mortality caused by measles. Indeed, estimates of 900,000 deaths annually in underdeveloped countries caused by measles are accepted as reasonably accurate. Hinman (1982) has compared and contrasted measles and smallpox as regards several factors which are relevant to worldwide eradication (Table 8.29). Note that measles shares six of the favourable characteristics with smallpox. Three important differing factors are that smallpox was of lower infectivity than measles and so did not spread so rapidly through susceptible populations. Therefore smallpox was sometimes eradicated from areas where overall immunization rates were low. In fact, a containment policy was used whereby once a smallpox case was discovered all contacts in the immediate area were vaccinated (see Chapter 15). In contrast, we know that measles may still be transmitted in communities with immunization levels of > 90%, but on the other hand, it is not clear if measles can persist indefinitely in these conditions of strong immunity pressure. A crucial remaining factor which could strongly influence such a campaign is the amount of international support for a global immunization campaign against measles. The answer here may be ambiguous and is not helped by countries where immunization rates are low (Table 8.30).

A satisfactory result in the USA, USSR or DDR however, would most likely stimulate international interest and already, another country, Canada, is considering a national programme of measles eradication. It is very worthwhile then to analyze the experience in one of these countries (USA) which hopefully will be repeated.

| TABLE 8.29. | Factors affecting eradication of smallpox and measles |
|-------------|------------------------------------------------------|
| Factor      | Smallpox    | Measles    |
| Animal reservoir | No          | No         |
| Long-term carrier state | No          | No         |
| Obvious illness | Yes         | Yes        |
| Immunity from disease | Lifelong    | Lifelong   |
| Immunity from vaccine | Long-term   | Long-term  |
| Effectiveness of vaccine | High       | High       |
| Stability of vaccine | Stable      | Labile     |
| Evidence of immunity | Visible    | Not visible |
| Infectivity   | Moderate    | High       |
| Universal vaccination | Not essential | Probably essential |
TABLE 8.30.  
Current status of measles vaccination campaigns

| Countries with high rate of measles vaccinations | Countries with low or very low rates of measles vaccination |
|--------------------------------------------------|----------------------------------------------------------|
| USA (99% reduction in cases)                      | UK                                                       |
| Canada                                           | West Germany, Scandinavia                                |
| Mexico                                           | France                                                   |
| Costa Rica                                       | Belgium                                                  |
| Czechoslovakia                                   | Holland                                                  |
| Albania                                          | Italy                                                    |
| Yugoslavia                                       | Greece                                                   |
| USSR                                             | Central and South American and African countries other than those detailed opposite |
| China (in certain target provinces)               |                                                          |
| Cuba                                             |                                                          |
| Brazil (certain areas)                           |                                                          |

Note that most vaccines are prepared in chick embryo cell cultures except for the Yugoslavian vaccine which is prepared in human diploid cells, the Iranian AIK-C strain prepared in MRC-5 human diploid cells and the USSR Leningrad-16 vaccine prepared in quail embryo cells. Many virus strains are used, although most of these were derived from the Edmonston B strain of virus. The virus is most often administered i.m. but earlier experiments in the USSR using intranasal administration have been reevaluated by Sabin et al. (1983) with good (preliminary) results. (see also Leon de Coto, 1983, Rissi, 1983, Assaad, 1983, Rey et al., 1983.)

in other countries. We shall also briefly review current progress of vaccination campaigns in the USSR and Sweden.

8.3.7. ERADICATION OF MEASLES CAMPAIGN IN THE USA

More than 90% immunization has been achieved in DDR, Alaska and USA, but since more recent data is available from the latter country it will be used as an example.

In 1978 a goal was established to eliminate indigenous measles from the USA by October 1st 1982 and the major elements in the strategy were high immunization levels, effective surveillance and aggressive outbreak controls. Obviously this precise goal has not yet been achieved but it may very well be reached this year. Since live measles vaccine was licensed in 1963, approximately 130 million doses have been used in the USA, including 19 million doses of Edmonston B vaccine and 111 million doses of ‘more attenuated’ virus strains. Since the early seventies, combined measles, mumps and rubella vaccines have been used and in 1980, for example, 75% of measles vaccine used was in the combined formulation. (Similarly, in the USSR between 1967 and 1972, 30 million doses of measles vaccine were used). The general setting or background of this measles eradication campaign in the USA involved the ‘Childhood Immunization Initiative’ which had two goals:
1. ensuring that at least 90% of children in the USA received vaccine against preventable diseases of childhood such as measles, rubella, mumps, polio, diphtheria, tetanus and pertussis, since even in 1976 it was estimated that as many as 20 million children under 15 years lacked protection against some of these agents in the USA.

