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CHAPTER 9

Infections caused by rubella, reoviridae, retro, Norwalk and coronaviruses

A number of RNA-containing viruses are brought together in this Chapter rather as 'leftovers' either because they fit uneasily into the existing classifying schemes or because we felt, like rubella, they deserve more attention than being submerged into the (non-arbovirus) Togavirus group! They form a fascinating chapter of infective agents both for the clinician and the virologist.

9.1. Rubella (German measles) infections

Although rubella is a common disease of childhood it is rather benign and probably would not have been considered even worthy of thought for vaccine had it not been for the dramatic discovery by Gregg (1941) in Australia of the teratogenic effect of the virus. In a classic retrospective study, initiated by his observation of an unusual number of cases of congenital cataract in Sydney, he linked this observation with an outbreak of rubella which had occurred 9 months previously. The case histories of 78 infants with congenital cataracts showed that 68 of their mothers had suffered from rubella in the first trimester of pregnancy. Later prospective studies worldwide, established that a syndrome of defects resulted from this intrauterine infection (congenital rubella syndrome) but that a triad of defects were most commonly detected in the infants: cataracts, cardiac deformities (causing cyanosis) and deafness. The so-called 'expanded rubella syndrome' (established by precise virological diagnosis in the mid-1960s) includes, additionally, low birth weight and failure to thrive, purpura, anaemia, brain changes, jaundice, dental defects and neurological manifestations (Table 9.1). Congenital rubella, therefore, often presents

TABLE 9.1.
Frequently encountered signs of congenital rubella

Ophthalmic defects
Cataracts
Glaucoma
Retinal cloudiness
Central nervous system defects
Sensorineural deafness
Speech defects
Mental retardation
Microcephaly
Cerebral calcification
Cardiovascular system
Persistent patent ductus arteriosus
Intraventricular septal defect
Haemopoietic system
Anaemia
Leucopenia
Thrombocytopenic purpura
Persistent lymphadenopathy
Bony system
Osseous malformation metaphyses of long bones
Miscellaneous
Intrauterine and postnatal growth retardation
Recurrent infections

For additional details see: Avery et al., 1965, Bellani et al., 1965, Cooper et al., 1965, Erickson, 1944, Hambridge et al., 1966, Horstmann et al., 1965, Keir, 1965, Lundström and Boström, 1958, Lundström et al., 1962, Manson et al., 1960, Menser et al., 1967.

as a complex clinical picture. If the virus infects the foetus during the process of organogenesis, in the first 3–4 months of pregnancy, then it is extremely likely that the infant will be born with gross defects. Nevertheless, some infants escape developmental defects but show other signs of infection, such as low birth weight or hepatosplenomegaly. The virus often persists in foetal tissues throughout gestation and can be recovered from the infant after birth (Menser et al., 1967, Monif et al., 1965). In the latter group, virus can be easily isolated from the throat, csf, urine and faeces and liver for up to 3 months after birth. These rubella syndrome children can, therefore, be an important infectious source for pregnant women. Prospective studies have established that when a mother is infected in the first 4 weeks of pregnancy a minimal figure of 60% of the infants have congenital defects, but other authorities would suggest a higher figure, approaching 90%. The incidence of congenital defects (principally deafness) resulting from intrauterine infection in the first 4 months of pregnancy has been estimated at between 1.5 and 50% (reviewed by Banatvala and Best, 1984). It should also be remembered that rubella infection of the

mother may result in spontaneous abortion in up to 20% of cases, usually when the infection occurs in the first eight weeks.

9.1.1. The virus and mode of replication

Rubella virus is a positive-stranded RNA virus, 40–70 nm in diameter with a lipoprotein envelope. It has been classified as a non-arthropod-borne togavirus and has been placed by itself in the genus Rubivirus. No antigenic relationship has been shown, for example, between rubella and more than 200 alpha and flaviviruses, and no cross reactions are detected with pestiviruses or equine arteritis virus (Brinton, 1980). The nucleocapsid is 30–40 nm in diameter and contains a single molecule of RNA which is infectious when extracted under appropriate conditions. The viral envelope is acquired by budding from the host cell. The pleomorphic character of the virus particle is presumably due to the non-rigid character of the lipid envelope; elliptical and oblong virus particles and particles bearing finger-like protrusions have been described (Fig. 9.1). The envelope bears poorly defined 5–6 nm surface spikes, presumably composed of viral glycoproteins, which carry the pH-dependent HA activity. At least four (see below) major polypeptides have been described, three glycoproteins associated with the envelope (E1, E2a and E2b), and a non-glycosylated core protein (C) which may represent viral structural proteins, precursor polypeptides, or host proteins which have remained associated with the purified virus (Oker-Blom et al., 1983, Alstyne et al., 1981, summarized in Table 9.2). The three glycoproteins have approximate molecular weights of 58K, 47K, and 42K respectively, whilst the molecular weight of the C protein is 37K. This would imply that the structural genes should have a coding capacity of 116K. Subgenomic 24S RNA species have been detected in virus infected cells (Oker-Blom et al., 1983) and, thus, rubella may be similar in this regard to the alphaviruses where a 26S mRNA encodes the 130K precursor of the structural proteins. It is quite likely that only three genes are required to code for the virus proteins, since E2a and E2b are very similar in their tryptic maps.

9.1.2. Clinical rubella in children and young adults

The attack is usually mild and is characterized by a 3 day rash, a few swollen and perhaps slightly tender lymph nodes, a slight temperature rise and some scarcely noticeable malaise. Christie (1980) summarizes it as a trifling ailment causing less inconvenience to the patient than the common cold. In as many cases again no rash is apparent at all and a subclinical infection proceeds silently. Regardless of the severity of rubella in the adult, the foetus can be infected, resulting in congenital infection. The incubation period for rubella is 14–16 days. The rash first appears on the face around the mouth and behind the ears. On the trunk, spots are at first discrete, about the size of a pin head. If the rash progresses then it spreads rapidly to cover

the arms and legs as well. Another tendency with a rubella rash is for it to come and go in an hour or so. Persons are most infectious for a few days before the rash appears and transmission is by virus aerosol and inhalation to the upper respiratory tract.

The most common complication of rubella in the adult is arthritis and arthralgia

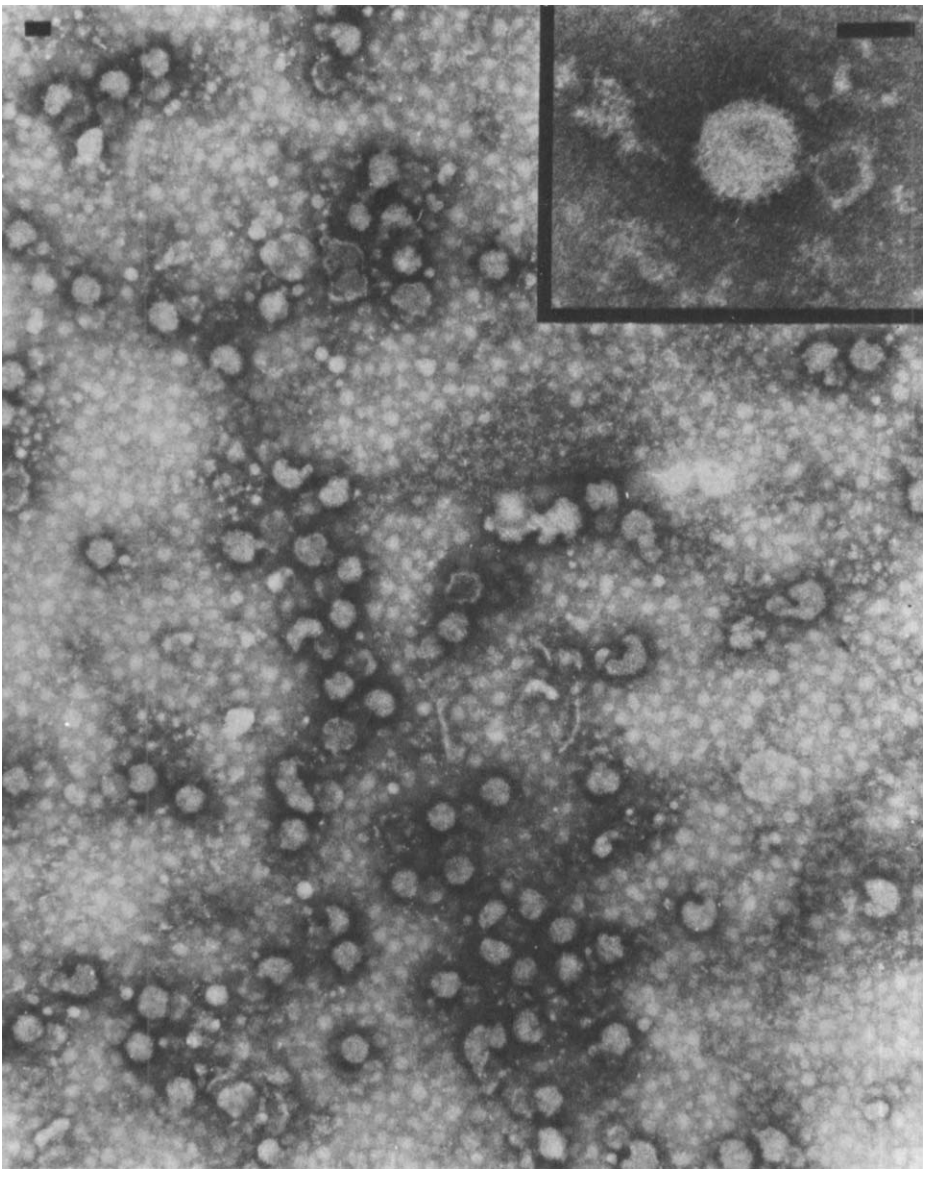

Fig. 9.1. Electron micrograph of rubella virus (kindly supplied by Dr I Chrystie, St Thomas Hospital).

TABLE 9.2.
Biochemical properties of rubella virus (after Banatvala and Best, 1984)

Virus Particle:	Diameter	40–70 nm			
	Buoyant Density	in sucrose	1.16–1.19 g/ml		
		in $CsCl_2$	1.20–1.23 g/ml		
	Sedimentation coefficient	240S (range 240–350S)			
Nucleocapsid:	Diameter	30–40 nm			
	Symmetry	Icosahedral			
	Sedimentation coefficient	150S			
	M.W.	$2.6–4.0 \times 10^6$			
Nucleic acid:		Single strand of RNA			
	Buoyant Density	1.634 g/ml			
	Sedimentation coefficient	38–40S			
	M.W.	$3.2–3.5 \times 10^6$			
Length of surface glycoprotein spikes		5–6 nm			
Chemical composition of virions		RNA	2.4%	Lipid	18.8%
		Proteins	74.8%	Carbohydrates	4 %
Major polypeptides:	E1, E2a, E2b	Envelope 55–62 and $46–50 \times 10^3$			
	C	Nucleo-capsid	31–35	$\times 10^3$	
Thermal stability:	4°C	Stable for 7 days			
	37°C	Inactivated at 0.1–0.4 $\log_{10}$ $TCID_{50}$/ml per hour			
	56°C	Inactivated at 1.5–3.5 $\log_{10}$ $TCID_{50}$/ml per hour			
pH Sensitivity:		Stable at pH 6.0–8.1			
		Unstable at more acid and alkaline pH			
UV Sensitivity:		Inactivated within 40 seconds			
	1350 W/cm^2	Inactivated at 7.0 $\log_{10}TCID_{50}$/0.1 ml per hour			
Photosensitivity:		Labile $K=0.07$ min^{-1} in PBS			

(in up to 1/5th of patients) which may persist for some weeks. Most commonly, the smaller joints are involved. Central nervous system complications are rare following rubella, and encephalitis incidence rates of approximately 1 in 5000 are generally assumed. The onset of encephalitis is usually sudden, within a day or two of the rash, and symptoms may vary from headache and drowsiness to convulsions and coma.

9.1.3. Prevention of rubella using vaccines

The important aspect with rubella is to prevent the congenital infection and this seemed an achievable goal following the introduction of several live attenuated vaccines in the mid-1960s. At this time, in the USA alone, an estimated 12.5×10^6 cases of rubella occurred with 20 000 malformed infants and 11 000 instances of foetal

wastage (reviewed by Hinman et al., 1983, Fig. 9.2). However, nearly two decades later the enthusiastic and high expectancies of those early days (Meyer et al., 1969, Weibel et al., 1969) have not been achieved, at least in many countries (reviewed by Banatvala, 1977, Clarke et al., 1979, 1980, 1983, Grenstein and Greares, 1982). This is not a reflection of the lack of efficacy of the vaccine, but rather a criticism of some national health authorities and general somnolence, and, to some extent, scientific disagreement about how best to use the vaccine.

