CHAPTER 8

Rotaviruses

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I. INTRODUCTION

Electron microscopy played an important role in the recent recognition of a large group of viruses associated with diarrheal disease in the young of humans, of many kinds of animals, and of birds, so it is fitting that the name chosen for them should be based on their particle morphology. Flewett *et al.* (1974a) derived the name *Rotavirus* from the Latin "rota" ("wheel") because in negatively stained preparations, the inner capsid subunits suggest the appearance of spokes supporting a smooth rim of outer capsid. In early publications, members of the group were referred to as "orbivirus-like" (Bishop *et al.*, 1973; Middleton *et al.*, 1974), "infantile gastroenteritis virus (orbivirus group)" (Petric *et al.*, 1975), and "reovirus-like agents" (Fernelius *et al.*, 1972; Kapikian *et al.*, 1974), and the term "Duovirus" was also proposed (Davidson *et al.*, 1975a), but *Rotavirus* came into more common use and was adopted officially for the genus (Matthews, 1979).

Historical aspects of the discovery of rotaviruses and of their role in diarrheal diseases have been reviewed by Flewett (1977), Flewett and Woode (1978), and Holmes (1979), so the stories will not be retold here, but the indebtedness of later investigators to the pioneering studies of J.S. Light and H.S. Hodes, L.M. Kraft, and C.A. Mebus will become evident as we discuss the basic properties of the viruses and diseases.

Characteristics and Classification. Early studies of the morphology and morphogenesis of the viruses of epizootic diarrhea of infant mice (EDIM) and of Nebraska calf scours suggested that they belonged with the reoviruses, and human infantile enteritis virus was also immediately

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recognized as orbivirus- or reoviruslike (Adams and Kraft, 1967; Banfield *et al.*, 1968; Bishop *et al.*, 1973; Flewett *et al.*, 1973; Middleton *et al.*, 1974).

Rotavirus particles are icosahedral, 65–75 nm in diameter, have two concentric layers of capsid, and contain a segmented genome of double-stranded RNA. The 11 segments range in molecular weight from about 0.2 to 2 $\times$ 10^6. Their growth is normally restricted to enterocytes of the small intestine of mammalian or avian species, but they can also be cultivated in kidney cells in the presence of trypsin. Rotaviruses cause diarrheal disease in all the species that they infect, especially in the young, but adult infections are also common. They are classified as a genus in the family Reoviridae (Matthews, 1979), and the type species is listed as "human rotavirus."

Since rotaviruses are not strictly host-specific (see Section VI.B) and more than one human serotype exists (Section VI.D.2), the type species will have to be more precisely designated. The WHO–FAO Committee for Comparative Virology suggests a bovine rotavirus [Nebraska calf diarrhea virus (NCDV)] as the type on the grounds that it had been studied in more detail (Derbyshire and Woode, 1978), but at present the choice on this criterion would be the simian rotavirus SA 11 (Malherbe and Strickland-Cholmley, 1967). Since SA 11 virus is related to a human rotavirus serotype (see Section VI.D.2) and is widely available throughout the world, it may well be the best eventual choice.

Until recently, all rotaviruses were thought to share common inner-capsid antigens that were detectable by immunofluorescence or complement fixation (Flewett and Woode, 1978), but serologically unrelated viruses that nevertheless seem to have all other rotavirus characteristics have now been found, though rather rarely, in pigs, turkeys, and humans. These "pararotaviruses" (Bohl *et al.*, 1982) are discussed further in Sections II.A, II.C, II.F, and VI.D.2.

II. VIRIONS

A. Morphology

Rotavirus particles are isometric and resemble those of reovirus, but by negative staining, the outer rim of complete particles appears smooth (Fig. 1A), whereas the periphery of reovirus particles is less sharply defined (Flewett *et al.*, 1974a; Holmes *et al.*, 1975). Rotavirus preparations usually also contain a significant proportion of particles that lack the outer capsid layer, and these single-shelled particles are often described as rough because their periphery shows projecting subunits of the inner capsid (Fig. 1B). Such particles closely resemble orbivirus inner capsids (Bishop *et al.*, 1974; Els and Lecatsas, 1972; Middleton *et al.*, 1974).