2. developing a permanent system to maintain this level of protection. A key factor in this campaign was identifying unvaccinated school children, and the medical records of 28 million school children were reviewed. To ensure that a high percentage of the population would continue to be protected, immunization requirements were enforced strictly as a condition for entering or attending school. By July 1979 all 50 states in the USA had laws requiring that children should have proof of immunity before entering school for the first time. In addition, 30 states required such proof for students at all levels from kindergarten to high school. Vigorous enforcement of school immunization laws seem to be the single most important factor in the immunization campaign, and students who do not provide documentary evidence of immunity to measles are excluded from school. In fact, experience has shown that in the successive school years 1978 – 1982, 93%, 94%, 96% and 96% of children respectively had been vaccinated against measles.

A constant pressure has had to be exerted to maintain the immunization programme as was shown in 1970–1971 when, because of the introduction of rubella vaccine, interest was diverted and measles cases increased from approximately 22,000 cases to 75,000 cases reported (Table 8.31). With increased public support, the figure was again forced down to 22,000 cases in 1974. However, it should be stressed that there is not universal agreement about the meaning behind these rises and falls. Sabin (1981), for example, attributes the periodic rise and fall in the number of reported measles cases from 1971 to 1977 to a build-up of susceptible persons, and not to a rise and fall in vaccinations. He suggests that if mass vaccination is carried out in a short time then the transmission of measles would be broken.

During the period 1960–81 reported measles deaths declined very significantly in the USA, from around 400 to less than 10 at present. Similarly, the number of reported cases of measles encephalitis has declined from around 330 in 1962 to around 3 at present. Since SSPE is considered to be a rare complication of natural measles, it was of prime importance to monitor cases which might have been caused by vaccine virus. However, since 1960 there has been a marked decline in reported SSPE cases in the USA. The estimated risk of SSPE following wild measles is much higher (6–22 cases per million measles cases) than the risk of SSPE following measles vaccine virus (0.48–1.13 per million measles vaccinations). In the UK, in contrast, 100,000 children a year suffer from measles, and 20 die.

8.3.8. Duration of vaccine immunity and vaccine failures

Immunity following administration of live measles vaccine appears to be long lasting, and in any case up to 10–14 years (Fig. 8.6). Even persons in whom antibody
TABLE 8.31.
Reported measles morbidity and mortality, United States, 1960–81 (from CDC Measles Surveillance Report, 1982)

| Year | Cases   | Cases per 100,000 population | Deaths  | Deaths per million population | Deaths per 1000 cases |
|------|---------|-------------------------------|---------|-------------------------------|-----------------------|
| 1960 | 441,703 | 245.4                         | 380     | 2.11                          | 0.860                 |
| 1961 | 423,919 | 231.7                         | 434     | 2.37                          | 1.023                 |
| 1962 | 481,530 | 259.2                         | 408     | 2.20                          | 0.847                 |
| 1963 | 385,156 | 204.3                         | 364     | 1.93                          | 0.945                 |
| 1964 | 458,083 | 239.7                         | 421     | 2.20                          | 0.919                 |
| 1965 | 261,904 | 135.3                         | 276     | 1.43                          | 1.054                 |
| 1966 | 204,136 | 104.4                         | 261     | 1.33                          | 1.279                 |
| 1967 | 62,705  | 31.8                          | 81      | 0.41                          | 1.292                 |
| 1968 | 22,231  | 11.1                          | 24      | 0.12                          | 1.080                 |
| 1969 | 25,826  | 12.8                          | 41      | 0.20                          | 1.588                 |
| 1970 | 47,351  | 23.2                          | 89      | 0.44                          | 1.880                 |
| 1971 | 75,290  | 36.5                          | 90      | 0.44                          | 1.195                 |
| 1972 | 32,275  | 15.5                          | 24      | 0.12                          | 0.744                 |
| 1973 | 26,690  | 12.7                          | 23      | 0.11                          | 0.862                 |
| 1974 | 22,094  | 10.5                          | 20      | 0.10                          | 0.905                 |
| 1975 | 24,374  | 11.4                          | 20      | 0.09                          | 0.821                 |
| 1976 | 41,126  | 19.2                          | 12      | 0.06                          | 0.292                 |
| 1977 | 57,345  | 26.5                          | 15      | 0.07                          | 0.262                 |
| 1978 | 26,871  | 12.3                          | 11      | 0.05                          | 0.409                 |
| 1979 | 13,597  | 6.2                           | —       | —                             | —                     |
| 1980 | 13,506  | 6.0                           | 6       | —                             | —                     |
| 1981 | 30,323  | 1.3                           | 2       | —                             | —                     |

Note that live attenuated measles vaccine was introduced in 1963.

is no longer detectable may still have immunological memory and so may be immune.

Vaccine failures result when patients do not serologically convert (primary failure) and when immunity is subsequently lost following a successful initial seroconversion (secondary failure). The primary failure rate in the USA is less than 5% and is attributed to impotent vaccine because of improper storage or handling or to interference with vaccine virus replication by pre-existing measles antibody. Secondary failure following live measles vaccine has not been demonstrated conclusively.

Measles importations are geographically widespread in the USA and a rising proportion of imported cases has occurred among US citizens returning from overseas travel. Between 1980–81, 209 cases of measles were reported to have been imported, representing 1.3% of the total measles cases during that period.