Following the isolation and cultivation of rubella virus in tissue culture in the early 1960s (Parkman et al., 1962, Weller and Neva, 1962, McCarthy et al., 1963) rapid progress was made in developing a number of simple attenuated vaccines. For example, virus was passaged 77 times in vervet monkey kidney cells (HVP-77 vaccine) (Meyer et al., 1966, 1968, Parkman et al., 1966) and 5 times in duck embryo

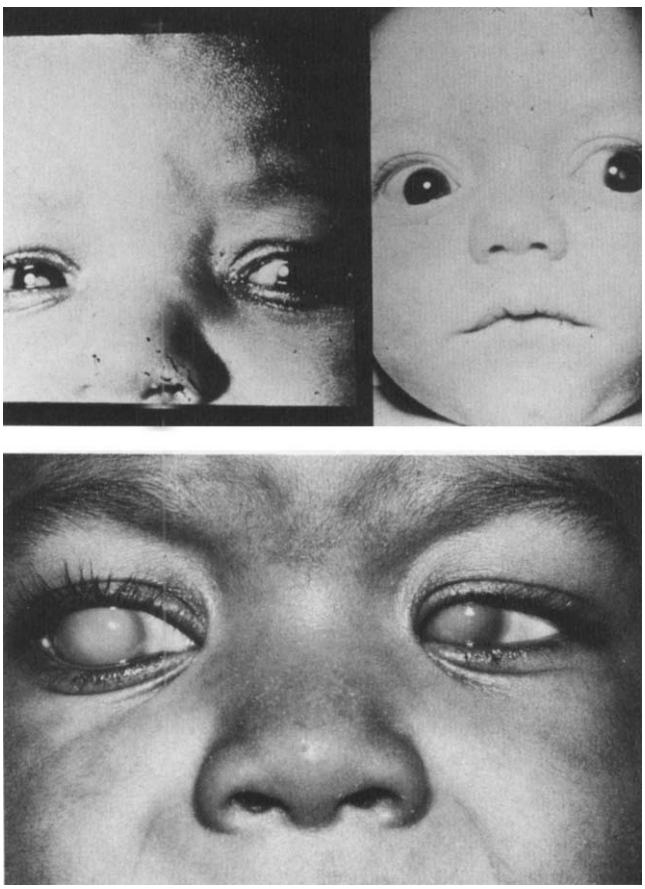

Fig. 9.2. Clinical aspects of rubella and rubella syndrome – cataracts in children with congenital rubella syndrome (courtesy of the late Dr. W. Marshall, Great Ormond Street Hospital for Sick Children.)

fibroblasts (HPV-77, DE5) and was found to be attenuated successfully for children and adults (Buynack et al., 1968, Weibel et al., 1968). Another strain of virus was passaged 51 times in primary rabbit kidney cells (Cendehill vaccine, Huygelen and Peetermans, 1967, Zygraich et al., 1971), whilst a third virus was passaged 4 times in HEK and 25 times in human diploid WI-38 cells (RA 27/3, Plotkin et al., 1973). Finally, and more recently, a Japanese vaccine virus was passaged in vervet MK cells (Table 9.3) 7 times and 20 times in primary guinea pig kidney cells (Best et al., 1974). All these vaccines when administered produced seroconversion in up to 95% of susceptible persons, with only limited side effects. Nevertheless, further experience indicated that the HPV-77 DK12 vaccine produced unacceptable side effects of arthralgia and arthritis and so it was withdrawn in the USA, for example. Later the HPV 77 DE5 vaccine was also withdrawn and replaced by the RA27/3 vaccine (Meruvax 2). Side effects including arthritis and arthralgia are usually mild (Best et al., 1974).

Early studies showed that the vaccine viruses were not transmitted to potentially susceptible sentinel volunteers (Halstead and Diwan, 1971), which was an essential requirement, because it was not known at first whether the vaccine viruses were teratogenic or not.

Immunity induced is long lived (Hoshino et al., 1982, Fig. 9.3) while experience over the last 15 years has shown the rubella vaccine viruses to have no teratogenic activity, although they have been isolated from foetal tissue and from infants whose mothers had been inadvertently vaccinated during pregnancy (Modlin et al., 1976). After experience accumulated with the different rubella vaccines it has become clearer that side effects are more associated with some, and the longevity of immune response induced might also differ. In short, it would now appear that the RA27/3 rubella vaccine strain has more positive features than some of the other viruses, and

TABLE 9.3.
Characteristics of some commonly used attenuated rubella vaccines

Vaccine	Origin of virus	Passage history for attenuation (Nos. of passages)
HPV77	Army recruit with rubella (1961)	Vervet monkey kidney (77)
HPV77, DE5	Army recruit with rubella (1961)	Vervet monkey kidney (77); duck embryo (5)
Cendehill	Urine from a case of postnatally acquired rubella (1963)	Vervet monkey kidney (3); primary rabbit kidney (51)
RA27/3	Kidney of rubella-infected fetus (1964)	Human embryonic kidney (4); WI-38 fibroblasts (17–25)
To-336	Pharyngeal secretion of child with postnatally acquired rubella Toyama, Japan (1967)	Vervet monkey kidney (7); primary guinea-pig kidney (20); primary rabbit kidney (3)
QEF (MEQ_{11})	Throat washing from patient in Osaka (1966) = Matsuura strain	Vervet monkey kidney (14); Chick amnion (65); Japanese quail embryo fibroblast cells (11)

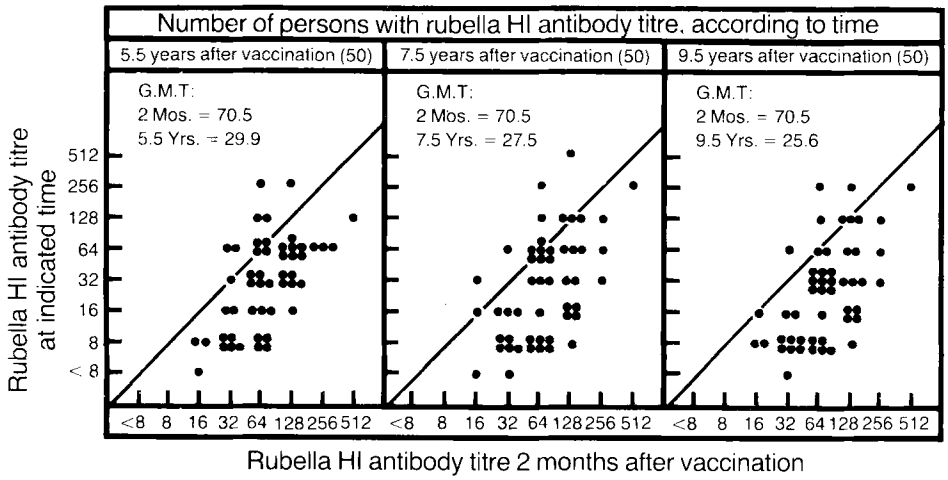

Fig. 9.3. Vaccine induced immunity to rubella is long lived. (after Weibel et al., 1975.)

is currently used on a rather wide scale. Immunity induced by RA27/3 more closely resembles the immune response to naturally acquired infection. This particular virus was first isolated in 1964 from an explant of a surgically aborted foetus by direct inoculation into WI-38 human diploid fibroblasts (reviewed by Plotkin et al., 1973). The virus was attenuated by low temperature passage and cloning in WI-38 cells, and was used for immunization between the 27th and 30th passage in these cells. A dose is 10^4 $TCID_{50}$ and can be administered intranasally or by s.c. injection (the latter technique is now favoured). In eight comparative studies performed in different countries, the mean HI antibody titre after immunization by either route was 2.4-fold higher with RA27/3 vaccine compared to Cendehill and HPV-77 vaccines (Plotkin et al., 1973). Similarly, higher levels of neutralizing antibody were detected following RA27/3 than Cendehill vaccine (Table 9.4) and higher levels of CF antibody than HPV-77 DE vaccine (Table 9.5). Nasal antibody (rubella specific IgA) is regularly detected after intranasal RA27/3 vaccine and frequently detected after s.c. injection with the same vaccine. In contrast, although specific IgA antibody is detected after Cendehill and HPV-77 DE5 vaccines, for example, it does not persist so well (Best et al., 1979) (Table 9.6). It should be noted here that there is no evidence of transmissibility from persons infected intranasally with RA27/3 vaccine.

An important aspect of rubella vaccines is whether recipients could become reinfected at later dates with wild rubella virus, which could still produce teratogenic effects. In early studies of persons receiving HPV-77 or Cendehill vaccines, and who were exposed to wild virus in nature or artificially, vaccines had re-infection rates ranging from 40–100% (Table 9.7). With RA27/3 vaccinees, re-infection rates of 3–10% were observed, which is close to figures obtained following natural infection. In a more recent study Best (personal communication) challenged a number of per-

TABLE 9.4.
Neutralizing antibody responses to 3 rubella vaccines (after Plotkin et al., 1973)

Investigator	Geometric mean neutralizing titres 6 to 8 weeks postimmunization		
	Cendehill	RA27/3 Subcutaneously	RA27/3 Intranasally
Plotkin (unpublished)	5 (6)[a]	15 (7)	13 (7)
Tobin	12 (28)	18 (29)	–
Fogel	6[b]/25	–	39[b]/43

[a] Number in parentheses is number of subjects tested.
[b] Number positive at 1 : 4 screening dilution per number tested.

TABLE 9.5.
Complement-fixation antibody responses to rubella vaccines (after Plotkin et al., 1973)

Vaccine	Percent seroconverting[a]	Geometric mean titre	Geometric mean titre of seroconverters[b]
HPV-77-DE	73	3.3	4.2 (16)
RA27/3 subcutaneously	100	6.0	6.0 (27)
RA27/3 intranasally	91	6.8	8.0 (22)

[a] Eight weeks immunization.
[b] Number in parentheses is number of seroconverters.

sons who had been vaccinated some 10 years earlier and viraemia was detected in 1 of 19 vaccinees who had antibody levels below 15 international units. Therefore, it is not yet known whether adequate levels of antibody *will* persist in all vaccinees for up to 30 years after vaccination, for example.

In summary, we have a number of successful rubella vaccines, developed empirically without any knowledge of genetics of the virus or the molecular basis of virulence or attenuation. Perhaps because of this absence of precise virological knowledge it has taken 10 years of field trials to establish that the various vaccine strains are not identical and vary, for example, in their ability to produce a long lived immune response and in side effects. The RA27/3 vaccine strain would appear to be a very successful one. It will be of particular interest, though, to analyze the genome of these different viruses and hence to determine, for example, how many mutations

TABLE 9.6.
Intranasal antibody after rubella vaccination (after Plotkin et al., 1973)

Investigator	Vaccine	No. positive/total
Plotkin	RA27/3-IN	5/6
	RA27/3-IN	9/15
	RA27/d-SC	4/9
	Cendehill	0/6
Bellanti	RA27/3-IN	8/8
Schiff-Ogra	Ra27/3-IN	8/10
	RA27/3-SC	4/10
Ogra	RA27/3-IN	13/15
	RA27/3-SC	2/5
	HPV-77-DK	0/30
Total	RA27/3-IN	43/54
	RA27/3-SC	10/24
	Others	0/36

TABLE 9.7.
Reinfection rates after artificial challenge of RA27/3 vaccines with intranasally given rubella virus

Investigator	Route of immunization	
	Subcutaneous	Intranasal
Plotkin (earlier data)	1/8	1/7
	0/7	–
	0/9	–
Liebhaber	1/10	1/9
Naficy	–	2/11
Ogra (unpublished data)	8/20	2/13
Smith, Kline and French (unpublished data)	–	1/10
Total	10/54 (18.5%)	7/50 (14%)

are required to change a rubella strain from a virulent one to an attenuated one. This could lead to the artificial 'construction' of a new generation of attenuated vaccines (see Chapter 2).

9.1.4. Side reactions and contraindications to rubella vaccination

Rubella vaccines are generally well tolerated, but lymphadenopathy, rash, arthralgia or arthritis may occur some two to four weeks after vaccination, although such reactions are usually less severe than those following naturally acquired disease (reviewed by Banatvala and Best, 1984, Best et al., 1974). In a study of 142 seronegative volunteers given Cendehill, HPV-77, DE5, RA27/3 or To-336 (Japanese) vaccines, joint symptoms were detected in up to 42% of vaccinees (Best et al., 1974) and persisted for up to eight days (Tables 9.8, 9).

9.1.5. Strategy for the prevention of rubella syndrome

When the vaccines were licensed in 1969, two strategies for use were discussed, but with a single aim, namely to prevent congenital rubella syndrome (RS). Widespread vaccination of adolescent girls and women would prevent RS, but would not influence virus transmission in the community. On the other hand, routine vaccination of children (boys and girls) early in life would increase herd immunity and interrupt transmission of the disease. The USA adopted the latter policy whilst the UK

TABLE 9.8.
Reactions occurring after vaccination in seroconverted vaccinees and controls (after Best et al., 1974)

	Cendehill	HPV77.DE-5	RA27/3	To-336	Controls
No. of subjects	35	31	36	34	39
Rubelliform rash	3 (8.6%)	1 (3.2%)	9 (25%)	0	1 (2.6%)
Lymphadenopathy	11 (31.4%)	17 (54.8%)	16 (44.4%)	13 (38.2%)	14 (35.9%)
Joint symptoms	8 (22.9%)	12 (38.7%)	15 (41.7%)	6 (17.6%)	0

TABLE 9.9.
Joint symptoms experienced by seroconverted vaccines (after Best et al., 1974)

	Cendehill	HPV77.DE-5	RA27/3	To-336	Total
No. seroconverted:	35	31	36	34	136
Joint symptoms	8 (22.9%)	12 (38.7%)	15 (41.7%)	6 (17.6%)	41 (30.1%)
Arthralgia only	7 (20%)	4 (12.9%)	9 (25%)	2 (5.9%)	22 (16.2%)
Arthritis	1 2.9%)	8 (25.8%)	6 (16.7%)	4 (11.8%)	19 (14.0%)
Multiple joint involvement (three joints or more)	4 (11.4%)	10 (32.3%)	8 (22.2%)	4 (11.8%)	26 (19.1%)
Symptoms lasting seven days or more	0	1 (3.2%)	2 (5.6%)	2 (5.9%)	5 (3.7%)

adopted the former policy. Recent epidemiological modelling studies have shown both countries to have taken the correct decision, because when the fractions of the susceptibles vaccinated is less than 80% it is better to let natural rubella spread (e.g. U.K.), whereas in countries where high rates of immunization are likely to be achieved (e.g. USA) then a policy of eradication can be followed with vaccine given to both boys and girls (Hinman et al., 1983, Anderson and May, 1983). In fact the latter assessment is perhaps rather diplomatic because a more severe critic would pronounce that, whilst the USA policy has at least prevented extensive outbreaks and reduced RS, the UK policy has, up to the present time, done neither.

