The Rotaviruses

Brief Review

By

T. H. Flewett and G. N. Woode*

Regional Virus Laboratory, Last Birmingham Hospital,
Birmingham, and Institute for Research on Animal Diseases,
Compton, Berkshire, England

Introduction

This review concerns a group of viruses causing acute gastroenteritis in young animals of various species. All members of the group share a common morphology differing, at least in detail, from other somewhat similar viruses. Other properties will be detailed below. The name 'Rotavirus' has been suggested for this group (41). The name 'Duovirus' has also been advanced (31). Some people still refer to them as 'reovirus-like agents' (56, 60), and some call them 'infantile gastroenteritis virus (orbivirus group)' (100). Most authors now use the name 'rotavirus'. For convenience we shall use the name 'rotavirus' but bearing in mind that no official designation of a name for this group has yet been made, and that no decision on its taxonomic status has been declared. We have tried to refer to at least the majority of important publications, but are well aware that some may have been missed and hope their authors, and the reader, will forgive us.

Historical

Epizootic Diarrhoea of Infant Mice (EDIM)

Outbreaks of acute diarrhoea of suckling mice were reported from several quarters in the United States before 1944, but had never been well characterized. Syverton and Olitsky (124) described one such in the Rockefeller Institute, but ascribed it to Salmonella infection. A very similar outbreak occurred in the animal stock of the Harvard Medical School in 1944. This time bacteriological investigations revealed occasional Salmonella infections; but the distribution of isolations did not support the thesis that these organisms were responsible for the outbreak.

---

* Present address: Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Ames, IA 50010, U.S.A.

The infection was apparently introduced by an animal attendant who had worked in another establishment where suckling mice were similarly afflicted. These events, the course of disease in the mice, and the appearance of cytoplasmic and also intranuclear inclusions were described in detail (26, 27, 97, 98, 99). Intranuclear inclusions were observed only in one batch of mice; but cytoplasmic inclusions appeared to be related to the disease (98), and were found only in the epithelium of the small intestine.

The disease appeared to be seasonal, occurring mainly in the autumn and early winter. Infection could be transmitted by feeding a collodion membrane filtrate of intestinal suspension. The disease first appeared between the 10th and 15th days of life, but sometimes as early as the third day. A yellowish watery diarrhoea appeared; mice usually recovered in a few days; death when it occurred was often due to impaction of hardened faeces in the rectum.

LIZBETH KRAFT, in a series of investigations over several years (2, 61, 62) established that the disease was caused by a rather heat-resistant virus, was highly infectious, and had an incubation period from 40 hours to 10 days. The virus was ether-resistant and was 65—75 nm in diameter, by its filtration end-point. It was different from reovirus type 3 and also from another mouse virus LIVIM (lethal intestinal virus of infant mice), which was ethersensitive, more sensitive to heat, and caused syncytial lesions in alimentary tracts of infected animals. In an important paper, ADAMS and KRAFT (3) showed, from an electron microscopic study of sections of mouse small bowel: i) that the EDIM virus replication was entirely cytoplasmic, ii) that virus particles were 'budded' from areas of 'viroplasm' into dilated endoplasmic vesicles where they were present in large numbers, iii) infected cells were shed, or burst into, the intestinal lumen, liberating very large numbers of virus particles, iv) that the cells affected were those on the sides and tips of the villi, cells in the crypts being spared. They observed that some, but not all, virus particles appeared to be surrounded by a membrane, and also that elongated forms were present. They also noted that the epithelial brush border appeared intact in the early stages, but became short, thick and irregular in the more severely infected cells.

Confirming and extending these findings, BANFIELD et al. (6) illustrated long tubular forms of the virus in thin sections of infected mouse gut, with an apparently helical structure on some. They observed that the EDIM virus did not conform precisely to patterns of intracellular growth set by other known cytoplasmic viruses, but that it most closely resembled the reoviruses.

No one had yet succeeded in isolating the virus in conventional tissue culture; RUBENSTEIN et al. (106) succeeded in taking the virus through three passages in organ cultures of mouse ileum and two passages in organ cultures of caecum, though all attempts at culture in mouse fibroblast monolayers failed. Somewhat surprisingly, attempts at isolation in organ cultures of mouse duodenum were also unsuccessful. MUCH and ZAJAK (88) characterized the virus further, establishing that it contained RNA and had a capsid resembling that of the bluetongue virus without its outer layer. They did not, however, illustrate forms of the virus with an outer capsid layer. They confirmed that the virus was very stable on storage and fairly acid-resistant; but found that it was less stable when carefully washed free of adventitious protein.

The 'SA.11' and '0' Viruses

The SA.11 virus was isolated in 1958 from a rectal swab from a vervet monkey (73). This virus produced eosinophilic inclusions in primary vervet monkey kidney tissue culture, and on further passages a cytopathic effect developed at 5—8 days after inoculation. A similar virus, giving rise to a rather slower cytopathic effect in vervet monkey kidney cells, also with cytoplasmic eosinophilic inclusions, was isolated on five occasions from pooled filtered washings of cattle and sheep intestines from the Johannesburg municipal abattoir. This virus could not be isolated in calf or lamb kidney tissue cultures and its species of origin is so far unknown, because pigs and equines were also slaughtered in this abattoir. These viruses (grown in tissue cultures) were shown to be morphologically identical, having a sharply defined outer capsid layer covering an inner capsid layer resembling that of bluetongue virus (35). Virus particles were often invested with envelopes derived from cell membranes. These viruses were found to be resistant to pH 4, but were slowly inactivated at pH 3 (63). These viruses were not isolated from diseased animals; but the SA.11 virus, though never tested in monkeys, has induced diarrhoea in young calves and gnotobiotic piglets (105).

Viruses in Bovine Diarrhoea

The Neonatal Calf Diarrhoea Virus

Epizootic (or Enzootic) diarrhoea of new-born calves has for centuries been a plague of stockbreeders. The disease attacks calves within four or five days of birth, causing at times a heavy mortality; 30 per cent loss is not uncommon. Although *Escherichia coli* has sometimes been incriminated, it has for long been clear that it has not been the usual cause. Attempts at experimental transmission had been made from time to time, but these had given equivocal results, probably because the virus, as will be seen, is ubiquitous in high titre on infected farms, so that controls as well as inoculated animals were liable to go down with the infection. [A virus called 'bovine viral diarrhoea virus' (BVD) has been known for some time; this is a togavirus which sometimes does cause diarrhoea in adult cattle; but it has nothing to do with epizootic diarrhoea of calves. Its name is misleading and confuses those not well acquainted with the veterinary literature].

Unequivocal success came from the use of gnotobiotic animals. MEBUS and his colleagues (77) first succeeded in transmitting infection by inoculating calves by intraduodenal tube with bacterium-free filtrates of diarrhoeal faeces. In later experiments calves were successfully infected by ordinary feeding. Animals developed diarrhoea usually within 24—48 hours; sometimes as early as 14—22 hours after inoculation. Virus particles 65 nm in diameter were found in large numbers in the faeces of infected animals (37). Cells in faeces could be stained by immunofluorescence. This method was found applicable to diagnosis of field outbreaks in infected herds. Virus, described as reovirus-like, was only twice isolated and adapted to continuous subculture in tissue cultures of bovine embryo kidney after many attempts (37, 78, 139).

This work was confirmed in England where two strains were adapted to tissue culture (15, 142), and more recently in other parts of the world. Another strain was adapted in N. Ireland (68).

Human Diarrhoea Viruses

As with calf diarrhoea, most bacteriological investigations of acute infectious diarrhoea in young children gave negative results. Early experimental transmissions to volunteers using bacterium-free filtrates have been reviewed by FLEWETT (46).

LIGHT and HODES (66) transmitted diarrhoea to calves using filtrates of human faeces in 1943; but others failed to confirm these results. It is, however, now known that rotaviruses were present in their material (80).

Although a small (28 nm) virus had been recovered from outbreaks of 'winter vomiting disease' in school children and adults by oral infection of adult volunteers (the 'Norwalk' agent) (58), this virus did not seem to be implicated to any great extent in most cases of acute infectious gastroenteritis of young children.

In the early days of virology, workers on human, animal, insect and plant viruses were all acquainted with each other — at any rate in England — and knew each other's work. But with proliferation of laboratories, diversification of interests, and multiplication of journals, this happy state has not existed for some time. Oblivious of the work of Mebus and his colleagues in Nebraska or not appreciating its importance, groups in Birmingham, Melbourne and Toronto were independently searching for virus particles in faeces or intestinal biopsies.

BISHOP et al. in Melbourne (9) took duodenal biopsies from young children with acute gastroenteritis and cut ultrathin sections. By electron microscopy they found dilated cytoplasmic vesicles containing large numbers of virus particles; these particles appeared to be budding into the vesicles from areas of 'viroplasm' in the cytoplasm. Because these particles resembled orbiviruses both in size and in their intracellular habit, BISHOP et al. suggested that they might be orbiviruses.

FLEWETT et al. (40) examined extracts of faeces from young children by electron microscopy using negative staining and found that in about half of these large numbers of virus particles were present. In faeces of 'control' children these particles were not present. FLEWETT et al. (40) illustrated particles with a double-layered capsid and a clearly defined rim. BISHOP et al. (10) confirmed the finding of virus in faeces, and SUZUKI and KONNO (123) confirmed the Melbourne work on electron microscopy of thin-sectioned biopsies.