Fig. 8.5. Clinical appearance of measles. a. quite severe rash and respiratory symptoms; b. milder measles case. (courtesy of the late Dr. W.C. Marshall, Great Ormond Street Hospital.)
The increasing proportion of measles cases in young adults in recent years (Table 8.32) has resulted in the initiation of vaccine programmes for adults, including military camps. Fortunately young adults do not appear to have an increased risk of serious illness from measles vaccine virus. Measles incidence in the armed forces in the US has dropped sharply as a result of a measles immunization programme initiated in 1980. Vaccination certificates are required for attendance at military schools and day-care centres, and all teachers have to be vaccinated or have proof of immunity.

Epidemiologists agree that complete eradication of indigenous measles will eventually have to be achieved through herd immunity and this might be achieved successfully by routinely giving a second dose of measles vaccine to all individuals particularly in cities or areas where there are many immigrants. Although the current nationwide vaccination rates are high in the USA (and the envy of many other
countries, and with accrued social and medical benefits) nevertheless they are not always above levels estimated to be necessary for herd immunity. When local herd immunity is achieved the vaccination programme still has to be continued indefinitely to prevent outbreaks of imported cases.

8.3.9. Mass measles vaccination in the USSR

Mass vaccination against measles virus was started in the USSR in 1967, although some republics had started as early as 1963–64 (Burgasov et al., 1973). The attenuated measles virus Leningrad-16 has been used in this campaign (Peradze and Smorodintsev, 1983). In the years before vaccination, a high morbidity due to measles was noted in the USSR, with an average incidence of 827 cases per 100,000 population. As the scale of vaccination increased a steady decline in morbidity followed and as early as 1969 measles incidence had dropped 4-fold. Serological analysis showed that virus induced immunity persisted for at least 7 years. Thus, of 4793 children’s sera analyzed over a period of 7 years at the end of the first year 88.2% of children had antibody and in subsequent years only a small proportion of children (2.5–6.4%) appeared to have lost antibodies. Actual antibody titres remained relatively constant over this period, however. A further point noted was that although no antibodies were detected in the blood of some vaccinated children, nevertheless they were resistant to actual infection. The authors estimated that during the 5 years of vaccination the USSR had saved more than $244 million dollars and the saving in the use of measles gamma globulin alone repaid all the expenses connected with the cost of the vaccine and its administration.

8.3.10. Measles eradication campaign in Sweden

General vaccination with a combined measles, mumps and rubella vaccine was introduced in Sweden in 1982 (see Christenson et al., 1983). The new immunization schedule comprises two vaccine injections, given at 18 months and 12 years of age, respectively (see Chapter 2). Vaccinating at 18 months only, was abandoned because of the risk of increasing numbers of people remaining unprotected among those left unvaccinated, or who had failed to seroconvert after the first inoculation. This has indeed occurred in the USA (see above). The aim of the new strategy is rapid elimination of all three diseases. A controlled field study was carried out in 150 children aged 18 months using two different batches of the vaccine. Seroconversion was seen in 99% against measles. Fever and rash as side effects of the vaccines were recorded five to 12 days after vaccination. Moderate fever (38.5–39.4 °C) was observed in 22 children, high fever (≥39.5 °C) in 33, and rash in 35. Even with high fever most of the children were not particularly ill, or fretful. Preliminary results obtained by follow up of routinely vaccinated schoolchildren aged 12 indicated a considerably lower rate of fever and rash during the postvaccination period, occurring in 3–10% of cases only.
The least costly way to deliver virus vaccines to the patient is in combination (Recommendation MMWR, 1972). For this approach to be successful there must be no interference between the viruses, the immunity for each virus should persist to the same extent as it would following administration of the virus alone (Weibel et al., 1972) and clinical reactions must be low or nonexistent. Combinations of live vaccines have been developed and used, particularly in the USA and USSR but are not used so extensively in the UK or the rest of Europe at present (Buynak et al., 1969, Stokes et al., 1971, Villarejos et al., 1971, Smorodintsev et al., 1970, Schiff, 1980, Sabin, 1981, Weibel et al., 1971). The serological conversion rate to four different combined virus vaccine was 94–99% (Hilleman et al., 1973) and, moreover, the quantity of antibody induced is comparable to the viruses given alone. The virus used is of great importance, since when a less attenuated measles vaccine was combined with the mumps virus, a lowered response to mumps was detected (Hilleman et al., 1973). Perhaps not unexpectedly, most of the fevers noted as side reactions were caused by the measles component.

The death-to-case ratio for infants less than one year of age is many times greater than it is for older children. The other factor leading to high mortality from measles in developing areas is malnutrition (Walsh, 1983). Severely malnourished children suffer more complications and are more likely to die than their better-nourished peers. During the Nigerian Civil War, observations made at a time of severe famine revealed that $\leq 15\%$ of the children infected with measles died, and 50% of those in a medical centre for treatment of kwashiorkor died (Smith and Foster, 1970). In India, among children hospitalized with measles 89% of those poorly nourished had more than one complication (e.g., pneumonia, otitis, stomatitis, diarrhoea) while only 20% of the well-nourished had more than one complication (Chen et al., 1980).