A summary of the USA experience (Schiff, 1980) has shown the following:

1. the incidence of RS has decreased significantly (Fig. 9.4);
2. the vaccine viruses are not teratogenic;
3. the vaccine of choice may be RA 27/3;
4. herd immunity has not stopped outbreaks, and outbreaks are occurring in unvaccinated groups e.g. young adults;
5. 20–35% of 18–25 year old females remain susceptible.

A much more vigorous campaign is therefore required even in the USA to immunize the susceptible women of child bearing age, and also to raise immunization levels in school children.

Sabin (1981) again takes a rather individual viewpoint by querying whether, in fact, the small number of RS cases justifies a large scale immunization programme. In any case he speculates that a mass immunization campaign over a *short* period of time would be necessary to break the chain of virus transmission.

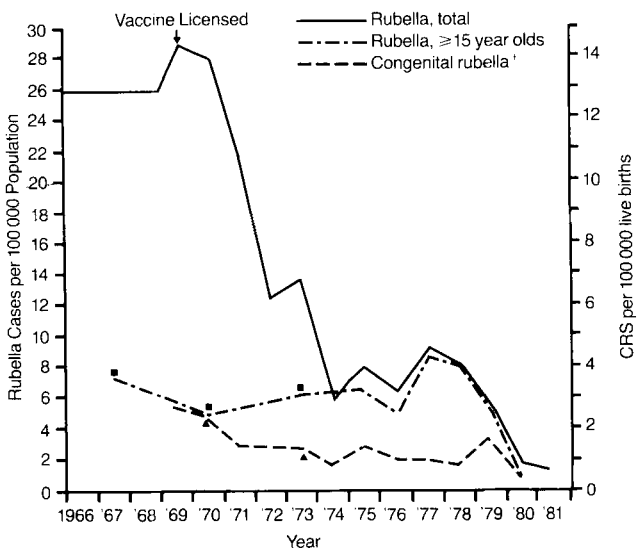

Fig. 9.4. Incidence of reported rubella cases and congenital rubella cases (U.S.A. 1966–81.)

Similarly to measles, since rubella vaccine is a live virus vaccine, it should not be given to patients whose immunity is deficient, whether as a result of disease (e.g. malignancy) or of treatment with corticosteroids, radiotherapy or cytotoxic drugs. Since rubella vaccine may be transmitted transplacentally, pregnancy is a contraindication and should be avoided for three months after vaccination. Examination of the products of conception of rubella-susceptible women vaccinated inadvertently during pregnancy has shown that rubella virus may be recovered from the placenta, kidney and bone marrow. Furthermore, histopathological studies have shown that changes in the placenta, decidua and foetal eye are similar to those occurring in naturally acquired infection. However, clinical and serological follow-up studies carried out on women in the USA, who elected to go to term after inadvertent rubella vaccination in early pregnancy, have shown that none of 277 infants delivered of mothers known to be rubella susceptible and given rubella vaccine within a period ranging from 3 months before to 3 months after conception had major malformations. Many of these infants have been followed up since, and though about 30% have serological evidence of intrauterine infection, none had defects compatible with congenital rubella. The risk of rubella-induced major malformations among infants delivered of susceptible mothers was calculated (based on a 95% confidence limit) as 3.3% – a figure similar to the risk in normal pregnancies (Table 9.10). The United States Immunisation Practices Advisory Committee still recommends that pregnant women should *not* be given rubella vaccine, but now states that inadvertent vaccination should no longer be a reason to recommend termination of pregnancy routinely.

TABLE 9.10.
Consequences of rubella vaccination during pregnancy, US 1969–1982 (MMWR, 32, 1983). Pregnancy outcome in susceptibles going to term

Vaccine	No.	To term	No. vaccinated between one week before to four weeks after conception	Evidence of infection[a]	Abnormalities[b]
			No. with date of conception known		
HPV77-DE5/ Cendehill	149	94	33/87 (38%)	8/194[d]	0[c]
RA27/3	111	81	28/81 (35%)	1/83	0
A11	260	175	61/168 (36%)	9/277 (3%)	0

[a] IgM present, IgG persisting beyond 6 months or isolation of rubella virus.
[b] Compatible with congenital rubella.
[c] Now aged 2–7 years.
[d] 149 infants whose mothers were susceptible at the time of vaccination and 45 whose mothers were of unknown status.

9.1.6. Serological surveys of rubella vaccine induced immunity

The results of the serological surveys in the UK indicate that the rubella vaccination programme has had an impact on the serological status of the *young adult females* in the study population. It is clear from fig. 9.5 that of women eligible for vaccination at school the proportions who were seronegative were much lower than those for men of the same age, and for older women. The low frequency of seronegative subjects among women born after 1956 was found consistently throughout the studies and relates chronologically to the expected effect of vaccination (Clarke et al., 1983).

In the first 4 years of a blood-donor study the seronegative proportion of women born before 1956 was consistently higher than that for men of the same age (Fig. 9.6). However, in 1980 the percentage who were seronegative of women born before 1951 was lower than that for men of the same age and for women born between 1951 and 1956. A possible explanation for this finding is that this age group included mothers who had been vaccinated at postnatal clinics after the birth of a child. Some indication of the persistence of rubella antibodies after vaccination can be obtained from the results. Women born in 1957 and 1958 would by 1976, the first year of the study, have been vaccinated up to 6 years previously, and by 1980 up to 10 years previously. If it is assumed that the differences in proportions of seronegative subjects between men and women born after 1956 were due to rubella vaccination, then the consequence of a rapid fall in the antibody due to vaccination would be that the proportion of seronegative women would tend to revert to that of men of the same age. There is no evidence that this occurred. Thus, it appears that over this 5-year period the antibody levels persisted from up to 6–10 years after vaccination. Other evidence has indicated that antibody can persist in the majority of vaccinees up to 16 years after vaccination (O'Shea et al., 1982).

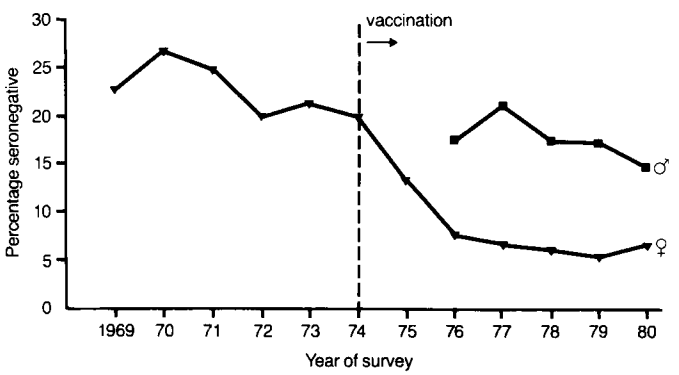

Fig. 9.5. Percentage of students found to be seronegative for rubella antibody in surveys carried out at Nottingham University. Dotted vertical line = earliest year in which student intake would have included females eligible for vaccination at school. (after Clarke et al., 1983.)

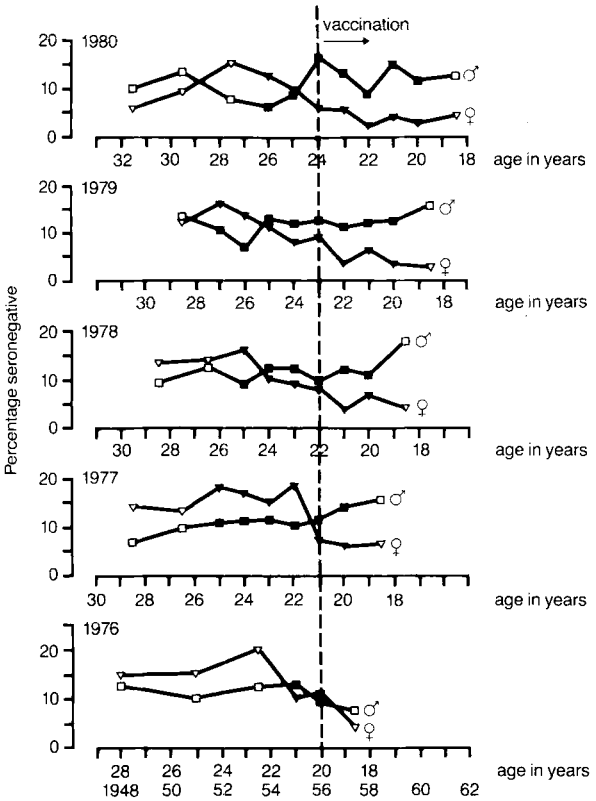

Fig. 9.6. Percentages of young adult blood donors found to be seronegative for rubella antibody in five annual surveys carried out from 1976 to 1980. Dotted vertical line = age group of females offered vaccine at school (i.e., those born from 1956 onwards). Age is based on year of birth. Points to the left of the dotted vertical line represent age groups of females who, because they were born before 1956 were not offered vaccine, points to the right of the dotted line represent age groups of females who were offered vaccination. Open symbols mean that data have been pooled for individuals with consecutive years of birth. (after Clarke et al., 1983.)

Hambling (1980) conducted an 8-year study from 1971 to 1978 in the UK, in which 150 000 serum samples from women of child-bearing age were tested for rubella antibody; he found that the seronegative proportion among women who had been eligible for vaccination at school was lower than that among older women. Other studies have reported similar trends, (Hutchinson et al., 1982) but two studies (Clubb et al., 1981 and Gilmour et al., 1982) failed to find any effect attributable to vaccination; the numbers of serum samples tested in the latter studies were much lower than in the other studies.

Surveys have shown that the frequencies of seronegative schoolchildren under 11 years and of seronegative young adult males in the UK are similar to that reported

before the introduction of vaccine. This finding would be expected because of the selective nature of the vaccination programme. These individuals constitute a susceptible group who could contribute to the spread of rubella in the community. Their influence on the incidence of congenital rubella may be discernible in the future when experience in the UK is compared with that in the USA, where both sexes are vaccinated in infancy (Hinman et al., 1983).

In a survey in 1977, (Peckham et al., 1977) 71% of 16-year old girls were reported to have received rubella vaccine, although uptake varied widely in different parts of the UK. After the rubella vaccination campaign in 1979, the uptake of vaccine increased to 84% in 1980 but it fell short of the 90% aimed at by the campaign. However, extensive local campaigns have achieved uptakes in excess of 90%. Further efforts are obviously needed to increase the uptake of rubella vaccine.

9.1.7. Combined measles–rubella immunization

Serological surveys in certain adolescent and young adult populations have indicated that susceptibility levels to rubella are approximately 15 to 20 percent (see above), not appreciably different from those reported in prevaccine years. Furthermore, adolescents and young adults now account for a high proportion of all reported measles (see Chapter 8) and rubella cases, particularly in the USA, and have the greatest risk of disease. These facts are illustrated by the measles and rubella outbreaks reported in secondary schools, colleges, military installations, and places of employment in the USA. This pattern of disease transmission has led to increased efforts to vaccinate older susceptible persons. Ideally, those in need of vaccination would be identified through a sensitive, specific, readily available, and inexpensive screening technique that provides immediate information. An obvious approach is a history of previous measles and rubella infection or vaccination; however, previous reports have indicated that such information obtained from prospective vaccinees or their parents may not be an accurate predictor of susceptibility. Preblud et al. (1982) in a recent study evaluated the sensitivity and specificity of *histories* of past infection or vaccination and determined the costs and effectiveness of three alternative strategies for vaccinating persons susceptible to measles, rubella, or both: (1) vaccinating *all* persons regardless of past history; (2) serologically screening all persons and vaccinating only those who were susceptible; (3) vaccinating all persons who do not have physician-documented proof of proper vaccination, past infection (measles only), or serological immunity.

These authors found that with few exceptions, any category of history response was associated with a measles susceptibility rate of approximately 5% or less. Maternal histories were statistically no more specific than cadet histories ($P > 0.06$), and identified at most only two thirds of susceptible persons. While both cadet and maternal histories for either infection or vaccination were sensitive (identifying 90% to 95% of all immune subjects), a history of neither was very nonspecific (identify-

ing at most 28% of susceptible persons). Positive histories for previous rubella infection and vaccination were associated with lower susceptibility rates than were negative histories ($P < 0.01$). However, *no* history successfully identified all persons susceptible to rubella. While cadet and maternal histories of rubella vaccination were more specific than other histories ($P < 0.01$), negative histories of vaccinations still failed to identify approximately 10% of the subjects susceptible to rubella. Comparison of discordant responses confirmed that maternal histories were generally no more reliable than cadet histories!

The costs and effectiveness of the three alternative vaccination strategies in a model cohort of 1000 subjects were investigated. Vaccination of *all* persons is the least expensive strategy (both in total cost and cost per susceptible person protected), unless records are available for 75% or more of the population in question, in which case vaccination after record review is less expensive. However, vaccination after record review is associated with a lower proportion of protected susceptible persons compared with the other two strategies, when 75% or more of the potential vaccinees have records (approximately 80% protected vs. 90%). These findings are not altered even if one assumes that records are only 80% accurate or that they are 100% accurate. In general, while there is little difference in the total cost of administering either measles or rubella vaccine alone or both in combination, the cost per susceptible person protected indicates that a combined measles and rubella vaccination programme is highly economical. A combined programme provides a 10% to 15% reduction in cost compared with a rubella-only vaccination programme (with the exception of the serological screening/vaccination strategy, in which case the combined programme costs 4.5% more) and approximately a fourfold to fivefold reduction compared with a measles-only vaccination programme.