MIDDLETON et al. (83) in Toronto had also been finding similar viruses in faeces, but also in duodenal aspirates and biopsies. Using frozen sections of biopsies they were able to test sera for the presence of antibody by the indirect fluorescent antibody method, and show that fluorescence could be found in intestinal cells distal to the nucleus. Most adults had antibodies. However, one volunteer did not; he was successfully infected when a filtrate of faeces from a patient was introduced by tube into his duodenum, although attempts to infect him orally were unsuccessful. Further work from many centres has since confirmed that a large proportion of cases of acute gastroenteritis in young children are apparently caused by infection with these viruses (18, 23, 29, 30, 31, 43, 44, 50, 54, 60, 67, 92, 93, 107, 110, 111, 113, 115, 126, 140).

Rotaviruses are apparently implicated in diarrhoea in about 25 per cent of cases under 1 year old; about 60 per cent between the age of 1 and 3; and 20 to 40 per cent between age 4 and 6 (7, 31, 125, 93, 18, 43, 56, 58, 59) and occasionally in older children (49).

The incubation period in infants appears to be about 24 hours, with frank diarrhoea after 48 hours. Maximum particle counts of virus are found in the faeces from the 3rd to the 5th day after onset. Shedding of virus is very small or absent after the 8th day; but rarely a child may continue to excrete virus for a month. This, however, does not indicate a severe infection; such patients have often had a mild disease (44). Particle counts in faeces are usually high, 10^9 particles/g faeces being usual, and counts of 10^{11} have been recorded. Virus excretion without symptoms has been described in outbreaks of infection in hospital nurseries (4, 11, 22, 24, 133).

Clinical Features of Rotavirus Infection in Children

The clinical features have been described in general (31, 46, 115). CARR et al. (25) described 100 consecutive cases admitted to hospital, all confirmed by electron microscopy of faeces. Initially, all were febrile, with temperatures up to $40°$ C; 36 presented with diarrhoea, 58 with vomiting, and diarrhoea with vomiting in 4. Two patients had vomiting only, without any diarrhoea. A search for rotaviruses in throat swabs of 20 proved negative. In 6 cases infection was complicated by enteropathic *Escherichia coli* infection. These suffered a much more prolonged illness, remaining febrile for more than a week. In another series of 32 cases (115) the one child with concomitant *E. coli* infection was the most seriously ill.

Similar Viruses in Other Species

Infectious diarrhoea has been studied in piglets (65, 69, 146) foals (45), lambs (116), rabbits (20), deer (135), and pronghorn antelope (103). In diarrhoeal faeces from all of these animals virus particles morphologically indistinguishable from the viruses of calves, monkeys, children and mice, have been found in large numbers. It seems likely, therefore, that similar viruses may well be found in faeces of the young of other species of mammals by anyone who cares to look for them.

Relationship Between Viruses of Different Species

Prompted by an unpublished report from Professor Mebus' laboratory in Nebraska that some workers had antibodies to the calf virus, a comparative investigation of the calf and human viruses was undertaken jointly between a veterinary and a medical laboratory (41). This showed that although both human and bovine convalescent sera reacted readily by immunofluorescence with tissue cultures infected with calf virus, human sera possessed low or undetectable titres of neutralizing antibody for the calf virus; whereas calf sera had high neutralizing antibody levels. By electron microscopy, antibodies in human convalescent sera could be seen adhering to and agglutinating both the human and the calf virus particles. Particles whose outer capsid layer was intact and also particles which had lost the outer capsid layer were agglutinated. Calf convalescent sera reacted similarly with the calf virus, but only agglutinated human virus particles without their outer capsid layer.

Because the morphology of the viruses differed clearly from that of the reoviruses, and also from the orbiviruses in that these do not possess a clearly defined outer capsid layer as seen in the electron microscope, FLEWETT et al. (41) suggested that these diarrhoea viruses were in a group separate from the reoviruses and the orbiviruses, and suggested the name 'rotavirus' for the new group. The name is

derived from the Latin *rota*, a wheel, because the complete virions in electron micrographs look like little wheels, having a wide hub with short spokes and a thin, clearly defined circular rim.

The word ROTA appeared in an ancient anagram, inscribed on a pillar discovered at the excavation of Pompeii:

<div align="center">

ROTAS

OPERA

TENET

AREPO

SATOR

</div>

Other Serological Reactions Between Rotaviruses

Complement Fixation Tests (CFT)

Complement fixing antigens can be made from any source of virus; some have used faeces from infected children or animals (115, 147) or from infected tissue cultures (55, 147), or from homogenized small bowel of mice infected with the EDIM virus (38). Nevertheless, no group of workers has been able by CFT to distinguish between rotaviruses from different species; WOODE *et al.* (147) found that they all cross-reacted to titre so that no distinction could be made; this was clearly evident if each antigen was titrated at the dilution found optimal for each particular serum.

Radial gel diffusion tests, using as antigens ultracentrifuged concentrates of faeces, and antisera from convalescent children and animals, have been made (47, 147); these revealed a single strong line common to all the rotaviruses and giving a 'reaction of identity' between all of them.

Methods of Detecting Rotavirus in Faeces

Rotavirus particles can usually be found without much difficulty using a simple procedure of differential centrifugation to concentrate them, followed by negative staining (92, 40, 42, 19). Some workers centrifuge through a sucrose gradient [e.g. BISHOP *et al.* (10)] to get 'cleaner' particles; we ourselves find this unnecessary except for occasional specimens: calf and pig faeces tend to be 'dirtier' than those of children and most other animals. Often particles are present in such numbers in faeces that they can be detected by direct electron microscopy of untreated faeces using a simple washing and staining method (39).

However, examination of a large number of specimens by electron microscopy (EM) is costly in time and equipment, and other methods have been explored. GRAUBALLE (47), SPENCE *et al.* (120), MIDDLETON *et al.* (85), BIRCH *et al.* (8) and TUFVESSON and JOHNSSON (134) have employed counter-current immunoelectro-osmophoresis to detect antigen in faeces. Although some (47) find the method more sensitive than EM provided that a sufficiently avid serum is used, most find it either about as good (134) or somewhat less sensitive (85). The method certainly does enable large numbers of specimens to be examined rapidly.

Others have used extracts of faeces as antigen in a complement fixation test (54, 115, 152). It is claimed that 'dry-cleaning' of faeces with fluorocarbons and careful differential centrifugation removes anticomplementary effects. ZISSIS (152) claims that the method is more sensitive than EM.

Radioimmunoassay (RIA) (86) and enzyme-linked immunoabsorbent assay (ELISA) test (108) have been applied to detection of human and calf rotaviruses respectively. Both groups claim that the method is at least as sensitive, and probably more sensitive than electron microscopy in detecting virus; but "it was difficult to be certain of a positive RIA diagnosis when the EM diagnosis was negative" (86).

Immunofluorescence has been used to stain cells or cell debris excreted in faeces from infected calves (77, 78), but non-specific staining has deterred many from applying the method to human faeces. However, YOLKEN et al. (151) have shown that it can be done if virus antigen is first separated by immune precipitations. The method is somewhat laborious and seems unlikely to compete with easier techniques.

Diagnosis by Isolation in Cell Culture

The reluctance of rotaviruses to grow in tissue culture has prevented the use of this method until recently (4, 149, 70). BANATVALA et al. (5) found that centrifugation of human faecal rotavirus samples on to preformed cell monolayers made the cells much more susceptible to infection, detectable by immunofluorescent staining. BRYDEN et al. (21) developed this discovery by the use of microtitre plates which allowed a large number of samples to be tested readily, with a sensitivity (in LLC MK2 cells) almost as great as that of EM. The method had the further advantage that infectivity could be titrated by counting the fluorescing infected cells as the virus did not spread from cell to cell.

In contrast to the difficulty of infecting cell cultures with the human rotavirus, rotaviruses isolated from calves, pigs, lambs and foals readily infect kidney cell cultures, primary or cell lines, prepared from calves, pigs, sheep and monkeys, using the methods described by WELCH et al. (139), WOODE et al. (142) and BRIDGER and WOODE (15). These authors found that the cell culture technique was as sensitive as EM and could be performed with flying cover slip cultures or microtitre-plate cultures, without the requirement for centrifugation. However, centrifugation increases the infectivity of these viruses also for tissue cultures. This technique is dependent, as are the techniques described above for antigen detection, on the monospecificity of the antiserum. Viruses have been discovered in faeces from calves with diarrhoea which produce a similar effect in cell culture, but are morphologically and antigenically different from rotaviruses, being approximately 30 nm in diameter.

Pathology and Pathogenesis

Little is known of the histopathology of human rotavirus infections. Histology of the bowel taken at autopsy is not worth much; available information is derived mainly from biopsy studies of duodenum or upper jejunum. The epithelium on the sides and tips of the villi is invaded by the virus. This has been demonstrated by electron microscopy of thin sections (9, 51, 123) and by immunofluorescent staining of biopsy material (32, 83) and of experimentally infected organ cultures of small intestine (148).

In the experimentally infected calf and pig and monkey detailed studies have been made (48, 80, 122, 150). Virus was demonstrable in the cytoplasm of the

brush-bordered epithelial cells throughout the small intestine; the lower part being affected later than the upper. Foci of 'viroplasm' were found in the cytoplasm, sometimes with virus particles apparently being formed within them. Clusters of particles occurred in dilated sacs of endoplasmic reticulum. The microvilli of the brush border, morphologically intact in the earliest stages, became shortened and ragged or disappeared completely. Cytoplasmic blebs protruded from the plasma membranes of some cells into the lumen. Scanning electron microscopy (82) showed villi shorter and broader than normal, with rounded epithelial cells upon them.

Cells on the sides and tips of the villi were most affected; cells in the crypts contained no detectable virus. In small bowel cells of mice infected with the EDIM virus similar changes were found (2, 6). In addition to spherical particles, filamentous forms were seen, possibly corresponding to the tubules of capsid protein frequently found in negatively stained preparations of mouse rotavirus (2, 38, 51), though found but rarely in human rotavirus preparations (43).