Measles in malnourished children differs markedly from the disease as usually seen in the United States and Europe. The rash becomes dark red-purple and exfoliates extensively, exposing large areas of epithelial surface to bacterial invasion. Se-
vere changes occur in the mouth, tracheal-bronchial tree, and intestine; extreme soreness of the mouth (stomatitis) prevents the child from eating or drinking; bronchopneumonia, persistent diarrhoea and protein-losing enteropathy then ensue (Axton, 1975). Xerophthalmia that results in blindness occurs in areas of the world where diets are deficient in vitamin A. In these areas, more than half of the incidence of blindness may be attributable to measles (Walsh, 1983). Viral excretion persists much longer in poorly nourished patients (12 days, rather than three days as in those well-nourished). The persistence of virus in lymphocytes and intestinal cells suggests that there is a delay in production of lymphocytes that are competent to destroy these cells. As a result, the virus multiplies for a longer period, more cells are infected, and the disease is more severe. In addition, the depressed cellular immune system increases the patient's susceptibility to secondary infection (Dossetor et al., 1977). Bronchopneumonia and diarrhoea are the most common causes of death from measles.

The heat lability of the measles vaccine greatly complicates efforts to distribute it in tropical climates. The freeze-dried vaccine has to be held at 2–8°C until minutes before injection and unless this temperature range is maintained, the vaccine rapidly loses its potency. However, in the last few years, new stabilizers have been developed that retard the heat inactivation (Heymann et al., 1979). In the presence of these stabilizers, titres of the attenuated virus remain adequate ($10^3$ median tissue culture-infective doses per dose) for up to three weeks at 37°C and up to six months at 20–25°C in the laboratory (McAleer et al., 1980). In field trials, >85% of children nine months of age or older seroconverted after receiving vaccine that was stored at 23–25°C for seven to eight days (Heymann et al., 1979).

The final problem in providing effective immunization for children in developing countries is reaching them during the brief interval between the loss of maternal antibodies and the acquisition of natural disease. As soon as maternal antibodies wane, the infants begin to acquire the disease. Ideally, each child should be vaccinated as soon as transplacental immunity fades and as soon as his/her immune system can adequately respond to the vaccine. At six months of age, at least 20% of children will have maternal antibodies, and in these infants, the vaccine virus will not multiply and no protection will be achieved. After immunization, only 60%–80% will seroconvert and develop protective immunity. However, in developing countries, 15%–20% of children may already have had measles if immunization is delayed until the age of nine months (Walsh, 1983, Medical Research Centre, Nairobi, 1982).

Measles vaccination is part of the World Health Organization Expanded Programme on Immunization. Vaccines against diphtheria, pertussis, tetanus, poliomyelitis, and other infections can be administered simultaneously with measles vaccine once a reliable and comprehensive delivery system is available. Prophylaxis for malaria and oral rehydration as well as health education are a few of the other measures that could easily be combined with measles immunization efforts (Walsh,
1983). At present rates of immunization of children against measles in South-east Asia are very low (0.15% of children immunized) compared to 63% in Europe. Recent observations in the United Republic of Cameroon support the WHO recommendations of one dose measles vaccination in tropical Africa at 9 months minimum age. Measles control activities in the capital city began in 1966 and mass vaccination campaigns were held every 2 years among children aged 6–36 months. However, in 1973 despite a sample survey showing that 78% of children in the city had been vaccinated there were over 4000 cases of measles among the under 12-months-old children. It was deduced that most of the vaccine given had been ineffective because of pre-existing immunity from the mother and from the use of impotent vaccine because of storage problems. Between 1975–79 new storage facilities were introduced and also the new WHO policy of vaccination at 9 months. By 1979 a 44% decrease in reported measles attack rates among children under 9 months of age was reported (Heymann et al., 1983).

8.3.13. MEASLES IMMUNIZATION USING AEROSOLS

Early experiments in the USSR (Terskikh et al., 1971) and Japan (Okuno et al., 1965) indicated that administration of aerosols of live measles vaccines could result in sero-conversion, even under circumstances of partial immunity which prevented sero-conversion by the same vaccine injected intramuscularly. Sabin et al. (1983) have adapted a simple aerosol apparatus that would be suitable for use in third world countries by large numbers of non-professional personnel.

Inhalation of undiluted, aerosolized measles vaccine was immunogenic in 100% of 4- to 6-month-old and older children with and without residual maternal antibody when the human diploid cell (HDC) vaccine containing the Ikic (Edmonston-Zagreb) strain and 1% human albumin was used. Prevaccination, residual, placently-transmitted, plaque-neutralizing antibody that can prevent an immune response after subcutaneous injection of measles vaccine, did not prevent an immune response after inhalation of aerosolized vaccine. There were no immediate clinical reactions in the 160 children who inhaled the aerosolized vaccines, and no significant subsequent reactions among the 96 children who were successfully immunized. There were no contact infections.