9.1.8. Prevention of rubella using immune globulin

Normal immune globulin may prolong the incubation period considerably but since inapparent infection is accompanied by viraemia, foetal damage is not prevented (Forrest and Honeyman, 1973, Polakoff, 1983). However, Peckham (1974) showed that infants of mothers given normal human immune globulin who experienced subclinical rubella in early pregnancy were less likely to be infected in utero than those infants whose mothers were not given immune globulin. It is therefore possible that the administration of normal human globulin reduces the level of foetal infection, damage, or both.

High-titred rubella immune globulin has been used experimentally to determine whether infection induced by rubella vaccine can be prevented (Urquhart et al., 1978). The results were encouraging in that 8 out of 20 volunteers (40%) given high-titre immune globulin and rubella vaccine simultaneously failed to sero-convert, and the remaining 12 exhibited delayed antibody responses compared with volunteers given vaccine alone. This preparation, which is in short supply, is available

via the Scottish Blood Transfusion Service, but has not been properly evaluated in the field. It may be recommended for the few susceptible pregnant women who come into contact with clinical rubella and for whom therapeutic abortion is unacceptable.

9.1.9. Chemoprophylaxis and rubella

Because of the success of the live attenuated rubella vaccines very few studies have been carried out of specific inhibition of rubella virus replication. Early in vitro and in vivo work was initiated in the 1960s with amantadine and other amines but was soon discontinued because of the rather low antiviral efficacy of the molecules investigated. Thus, Oxford and Schild (1965) described the inhibitory effects of amantadine on microplaque formation by several strains of rubella virus (Fig. 9.7 and Table 9.11). Amantadine at 100 μg/ml exerted some toxic effects in the line of rabbit kidney cells used (RK-13), whereas 20 μg/ml amantadine was required to inhibit microplaque formation by 90% – the therapeutic index therefore was low, particularly compared to the in vitro activity against strains of influenza A virus (see Chapter 7). Amantadine was also active as an antiviral in organ cultures of ferret trachea infected with rubella virus strains (Figs. 9.8 and 9.9) but no antiviral effect was noted in prophylactic experiments in laboratory animals (Table 9.11) infected intranasally with rubella virus and given relatively high dosages of the compound (50–100 mg/kg).

Three infants with congenital rubella syndrome were given human leukocyte (al-

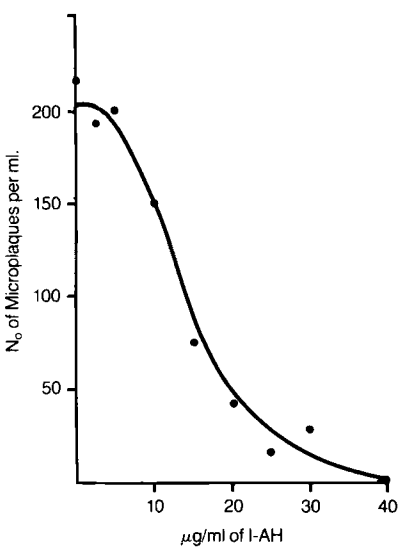

Fig. 9.7. Dose response curve for amantadine and rubella virus.

TABLE 9.11.
Comparison of antirubella virus activity of aminoadamantane (amantadine) in different test systems (after Oxford and Schild, 1965)

Animal species	Inhibition of rubella virus multiplication[a] in:		
	Cell cultures (25 μg/ml aminoadamantane)	Organ cultures (25 μg/ml aminoadamantane)	Experimental animals (50–100 mg/kg aminoadamantane)
Rabbit	0.8–1.0[b]	0.5–1.0 (lung)	0
Ferret	not tested	0.5–1.0 (trachea)	0
Hamster	not tested	0.5–1.5 (lung)	0

[a] $\log_{10}$ $TCID_{50}$/ml reduction of virus titre in cultures treated with aminoadamantane compared to control cultures.
[b] RK-13 cells, continuous rabbit kidney.

pha) interferon at doses of 2×10^5 to 7×10^5 U/kg per day for 10 days, by Arvin et al. (1982). A transient decrease in pharyngeal virus excretion was observed with treatment. No significant side effects were associated with the administration of human leukocyte interferon to these infants (Table 9.12) but no beneficial effect was noted.

9.1.10. Summary

A number of live, attenuated rubella vaccines have been developed empirically and tested over the last two decades, and although all are efficacious, nevertheless, differences in reactivity and immunogenicity have been detected. The rubella vaccine designated RA27/3 (human diploid cell) is now used widely. There appears little or no need for a chemotherapeutic approach or new vaccines, but rather a more intensified vaccination campaign is required so that rubella epidemics and rubella syndrome can be prevented. Much remains to be discovered about genetic and antigenic variation among rubella field viruses, virus structure and biochemistry.

9.2. Reoviridae infections

The family *reoviridae* (the name reo arose from *re*spiratory, *e*nteric, *o*rphan) consists of reovirus, orbivirus and rotavirus as shown in Table 9.13. Viruses from this family seem to infect most humans and vertebrates, and antibodies have been found in all investigated human populations.

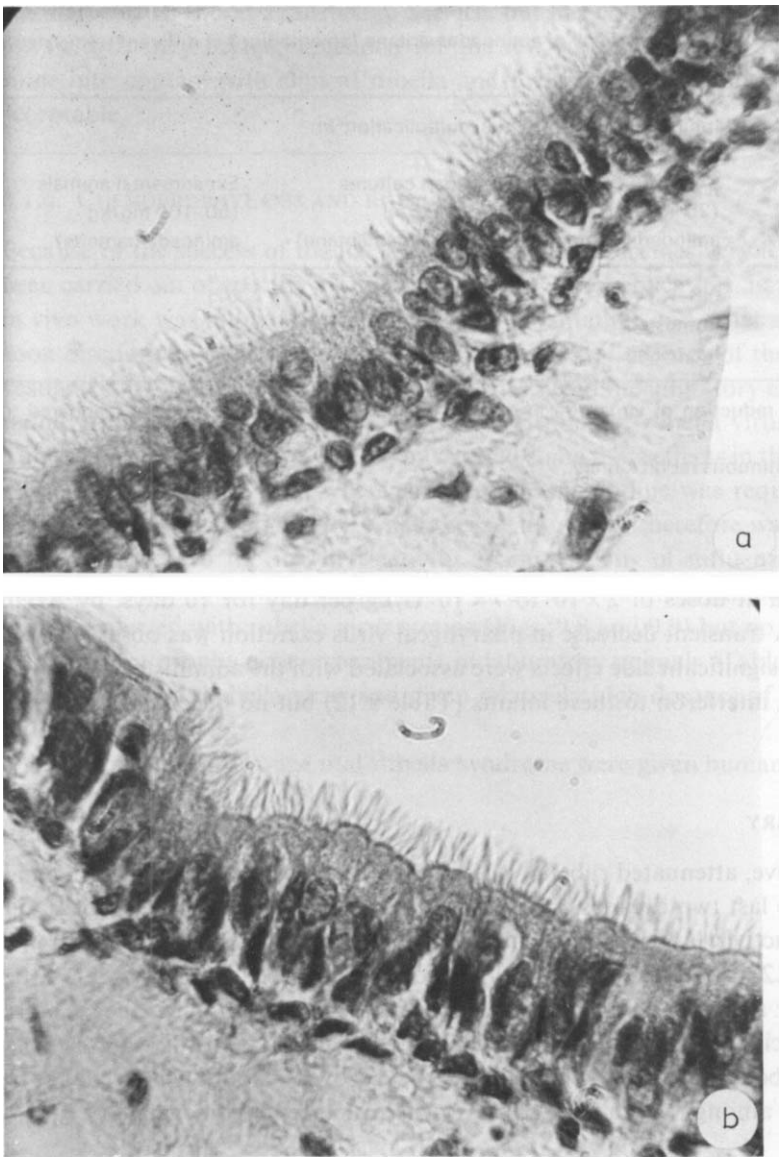

Fig. 9.8. Multiplication of rubella virus in organ cultures of ferret trachea. a, normal trachea; b, rubella virus infected trachea. No overt signs of infection can be seen, but infective virus is released into the medium (see Fig. 9.9.).

9.2.1. The viruses

The virus particles are 60–80 nm in diameter and contain double-stranded RNA, in reoviruses 10 segments, divided in 3 size classes, with a total molecular weight of 15×10^6, in orbiviruses 10 segments with a total molecular weight of 12×10^6 and

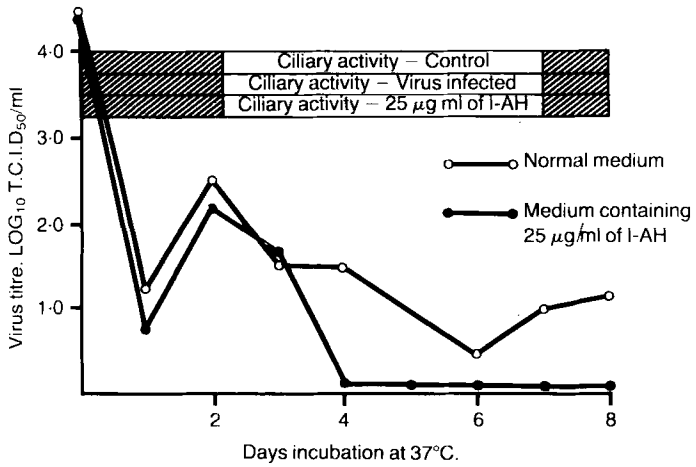

Fig. 9.9. Ferret trachea organ cultures infected with rubella in the presence and absence of amantadine (I-AH).

TABLE 9.12.
Clinical findings in infants with congenital rubella treated with interferon (after Arvin et al., 1982)

Infant	Birth wt (g)	Gestational age (wks)	Status at treatment		Current status	
			Age (mos)	Clinical problems	Age (mos)	Clinical problems
A	1680	34	3	Microophthalmia, cataract, pulmonic stenosis	48	Deafness, impaired vision, delayed growth and development
B	2340	42	5	Cataract, chronic pneumonia, failure to thrive	24	Deafness, chronic pneumonia, delayed growth and development
C	2320	40	3	Microophthalmia, cataract, probable ventricular septal defect	12	Deafness, impaired vision, delayed growth and development

in rotaviruses 11 segments with a total molecular weight of 10×10^6. Fig. 9.10 shows the morphology of the human rotavirus. The particles, which lack an envelope, have icosahedral symmetry, and are made up of two distinct capsid shells. The outer shell can be removed by chymotrypsin treatment to reveal a core. This core contains an RNA dependent RNA polymerase coded by the viral genome and a poly A polymerase activity. The virions also contain 5′-terminal cap-forming enzymes (Yamakawa et al., 1982).

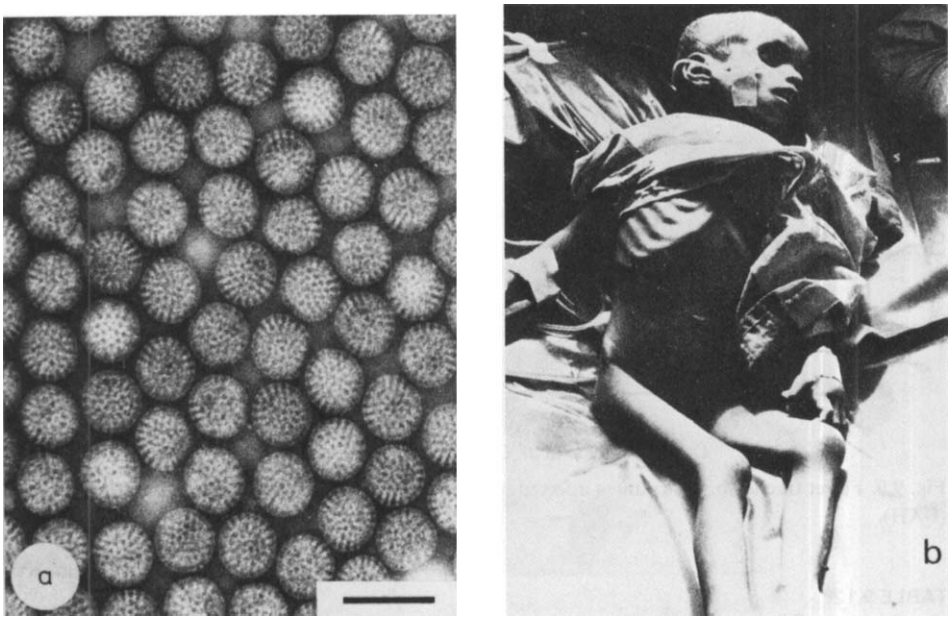

Fig. 9.10. a, Human rotavirus. EM picture of human stool isolate. The bar is 100 nm. (Courtesy of Dr. L. Svensson.). b, Child in a Bangladesh hospital suffering from chronic malnutrition and acute diarrhoeal disease. Development of virus vaccines and also satisfactory nutrition are both urgently required. (From 'World Health', a WHO publication.)

9.2.2. Replication and molecular biology

The virus particles are bound to receptors on the cell, transported into the cytoplasm and uncoated within lysosomes. After removal of the outer capsid by proteolytic enzymes, the core-associated RNA transcriptase enzyme transcribes mRNA from the RNA genome in the virus core. The virus core also contains the viral enzymes necessary for the formation of caps on the viral mRNA (Yamakawa et al., 1982). The cap formation involves the following viral reactions:

1. transcriptase
   $pppG + pppC \rightarrow pppGpC + PPi$
2. nucleotide phosphorylase
   $pppGpC \rightarrow ppGpC + Pi$
3. guanylyltransferase
   $pppG + ppGpC \rightleftarrows GpppGpC + PPi$
4. methyltransferase 1
   $GpppGpC + \text{Ado Met} \rightarrow m^7GpppGpC + \text{Ado Hey}$
5. methyltransferase 2
   $m^7GpppGpGpC + \text{Ado Met} \rightarrow m^7GpppG^mpC + \text{Ado Hey}$

The viral mRNA does not contain any poly-A sequence at the 3′-terminal.