Similar cytoplasmic changes have been found in tissue cultures infected with a tissue culture adapted strain of the calf virus (71) and in cells infected with the SA.11 monkey virus (63).

In the experimentally infected calf and pig, killed at the height of disease, the intestinal damage was often severe, with almost complete destruction of the villi in some areas. But regeneration in surviving animals was very rapid; the intestinal structure returned almost to normal about 8—10 days after the onset.

Fatal cases—sometimes the mortality in farm outbreaks has been 30 to 40 per cent—do happen in the field. The virus may also be lethal in the gnotobiotic animal; filtrates of faeces in dilutions up to 10^{-7} have infected gnotobiotic piglets with 30 per cent mortality (146).

A Mechanism of Diarrhoea

Infection of the brush-bordered epithelium destroys the cells which synthesise disaccharidases. Lack of these enzymes causes lactose or other disaccharides to remain in the lumen of the bowel—only monosaccharides are absorbed by the healthy bowel—and so causes an osmotic drain, attracting body fluid into the bowel lumen (46). Absorption of xylose is also impaired (76); this test is much used by paediatricians as an index of upper small bowel function. The persistence of the lactose is the main cause of the diarrhoea; stopping milk or other disaccharide source and giving water, or better, dilute electrolyte solution, usually stops the diarrhoea dramatically, both in infants and animals. As xylose and glucose are absorbed by the same pathway, glucose in excess can also make diarrhoea worse. The effect is compounded because bacteria in the colon break down glucose and lactose into short-chain acids (up to 6/mol of lactose) thus greatly increasing the osmolarity of the colon contents.

Cetaceae (whales, dolphins, etc.) have practically no lactose in their milk; it will be interesting to learn whether they suffer from rotavirus infection and, if so, whether this causes diarrhoea in their young.

Immunity in Man

Various studies (12, 32, 33, 55, 87, 94) have shown that antibodies, mainly IgM, appear very rapidly; IgG soon replaces the IgM. Over 90 per cent of children

have antibodies by the age of 6; CF antibodies diminished rapidly after infection (34, 58), but neutralizing (NT) and immunofluorescence (FA) antibodies reached their highest average levels at 6 years of age and thereafter diminished gradually until by the age of 80 antibodies were often not detectable by FA, though NT antibodies could still be detected—because the N test is more sensitive than FA. The progressive decline of antibody levels with age suggests that reinfections either are not common or do not boost serum antibody levels. Reinfection occurred in two adults in contact with infected children (95). The adults both had antibodies before becoming infected, but the levels were boosted by their reinfection.

Antibodies are transmitted across the placenta and can be detected in infants in the first weeks of life by FA and NT. Subclinical rotavirus infections have been reported from hospital nurseries (11, 28, 72); it may be that circulating antibodies are, at this age, protective, but the weight of evidence from observation of rotavirus infection in animals is against this (118, 143, 119).

Immunity in Animals

In one survey in the U.K. 80 per cent of outbreaks of calf diarrhoea were associated with rotavirus infection and from 66 per cent of the faecal samples received rotavirus was isolated (145). Fifty-nine herds of cattle, distributed throughout the U.K. and Northern Ireland, most with a history of diarrhoea incidence, were serologically positive for rotavirus infection.

Two farm herds have been studied in detail. The serum neutralizing titres of yearling and adult cattle to calf rotavirus were determined. 44 out of 445 had titres of 40 or less and 260/445 had titres of 320 or greater, these two extremes representing high and low levels of immunity. Thus an important proportion of animals, including adults, may well be susceptible to infection and unable to provide passive protection for their young. [A similar situation appears to obtain in the human (127).]

In Canada, 237/300 cattle from 29/30 herds had rotavirus antibody (Babiuk and Acres, personal communication). The source of virus for outbreaks of diarrhoea is not known, but wild animals and human contacts are possible sources. However, rotavirus excretion from adult cattle (141) and adult humans (13, 152) has been reported, and many outbreaks in calves can be associated with diarrhoea in an occasional cow.

Although rotavirus infection can cause clinical illness at all ages, it is most severe in calves of less than seven weeks of age, and commonly occurs within the first 7 days of life. Passive protection afforded by colostrum has limited value and vaccination of the dam has not resulted in much improvement, although early results suggested this might be advantageous (79, 143, 118, 119):

An attenuated vaccine has been developed in the U.S.A. from the Nebraska isolate which gives good protection under experimental and trial conditions (79), but requires seven days between vaccination and challenge for protection to develop against the U.K. rotavirus isolate (WOODE, unpublished). However, under strictly controlled vaccination trials this vaccine has shown little protection against incidence of calf diarrhoea (1, 89). The failure to protect calves may be due to numbers of aetiological causes, as ACRES and RADOSTITS (1) suggest; perhaps due to interference with the vaccine virus by colostral antibody, or to the

overwhelming challenge dose calves receive if exposed to diarrhoeic faeces from other calves. Thus in sequential vaccine trials, in contrast to double blind trials, THURBER et al. (129) claimed that protection could only be achieved when all calves were vaccinated.

The recognition that rotavirus isolates from naturally infected animals of one species are poorly neutralized by convalescent sera from animals of other species, has introduced a new dimension into the problem (128). Woode (unpublished) has found that there is limited cross protection in calves and pigs between human, calf, pig and foal rotaviruses. As evidence of calf type rotavirus in pigs has been reported (146), it is probable that humans and other mammals may sometimes be infected naturally with a variety of antigenically different rotaviruses (16, 80, 150).

Subclinical Infections

Subclinical infections have been recorded (144). In an attempt to investigate this WOODE studied seroconversion in weaned pigs on a farm both during severe epidemics of rotavirus associated diarrhoea and during periods when no disease occurred. At both times 85 per cent of litter groups sero-converted between weaning at 5 weeks of age and three weeks later. The rotavirus isolated from diseased pigs was shown to be pathogenic, but no isolate was obtained from pigs seroconverting without disease (WOODE, unpublished). Subclinical infection can occur when calves and lambs are protected by colostral antibody in the gut lumen (118, 143), but probably not by antibody in milk. It appears probable that the pigs were infected with avirulent strains of rotavirus, perhaps derived from man or some other animal (16, 141). Pig and calf rotaviruses isolated in the U.K. are virulent to gnotobiotic piglets, resulting in death if fed within a day of birth. If given at 7 days of age these viruses cause a 25 per cent loss of body weight, but a low mortality. In contrast, unlike the findings of MEBUS et al. (81, 132) in the U.S.A., U.K. isolates of lamb, foal and human rotavirus multiply without causing clinical disease in pigs, although the lamb and foal isolates caused disease in lambs and a foal (116, 117, 144).

Characterization of Rotaviruses

Rotavirus Morphology

The diameter of the complete virion has been estimated by different authors to be between 65 (147) and 70—75 nm (51); intermediate estimates were 72 (35) for the SA.11 and '0' viruses; MARTIN et al. (74) estimated 70 nm; PALMER et al. (96) estimated 67—68 nm. As the spherical virus particles become flattened to some extent when drying down in the negative stain—this can easily be seen with the aid of a eucentric goniometer stage—the estimates from different laboratories correspond remarkably closely. The following description is based on MARTIN et al. and PALMER et al. (74, 96).

Around an icosahedral core bounded by a thin membrane are attached radially arranged capsomeric subunits forming an inner capsid layer. These can be detached by trypsin, but not by chymotrypsin, which removes reovirus capsomeres. These subunits are believed to be wedgeshaped trimers arranged so as to make 180 units,

each of 3 components. On the structural level, the particle follows the structure of $T = 9$ icosadeltahedron; but in the clustering of these units into 32 capsomeres (or 'megameres') it conforms to a $T = 3$ morphology. Surmounting the inner layer of sub-units is an outer layer which gives in negatively stained preparations the appearance of a smooth rim on the ends of 20 short spokes radiating from a wide hub.

In their interpretation of the morphology of the subunits in the outer capsid layer PALMER et al. (96) differ from FLEWETT et al. (43). It seems unlikely that precise details of the structure of the subunits can be established until these are isolated in an identifiable form and perhaps crystallized. There is a limit to what can be deduced from negatively stained whole virions, especially as the outer layer is susceptible to damage by the electron beam (Flewett, Davies and Sheldon, unpublished observations); perhaps because this layer contains glycoproteins (105).

The density of the human virus was 1.36—1.38 (57).

Relationship of Morphology to Infectivity

The presence of the outer capsid layer appears to be necessary for infectivity. BRIDGER and WOODE (17) separated calf virus particles with intact outer capsid layers ('smooth' particles) from those lacking the outer capsid layer ('rough') particles by centrifugation in a CsCl gradient. The 'smooths' banded at density 1.36 g/ml and the 'roughs' at 1.38 g/ml. The particle/infectivity ratio was about 1000-fold higher for the smooth than the rough. ELIAS (33) found similar densities and infectivity ratios for the human virus. But because it was not possible completely to free the 'rough' fraction from 'smooth' particles, it could not be determined whether the 'rough' particles had a very low infectivity or none at all.

Rotavirus RNA

Several groups of workers have endeavoured to characterize rotavirus RNA (137). The work has been done by chromatographic or gel electrophoretic separation of RNA segments isolated from rotaviruses of calves (90, 104, 130, 91, 136) infants (104, 105, 109, 91, 53), piglets and lambs (131, 132) and the SA. 11 virus (105).