This compares very favourably, for example, with a 1974–1981 study in Nairobi, Kenya, which showed measles vaccine failures in 85%, 65%, and 48% of infants vaccinated subcutaneously at 4, 5 and 6 months of age, respectively (MRC, 1982). A study in South America showed failure of seroconversion in 42%, 31%, and 18% of infants 6, 7, and 8 months of age, respectively, after subcutaneous injection of measles vaccine. In the Sabin study (1983), it was found that inhalation of the aerosolized HDC vaccine, containing the Ikic (Edmonston-Zagreb) plaque-purified strain of measles virus (Ikic et al., 1968), further attenuated in HDC, and 1% human albumin, immunized all of 39 infants 4 to 6 months of age and all of 21 children
12 to 24 months of age without reference to the amount of maternal antibody measured before vaccination. Sero-conversion was evident at six weeks after vaccination in all but four infants, 4 and 5 months of age, with residual maternal antibody titres of 130 to 512, in whom sero-conversion became evident only in the blood obtained six months after vaccination. However, a rather worrying observation was that another measles vaccine (but cultivated in CEF cells) did not achieve 100% sero-conversion when given by aerosol, even although it contained ten times more virus (but no added protein). Further studies are required to determine whether the human diploid vaccine was intrinsically more immunogenic or whether the 1% human albumin stabilized the virus during the aerosol infection.

8.3.14. CHEMOPROPHYLAXIS AND THERAPY OF MEASLES INFECTIONS

In general it can be stated that the practical and economic success of the measles vaccine (Table 8.33) has reduced the need for effective prophylactic agents. Nevertheless such compounds, if cheaply produced, could be useful in third world countries or in developed countries in special groups of children at high risk but who have not been immunized. Measles virus is inhibited in vitro by the nucleoside analogue ribavirin (Table 8.34) and two small uncontrolled clinical trials have shown the compound administered orally at 10 mg/kg for 7 days at the rash stage to reduce the severity of symptoms (Fernandez, 1980).

Early studies of certain carbobenzoxy tripeptides (carbobenzoxy-D-phe-L-phe-nitro-L-arg) by Norrby (1971) showed that 1 µg/ml was sufficient to inhibit penetration of measles virus into new cells. Cell fusion and haemolytic properties of the virus were also inhibited but no effect was noted on virus adsorption. One hundred-fold higher concentrations showed inhibition effects on mumps virus, but no anti-

| Number |
|---------|
| Cases averted | 48420000 |
| Lives saved | 4840 |
| Cases of mental retardation averted | 16100 |
| Additional years of normal and productive life by preventing premature death and retardation | 1439000 |
| School days saved | 159309000 |
| Physician visits saved | 24880000 |
| Hospital days saved | 2762000 |
| Net benefits achieved | $4448000000 |

(See Chapter 2 for a more extensive discussion of economic benefits of vaccination.)
viral effects were detected against Sendai or RSV. The possible biochemical basis of the antiviral action was not appreciated at the time but re-evaluation of the general approach by Choppin et al. (1983) has highlighted what could be an interesting point of action of antivirals. Choppin et al. (1983) noted the similarity in N-terminal sequences of the F2 polypeptide between paramyxoviruses and many of the short peptides which had been shown earlier to be active virus inhibitors in vitro. Therefore, it was proposed that the fusion activity of F protein might be inhibited by oligopeptides which mimicked this region of the protein. It was found that oligopeptides with the appropriate amino acid sequence were highly effective specific inhibitors of virus infectivity at the level of virus penetration and of virus induced fusion and haemolysis (i.e., activities that reflect the membrane fusing activity of the F protein). Oligopeptides were found which inhibited SV5, Sendai, canine distemper virus as well as measles (Table 8.35). However, measles virus was much more inhibited than the other viruses.

From such studies, several conclusions have been drawn. In general terms it was noted that the oligopeptides with the correct amino acid sequence were specific inhibitors, and the longer the peptide, the more active they were. The most effective peptide tested was a heptapeptide with the sequence of the Sendai virus F1 N-terminus and a 50% effective concentration of 0.02 μM. A carbobenzyoxy (Z) group on the N-terminal amino acid increased activity as compared to the same oligopeptide with an unblocked N-terminal residue. Other hydrophobic additions also increased inhibitory activity, such as a dansyl (DNS) or a t-butyloxycarbonyl (t-BOC) group. The steric configuration of the N-terminal phenylalanine also significantly affected activity, e.g., Z-D-Phe-L-Gly was more active than Z-L-Phe-L-Phe-Gly and finally