Reovirus proteins are synthesized and cleaved in the cytoplasm as indicated in Figure 9.11. Synthesis of minus strands to form new double stranded RNA is also carried out by viral RNA polymerase in the cytoplasm using replicative complexes consisting of one of each of the ten or eleven plus stranded RNA molecules (for a review see Joklik, 1980). This detailed knowledge of the replicative events has been established using reovirus and it is not entirely clear if orbi- and rotaviruses differ from this pattern in any respect. However, there seems to be several viral enzymes in the reoviridae family suitable as targets for antiviral drugs.

9.2.3. Clinical aspects

Table 9.13 lists the main syndromes caused by reo-, orbi- and rotaviruses. Reovirus infections in humans seem to result in very mild, or no symptoms. In contrast, rota-

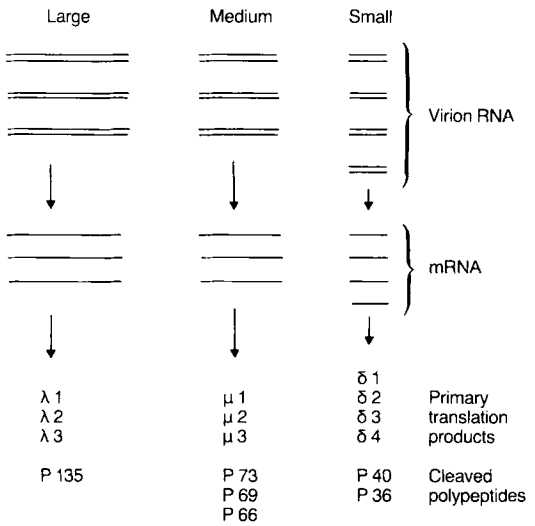

Fig. 9.11. Transcription and translation of reovirus RNA.

TABLE 9.13.
Reoviridae affecting humans

Virus	Disease
Reovirus 1, 2 and 3	Mild fever, diarrhoea and respiratory disease
Orbivirus, Colorado tick fever, and some other tick-borne viruses	Mild fevers
Rotavirus, 4 serotypes, each comprised of sub-groups	Diarrhoea, mainly in infants and young children. Can be severe and is common

viruses are the most common cause of acute gastroenteritis in infants and children, being responsible for about half the cases (Davidson et al., 1975). The symptoms include diarrhoea, fever, abdominal pain and vomiting, resulting in dehydration. This can be severe, especially in third world countries with inadequate medical care, and the result can be a very considerable mortality (see Chapter 1 and Fig. 9.10b). It has been estimated that 3–5 billion cases and 5–18 million childhood deaths occur each year due to rotaviruses in Asia, Africa and South America, making it the single largest cause of disease and death in these areas of the world (Elliot, 1976, Kapikian et al., 1980). Gastroenteritis caused by rotavirus is also found in adults and can be severe in elderly patients (Halvorsrud and Örstavik, 1980).

The orbiviruses, such as Colorado tick fever, cause a mild human disease with fever.

9.2.4. Epidemiology

The faecal-oral route seems to be the main way of transmission of these viruses and the incubation period is 2–4 days. This incubation period has also been found in volunteers challenged orally with rotavirus (Kapikian et al, 1983). In most cases rotavirus infections occur in infants and young children as shown in Table 9.14. A seasonal variation in the prevalence, with the highest figures during the cold season, has been reported in several studies (Middleton, 1982).

High frequencies of antibodies against rotavirus in several populations show that this is a very common infection. About 90% of children at the age of two have antibodies both to type 1 and type 2 human rotavirus (Wyatt et al., 1978) and about 70% of the adult population in industrialized countries have antibodies to rotaviruses (Gust et al., 1977, Middleton et al., 1976). Using a sensitive ELISA method to determine the presence of antibodies to rotaviruses the figures seem to approach 100% in different populations (Ghose et al., 1978, Yolken et al., 1978). The distribution of rotavirus types and subgroups is presently not clear (see Chapter 17 for a discussion of molecular epidemiology).

TABLE 9.14.
Age range of 326 consecutive rotavirus patients in Toronto (after Middleton, 1982)

Patients (No.)	Age groups (%)					
	0–6 months	6–12 months	12–18 months	18–24 months	2–3 yrs	>3 yrs
Patients admitted with enteritis (257)	21.4	40.1	18	5	8.2	7.3
Nosocomial enteritis patients (69)	56	34	6	1	3	–
Total patients (326)	29	39	15	4	7	6

9.2.5. Vaccination and immunotherapy

Different approaches to immunization against rotavirus gastroenteritis have been discussed and the reader is referred to these excellent reviews (Kapikian et al., 1980, McLean and Holmes, 1980, Saulsbury et al., 1980, Chanock, 1981, Kapikian et al., 1981, Wyatt et al., 1981, Kapikian et al., 1983). There are two pieces of information that are important in the context of rotavirus immunization and that we should emphasize here. One is the number of different strains and their antigenic diversity and the other the relative importance of antibodies in serum and in the intestinal fluid. The results of Kapikian et al. (1983) indicate that the presence of serum antibodies could prevent a symptomatic infection by challenge virus. The presence of local neutralizing antibodies in the intestinal fluid has been shown by Davidson et al. (1983) and could also be of importance.

The possibility of using an attenuated live vaccine seems promising and a human type 2 rotavirus strain has been serially propagated in gnotobiotic piglets to yield an attenuated strain WA, which is presently being tested for safety and antigenicity in volunteers (Edelman, 1982, See Chapter 2). Using a live attenuated bovine rotavirus vaccine Vesikari et al. (1984) have recently achieved an 88% protection rate in children during a rotavirus epidemic. The development of a vaccine by hybrid DNA technology or using immunogenic small polypeptides will certainly be investigated against rotavirus in the future.

Passive immunization through antibodies in milk given to infants should also be considered, and is an important argument in favour of breast feeding. Passive immunization has also been used in immunodeficient patients with chronic rotavirus infections (Saulsbury et al., 1980).

When considering the present status of immunotherapy and prophylaxis against rotavirus it should be kept in mind that these viruses have been known only for a short period of time. Vaccination against reo- or orbiviruses seems not to be particularly important in comparison to the very great need for vaccination against rotaviruses, especially in underdeveloped countries.

9.2.6. Chemotherapy

No clinically effective chemotherapy of infections caused by *reoviridae* has been reported. Restoration of electrolyte balance and replacement of fluids by the oral or parenteral route is important in cases of rotavirus infections resulting in diarrhoea.

Ribavirin will, in cell culture, inhibit the growth of rotavirus (Chang and Heel, 1981) but was not active in rotavirus infection in mice (Schoub and Prozesky, 1977). Similar results using ribavirin and some other compounds in cell culture were reported by Smee et al. (1982) and the results are shown in Table 9.15. Although some antiviral effects were shown, none of the compounds or ribavirin mono- and triphosphate had any effect on the rotavirus RNA polymerase in a cell-free assay. Also, these compounds showed very little activity on a murine rotavirus infection in mice.

TABLE 9.15.
Effect of antiviral substances on infectious simian rotavirus yields in MA-104 cells (after Smee et al., 1982)

Drug. conc. μg/ml	Virus yield ($log_{10}CCID_{50}$/0.1 ml)			
	Ribavirin	3-DG	3-DU	(S)-DHPA
10	5.5	4.6	5.5	5.7
100	5.0	2.3	5.5	3.7
1000	3.3	1.7	4.5	2.7

Untreated samples had yields of 5.5–5.7. 3-DG, 3-deazaguanine; 3-DU, 3-deazauridine; (S)-DHPA, 9-(S)-(2,3-dihydroxypropyl)adenine

TABLE 9.16.
Retroviridae

Subfamily	Human viruses
RNA tumour virus group (Oncovirinae)	Human T-cell leukaemia/lymphoma virus (HTLV)
Foamy virus group (Spumavirinae)	Human foamy virus? (not causing tumours)
Meadi/visna virus group (Lentivirinae)	-

The presence of several viral enzymes should make it feasible to synthezise inhibitors and useful animal models are available for rotavirus infections, and so this could be a fertile area for investigation in the future.

9.3. Retrovirus infections

The impact of retroviruses on human diseases is far from clear but they are involved in several animal tumours and probably in some human malignancies as well as implicated in AIDS.

9.3.1. The viruses

The retroviruses can be divided into three main groups as shown in Table 9.16. The unequivocal identification of a human retrovirus (HTLV, see later) is recent although this group of viruses has been studied for many years. The retroviruses contain a dimeric single-stranded viral RNA and a reverse transcriptase enzyme. The enveloped virus particles are 80–100 nm in diameter and have an internal icosahedral capsid containing RNA with a monomeric M.W. of 3×10^6. The viral pro-

teins are glycosylated and the envelope contains lipids derived from the plasma membrane. The virus particles contain both type-specific and group-specific antigens.

9.3.2. Replication and molecular biology

Retrovirus replication starts with an interaction between an envelope glycoprotein and a specific receptor on the cell-surface. After uncoating, the virion RNA is transcribed into DNA by a reverse transcriptase enzyme. Transfer RNA functions as a primer in this process. A linear DNA is formed containing terminal repeats and the dsDNA can circularize. The retroviral DNA integrates into cellular DNA in a way similar to the integration of transposons. Integration seems to be required for virus replication and the integrated viral DNA is transcribed by cellular RNA polymerase II into virion RNA and mRNA. The viral genome contains genes for the group antigen (gag), the reverse transcriptase (pol), the envelope glycoprotein (env) and can also have onc and sarc genes. The integrated viral DNA can enter the germ line and be transmitted as a provirus in a vertical manner.

9.3.3. Clinical aspects

The retroviruses have been associated in animals with leukaemias, lymphomas, sarcomas and carcinomas. In humans a T-cell leukaemia/lymphoma virus (HTLV) has recently been isolated from patients with cutaneous T-cell lymphomas (Poiesz et al., 1980, Poiesz et al., 1981, Miyoshi et al., 1981, Popovic et al., 1982, Reitz et al., 1983, Sarin et al., 1983). The integration of the HTLV genome at the same site in cells from 3 out of 13 patients with mature T-cell leukaemia-lymphoma indicates that integration next to specific cellular genes is important in neoplastic transformations (Hahn et al., 1983). This disease has an aggressive course with poor prognosis and has both skin manifestations and visceral involvement with hypercalcaemia, hepatosplenomegaly and lymphadenopathy. This virus-associated malignancy shows a geographic clustering to parts of the USA, the Caribbean and Japan (Blattner et al., 1983) and the presence of disease is clearly correlated to areas were HTLV infection is prevalent.

No chemotherapy or vaccination against HTLV is presently available but the possible association of HTLV with some cases of AIDS (acquired immune deficiency syndrome) will certainly arouse considerable additional interest (Essex et al., 1983, Gelman et al., 1983, Gallo et al., 1983, Barré-Sinoussi et al., 1983) in prevention and treatment of infections and malignancies caused by HTLV. Several viral enzymes and especially the reverse-transcriptase could be suitable targets for inhibitors. The activity of foscarnet against retroviruses (See Öberg, 1983) gives one example of a drug which should be evaluated in this context.

9.4. Norwalk virus infections

After an outbreak of gastroenteritis (winter vomiting disease) among students and teachers in an elementary school in Norwalk, Ohio, stool filtrates from secondary cases were able subsequently to induce disease in volunteers. By the use of immune electron microscopy (IEM) Kapikian et al (1972) identified the Norwalk agent as 27 nm virus particles. Since then several similar infectious agents have been described.

The classification of the Norwalk group of viruses is uncertain and it has been regarded either as a parvo- or as a calcivirus. The present information seems to favour the latter classification and it is, therefore, discussed in this 'mixed bag' chapter on RNA viruses. The Norwalk group of viruses has recently been the subject of an excellent review by Kapikian et al. (1982).

9.4.1. The viruses

The Norwalk-like agents have a diameter of 25–27 nm, a density of 1.37–1.41 g/cm^3 in CsCl, possess one major capsid polypeptide and have a morphology by electron microscopy similar to that of picorna-, parvo- or calcivirus. A problem with the identification has been that the Norwalk viruses have been impossible to grow in cell culture. An overview of the possible agents included in the Norwalk virus group is given in Table 9.17. This group of agents has been found by EM and IEM in

TABLE 9.17.
Characteristics of Norwalk virus and related agents associated with acute epidemic, nonbacterial gastroenteritis in humans (after Kapikian et al., 1982)

Agent		Induces illness in		
	Size, nm	Humans	Animals	Antigenic relationship
Norwalk	27×32	Yes	No	Distinct
Hawaii	26×29	Yes	No	Distinct
Montgomery County	27×32	Yes	No	Related to Norwalk agent
Ditchling	25–26	Not tested	No	Ditchling and W agents related
W	25–26	Yes	Not tested	to each other but not to Norwalk or Hawaii agents
Cockle	25–26	Not tested	Not tested	Distinct from Norwalk and Hawaii agents
Paramotta	23–26	Not tested	Not tested	Distinct from Norwalk agent
Colorado	27–32	Yes	Not tested	Distinct from Norwalk, Hawaii and Marin county agents
Marin County	27	?	No	Distinct from Norwalk, Hawaii and Colorado agents

None of the viruses has been grown in cell culture.