RNA (Human) (Extracted From Faeces)

OBIJESKI et al. (91) purified rotaviruses from human faeces by glycerol-tartrate gradient separation and treatment with fluorocarbon and Nonidet P-40. After disruption by conventional means the nucleic acids and proteins were run on polyacrylamide gels. They found 8 RNA bands and estimated mol. wts. of $10^6 \times$: 2.3, 1.66, 1.5, 0.85, 0.76, 0.50, 0.30, 0.24. From the density of bands 2 and 6 they calculated that these bands respectively contained three and six genome segments, thus giving a total of 15 segments. They compared these results with their findings with reovirus RNA run in a similar system. They found slightly different molar ratios from those reported by SCHNAGL and HOLMES (109). SCHNAGL and HOLMES found probably eleven, possibly twelve, segments in the human rotavirus. They observed slight variation in migration rates of one or two components when comparing viruses isolated from two different cases.

Like SCHNAGL and HOLMES (109), KALICA et al. (53) also found eleven segments of RNA in rotavirus from human faeces and in the 'Lincoln' tissue-culture adapted

strain of calf virus isolated in Nebraska. They compared these with RNAs isolated from the SA.11 and '0' viruses grown in monkey kidney cultures. They examined each RNA sample by electron microscopy and found no evidence of denaturation. They measured contour length of the molecules and illustrated them in histograms. The four rotaviruses studied had four contour length classes, differing from the reoviruses, which have three RNA length classes. Five out of eleven segments of the human rotavirus RNA migrated at slightly different rates from the corresponding segments of calf rotavirus RNA.

In pig rotavirus TODD and McNULTY (130) found 11 (or perhaps 12) RNA segments migrating in 9 bands. They were not able to distinguish between RNAs from the pig rotavirus and two isolates of calf virus by co-electrophoresis. (Either the calf and pig RNAs are indeed identical, or the authors' 'pig' rotavirus may have been a bovine strain isolated from pigs; some field outbreaks in pigs are now known to be caused by virus serologically identical with the bovine and differing from 'true' pig isolates.) TODD and McNULTY (131) found that the lamb virus RNA differed in one segment from the pig virus RNA.

VERLY and COHEN (136) also found eleven RNA segments in calf rotavirus in eleven bands using polyacrylamide slab gels. They found differences of up to 6 per cent in mobilities for bands 3, 4, 5 and 6 between different field isolates and between these and the tissue culture-adapted Nebraska isolate.

Using isotope-labelled (^{3}H-uridine or ^{3}H-thymidine) tissue culture-adapted calf virus—the Compton strain—NEWMAN et al. (90) separated 8 peaks of radioactivity in polyacrylamide gels, deducing 11 or 12 segments. It thus appears that the weight of evidence indicates 11 (or possibly 12) RNA segments. The molecular weights quoted below are for the Nebraska tissue culture-adapted calf virus, as it seems likely that this will be adopted as the prototype rotavirus, and are taken from the most recent publication (136). (Other authors' estimates are similar, but not identical.) They are ($\times 10^6$) 2.2; 1.85; 1.70; 1.55; 1.00; 0.82; 0.51; 0.51; 0.51; 0.26; 0.20.

The differences in molecular weight estimates found in different laboratories should not all be ascribed to experimental error. RAMIG et al. (102) found electrophoretic differences between RNAs in different strains of reovirus type 3, and similar differences among different rotavirus strains are only to be expected.

Cross-hybridization studies will be required to determine how the RNAs of different rotaviruses are related; but we shall probably have to wait until the different strains can be grown in tissue culture so that the nucleic acids can be labelled. Attempts to label mouse rotavirus by giving ^{32}P-labelled phosphate orally or parenterally to suckling mice, even in large amounts, have given very disappointing results (Flewett et al., unpublishable, and some sad personal communications). However, PETRIC et al. (100) were able to label RNA in human virus grown in newborn piglets with ^{3}H-uridine, but not with ^{3}H-deoxythymidine. In a further paper (101) they found a buoyant density in Cs_2SO_4 of 1.57 g/ml for the RNA.

Polypeptides

NEWMAN et al. (90) grew the Compton strain of tissue culture adapted calf rotavirus in calf kidney cells, but did not incorporate an isotope-labelled amino acid in the medium. They analysed the polypeptides from purified virions using

a conventional 8 M-urea-SDS-mercaptoethanol PAGE system. They found 2 major polypeptide bands and 3 minor bands.

RODGER et al. (104) compared polypeptides of human and calf rotaviruses purified from faeces by fluorocarbon extraction and centrifugation through a variety of gradients. Using a discontinuous electrophoresis system according to LAEMMLI they found nine polypeptides having mol. wts. ranging from 131,000 to 14,500 for the calf virus, and very similar mol. wts. for the human virus, though a polypeptide analogous to the smallest calf polypeptide was not found.

BRIDGER and WOODE (17) separated particles of calf rotavirus with, and without, the outer capsid layer by isopyknic banding in caesium chloride. Finding that infectivity was associated wholly, or almost wholly, only with the complete virion, they examined the polypeptides, using a similar method to that of NEWMAN et al. (90). They found that a major polypeptide, mol. wt. 63,000, was absent in the incomplete particles.

OBIJESKI et al. (91) separated 10 structural polypeptides with mol. wts. $\times 10^3$: 155, 137, 130, 118, 98, 67, 60, 40, 32, 24; total about 850,000. Most of the total protein (about 87 per cent) was in bands 2 and 8. More recently, TODD and McNULTY (131) have found four polypeptides present in complete, but not in incomplete, lamb rotaviruses; i.e. located presumably in the outer capsid layer. RODGER et al. (105) have compared polypeptides in human, calf and SA.11 viruses. Like TODD and McNULTY they found four polypeptides in the outer capsid layer; the major polypeptide was glycosylated. For the calf virus they found molecular weights as follows: ($\times 10^3$) Inner layer: 131; 103; 97; 92; 32. Outer layer: 58; 22; 16.5; 14.5.

If all these structural proteins are indeed virus proteins—and it is indeed difficult to be certain that one has cleaned virions from faeces free from all extraneous protein—the RNA segments described seem hardly enough to specify many additional non-structural proteins. It was calculated by OBIJESKI et al. (91) that there wasn't quite enough RNA to specify all the polypeptides found in the virion.

Other Characteristics

Multiplication of the calf virus was not inhibited by 5-iodo-2-deoxyuridine up to 500 μg/ml. The virus was resistant to lipid solvents and was stable at pH 3 (138, 139). Unlike reoviruses, however, it was not stabilized by molar $MgCl_2$ at pH 6—7 when heated for 1 hour at 50° C. The SA.11 and '0' viruses were rather less stable at pH 3, but were stable at pH 4 (73), unlike the members of the orbivirus group (14) which are all very acid-labile. Stabilities of the human virus have not yet been published.

Cryptogram

It now becomes possible to attempt to construct a cryptogram for the calf rotavirus, the most extensively investigated member of the group. Such information as is available about the other members of the group indicates that they probably will fit into the same cryptogram:

$$R/2 : \Sigma \ (11—14)/* : S/S : V/0$$

Isometric two-layered capsid, occasionally surrounded by a pseudomembrane, probably of host origin. BD (CsCl) 1.36 g/cm³. Capsid diameter 60—70 nm, usually

about 65 nm. Resists lipid solvents. Virus synthesis and maturation in cytoplasm with formation of small granular inclusions. Virus particles usually found in endoplasmic vesicles.

Haemagglutinin

An antigen prepared from African green monkey cells infected with the Nebraska strain of calf virus was found to agglutinate human group 0 cells over a wide pH range (5.7—7.4) (121). The haemagglutinin was associated with the virus particle and was inhibited by serum from a gnotobiotic calf which had been inoculated with the Nebraska virus. Serum from a guinea-pig immunized with human rotavirus also inhibited. (The U.K. isolate lacks the haemagglutinin.)

Polymerase

By analogy with reoviruses one would expect to find an RNA-dependent RNA polymerase in the virion. No one has yet published any account of this.

Taxonomy of Rotaviruses: A Separate Genus of the Reoviridae?

The neonatal calf diarrhoea agent of MEBUS et al. (77) was desciibed as reoviruslike (37, 137). Subsequently, it was proposed that the human and calf viruses should be included in the family Reoviridae as an orbivirus (84) or that they constituted a separate and distinct group for which the names 'rotavirus' (41) or duovirus (31) were proposed.

The inclusion by FENNER (36) of the rotavirus group in the family Reoviridae as a separate genus, to be named, is consistent with the published data of the many authors cited above. Table 1 summarises the comparative properties of reovirus, orbivirus, and rotavirus groups.

Table 1. *Comparative properties of Reovirus, Orbivirus and Rotavirus groups*

General	RNA segments double stranded	RNA mw	Density CsCl	Sedimentation Constant	Polypeptides	Virion size (nm)	Structure capsomeres	Acid stability pH 3
Reovirus	10	$12—15 \times 10^6$	1.38	630	7	60—75	92	Stable
Orbivirus	10	12×10^6	1.36	550	10	55—80	32	Labile
(Rotavirus)	11—12	$11—14 \times 10^6$	1.36 and 1.38	500—530[a]	5—10	55—66	32	Stable

[a] Ref. 125

Clearly, the similarities of the rotavirus group to the genera Reovirus and Orbivirus are close enough to place the rotavirus group in the family Reoviridae. However, the question to be decided is whether the rotaviruses are sufficiently different to be classified in a separate genus. In morphogenesis and morphology they are closer to the orbiviruses than to the reoviruses. Orbiviruses and rotaviruses, but not reoviruses, bud through into vesicles of endoplasmic reticulum to form double-layered particles. However, the outer layer of the orbiviruses is

amorphous or diffuse, at any rate as seen in negatively stained preparations, whereas the outer layer of the rotaviruses is sharp and clear. When the outer capsid layer is lost the viruses are morphologically very similar (51, 74), though the orbiviruses are larger in diameter (64, 74, 51). Reoviruses are uncoated by chymotrypsin, which does not affect the double-shelled calf rotavirus (52) suggesting that the outer protein may be a glycoprotein (this has now been confirmed) (105).