| Ribavirin concentration (μg/ml) | CPE inhibition (%) | Cytotoxicity |
|-------------------------------|-------------------|-------------|
| 1000                          | 100               | +           |
| 320                           | 100               | +           |
| 100                           | 100               | -           |
| 32                            | 94                | -           |
| 10                            | 54                | -           |
| 3.2                           | 50                | -           |
| 1.0                           | 34                | -           |
| 0.32                          | 29                | -           |
| 0.1                           | 26                | -           |
| 0.032                         | 21                | -           |
| 0.01                          | 20                | -           |
| 0.0032                        | 15                | -           |
| 0.001                         | 0                 | -           |
TABLE 8.35.
Inhibition by oligopeptides of plaque formation by paramyxoviruses and myxoviruses (after Choppin et al., 1983)

| Virus                  | Peptide                                                                 | 50% effective concentration (μM) |
|------------------------|-------------------------------------------------------------------------|----------------------------------|
| Measles                | Z-d-Phe-L-Phe-Gly-d-Ala-d-Val-d-Ile-Gly                                   | 0.02                             |
|                        | Z-d-Phe-L-Phe-Gly                                                       | 0.20                             |
|                        | Z-d-Phe-L-Phe-L-(NO₂)Arg                                               | 0.20                             |
|                        | Z-d-Phe-L-Phe-Gly(chloromethylketone)                                   | 0.20                             |
|                        | Z-d-Phe-L-Phe-L-Tyr                                                    | 0.20                             |
|                        | Z-d-Phe-L-Phe-L-(Azido-Phe)                                            | 0.28                             |
|                        | DNS-o-Phe-L-Phe-Gly                                                    | 0.34                             |
|                        | Z-d-Phe-L-(pBr)Phe-Gly                                                 | 0.52                             |
|                        | t-BOC-o-Phe-L-Phe-Gly                                                  | 2.0                              |
|                        | Z-d-Phe-L-Tyr-Gly                                                      | 9.3                              |
|                        | Z-d-Phe-d-Phe-Gly                                                     | 10                               |
|                        | Z-d-Phe-L-Phe-Gly(methyl ester)                                        | 20                               |
|                        | Z-d-Phe-L-(Benzy1)Tyr-Gly                                              | 20                               |
|                        | Z-L-Phe-L-Phe-Gly                                                      | 23                               |
|                        | -d-Phe-L-Phe-Gly                                                       | 130                              |
|                        | Ac-d-Phe-L-Phe-Gly                                                     | 20                               |
|                        | Z-Gly-L-Phe-L-Phe-Gly                                                  | 870                              |
| Sendai                 | Z-d-Phe-L-Phe-Gly                                                      | 320                              |
|                        | Z-d-Phe-L-Phe-L-(NO₂)Arg                                               | 540                              |
|                        | Z-Gly-L-Phe-L-Phe-Gly                                                  | >1000                            |
| SV5                    | Z-d-Phe-L-Phe-Gly                                                      | 320                              |
|                        | Z-d-Phe-L-Phe-L-(NO₂)Arg                                               | 500                              |
| Measles mutant R93     | Z-d-Phe-L-Phe-Gly                                                      | 20                               |
|                        | Z-d-Phe-L-Phe-L-(NO₂)Arg                                               | >1000                            |
| Canine distemper       | Z-d-Phe-L-Phe-Gly                                                      | 1.50                             |
|                        | Z-d-Phe-L-Phe-L-(NO₂)Arg                                               | >1000                            |
| Influenza A (WSN)      | Z-Gly-L-Leu-L-Phe-Gly                                                  | 20                               |
|                        | Z-Gly-d-Phe-L-Phe-Gly                                                  | 23                               |
|                        | Z-Gly-L-Phe-L-Phe-Gly                                                  | 53                               |
|                        | Z-d-Phe-L-Phe-Gly                                                      | 290                              |

Z denotes a carbobenzoxy group; DNS, a dansyl group; t-BOC, a tertiary butyloxy group; and Ac, an acetyl group.

Esterification of the C-terminal amino acid decreased activity. The effects of the substitutions at the termini of the oligopeptides may be related to the positioning of the inhibitor at the site of action. Thus, the carbobenzoxy or dansyl groups add hydrophobicity to the N-terminus, whereas esterification of the C-terminus decreases the polarity of the peptide, and such changes could affect the orientation of the peptide. The peptide may also be protected from proteolytic activity.

The specificity of the oligopeptide inhibitors and the importance of the correct
TABLE 8.36.
Inhibition of wild type and mutant measles viruses by oligopeptides (after Choppin et al., 1983)

| Peptide                | 50% effective concentration (μM) |
|------------------------|----------------------------------|
|                        | wt         | mutant    |
| Z-D-Phe-L-(NO₂)Arg     | 0.2        | >1000     |
| Z-D-Phe-L-Phe-Gly      | 0.2        | 21        |

An amino acid sequence was demonstrated by the selection of a mutant of measles virus that was resistant to the action of a tripeptide (Z-D-Phe-L-Phe-L-(NO₂)Arg) by repeated passage of the virus in the presence of this peptide. Although the mutant became resistant to this peptide, it remained relatively sensitive to Z-D-Phe-L-Phe-Gly, which differed only in the third amino acid. (Table 8.36).