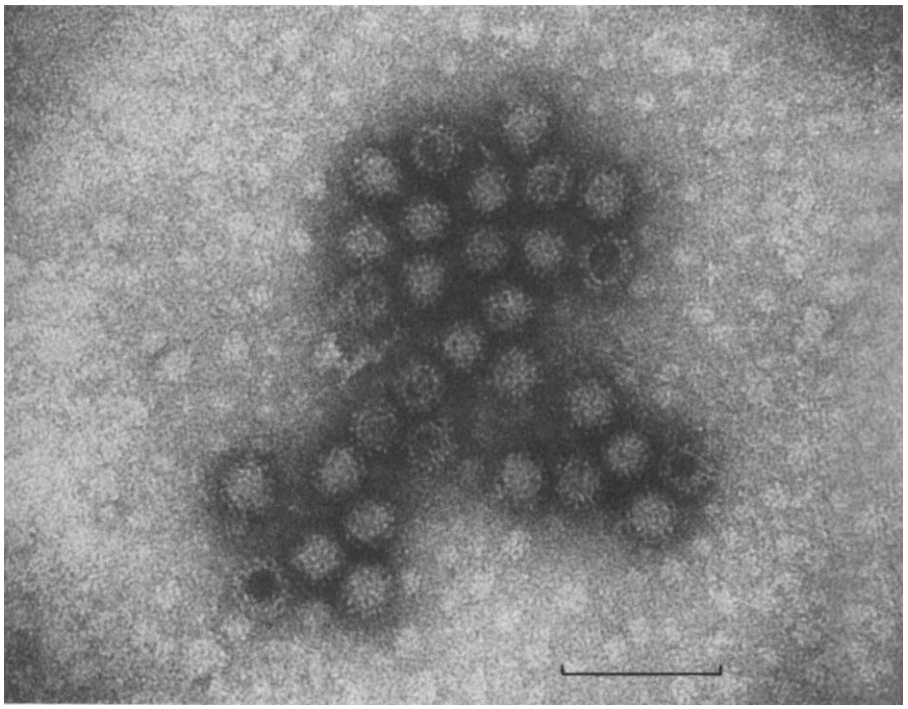

Fig. 9.12. Norwalk-like virus. EM picture from human stool isolate. The bar on the micrograph represents 100 nm. (courtesy of Dr. E.O. Caul.)

stools (Fig. 9.12). Calciviruses, to which group Norwalk virus might belong, contain infectious single-stranded RNA, M.W. $2.6–2.8 \times 10^6$, have a density of 1.36–1.39 g/cm^3 in CsCl, a diameter of 35–39 nm and possess one major capsid polypeptide.

9.4.2. Clinical aspects

The disease induced by Norwalk virus is a mild, self-limiting, gastroenteritis with vomiting and diarrhoea, lasting for 1–2 days. From studies on volunteers the incubation time has been found to be 48 hours and the virus shedding in stools was maximal during the acute illness (Thornhill et al., 1975). The acute infection results in histopathological lesions in the jejunum and a broadening of the villi in the small intestine.

9.4.3. Epidemiology

In healthy adults the prevalence of antibodies against Norwalk virus is between 50 and 90% in different parts of the world (Greenberg et al., 1979). Antibodies are gra-

dually acquired over a period of years in industrialized countries while in developing countries a high prevalence of antibodies is found in children. The acquisition of antibodies against Norwalk virus takes place later than against rotavirus (Black et al., 1982). Contaminated water seems often to be responsible for spreading the virus (Taylor et al., 1981, Baron et al., 1982, Kaplan et al., 1982). Only a short term immunity has been observed in volunteers after a challenge with Norwalk virus although this immunity prevented illness after a second challenge with virus 6–14 weeks later. However, the immunity after one challenge did not prevent illness when the rechallenge was delayed 27–42 months (Parrino et al., 1977). There seems to be no clear correlation between antibody level and development of clinical symptoms after challenge with Norwalk virus (Blacklow et al., 1979, Greenberg et al., 1981). Further studies on the mechanism of immunity and the possible influence of genetic factors are required before immunoprophylaxis can be discussed. No specific antiviral treatment of the disease is presently known.

9.5. Conclusions about reoviridae, retrovirus and Norwalk virus infections

The high incidence of acute gastroenteritis caused by rotaviruses calls for prophylactic and therapeutic measures. Although no vaccine is presently available it seems

TABLE 9.18.
Coronaviruses (see also Wege et al., 1982)

Virus	Host	Disease
Infectious bronchitis virus (IBV)	Chicken	Respiratory disease, nephritis, gonaditis
Murine hepatitis virus (MHV)	Mouse	Hepatitis, encephalomyelitis, enteritis, vasculitis
Bovine coronavirus (BCV)	Cattle	Enteritis
Human coronavirus (HCV)	Man	Respiratory disease
Transmissible gastroenteritis virus (TGEV)	Pig	Enteritis
Haemagglutinating encephalomyelitis virus (HEV)	Pig	Vomiting and wasting disease encephalomyelitis
Probable virus member		
Canine coronavirus (CCV)	Dog	Enteritis
Feline infectious peritonitis virus (FIPV)	Cat	Peritonitis, granulomatous inflammations in many organs
Possible virus member		
Rat coronavirus, sialodacryoadenitis virus (RCV, SDAV)	Rat	Respiratory disease, adenitis
Turkey coronavirus (TCV)	Turkey	Enteritis
Porcine epidemic diarrhoea virus (PEDV)	Pig	Enteritis

likely that vaccines will be developed in the next few years. There are also several rotavirus enzymes useful as targets for antiviral drugs. However, no antiviral drugs have shown therapeutic effects against rotavirus infections.

The newly discovered human retrovirus (HTLV) has not yet been investigated in such detail as to predict the usefulness of vaccine or antiviral drugs. Several compounds are known to inhibit other retrovirus enzymes but the implication of this for chemotherapy of HTLV infection is unknown at present.

The possibility and need for vaccination or chemotherapy against Norwalk virus and related agents is unclear.

9.6. Coronaviruses

Coronaviruses are a group of eleven pleomorphic, positive stranded, RNA-containing enveloped viruses (reviewed by Siddell et al., 1983, Tyrrell et al., 1968, Robb and Bond, 1979) infecting humans, animals and birds (Tables 9.18, 19). Human strains mainly infect the respiratory tract and are confined, normally, to the ciliary epithelium of the trachea, nasal mucosa and alveolar cells of the lungs.

9.6.1. Virus structure and replication

Coronavirions are pleomorphic, 60 to 220 nm in diameter and have club-shaped surface projections about 20 nm in length (Fig. 9.13). In thin sections the virion envelope may be visualized as inner and outer shells separated by a translucent space. The internal ribonucleoprotein (RNP) component of coronavirions has been visualized as a long strand of 1 to 2 nm diameter, or as a helical RNP condensed into coiled structures of varying diameter, normally 10 to 20 nm.

The coronavirion nucleocapsid contains a non-glycosylated protein of 50K to 60K M.W. This protein is phosphorylated and purified Murine Hepatitis Virus (MHV) virions have been shown to contain a protein kinase activity. Coronavirions contain two major envelope proteins. The matrix protein is a transmembrane glyco-

TABLE 9.19.
Antigenic relationships of coronaviruses

	Group 1	Group 2
Mammalian	HCV 229E and other isolates	HCV OC43 and other isolates
	TGEV (1 serotype)	MHV (many serotypes)
	CCV (1 serotype)	RCV (SDAV) (1 serotype)
	FIPV (1 serotype)	BCV (1 serotype)
		HEV (1 serotype)
Avian	IBV (at least 8 serotypes)	TCV (1 serotype)

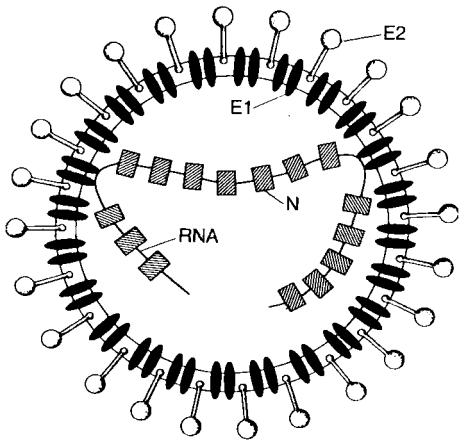

Fig. 9.13. A model of coronavirion structure. Genome (RNA), nucleocapsid protein (N), matrix protein (E1) and peplomer protein (E2) are shown. (after Siddell et al., 1983.)

protein of 20K to 35K. The glycosylated region of the protein is exterior to the virion envelope and in many cases matrix proteins with different degrees of glycosylation are incorporated into virions. The second corona virion envelope protein, which constitutes the surface peplomer, is responsible for eliciting neutralizing antibodies during infection and is thus vital for incorporation into any vaccine (Schmidt and Kenny, 1981). In many cases different molecular weight forms (80K to 200K) of the protein are incorporated into virions. The protein is acylated and complex and mannose-rich carbohydrate side-chains are N-glycosydically linked to the polypeptide. Virions grown in cells treated with tunicamycin lack the peplomer protein and are unable to attach to cells or initiate infection. There are indications that for some coronaviruses proteolytic processing of the peplomer protein occurs during morphogenesis and may be involved in activating functions such as virus-induced cell fusion (Collins et al., 1982).

The coronavirus genome is a linear molecule of single-stranded RNA, which is polyadenylated and infectious. The genome RNA has a M.W. of 5×10^6 to 7×10^6, corresponding to about 15000 to 20000 nucleotides (Fig. 9.14). T_1-resistant oligonucleotide fingerprinting of genome RNA and intracellular viral mRNA confirms the positive polarity of the genome and indicates that it does not have extensive sequence reiteration (Macnaughton and Madge, 1978, Siddell et al., 1983).

As regards early events of virus-cell interaction, Patterson and Macnaughton (1981) have shown that virions initially attach over the whole cell surface but are then rapidly redistributed away from the cell periphery by an energy-requiring process. The reason for this redistribution is unknown. Krzytyniak and Dupuy (1981) have shown that MHV3 uptake into cells is rapid and temperature-dependent. Uptake is not related to the phagocytic capacity of the cells and may, therefore, involve a mechanism such as receptor-mediated endocytosis, as has been reported for other

virus-cell systems (Helenius et al., 1980, and see Chapter 7 for example). The essential features of virus genome replication are: (i) the expression of coronavirus information in the cell is mediated through multiple subgenomic mRNAs, which form a 3′ co-terminal nested set; (ii) as far as is known, each mRNA directs the translation of only one protein; (iii) the size of the translation product for each RNA corresponds approximately to the coding potential of the 5′ sequences which are absent from the next smallest RNA. Although it has not been proven, these features and the inability of ribosomes to initiate translation at internal sites on eukaryotic mRNA suggest that only the 5′ sequences of each mRNA (depicted as genes A, B, C, etc. in Fig. 9.14) are translated into protein. This strategy has many parallels with the strategies of other positive-stranded RNA viruses (see Chapter 4). Also, the coronavirus strategy appears to be a flexible one, allowing for the control of viral protein synthesis at the levels of both transcription and translation. (For additional details see Stern and Kennedy, 1980).

In general, the smallest RNA, RNA7, encodes the intracellular nucleocapsid polypeptide (60K). The next smallest, RNA6, encodes the matrix protein polypeptide (23K) in vitro, or its glycosylated counterpart (25K) in oocytes, and the third major intracellular RNA, RNA3, encodes the peplomer protein core (120K) in vitro or the co-translationally glycosylated peplomer precursor (150K) in oocytes. The translation products of two further MHV RNAs, RNA2 and RNA4/5, have been identified as corresponding to 30K and 14K to 17K intracellular viral polypeptides, respectively. Finally, morphogenic studies on the maturation of coronaviruses have revealed that virus assembly is restricted to the cytoplasm, where progeny virions are formed by a budding process from membranes of the rough endoplasmic reticulum. The virions acquire their lipid envelope from the cells, excluding host cell proteins in the process, and are subsequently transported through and accumulate in the Golgi complex and smooth walled vesicles. There is an absence of budding from the plasmalemma (Beesley and Hitchcock, 1982, Ducatelle et al., 1981, Holmes et

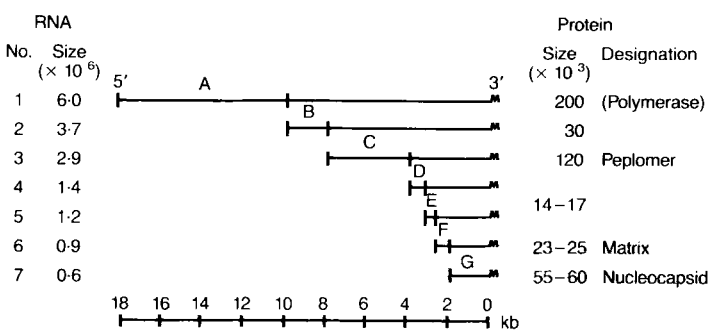

Fig. 9.14. The structure and expression of the murine hepatitis (coronavirus) genome. (after Sidell et al., 1983.)

al., 1981, Massalski et al., 1981) but the precise mechanism of virus release, as with many other viruses, has not been elucidated to date.

9.6.2. Prevention of human disease

Very little work has been carried out to date with *human* coronaviruses, either from the point of view of vaccine development or specific antivirals. Both approaches may be usefully investigated in the future. Genetic cloning may be particularly useful for development of inactivated vaccines, since the virus itself would be difficult to replicate and purify in large quantities for conventional vaccines.