Rotaviruses are almost as resistant to pH 3 as reoviruses, whereas orbiviruses are very acid-labile. Although basically similar to those of reoviruses and orbiviruses, rotavirus genomes possess 11 or 12 segments [or even 15 (91)]; whereas orbiviruses and reoviruses have 10 (109).

Antigenic Relationships

All the known rotaviruses share a common antigen demonstrable by immunofluorescence, complement fixation, gel diffusion and immunoelectron microscopy. This group reaction is associated with the inner capsid layer (41, 75, 147). They are, however, distinguishable from each other by serum neutralization, though there is some cross-reaction (112, 128, 147). The rotaviruses thus form a coherent interrelated group.

The bovine and human rotaviruses are not related antigenically to reovirus types 1, 2 and 3, dog reovirus, or to bluetongue virus and other orbiviruses (15, 55, 139, 141).

Rotaviruses are all intestinal pathogens transmitted, as far as is known, by the faecal-oral route; whereas orbiviruses are systemic pathogens transmitted often, if not always, by arthropod vectors. (We mention this as additional evidence, but bearing in mind that host and tissue affinities are not good taxonomic criteria.)

These similarities and differences lead us to propose that the rotavirus group be placed in a separate genus of the *Reoviridae*, the type species to be the Nebraska isolate of the calf virus. This, though not the first rotavirus known (the EDIM virus has this distinction) is the best chracterized. The genus name of Rotavirus has been proposed by the Reovirus Working Teams Report of the WHO/FAO Committee for Comparative Virology, 1976: we certainly would not dissent from this proposal.

Because rotaviruses from one species can infect other species one cannot assert that an isolate from an animal is the rotavirus of that species. It will, therefore, be necessary to subtype individual isolates by cross neutralization, using, preferably, antisera raised in gnotobiotic animals, or failing that, in animals devoid of rotavirus antibody.

Conclusions

Much has been accomplished in the last few years. But before the knowledge so far gained can be put to the practical purpose of preventing infection in children and other young animals, some important problems need to be solved:

1. Why won't these viruses replicate in serial subculture—except very rarely? The first step in making any vaccine is to propagate the virus.

2. To confer immunity, is it sufficient to raise circulating antibodies, or is local alimentary tract antibody essential for protection? Veterinary experience clearly suggests that circulating IgG is insufficient; it *may* not be so in man, but evidence is lacking.

3. Are there different serotypes of rotaviruses attacking one species? Do we need to protect children—or calves—or lambs—against more than one serotype of virus?

4. If we need a live attenuated virus vaccine—and the evidence suggests that we shall—even if we can propagate the human virus in serial sub-culture, how are we to determine whether the virus has been attenuated? The experiment will have to be made in children; we have seen that virus virulent in children is avirulent in piglets, so animal tests won't do.

5. Rotavirus infections are undoubtedly of great economic importance to farmers and stock breeders; but although they cause much illness in children, they don't kill many, at least in England. How important are they in tropical countries where many children die of infective diarrhoea? We know rotavirus infections exist in some tropical countries, but do they really matter?

There has been some optimistic talk of rotavirus vaccines 'just around the corner' for children. Until these and other difficult problems are solved, prospects for a vaccine remain distant.

Addendum

Since this review was written COHEN (153) has demonstrated an RNA-dependent RNA polymerase in purified rotavirus particles. The enzyme was activated with 1 mM-EDTA treatment or by heat shock. This treatment removed the outer capsid layer and unmasked the virus-associated RNA polymerase. Chymotrypsin did not unmask polymerase, whereas it does so in reoviruses; but the rotavirus enzyme, like that of reoviruses, required magnesium (optimum activity at 10 mM concentration); and optimum pH was 8.0.

References

1. ACRES, S. D., RADOSTITS, O. M.: The efficacy of a modified live reo-like virus vaccine and an *E. coli* bacteria for prevention of acute undifferentiated neonatal diarrhoea of beef calves. Canad. vet. J. 17, 197—212 (1976).
2. ADAMS, W. R., KRAFT, L. M.: Epizootic diarrhea of infant mice: identification of the etiologic agent. Science 141, 359—360 (1963).
3. ADAMS, W. R., KRAFT, L. M.: Electron-microscopic study of the intestinal epithelium of mice infected with the agent of epizootic diarrhea of infant mice (EDIM virus). Amer. J. Pathol. 51, 39—44 (1967).
4. ALBREY, M. B., MURPHY, A. M.: Rotavirus growth in bovine monolayers. Lancet i, 753 (1976).
5. BANATVALA, J. E., TOTTERDELL, BARBARA, CHRYSTIE, I. L., WOODE, G. N.: *In-vitro* detection of human rotaviruses. Lancet ii, 821 (1975).
6. BANFIELD, W. G., KASNIC, G., BLACKWELL, J. H.: Further observations on the virus of epizootic diarrhoea of infant mice. An electron microscopic study. Virology 36, 411—421 (1968).
7. BARRETO, J. G., PALMER, E. L., NAHMIAS, A. J., HATCH, M. H.: Acute enteritis associated with reovirus-like agents. J. Amer. med. Ass. 235, 1857—1860 (1976).
8. BIRCH, C. J., LEWIS, F. A., KENNETT, M. L., HOMOLA, M., PRITCHARD, H., GUST, I. D.: A study of the prevalence of rotavirus infection in children with gastroenteritis admitted to an infectious diseases hospital. J. med. Virol. 1, 69—77 (1977).
9. BISHOP, R. F., DAVIDSON, G. P., HOLMES, I. H., RUCK, B. J.: Virus particles in epithelial cells of duodenal mucosa from children with acute non-bacterial gastroenteritis. Lancet ii, 1281—1283 (1973).

10. BISHOP, R. F., DAVIDSON, G. P., HOLMES, I. H., RUCK, B. J.: Detection of a new virus by electronmicroscopy of faecal extracts from children with acute gastroenteritis. Lancet **i**, 149—151 (1974).
11. BISHOP, R. F., HEWSTONE, A. S., DAVIDSON, G. P., TOWNLEY, R. R. W., HOLMES, I. H., RUCK, B. J.: An epidemic of diarrhoea in human neonates involving a reovirus-like agent and 'enteropathogenic' serotypes of *Escherichia coli*. J. clin. Pathol. **29**, 46—49 (1976).
12. BLACKLOW, N. R., ECHEVERRIA, P., SMITH, D. H.: Serological studies with reovirus-like enteritis agent. Infect. Immun. **13**, 1563—1566 (1976).
13. VON BONSDORFF, C. H., HOVI, T., MAKELA, P., HOVI, L., TEVALVOTO-AARNIO, M.: Rotavirus associated with acute gastroenteritis in adults. Lancet **ii**, 423 (1976).
14. BORDEN, E. C., SHOPE, R. E., MURPHY, F. A.: Physico-chemical and morphological relationships of some arthropod-borne viruses to blue-tongue virus—A new taxonomic group. Electron microscopic studies. J. gen. Virol. **13**, 261—271 (1971).
15. BRIDGER, JANICE C., WOODE, G. N.: Neonatal calf diarrhoea: Identification of reovirus-like (rotavirus) agent in faeces by immunofluorescence and immune electron microscopy. Brit. vet. J. **131**, 528—535 (1975).
16. BRIDGER, JANICE C., WOODE, G. N., JONES, JOANNA M., FLEWETT, T. H., BRYDEN, A. S., DAVIES, HEATHER: Transmission of human rotaviruses to gnotobiotic piglets. J. med. Microbiol. **8**, 565—569 (1975).
17. BRIDGER, J. C., WOODE, G. N.: Characterization of two particle types of calf rotavirus. J. gen. Virol. **31**, 245—250 (1976).
18. BRYDEN, A. S., DAVIES, H. A., HADLEY, R. E., FLEWETT, T. H.: Rotavirus Enteritis in the West Midlands during 1974. Lancet **ii**, 241—243 (1975).
19. BRYDEN, A. S., DAVIES, H. A.: The laboratory diagnosis of epizootic diarrhoea of infant mice. J. inst. Anim. Technicians **26**, 63—67 (1975).
20. BRYDEN, A. S., THOULESS, M. E., FLEWETT, T. H.: A rabbit rotavirus. Vet. Rec. **99**, 323 (1976).
21. BRYDEN, A. S., DAVIES, H. A., THOULESS, M. E., FLEWETT, T. H.: Diagnosis of rotavirus infection by cell culture. J. med. Microbiol. **10**, 121—125 (1977).
22. BUFFET-JANVRESSE, C., BERNARD, E., MAGARD, H.: Responsabilité des rotavirus dans les diarrhées du nourrisson. Nouv. Pr. med. **5**, 1249—1251 (1976).
23. BUFFET-JANVRESSE, C., BARBOTIN, J.-N., MAGARD, H.: Intérét de la microscopie électronique pour la mise en évidence de structures virales dans les selles. Nouv. Pr. med. **4**, 1599 (1975); **10**, 121—125 (1977).
24. CAMERON, D. J. S., BISHOP, R. F., DAVIDSON, G. P., TOWNLEY, R. R. W., HOLMES, I. H., RUCK, B. J.: New virus associated with diarrhoea in neonates. Med. J. Aust. **1**, 85—86 (1976).
25. CARR, M. E., MCKENDRICK, D. W., SPYRIDAKIS, T.: The clinical features of infantile gastroenteritis due to rotavirus. Scand. J. inf. Dis. **8**, 241—243 (1976).
26. CHEEVER, F. S., MUELLER, J. H.: Epidemic diarrhoeal disease of suckling mice. I. Manifestation, epidemiology, and attempts to transmit the disease. J. exp. Med. **85**, 405—416 (1947).
27. CHEEVER, F. S.: Epidemic diarrheal disease of suckling mice. Ann. N.Y. Acad. Sci. **66**, 196—203 (1956).
28. CHRYSTIE, I. L., TOTTERDELL, B., BAKER, M. J., SCOPES, J. W., BANATVALA, J. E.: Rotavirus infections in a maternity unit. Lancet **ii**, 79 (1975).
29. CONKLIN, R. H., DUPONT, H. L., GOLDSCHMIDT, M. C., RODRIGUEZ, J. T.: Occurrence of viral particles in diarrhoea: Houston, Texas and Guatemala. New Engl. J. Med. **292**, 644—645 (1975).
30. CRUICKSHANK, J. G., EXTON, J. H. M., WEBSTER, O. F.: Viruses in gastroenteritis. Lancet **i**, 1353 (1974).
31. DAVIDSON, G. P., BISHOP, R. F., TOWNLEY, R. R. W., HOLMES, I. H., RUCK, B. J.: Importance of a new virus in acute sporadic enteritis in children. Lancet **i**, 242—246 (1975).