The oligopeptides did not appear to have significant toxic effects on the cells used in these studies and cells survived for days with no detectable cytopathic changes, and multiplied normally in the presence of the peptides. Preliminary experiments also suggest the oligopeptides are not highly toxic in mice. However, a problem with the compounds is their relative insolubility and this factor makes precise quantitation of antiviral effects difficult. To investigate their site of action, oligopeptides have been synthesized with radioactive or fluorescent (dansyl) labels to monitor their site of binding. Radioactively-labelled oligopeptides were added to purified virus, to mock infected cells, or to infected cells, and after washing, specific binding was calculated. Such studies suggested that the oligopeptides bind to the cell and not to the virus.

8.3.15. SUMMARY

Attenuated measles vaccines have been developed empirically by selection of 'host range' mutants, and are widely and successfully used throughout the world. Using the vaccine, some countries may soon eliminate measles as an endogenous virus but continued problems are anticipated, particularly in adults with viruses re-introduced by visitors from abroad. Basic studies on new antivirals are continuing (particularly with oligopeptides) but antiviral compounds are unlikely to have extended use in the clinic, except perhaps in tropical areas where the disease may be life threatening. However, a vaccination programme in these areas is preferable, and is an urgent need.
8.4. Parainfluenza viruses

8.4.1. Parainfluenza viruses types I–IV

These respiratory viruses are worldwide in their distribution and affect all age groups (Hamre et al., 1961, McLean et al., 1961, Parrott et al., 1959, Kapikian et al., 1960, Smorodintsev, 1962, Chanock et al., 1958, 1959, Andrewes et al., 1959, Bloom et al., 1961, Kelen and McLeod, 1977, Tyrrell et al., 1959, Birkum-Peterson, 1958). A feature of the epidemiology of these viruses is that re-infections are common, although they may often be subclinical. These subclinical infections maintain a huge and active reservoir of infective virus. The viruses are transmitted by airborne droplets and particularly rapid spread is noted in institutionalized children. It has been estimated that parainfluenza viruses constitute up to one third of all respiratory tract infections of humans and 40% of respiratory infection in preschool children and infants.

8.4.2. Clinical aspects

The incubation period varies from 2–4 days in children and 3–6 days in adults. In pre-school children, particularly, a severe respiratory infection often develops and types I and II are most often associated with laryngo tracheobronchitis (croup), whereas types III and IV cause infection of the lower respiratory tract such as bronchitis and pneumonia. When the lower respiratory tract is involved bronchospasms are a predominant clinical sign.

In adults hoarseness is often the main symptom and, in general, all four virus types cause a range of respiratory illness.

In a recent study in Chapel Hill (USA) involving a study of 7000 cases of lower respiratory tract infection in a pediatric practice 18%, 4% and 15% of virus isolates were parainfluenza I, II and III respectively (summarized by Tyeryar, 1983). Of children from whom parainfluenza virus type I and II was isolated, almost 60% had croup. Parainfluenza type III was closely associated with tracheobronchitis. The risk for hospitalization for illnesses associated with parainfluenza type III was much less than for RSV, and the risk for infection with the former virus during the first four months of life was inversely related to the level of neutralizing antibody measured in the cord serum at birth. Interestingly, patterns of antibody to haemagglutinin and to neuraminidase correlated with antibody patterns by viral neutralization assays. The ability of the assay to detect rises of antibody levels to fusion (F) protein resulted in serological recognition of infection not discernible by antibody responses to HN. Primary infections produced greater antibody rises to HN than to F protein, but peak titres to HN were characteristically delayed up to 10 months.

Secondary infections with parainfluenza virus type III infrequently provided any further immunological responses to the HN protein. The data suggested that a sec-
ondary infection was important for stimulation of antibody to F protein and, in some cases, the rise in level of antibody to F protein was the only marker of secondary infection.

8.4.3. Virus structure and replication
These viruses are typical paramyxoviruses and have a replicative strategy similar to that already described for negative strand viruses such as measles (q.v.).

8.4.4. Prevention of parainfluenza virus infections using vaccines
It might be an obvious deduction from the above comments on natural re-infection in children with parainfluenza viruses that effective vaccines would be difficult to produce. Indeed, earlier studies of experimental vaccines showed that immunized children, when later encountering a wild virulent parainfluenza virus, could still have a severe infection. This again indicates the absence of knowledge about important antigenic determinants on the virus and, in particular, the absence of data on the function of the fusion (F) glycoprotein of these viruses. As with inactivated measles and RSV vaccines, with experimental parainfluenza vaccines the inactivation process during vaccine production very probably inactivated the F protein, and so although some neutralizing antibodies were produced nevertheless, in the absence of antibodies to F protein, immunized children were subsequently not protected against infection.