References: Rubella

Alstyne, van D., Krystal, G., Kettyls, G.D. and Bohn, E.M. (1981) The purification of rubella virus (RV) and determination of its polypeptide composition. Virology 108, 491–498.

Anderson, R.M. and May, R.M. (1983) Vaccination against rubella and measles: quantitative investigations of different policies J. Hyg. 90, 259–325.

Arvin, A.M., Schmidt, N.J., Cantell, K. and Merigan, T.C. (1982) Alpha interferon administration to infants with congenital rubella. Antimicrob. Agents and Chemother. 21, 259–261.

Avery, G.B., Monif, G.G.R., Sever, J.L. and Leikin, S.L. (1965) Rubella syndrome after inapparent maternal illness. Am. J. Dis. Child. 110, 444.

Banatvala, J.E. (1977) Rubella vaccines. In Recent advances in clinical virology, Ed. A.P. Waterson. Churchill Livingstone, Edinburgh.

Banatvala, J.E. and Best, J.M. (1984) In Principles of Bacteriology, Virology and Immunity, Seventh Edition, Eds. Topley and Wilson.

Bellani, J.A., Artenstein, M.S., Olson, L.M., Buescher, E.L., Luhrs, C.E. and Milstead K.L. (1965) Congenital rubella. Clinicopathologic, virologic and immunologic studies. Am. J. Dis. Child. 110, 464.

Best, J.M., Banatvala, J.E. and Bowen, J.M. (1974) New Japanese rubella vaccines: comparative trials. Brit. Med. J. 3, 221–224.

Best, J.M., Harcourt, G.C., O'Shea, S., and Banatvala, J.E. (1979) Rubella vaccines. Lancet ii, 690–691.

Brinton, M.A. (1980) Non arbo Togaviruses. In The Togaviruses, Biology Structure and Replication, Ed. R.W. Schlesinger, pp 623–659. Academic Press, New York.

Böttiger, M. and Heller, L. (1976) Experience from vaccination and revaccination of teenage girls with three different rubella vaccines. J. Biol. Standard. 4, 107.

Buynak, E.B., Hilleman, M.R., Weibel, R.E. and Stokes, J. Jr. (1968) Live attenuated rubella virus vaccines prepared in duck embryo cell culture. I. Development and clinical testing. J.A.M.A. 204, 195–200.

Christie, A.B. (1980). Infectious Diseases: Epidemiology and Clinical Practice. Churchill, Livingstone, Edinburgh.

Clarke, M., Boustred, J., Schild, G.C. et al. (1979) Effect of rubella vaccination programme on serological status of young adults in United Kingdom. Lancet i, 1224–1226.

Clarke, M., Stitt, J., Seagroatt, V. et al. (1980) Rubella antibody status of young adults in U.K. Lancet i, 537–538.

Clarke, M., Schild, G.C., Miller, C., Seagroatt, V., Pollock, R.M. Finlay, S.E. and Barbara J.A.J. (1983) Surveys of rubella antibodies in young adults and children. Lancet i, 667–669.

Clubb, R.A., Dove, G.A.W., Macinnes, I.E. and Hind, S. (1981) Factors influencing rubella immunity in women. Br. Med. J. 282, 275–277.

Cooper, L., Green, R.H., Krugman, S., Giles, J.P. and Mirick, G.S. (1965) Neonatal thrombocytopenic purpura and other manifestations of rubella contracted in utero. Am. J. Dis. Child. 110, 416.

Erickson, C.A. (1944) Rubella early in pregnancy causing congenital malformation of the eyes and the heart. J. Pediat. 25, 281.

Forrest and Honeyman (1973) Med. J. Aust. i, 745.

Gilmore, D., Robinson, E.T., Gilmour, W.H. and Urquhart, G.E.D. (1982) Effect of rubella vaccination programme in schools on rubella immunity in a general practice population. Br. Med. J. 284, 628–630.

Gregg, N.M. (1941) Congenital cataract following german measles in the mother. Trans. Ophthal. Soc. Aust. 3, 35.

Halstead, S.B. and Diwan, A.R. (1971) Failure to transmit rubella vaccine virus. A close-contact study in adults. J. Am. Med. Ass. 215, 634.

Hambidge, R.M., Shaffer, D., Marshall, W.C. and Hayes, K. (1966) Congenital rubella. Report of two cases with generalized infection. Br. Med. J. 1, 650.

Hambling, M.H. (1980) Changes in the distribution of rubella antibodies in women of childbearing age during the first eight years of a rubella vaccination programme. J. Infection 2, 341–346.

Hethcote, H.W. (1983) Measles and rubella in the United States. Am. J. Epidemiol. 117, 2–13.

Hinman, A.R., Bart, K.J., Orenstein, W.A. and Preblud, S.R. (1983) Rational strategy for rubella vaccination. Lancet i, 39–41.

Horstmann, D.M., Banatvala, J.E., Riordan, J.T., Payne M.C., Whittemore, R., Opton, E. and Florey, C. du Ve (1965) Maternal rubella and the rubella syndrome in infants. Am. J. Dis. Child. 110, 408.

Hoshino, M., Oka, Y., Deguchi, M., Hirayama, M. and Kono, R. (1982) The ten year follow-up of the persistence of humoral antibody to rubella virus acquired by vaccination with the Japanese To 336 vaccine. J. Biol. Standardiz. 10, 213–220.

Hutchinson, A. and Thompson, J. (1982) Rubella prevention, two methods compared. Br. Med. J. 284, 1087–1089.

Huygelen, C. and Peetermans, J. (1967) Attenuation of rubella virus by serial passage in primary rabbit kidney cell cultures. II. Experiments in animals. Arch. Ges. Virusforsch. 21, 357.

Keir, E.H. (1965) Results of rubella in pregnancy. II. Hearing defects. Med. J. Aust. 2, 691.

Lundström, R. and Boström, I.-L. (1958) Rubella in pregnancy and the incidence of developmental anomalies of the eyes. Acta Ophthalmol. 36, 782.

Lundström, R., Leysell, L. and Berghagen, N. (1962) Dental development in children following maternal rubella. Acta Paediat. Stockh. 51, 155.

Manson, M.M., Logan, W.P.D., Loy, R.M. (1960) Rubella and other virus infection during pregnancy. Minist. Hlth. Rep. on Publ. Hlth. Med. Subj. 101, HMSO, London.

McCarthy, K., Taylor-Robinson, C.H. and Pillinger, S.E. (1963) Isolation of rubella virus from cases in Britain. Lancet ii, 593.

Menser, M.A., Harley, J.D., Hertzberg, R., Dorman, D.G. and Murphy, A.M. (1967) Persistence of virus in lens for three years after prenatal rubella. Lancet ii, 387.

Meyer, H.M. Jr., Parkman, P.D., and Panos, T.C. (1966) Attenuated rubella virus: II. Production of an experimental live virus vaccine and clinical trial. N. Engl. J. Med. 275, 575.

Meyer, H.M., Jr., Parkman, P.D., Hobbins, T.E., and Ennis, F.A. (1968) Clinical studies with experimental live rubella virus vaccine (strain HPV-77) and an evaluation of vaccine-induced immunity, Am. J. Dis. Child. 115, 648.

Meyer, H.M., Jr., Parkman, P.D. and Hopps, H.E. (1969) The control of rubella. Pediatrics 44, 5.

Modlin, J.F., Herrmann, K., Brandling-Bennett, A.D., Eddins, D.L. and Hayden, G.F. (1976) Risk of congenital abnormality after inadvertent rubella vaccination of pregnant women. New. Engl. J. Med. 294, 972–974.

Monif, G.R.G., Avery, G.B., Korones, G.B. and Sever J.L. (1965) Postmortem isolation of rubella virus from three children with rubella syndrome defects. Lancet i, 723.

Oker-Blom, C., Kalkkinen, N., Kääriäinen, L. and Pettersson, R.F. (1983) Rubella virus contains one capsid protein and three envelope glycoproteins E1, E2a and E2B. J. Virol. 46, 964–973.

Orenstein, W.A. and Greaves, W.L. (1982) Congenital rubella syndrome: a continuing problem. J.A.M.A. 247, 1174–1175.

O'Shea, S., Best, J.M., Banatvala, J., Marshall, W.C. and Dudgeon, J.A. (1982) Rubella vaccination: persistence of antibodies for up to 16 years. Br. Med. J. 285, 253–255.

Oxford, J.S. and Schild, G.C. (1965) In vitro inhibition of rubella virus by l-adamantanamine hydrochloride. Arch. Ges. Virusforsch. 17, 313–329.

Parkman, P.D., Buescher, E.L. and Artenstein, M.S. (1962) Recovery of rubella virus from army recruits. Proc. Soc. Exp. Biol. Med. 111, 225.

Parkman, P.D., Meyer, H.M., Jr., Kirschstein, R.L., and Hopps, H.E. (1966) Attenuated rubella virus: I. Development and laboratory characterization. N. Engl. J. Med. 275, 569.

Peckham, C.S. (1974) Clinical and serological assessment of children exposed in utero to confirmed maternal rubella. Br. Med. J. 1, 259–261.

Peckham, C.S., Marshall, W.C. and Dudgeon, J.A. (1977) Rubella vaccination of schoolgirls: factors affecting vaccine uptake. Br. Med. J. 1, 760–761.

Plotkin, S., Farquhar, J.D. and Ogra, P.L. (1973) Immunologic properties of RA27/3 rubella virus vaccine. A comparison with strains presently licensed in the United States. J. Am. Med. Assoc. 225, 585–590.

Polakoff, S. (1983) The use of immunoglobulins in virus infections. In Recent Advances in Clinical Virology, Ed. A.P. Waterson. Churchill Livingstone, Edinburgh.

Preblud, S.R., Gross, F., Halsey, N.A., Hinman, A.R., Hermann, K.L. and Koplan, J.P. (1982) Assessment of susceptibility to measles and rubella. J.A.M.A. 247, 1134–1137.

Sabin, A.B. (1981) Evaluation of some currently available and prospective vaccines. J.A.M.A. 246, 236–241.

Schiff, G.M. (1980) Active immunization for adults. Ann. Rev. Med. 31, 441–451.

Urquhart, G.E.D., Crawford, R.J., and Wallace J. (1978) Trial of high titre rubella immunoglobulin. Br. Med. J. 2, 1331.

Weibel, R.E., Stokes, J., Jr., et al. (1968) Live attenuated rubellavirus vaccines prepared in duck embryo cell culture. II. Clinical tests in families and in an institution. J.A.M.A. 205, 554–558.

Weibel, R.E., Stokes, J., Buynak, E.B. and Hilleman, M.R. (1969) Live rubella vaccination in adults and children. Am. J. Dis. Child. 118, 226.

Weibel, R.E., Buynak, E.B., McLean, A.A. and Hilleman, M.R. (1975) Long-term follow-up for immunity after monovalent or combined live measles, mumps and rubella vaccines. Pediatrics 56, 380.

Weller, T.H. and Neva, F.A. (1962) Propagation in tissue culture of cytopathic agents from patients with rubella-like illness. Proc. Soc. Exp. Biol. Med. 111, 215.

Zygraich, N., Peetermans, J. and Huygelen, C. (1971) In vivo properties of attenuated rubella virus Cendehill strain. Arch. Ges. Virusforsch, 33, 225.

References: Reoviridae, Retrovirus and Norwalk viruses

Baron, R.C., Murphy, F.D., Greenberg, H.B., Davis, C.E., Bregman, D.J., Gary, G.W., Huges, J.M. and Schonberger, L.B. (1982) Norwalk gastroenteritis illness: an outbreak associated with swimming in a recreational lake and secondary person-to-person transmission. Am. J. Epidemiol. 115, 163–172.

Barré-Sinnoussi, F., Chermann, J.C., Rey, F., Nugeyere, M.T., Chamaret, S., Gruest, J., Danguet, C., Axler-Blin, C., Vézinet-Brun, F., Rouzioux, C., Rozenbaum, W. and Montagnier, L. (1983) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 220, 868–871.

Black, R.E., Greenberg, H.B., Kapikian, A.Z., Brown, K.H. and Becker, S. (1982) Acquisition of serum antibody to Norwalk virus and rotavirus and relation to diarrhea in a longitudinal study of young children in rural Bangladesh. J. Inf. Dis. 145, 483–489.

Blacklow, N.R., Cukor, G., Bedigian, M.K., Echeverria, P., Greenberg, H.B., Schreiber, D.S. and Trier, J.S. (1979) Immunoresponse and prevalence of antibody to Norwalk enteritis virus as determined by radioimmunoassay. J. Clin. Microbiol. 10, 903–909.

Blattner, W.A., Blayney, D.W., Robert-Guroff, M., Sarngadharan, M.G., Kalyanaraman, V.S., Sarin, P.S., Jaffe, E.S. and Gallo, R.C. (1983) Epidemiology of human T-cell leukemia/lymphoma virus. J. Inf. Dis. 147, 406–416.

Chang, T.-W. and Heel, R.C. (1981) Ribavirin and inosiplex: A review of their present status in viral diseases. Drugs 22, 111–128.

Chanock, R.M. (1981) Strategy for development of respiratory and gastrointestinal vaccines in the 1980s. Joseph E. Smadel Memorial Lecture. J. Inf. Dis. 143, 364–374.

Davidson, G.P., Bishop, R.F., Townley, R.R.W., Holmes, I.H. and Ruck, B.J. (1975) Importance of a new virus in acute sporadic enteritis in children. Lancet i, 242–245.

Davidson, G.P., Hogg, R.J. and Kirubakaran, C.P. (1983) Serum and intestinal immune response to rotavirus enteritis in children. Infect. Immun. 40, 447–452.

Edelman, R. (1982) Enteric infections. Inf. Dis. 12, 4–9.