32. DAVIDSON, G. P., GOLLER, I., BISHOP, R. F., TOWNLEY, R. R. W., HOLMES, I. H., RUCK, B. J.: Immunofluorescence in duodenal mucosa of children with acute enteritis due to a new virus. J. clin. Pathol. **28**, 263—266 (1975).

33. ELIAS, M. M.: Separation and infectivity of two particle types of human rotavirus. J. gen. Virol. **37** (in press, 1977).

34. ELIAS, M. M.: Distribution and levels of rotavirus antibodies in different age groups. J. Hyg. (Camb.), (in press, 1977).

35. ELS, H. J., LECATSAS, G.: Morphological studies on Simian virus SA.11 and the "related" 0 agent. J. gen. Virol. **17**, 129—132 (1972).

36. FENNER, F.: Classification and nomenclature of viruses. Second report of the International Committee on Taxonomy of Viruses. Intervirology **7**, 1—116 (1976).

37. FERNELIUS, A. L., RITCHIE, A. E., CLASSICK, L. G., NORMAN, J. O., MEBUS, C. A.: Cell culture adaptation and propagation of a reovirus-like agent of calf diarrhoea from a field outbreak in Nebraska. Arch. ges. Virusforsch. **37**, 114—130 (1972).

38. FLEWETT, T. H.: Implications of recent virological researches. In: ELLIOTT, K., KNIGHT, J. (eds.), Acute diarrhoea in childhood, Ciba Symposium No. 42. Amsterdam: Elsevier 1976.

39. FLEWETT, T. H., BOXALL, E. H.: The hunt for viruses in infections of the alimentary system: an immuno-electron microscopical approach. Clinics in Gastroenterology **5**, 359—385 (1976).

40. FLEWETT, T. H., BRYDEN, A. S., DAVIES, H. A.: Virus particles in gastroenteritis. Lancet **ii**, 1497 (1973).

41. FLEWETT, T. H., BRYDEN, A. S., DAVIES, H. A., WOODE, G. N., BRIDGER, J. C., DERRICK, J.: M. Relation between viruses from acute gastroenteritis of children and new born calves. Lancet **ii**, 61—63 (1974).

42. FLEWETT, T. H., BRYDEN, A. S., DAVIES, H. A.: Diagnostic electron microscopy of faeces. I. The viral flora of the faeces as seen by electron microscopy. J. clin. Pathol. **27**, 603—608 (1974).

43. FLEWETT, T. H., DAVIES, H. A., BRYDEN, A. S., ROBERTSON, M. J.: Diagnostic electron microscopy of faeces. II. Acute gastroenteritis associated with reovirus-like particles. J. clin. Pathol. **27**, 608—614 (1974).

44. FLEWETT, T. H., BRYDEN, A. S., DAVIES, H. A., MORRIS, C. A.: Epidemic viral enteritis in a long-stay children's ward. Lancet **i**, 4 (1975).

45. FLEWETT, T. H., BRYDEN, A. S., DAVIES, H. A.: Virus diarrhoea in foals and other animals. Vet. Rec. **96**, 477 (1975).

46. FLEWETT, T. H.: Acute non-bacterial infectious gastroenteritis. An essay in comparative virology. In: WATERSON, A. P. (ed.), Recent advances in clinical virology, 151—169. Edinburgh-London-New York: Churchill Livingstone 1977.

47. GRAUBALLE, P. C., GENNER, J., MEYLING, A., HORNSLETH, A.: Rapid diagnosis of rotavirus infections: comparison of electron microscopy and immunoelectroosmophoresis for the detection of rotavirus in human infantile gastroenteritis. J. gen. Virol. **35**, 203—218 (1977).

48. HALL, G. A., BRIDGER, J. C., CHANDLER, R. L., WOODE, G. N.: Gnotobiotic piglets experimentally inoculated with neonatal calf diarrhoea reovirus-like agent (rotavirus). Vet. Pathol. **13**, 197—210 (1976).

49. HARA, M., MUKOYAMA, J., TSURUHARA, T., SAITO, Y., TAGAYA, I.: Duovirus in schoolchildren with gastroenteritis. Lancet **i**, 311 (1976).

50. HOLMES, I. H., MATHAN, M., BHAT, P., ALBERT, M. J., SWAMINATHAN, S. P., MAIYA, P. P., PEREIRA, S. M., BAKER, S. J.: Orbiviruses and gastroenteritis. Lancet **ii**, 658—659 (1974).

51. HOLMES, I. H., RUCK, B. J., BISHOP, R. F., DAVIDSON, G. P.: Infantile enteritis viruses: Morphogenesis and morphology. J. Virol. **16**, 937—943 (1975).

52. HOLMES, I. H., RODGER, S. M., SCHNAGL, R. D., RUCK, B. J.: Is lactose the receptor and uncoating enzyme for infantile enteritis (Rota) viruses? Lancet **i**, 1387—1388 (1976).

53. KALICA, A. R., GARON, C. F., WYATT, R. G., MEBUS, C. A., VAN KIRK, D. H., CHANOCK, R. M., KAPIKIAN, A. Z.: Differentiation of human and calf reovirus-

like agents associated with diarrhoea using polyacrylamide gel electrophoresis of RNA. Virology **74**, 86—92 (1976).

54. KAPIKIAN, A. Z., KIM, H. W., WYATT, R. G., RODRIGUEZ, W. J., ROSS, S., CLINE, W. L., PARROTT, R. H., CHANOCK, R. M.: Reovirus-like agent in stools: Association with infantile diarrhoea and development of serologic tests. Science **185**, 1049—1053 (1974).

55. KAPIKIAN, A. Z., CLINE, W. L., MEBUS, C. A., WYATT, R. G., KALICA, A. R., JAMES, H. D., VAN KIRK, D. H., CHANOCK, R. M., KIM, H. W.: New complement fixation test for the human reovirus-like agent of infantile gastroenteritis. Lancet **i**, 1056—1061 (1975).

56. KAPIKIAN, A. Z., KIM, H. W., WYATT, R. G., CLINE, W. L., ARROBIO, J. O., BRANDT, C. D., RODRIGUEZ, W. J., SACK, D. A., CHANOCK, R. M., PARROTT, M. D.: Human reovirus-like agent as the major pathogen associated with 'winter' gastroenteritis in hospitalized infants and young children. New Engl. J. Med. **294**, 965—972 (1976).

57. KAPIKIAN, A. Z., KALICA, A. R., WAI-KUO SHIH, J., CLINE, W. L., THORNHILL, T. S., WYATT, R. G., CHANOCK, R. M., KIM, H. W., GERIN, J. L.: Buoyant density in caesium chloride of the human reovirus-like agent of infantile gastroenteritis by ultracentrifugation, electron microscopy and complement fixation. Virology **70**, 564—569 (1976).

58. KAPIKIAN, A. Z., KIM, H. W., WYATT, R. G., CLINE, W. L., PARROTT, R. H., CHANOCK, R. M., ARROBIO, J. O., BRANDT, C. D., RODRIGUEZ, E. J., KALICA, A. R., VAN KIRK, D. H.: Recent advances in the aetiology of viral gastroenteritis. In: ELLIOTT, K., KNIGHT, J. (eds.), Acute Diarrhoea in Childhood, Ciba Symposium No. 42. Amsterdam: Elsevier 1976.

59. KONNO, T., SUZUKI, H., ISHIDA, N.: Reovirus-like agent in Japanese infants with gastroenteritis. Lancet **i**, 918—919 (1975).

60. KONNO, T., SUZUKI, H., IMAI, A., ISHIDA, N.: Reovirus-like agent in acute epidemic gastroenteritis in Japanese infants: fecal shedding and serologic response. J. inf. Dis. **135**, 259—266 (1977).

61. KRAFT, L. M.: Studies on the etiology and transmission of epidemic diarrhoea of infant mice. J. exp. Med. **106**, 743—755 (1957).

62. KRAFT, L. M.: Epizootic diarrhea of infant mice and lethal intestinal infection of infant mice. In: Viruses of laboratory rodents. Nat. Cancer Inst. Monogr. **20**, 55—61 (1966).

63. LECATSAS, G.: Electron microscopic and serological studies on simian virus SA.11 and the "related" 0 agent. Onderstepoort J. vet. Res. **39**, 133—138 (1972).

64. LECATSAS, G. J.: Virus particles in acute gastroenteritis. Lancet **ii**, 524 (1974).

65. LECCE, J. G., KING, M. W., MOCK, R.: Reovirus-like agent associated with fatal diarrhoea in neonatal pigs. Infect. Immun. **14**, 816—825 (1976).

66. LIGHT, J. S., HODES, H. L.: Studies on epidemic diarrhoea in the newborn: isolation of filterable agent causing diarrhoea in calves. Amer. J. publ. Hlth. **33**, 1451—1454 (1943).