Fulginiti et al. (1967a, b, 1969) described a vaccine trial in children with parainfluenza virus types I, II and III. The vaccine was prepared by formalin treatment of virus harvested from the amniotic cavity of embryonated hens' eggs. 537 children were immunized and ranged in age from 6 months to 6 years. The antibody response to immunization is summarized in Table 8.37 and in general was satisfactory since most seronegative children developed HI or neutralizing antibodies. Parainfluenza virus infection occurred in the community in the months following immunization and so the protective efficacy of the vaccine was assessed, but no evidence of protection could be established. Indeed, attack rates for subsequent parainfluenza illness were rather higher in the vaccinated group than the control groups (Table 8.38).

An innovative approach to a parainfluenza type III vaccine has been to isolate F and HN glycoproteins in their native antigenic forms and to assemble them into a multivalent structure (reviewed by Tyeryar, 1983). The virus is disrupted with the non-ionic detergent octylglucoside, the glycoproteins separated by ultracentrifugation and the detergent dialysed off, when the glycoproteins reassemble.
TABLE 8.37.
Summary of fourfold or greater parainfluenza HI rise in parainfluenza vaccinees (TPV) compared to controlsa (after Fulginiti et al., 1969)

| Pre-vaccine III antibody status | >4 × antibody rise 30 days post 3rd dose of vaccine | Para 1 No./Total (%) | Para 2 No./Total (%) | Para 3 No./Total (%) |
|-------------------------------|-----------------------------------------------------|----------------------|----------------------|----------------------|
| Seronegative (<1:8)           |                                                     |                      |                      |                      |
| TPV                           |                                                     | 47/47 (100)          | 38/43 (88)           | 10/11 (91)           |
| Controla                      |                                                     | 4/35 (11)            | 11/27 (40)           | 0/6 (0)              |
| Seropositive (≥1:8)           |                                                     |                      |                      |                      |
| TPV                           |                                                     | 10/34 (29)           | 12/38 (32)           | 20/70 (29)           |
| Controla                      |                                                     | 0/18 (0)             | 2/26 (8)             | 5/47 (11)            |
| Totals                         |                                                     | 57/81 (70)           | 40/81 (40)           | 30/81 (37)           |

a Controls are RS virus vaccine recipients.

TABLE 8.38.
Attack rates for hospitalized illness due to parainfluenza viruses (after Fulginiti et al., 1969)

| Vaccine group                  | No. with para illness | No. with croup | No. virus isolated | No. >4 × rise antibody | Attack rate for para illness per 100 at risk |
|-------------------------------|-----------------------|----------------|--------------------|-------------------------|---------------------------------------------|
| TPV                           | 6                     | 7              | P-3 0/1            | 1/1                     | 1.1 (6/567)                                |
|                               |                       |                | P-2 1/3            | 3/3                     |                                            |
|                               |                       |                | P-1 2/2            | 2/2                     |                                            |
| Control (RSV)                 | 2                     | 1              | P-3 0/1            | 1/1                     | 0.43 (2/464)                              |
|                               |                       |                | P-1 0/1            | 1/1                     |                                            |
| Control (non-vaccinee)        | 3                     | 2              | P-3 1/1            | 1/1                     | 0.3 (3/1001)                              |
|                               |                       |                | P-2 0/1            | 1/1                     |                                            |
|                               |                       |                | P-1 1/1            | 1/1                     |                                            |

TPV, trivalent parainfluenza vaccine; P, parainfluenza type.

8.4.5. CHEMOPROPHYLAXIS OF PARAINFLUENZA VIRUSES

Amantadine was shown in earlier studies (Davies et al., 1964) to have some mild inhibitory effect against RSV, parainfluenza types II and III and Sendai virus, although this was not equivalent to its marked inhibitory effects against influenza (Chapter 7).

Thirty adult volunteers were given 100 mg capsules of amantadine and placebo
twice daily for 12 days. On the fourth day the men were challenged intranasally with 10^4.5 TCCD50 of parainfluenza virus (Smith et al., 1967). A mild upper respiratory tract infection developed in 17 of the 30 men and no difference in the number developing illness was detected between the two groups. Also no difference in severity or duration of illness or virus isolation rate was detected. Finally, the serum antibody titres were not different in the two groups and the authors concluded that the compound was not active, with the reservation that a large virus challenge dose was used. Nevertheless, the concentrations of drug detected in the nasal wash (approx. 0.3 μg/ml allowing for dilution) would not be high enough to inhibit replication of parainfluenza viruses (based on in vitro data), although they would be high enough to inhibit replication of influenza A viruses.

In vitro, parainfluenza viruses are inhibited by certain benzimidazole compounds (Bucknall, 1967) a biguanide also has some virus inhibitory effect in cell cultures (Tobita, 1968), and ribavirin inhibits Sendai virus in vivo (page 315).

More extensive studies for new antivirals against parainfluenza viruses are urgently required and could probably be initiated now with good prospects for success.

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