Elliot, K.M. (1976) Acute diarrhoea in childhood. Ciba Foundation Symposium No. 42, p. 1, Elsevier, Excerpta Medica, Amsterdam.

Essex, M., McLane, M.F., Lee, T.H., Falk, L., Howe, C.W.S., Mullins, J.I., Cabradilla, C. and Francis, D.P. (1983) Antibodies to cell membrane antigens associated with human T-cell leukemia virus in patients with AIDS. Science 220, 859–862.

Gallo, R.C., Sarin, P.S., Gelmann, E.P., Robert-Guroff, M., Richardson, E., Kalyanaraman, V.S., Mann, D., Sidhu, G.D., Stahl, R.E., Zolla-Pazner, S., Leibowitch, J. and Popovic, M. (1983) Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). Science, 220, 865 867.

Gelmann, E.P., Papovic, M., Blayney, D., Masur, H., Sidhu, G., Stahl, R.E. and Gallo, R.C. (1983) Proviral DNA of a retrovirus, human T-cell leukemia virus, in two patients with AIDS. Science 220, 862–865.

Ghose, L.H., Schnagl, R.D. and Holmes, I.H. (1978) Comparison of an enzyme-linked immunosorbent assay for quantitation of rotavirus antibodies with complement fixation in an epidemiological survey. J. Clin. Microbiol. 8, 268–276.

Greenberg, H.B., Valdesuso, J., Kapikian, A.Z., Chanock, R.M., Wyatt, R.G., Szmuness, W., Larrick, J., Kaplan, J., Gilman, R.H. and Sack, D.A. (1979) Prevalence of antibody to the Norwalk virus in various countries. Inf. Immunol. 26, 270–273.

Greenberg, H.B., Wyatt, R.G., Kalica, A.R., Yolken, R.H., Black, R., Kapikian, A.Z. and Chanock, R.M. (1981) New insights in viral gastroenteritis. Perspect. in Virol. 11, 163–187.

Gust, I.D., Pringle, R.C., Barnes, G.L., Davidson, G.P. and Bishop, R.F. (1977) Complement-fixing antibody response to rotavirus infection. J. Clin. Microbiol. 5, 125–130.

Hahn, B., Monzari, V., Colombini, S., Franchini, G., Gallo, R.C. and Wong-Staal, F. (1983) Common site of integration of HTLV in cells of three patients with mature T-cell leukemia-lymphoma. Nature 303, 253–256.

Halvorsrud, J. and Örstavik, I. (1980) An epidemic of rotavirus-associated gastroenteritis in a nursing home for the elderly. Scand. J. Inf. Dis. 12, 161–164.

Joklik, W.K. (1980) The structure and function of the reovirus genome. Ann. N.Y. Acad. Sci. 354, 107–124.

Kapikian, A.Z., Wyatt, R.G., Dolin, R., Thornhill, T.S., Kalica, A.R. and Chanock, R.M. (1972) Visualization by immune electron microscopy of a 27 nm particle associated with acute infectious non-bacterial gastroenteritis. J. Virol. 10, 1075–1081.

Kapikian, A.Z., Wyatt, R.G., Greenberg, H.B., Kalica, A.R., Kim, H.W., Brandt, C.D., Rodriguez, W.J., Parrott, R.H. and Chanock, R.M. (1980) Approaches to immunization of infants and young children against gastroenteritis due to rotaviruses. Rev. Inf. Dis. 2, 459–469.

Kapikian, A.Z., Greenberg, H.B., Kalica, A.R., Wyatt, R.G., Kim, H.W., Brandt, C.D., Rodriguez, W.J., Flores, J., Singh, N., Parrott, R.H. and Chanock, R.M. (1981) New developments in gastroenteritis. In Proceedings of the Third Nobel Conference. Acute enteric infections in children. New Prospects for treatment and prevention, Eds. T. Holme, J. Holmgren, M.H. Merson, and R. Mollby, pp 9–57. Elsevier, New York.

Kapikian, A.Z., Greenberg, H.B., Wyatt, R.G., Kalica, A.R. and Chanock, R. (1982) The Norwalk group of viruses – agents associated with epidemic viral gastroenteritis. In Virus Infections of the Gastrointestinal Tract, Eds. D.A.J. Tyrrell and A.Z. Kapikian, pp 147–177. Marcel Dekker Inc., New York, Basel.

Kapikian, A.Z., Wyatt, R.G., Levine, M.M., Yolken, R.H., VanKirk, D.H., Dolin, R., Greenberg, H.B. and Chanock, R.M. (1983) Oral administration of human rotavirus to volunteers: induction of illness and correlates of resistance. J. Inf. Dis. 147, 95–106.

Kaplan, J.E., Goodman, R.A., Schonberger, L.B., Lippy, E.C. and Gary, G.W. (1982) Gastroenteritis due to Norwalk virus: An outbreak associated with a municipal water system. J. Inf. Dis. 146, 190–197.

McLean, B.S. and Holmes, I.H. (1980) Transfer of antirotaviral antibodies from mothers to their infants. J. Clin. Microbiol. 12, 320–325.

Middleton, P.J. (1982) Role of viruses in pediatric gastrointestinal disease and epidemiologic factors. In Virus Infections of the Gastrointestinal Tract, Eds. D.A.J. Tyrrell and A.Z. Kapikian, pp. 211–225. Marcel Dekker.

Middleton, P.J., Petric, M. Hewitt, C.M., Szymanski, M.T. and Tam, J.S. (1976) Counter-immunoelectro-osmophoresis for the detection of infantile gastroenteritis virus (orbi-group) antigen and antibody. J. Clin. Pathol. 29, 191–197.

Miyoshi, I., Kubonishi, I., Yoshimoto, S., Akagi, T., Ohtsuki, Y., Shiraishi, Y., Nagata, K. and Hinuma, Y. (1981) Type C virus particles in a cord T-cell derived by cocultivating normal human cord leucocytes and human leukemic T cells. Nature 294, 770–771.

Öberg, B. (1983) Antiviral effects of phosphonoformate (PFA, foscarnet sodium). Pharmc. Ther. 19, 387–415.

Parrino, T.A., Schreiber, D.S., Trier, J.S., Kapikian, A.Z. and Blacklow, N.R. (1977) Clinical immunity in acute gastroenteritis caused by the Norwalk agent. N. Engl. J. Med. 297, 86–89.

Poiesz, B.J., Ruscetti, F.W., Gazdar, A.F., Bunn, P.A., Minna, J.D. and Gallo, R.C. (1980) Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc. Natl. Acad. Sci. U.S.A. 77, 7415–7419.

Poiesz, B.J., Ruscetti, F.W., Reitz, M.S., Kalyanaraman, V.S. and Gallo, R.C. (1981) Isolation of a new type C retrovirus (HTLV) in primary uncultured cells of a patient with Sézary T-cell leukemia. Nature 294, 268–271.

Popovic, M., Reitz, M.S. Jr., Sarngadharan, M.G., Robert-Guroff, M., Kalyanaraman, V.S., Nakao, Y., Miyoshi, I., Minowada, J., Yoshida, M., Ito, Y. and Gallo, R.C. (1982) The virus of Japanese adult human T-cell leukemia is a member of the human T-cell leukemia virus group. Nature 300, 63–66.

Reitz, M.S. Jr., Kalyanaraman, V.S., Robert-Guroff, M., Popovic, M., Sarngadharan, M.G., Sarin, P.S. and Gallo, R.C. (1983) Human T-cell leukemia/lymphoma virus. The retrovirus of adult T-cell leukemia/lymphoma. J. Inf. Dis. 147, 399–405.

Sarin, P.S., Aoki, T., Shibata, A., Ohnishi, Y., Aoyagi, Y., Miyakoshi, H., Emura, I., Kalyanaraman, V.S., Robert-Guroff, M., Popovic, M., Sarngadharan, M., Nowell, P.C. and Gallo, R.C. (1983) High incidence of human type-C retrovirus (HTLV) in family members of a HTLV-positive Japanese T-cell leukemia patient. Proc. Natl. Acad. Sci. U.S.A. 80, 2370–2374.

Saulsbury, F.T., Winkelstein, J.A. and Yolken, R.H. (1980) Chronic rotavirus infection in immunodeficiency. J. Ped. 97, 61–65.

Schoub, B.D. and Prozesky, O.W. (1977) Antiviral activity of ribavirin in rotavirus gastroenteritis of mice. Antimicrob. Agents Chemother. 12, 543–544.

Smee, D.F., Sidwell, R.W., Clark, S.M., Barnett, B.B. and Spendlove, R.S. (1982) Inhibition of rotaviruses by selected antiviral substances: Mechanism of viral inhibition and in vivo activity. Antimicrob. Agents. Chemother. 21, 66–73.

Taylor, J.W., Gary, G.W. Jr. and Greenberg, H.B. (1981) Norwalk related viral gastroenteritis due to contaminated drinking water. Am. J. Epidemiol. 114, 584–592.

Thornhill, T.S., Kalica, A.R., Wyatt, R.G., Kapikian, A.Z. and Chanock, R.M. (1975) Pattern of shedding of the Norwalk particle in stools during experimentally induced gastroenteritis in volunteers as determined by immune electron microscopy. J. Inf. Dis. 132, 28–34.

Vesikari, T., Isolauri, E., D'Hondt, E., Delem, A., André, F.E. and Zissis, G. (1984) Protection of infants against rotavirus diarrhoea by RIT 4237 attenuated bovine rotavirus strain vaccine. Lancet i, 977–980.

Wyatt, R.G., Kalica, A.R., Mebus, C.A., Kim, H.W., London, W.T., Chanock, R.M. and Kapikian, A.Z. (1978) Reovirus-linked agents (rotaviruses) associated with diarrheal illness in animal and man. Perspect. Virol. 10, 121–145.

Wyatt, R.G., Kapikian, A.Z., Greenberg, H.B., Kalica, A.R. and Chanock, R.M. (1981) Prospects for development of a vaccine against rotavirus diarrhea. In Proceedings of the Third Nobel Conference. Acute enteric infections in children. New prospects for treatment and prevention, Eds. T. Holme, J. Holmgren, M.H. Merson and R. Mollby, pp 505–522. Elsevier, New York.

Yamakawa, M., Furuichi, Y. and Shatkin, A.J. (1982) Reovirus transcriptase and capping enzymes are active in intact virions. Virology 118, 157–168.

Yolken, R.H., Wyatt, R.G., Zissis, G., Brandt, C.D., Rodriguez, W.J., Kim, H.W., Parrott, R.H., Urrutia, J.J., Mata, L., Greenberg, H.B., Kapikian, A.Z. and Chanock, R.M. (1978) Epidemiology of human rotavirus types 1 and 2 as studied by enzyme-linked immunosorbent assay. N. Engl. J. Med. 299, 1156–1161.

References: Coronaviruses

Beesley, J.E. and Hitchcock, L.M. (1982) The ultrastructure of feline infectious peritonitis virus in feline embryonic lung cells. J. Gen. Virol. 59, 23–28.

Collins, A.R., Knobler, R.L., Powell, H. and Buchmeier, M.J. (1982) Monoclonal antibodies to murine hepatitis virus 4 (strain JHM) define the viral glycoprotein responsible for attachment and cell fusion. Virology, 119, 358–371.

Ducatelle, R., Coussement, W., Pensaert, M.B., Debouck, P. and Hoorens, J. (1981). In vivo morphogenesis of a new porcine enteric coronavirus CV777. Arch. Virol. 58, 35–44.

Helenius, A., Fries, E., Garoff, H. and Simons, K. (1980) On the entry of Semliki Forest virus into BHK-21 cells. J. Cell. Biol. 84, 404–420.

Holmes, K.V., Doller, E.W. and Sturman, L.S. (1981) Tunicamycin resistant glycosylation of a coronavirus glycoprotein: demonstration of a novel type of viral glycoprotein. Virology 115, 334–344.

Krzystyniak, K. and Dupuy, J.M. (1981) Early interaction between mouse hepatitis virus 3 and cells. J. Gen. Virol. 57, 53–61.

MacNaughton, M.R. and Madge, M.H. (1978) The genome of human coronavirus strain 229E. J. Gen. Virol. 39, 497–504.

Massalski, A., Coulter-Mackie, M. and Dales, S. (1981) Assembly of mouse hepatitis virus strain JHM. Adv. Exp. Med. Biol. 142, 111–118.

Patterson, S. and MacNaughton, M.R. (1981) The distribution of human coronavirus strain 229E on the surface of human diploid cells. J. Gen. Virol. 53, 267–273.

Robb, J.A. and Bond, C.W. (1979) Coronaviridae. In Comprehensive Virology, Vol. 14, Eds. H. Fraenkel-Conrat and R.W. Wagner, pp. 193–247. Plenum Press, New York.

Schmidt, O.W. and Kenny, G.E. (1981) Immunogenicity and antigenicity of human coronavirus 229E and OC43. Infect. Immun. 32, 1000–1006.

Siddell, S., Wege, H. and Ter Meulen, V. (1983) The biology of coronaviruses. J. Gen. Virol. 64, 761–776.

Stern, D.F. and Kennedy, S.I.T. (1980) Coronavirus multiplication strategy. I. Identification and characterization of virus-specified RNA. J. Virol. 34, 665–674.

Tyrrell, D.A.J., Almeida, J.D., Berry, D.M., Cunningham, C.H., Hamre, D., Hofstad, M.S., Mallucci, L. and McIntosh, K. (1968) Corona viruses. Nature (London) 220, 650.

Wege, H., Siddell, S. and Ter Meulen, V. (1982) The biology and pathogenesis of coronaviruses. Curr. Top. in Microbiol. Immunol. 99, 165–200.