67. LOMBARDI, G. H., ROSETO, A. M., STAMBOULIAN, D., BARRERA ORO, J. G.: Virus of infantile gastroenteritis. Lancet **ii**, 131 (1975).

68. MCNULTY, M. S., ALLAN, G. M., MCFERRAN, J. B.: Isolation of a cytopathic calf rotavirus. Res. vet. Sci. **21**, 114—115 (1976).

69. MCNULTY, M. S., PEARSON, G. R., MCFERRAN, J. B., COLLINS, D. S., ALLAN, G. M.: A reovirus-like agent (rotavirus) associated with diarrhoea in neonatal pigs. Vet. Microbiol. **1**, 55—63 (1976).

70. MCNULTY, M. S., ALLAN, G. M., CURRAN, W. L., MCFERRAN, J. B.: Comparison of methods for diagnosis of rotavirus infection of calves. Vet. Rec. **98**, 463—464 (1976).

71. MCNULTY, M. S., CURRAN, W. L., MCFERRAN, J. B.: The morphogenesis of a cytopathic bovine rotavirus in Madin-Darby bovine kidney cells. J. gen. Virol. **33**, 503—508 (1976).

72. MADELEY, C. R., COSGROVE, B. P., BELL, E. J., FALLON, R. J.: Stool viruses in babies in Glasgow. J. Hyg. (Camb.) **78**, 261—273 (1977).

73. Malherbe, H. H., Strickland-Cholmley, M.: Simian virus SA.11 and related 0 agent. Arch. ges. Virusforsch. **22**, 235—245 (1967).
74. Martin, M. L., Palmer, E. L., Middleton, P. J.: Ultrastructure of infantile gastroenteritis virus. Virology **68**, 146—153 (1975).
75. Mathan, M., Almeida, J. D., Cole, J.: An antigenic subunit present in rotavirus infected faeces. J. gen. Virol. **34**, 325—329 (1977).
76. Mavromichalis, J., Evans, N., McNeish, A. S., Bryden, A. S., Davies, H. A., Flewett, T. H.: Intestinal damage in rotavirus and adenovirus gastroenteritis assessed by D-xylose malabsoprtion. Arch. Dis. Child. 589—591 (1977).
77. Mebus, C. A., Underdahl, N. R., Rhodes, M. B., Twiehaus, H. J.: Calf diarrhoea (scours) reproduced with a virus from a field outbreak. Bull. Neb. Agric. Exp. Statn. **233**, 1—16 (1969).
78. Mebus, C. A., Kono, M., Underdahl, N. R., Twiehaus, M. J.: Cell culture propagation of a neonatal calf diarrhoea (scours) virus. Canad. vet. J. **12**, 69—72 (1971).
79. Mebus, C. A., White, R. G., Bass, E. P., Twiehaus, M. J.: Immunity to neonatal calf diarrhoea virus. J. Amer. vet. med. Ass. **163**, 880—883 (1973).
80. Mebus, C. A., Wyatt, R. G., Sharpee, R. L., Sereno, M. M., Kalica, A. R., Kapikian, A. Z., Twiehaus, M. J.: Diarrhoea in gnotobiotic calves caused by the reovirus-like agent of human infantile gastroenteritis. Infect. Immun. **14**, 471—474 (1976).
81. Mebus, C. A., Wyatt, R. G., Kapikian, A. Z.: Intestinal lesions induced in gnotobiotic calves by the virus of human infantile gastroenteritis. Vet. Pathol. **14**, 273—282 (1977).
82. Mebus, C. A., Newman, L. E.: Scanning electron, light, and immunofluorescent microscopy of intestine of gnotobiotic calf infected with reovirus-like agent. Amer. J. vet. Res. **38**, 553—558 (1977).
83. Middleton, P. J., Szymanski, M. T., Abbott, G. D., Bartolussi, R., Hamilton, J. R.: Orbivirus acute gastroenteritis of infancy. Lancet i, 1241—1244 (1974).
84. Middleton, P. J., Petric, M., Szymanski, M. T.: Propagation of infantile gastroenteritis virus (orbi-group) in conventional and germfree piglets. Infect. Immun. **12**, 1276—1280 (1975).
85. Middleton, P. J., Petric, M., Hewitt, C. M., Szymanski, M. T., Tam, J. S.: Counter-immunoelectro-osmophoresis for the detection of infantile gastroenteritis virus (orbi-group) antigen and antibody. J. clin. Pathol. **29**, 191—197 (1976).
86. Middleton, P. J., Holdaway, M. D., Petric, M., Szymanski, M. T., Tam, J. S.: Solid phase radioimmunoassay for the detection of rotavirus. Infect. Immun. **16**, 439—444 (1977).
87. Morishima, T., Nagayoshi, S., Ozaki, T., Isomura, S., Suzuki, S.: Immuno-fluorescence of human reovirus-like agent of infantile diarrhoea. Lancet ii, 695—696 (1976).
88. Much, D. H., Zajac, I.: Purification and characterisation of epizootic diarrhoea of infant mice virus. Infect. Immun. **6**, 1019—1024 (1972).
89. Newman, F. S., Myers, L. L., Firehammer, B. D., Catlin, J. E.: Licensing and use of the calf scours vaccine — Part II. An Analysis of scourvax — reo protection. Proc. 77th Ann. Mtg. U.S. Anim. Hlth. Ass. 59—64 (1973).
90. Newman, J. F. F., Brown, F., Bridger, J. C., Woode, G. N.: Characterization of a rotavirus. Nature (Lond.) **258**, 631—633 (1975).
91. Obijeski, J. F., Palmer, E. L., Martin, M. L.: Biochemical characterization of infantile gastroenteritis virus (IGV). J. gen. Virol. **34**, 485—497 (1977).
92. Ørstavik, I., Figenschau, K. J., Ulstrup, J. C.: Rotavirus in stored specimens of faecal extracts. Lancet ii, 1083 (1974).
93. Ørstavik, I., Figenschau, J. K., Haug, K. W., Ulstrup, J. C.: A reovirus like agent (rotavirus) in gastroenteritis of children; virus detection and serological studies. Scand. J. inf. Dis. **8**, 1—5 (1976).
94. Ørstavik, I., Haug, K. W.: Virus-specific IgM antibodies in acute gastroenteritis due to a reovirus-like agent (rotavirus). Scand. J. inf. Dis. **8**, 237—240 (1976).

95. ØRSTAVIK, I., HAUG, K. W., SØVDE, A.: Rotavirus-associated gastroenteritis in two adults probably caused by virus reinfection. Scand. J. inf. Dis. **8**, 277—278 (1976).

96. PALMER, E. L., MARTIN, M. L., MURPHY, F. A.: Morphology and stability of infantile gastroenteritis virus: Comparison with reovirus and blue-tongue virus. J. gen. Virol. **35**, 403—414 (1977).

97. PAPPENHEIMER, A. M., ENDERS, J. F.: An epidemic diarrhoeal disease of suckling mice. II. Inclusions in the intestinal epithelial cells. J. exp. Med. **85**, 417—421 (1947).

98. PAPPENHEIMER, A. M., CHEEVER, F. S.: Epidemic diarrhoeal disease of suckling mice. IV. Cytoplasmic inclusion bodies in intestinal epithelium in relation to the disease. J. exp. Med. **88**, 317—324 (1948).

99. PAPPENHEIMER, A. M.: Epidemic diarrheal diseases of suckling mice. J. nat. Cancer Inst. **20**, 861—864 (1958).

100. PETRIC, M., SZYMANSKI, M. T., MIDDLETON, P. J.: Purification and preliminary characterization of infantile gastroenteritis virus (orbivirus group). Intervirology **5**, 233—238 (1975).

101. PETRIC, M., TAM, J. S., MIDDLETON, P. J.: Preliminary characterization of the nucleic acid of infantile gastroenteritis virus (orbivirus group). Intervirol. **7**, 176—180 (1976).

102. RAMIG, R. F., CROSS, R. K., FIELDS, B. N.: Genome RNA's andpoly peptides of reovirus serotypes 1, 2 and 3. J. Virol. **22**, 726—733 (1977).

103. REED, D. E., DALEY, C. A., SHANE, H. J.: Reovirus-like agent associated with neonatal diarrhea in pronghorn antelope. J. Wild. Dis. **12**, 488—491 (1976).

104. RODGER, S. M., SCHNAGL, R. D., HOLMES, I. H.: Biochemical and biophysical characteristics of diarrhoea viruses of human and calf origin. J. Virol. **16**, 1229—1235 (1975).

105. RODGER, S. M., SCHNAGL, R. D., HOLMES, I. H.: Further biochemical characterization of rotaviruses including the detection of surface glycoproteins. J. Virol. (in press, 1977).

106. RUBENSTEIN, D., MILNE, R. G., BUCKLAND, R., TYRRELL, D. A. J.: The growth of the virus of epidemic diarrhoea of infant mice (EDIM) in organ cultures of intestinal epithelium. Brit. J. exp. Pathol. **52**, 442—445 (1971).

107. RYDER, R. W., SACK, D. A., KAPIKIAN, A. Z., McLAUGHLIN, J. C., CHAKRABORTY, J., MIZANUR RAHMAN, A. S. M., MERSON, M. H., WELLS, J. G.: Enterotoxigenic *Escherichia coli* and reovirus-like agent in rural Bangladesh. Lancet **i**, 659—662 (1976).

108. SCHERRER, R., BERNARD, S.: Application d'une technique immunoenzymologique (ELISA) à la détection du rotavirus bovin et des anticorps dirigés contre lui. Ann. Microbiol. (Inst. Pasteur) **128 A**, 499—510 (1977).

109. SCHNAGL, R. D., HOLMES, I. H.: Characteristics of the genome of human infantile enteritis virus (rotavirus). J. Virol. **19**, 267—270 (1976).

110. SCHOUB, B. J., KOORNHOF, H. J., LECATSAS, G., PROZESKY, O. W., FRERNA, I., HARTMAN, E., KASSEL, H.: Viruses in acute summer gastroenteritis in black infants. Lancet **i**, 1093 (1975).

111. SCHOUB, B. D., GREEF, A. S., LECATSAS, G., PROZESKY, O. W., HAY, I. T., PRINSLOO, J. G., BALLARD, R. G.: A microbiological investigation of acute summer gastroenteritis in black South African infants. J. Hyg. (Camb.) **78**, 377—385 (1977).

112. SCHOUB, B. D., LECATSAS, G., PROZESKY, O. W.: Antigenic relationship between human and simian rotaviruses. J. med. Microbiol. **10**, 1—6 (1977).

113. SEXTON, M., DAVIDSON, G. P., BISHOP, R. F., TOWNLEY, R. R. W., RUCK, B. J., HOLMES, I. H.: Viruses in gastroenteritis. Lancet **ii**, 355 (1974).

114. SHARPEE, R., MEBUS, C. A.: Rotaviruses of man and animals. Lancet **i**, 639 (1975).

115. SHEPHERD, R. W., TRUSLOW, S., BIRD, R., CUTTING, W., DARNELL, R., BARKER, C. M.: Infantile gastroenteritis: A clinical study of reovirus-like agent infection. Lancet **ii**, 1082—1083.

116. Snodgrass, D. R., Smith, W., Gray, E. W., Herring, J. A.: A rotavirus in lambs with diarrhoea. Res. vet. Sci. **20**, 113—114 (1976).

117. Snodgrass, D. R., Herring, J. A., Gray, E. O.: Experimental rotavirus infection in lambs. J. comp. Pathol. **86**, 637—642 (1976).

118. Snodgrass, D. R., Wells, P. W.: Rotavirus infection in lambs: Studies on passive protection. Arch. Virol. **52**, 201—205 (1976).

119. Snodgrass, D. R., Madeley, C. R., Wells, P. W., Angus, K. W.: Human rotavirus in lambs: infection and passive protection. Infect. Immun. **16**, 268—270 (1977).

120. Spence, L., Fauvel, M., Bouchard, S., Babuik, L., Saunders, J. R.: Test for reovirus-like agent. Lancet **ii**, 322 (1975).

121. Spence, L., Fauvel, M., Petro, R., Bloch, S.: Haemagglutinin from rotavirus. Lancet **ii**, 123 (1976).

122. Stair, E. L., Mebus, C. A., Twiehaus, M. J., Underdahl, N. R.: Electron microscopy of intestines infected with a reovirus-like agent. Vet. Path. **10**, 155—170 (1973).

123. Suzuki, H., Konno, T.: Reovirus-like particles in jejunal mucosa of a Japanese infant with acute infectious non-bacterial gastroenteritis. Tohoku J. exp. Med. **115**, 199—211 (1975).

124. Syverton, J. T., Olitsky, P. K.: Bacteriological studies on an epizootic of intestinal disease in suckling and newly weaned mice. J. exp. Med. **60**, 385—394 (1934).

125. Tam, J. S., Szymanski, M. T., Middleton, P. J., Petric, M.: Studies on the particles of infantile gastroenteritis virus (orbivirus group). Intervirol. **7**, 181 to 191 (1976).

126. Tan, G. S., Townley, R. R. W., Davidson, G. P., Bishop, R. F., Holmes, I. H., Ruck, B. J.: Virus in faecal extracts from children with gastroenteritis. Lancet **i**, 1109 (1974).

127. Thouless, M. E., Bryden, A. S., Flewett, T. H.: Rotavirus neutralization by human milk. Brit. med. J. (in press, 1977).

128. Thouless, M. E., Bryden, A. S., Flewett, T. H., Woode, G. N., Bridger, J. C., Snodgrass, D. R., Herring, J. A.: Serological relationships between rotaviruses from different species as studied by complement fixation and neutralisation. Arch. Virol. **53**, 287—294 (1977).

129. Thurber, E. T., Bass, E. P., Beckenhauer, W. H.: Field trial evaluation of a reo-coronavirus calf diarrhea vaccine. Canad. J. comp. Med. **41**, 131—136 (1977).

130. Todd, D., McNulty, M. S.: Characterization of pig rotavirus RNA. J. gen. Virol. **33**, 147—150 (1976).

131. Todd, D., McNulty, M. S.: Biochemical studies on a reovirus-like agent (rotavirus) from lambs. J. Virol. **21**, 1215—1218 (1977).

132. Torres-Medina, A., Wyatt, R. G., Mebus, C. A., Underdahl, N. R., Kapikian, A. Z.: Diarrhea caused in gnotobiotic piglets by the reovirus-like agent of human infantile gastroenteritis. J. inf. Dis. **133**, 22—27 (1976).

133. Totterdell, B. M., Chrystie, I. L., Banatvala, J. E.: Rotavirus infections in a maternity unit. Arch. Dis. Child. **51**, 924 (1976).

134. Tufvesson, B., Johnsson, T.: Immunoelectro-osmophoresis for detection of reo-like virus: methodology and comparison with electron microscopy. Acta path. Microbiol. Scand. **B84**, 225—228 (1976).

135. Tzipori, S., Caple, I. W., Butler, K.: Isolation of a rotavirus from deer. Vet. Rec. **99**, 398 (1976).

136. Verly, E., Cohen, J.: Demonstration of size variation of RNA segments between different isolates of calf rotavirus. J. gen. Virol. **35**, 583—586 (1977).

137. Welch, A. B.: Purification, morphology and partial characterization of a reovirus-like agent associated with neonatal calf diarrhea. Canad. J. comp. Med. **35**, 195—202 (1971).

138. Welch, A. B., Thompson, T. L.: Physicochemical characterization of a neonatal calf diarrhea virus. Canad. J. comp. Med. **37**, 295—301 (1973).

139. WELCH, A. B., TWIEHAUS, M. J.: Cell culture studies of a neonatal calf diarrhea virus. Canad. J. comp. Med. **37**, 287—294 (1973).
140. WHITE, G. B. B., ASHTON, C. I., ROBERTS, C., PARRY, H. E.: Rotavirus in gastroenteritis. Lancet ii, 726 (1974).
141. WOODE, G. N., BRIDGER, J. C.: Viral enteritis of calves. Vet. Rec. **96**, 85—88 (1975).
142. WOODE, G. N., BRIDGER, J. C., HALL, G. A., DENNIS, M. J.: The isolation of a rotavirus-like agent associated with diarrhoea in colostrum deprived calves in Great Britain. Res. vet. Sci. **16**, 102—105 (1974).
143. WOODE, G. N., BRIDGER, J. C.: Levels of colostral antibodies against neonatal calf diarrhoea virus. Vet. Res. **97**, 148—149 (1975).
144. WOODE, G. N.: Pathogenic rotaviruses isolated from pigs and calves. In: ELLIOTT, K., KNIGHT, J. (eds.), Acute diarrhoea in Childhood, Ciba Symposium No. 42. Amsterdam: Elsevier 1976.
145. WOODE, G. N.: Viral diarrhoea in calves. Vet. Ann. **16**, 30—34 (1976).
146. WOODE, G. N., BRIDGER, J., HALL, G. A., JONES, J. M., JACKSON, G.: The isolation of reovirus-like agents (rotaviruses) from acute gastroenteritis of piglets. J. med. Microbiol. **9**, 203—209 (1976).
147. WOODE, G. N., BRIDGER, J. C., JONES, J. M., FLEWETT, T. H., BRYDEN, A. S., DAVIES, H. A., WHITE, G. B. B.: Morphological and antigenic relationships between viruses (Rotaviruses) from acute gastroenteritis of children, calves, piglets mice and foals. Infect. Immun. **14**, 804—810 (1976).
148. WYATT, R. G., KAPIKIAN, A. Z., THORNHILL, T. S., SERENO, M. M., KIM, H. W., CHANOCK, R. M.: *In vitro* cultivation in human fetal intestinal organ culture of a reovirus-like agent associated with non-bacterial gastroenteritis in infants and children. J. inf. Dis. **130**, 523—528 (1974).
149. WYATT, R. G., GILL, V. W., SERENO, M. M., KALICA, A. R., VAN KIRK, D. H., CHANOCK, R. M., KAPIKIAN, A. Z.: Probable *in vitro* cultivation of human reovirus-like agent of infantile diarrhoea. Lancet i, 98—99 (1976).
150. WYATT, R. G., SLY, D. L., LONDON, W. T., PALMER, A. E., KALICA, A. R., VAN KIRK, D. H., CHANOCK, R. M., KAPIKIAN, A. Z.: Induction of diarrhea in colostrum-deprived newborn rhesus monkeys with the human reovirus-like agent of infantile gastroenteritis. Arch. Virol. **50**, 17—27 (1976).
151. YOLKEN, R. H., WYATT, R. G., KALICA, A. R., KIM, H. W., BRANDT, C. D., PARROTT, R. H., KAPIKIAN, A. Z., CHANOCK, R. M.: Use of a free viral immunofluorescence assay to detect human reovirus-like agent in human stools. Infect. Immun. **16**, 467—470 (1977).
152. ZISSIS, G., LAMBERT, J. P., FONTEYNE, J., DE KEGAL, D.: Child-mother transmission of rotavirus. Lancet i, 96 (1976).
153. COHEN, J.: Ribonucleic acid polymerase associated with purified calf rotavirus. J. gen. Virol. **36**, 395—402 (1977).

Authors' address: Dr. T. H. FLEWETT, Regional Virus Laboratory, East Birmingham Hospital, Bordesley Green East, Birmingham, B 9 5ST, England.