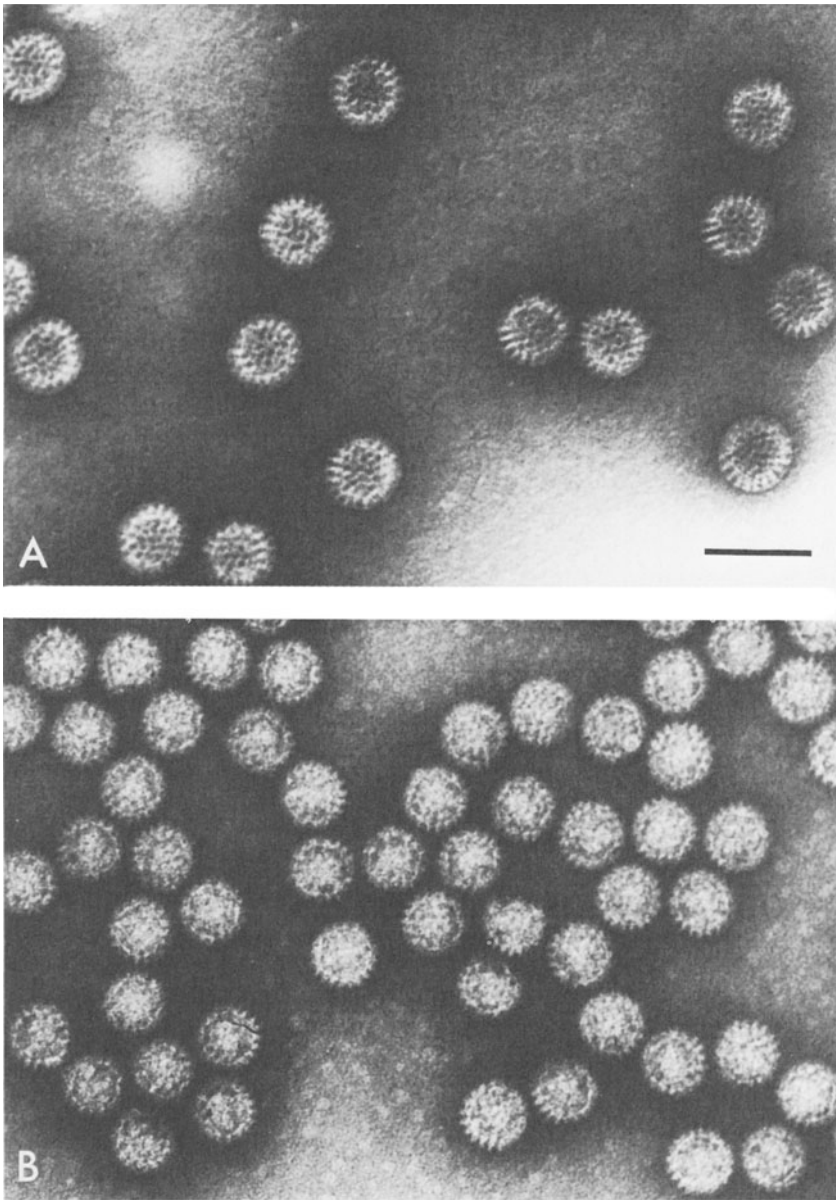

FIGURE 1. Particles of simian rotavirus SA 11, negatively stained with ammonium molybdate. (A) Complete or double-shelled particles; (B) innercapsid or single-shelled particles. Scale bar: 100 nm.

Differences in negative-staining technique probably account for most of the variation in reported size estimates; for example, Woode *et al.* (1976b) showed that particles on formvar–carbon films appeared 10% larger than those on pure carbon supports, when negatively stained with phosphotungstate. According to figures published on murine, bovine, human, porcine, simian, and avian rotaviruses, the diameter of the inner capsid is 55–65 nm and that of the outer capsid 65–75 nm (McNulty, 1979; Woode *et al.*, 1976b). Particles penetrated by negative stain reveal an inner core (38 nm), and isolated, thin-walled cores are sometimes seen in virus preparations treated with EDTA and trypsin (Palmer *et al.*, 1977) or in human stools (Payne *et al.*, 1981). They can also be prepared by treatment of rotaviruses with sodium thiocyanate (Almeida *et al.*, 1979).

Tubules of diameter 50–100 nm and consisting of hexagonally packed subunits (spacing 10 nm) are found in association with some but not all strains of murine, human, bovine, and equine rotaviruses (Banfield *et al.*, 1968; Flewett *et al.*, 1974c; Holmes *et al.*, 1975; Kimura, 1981; Suzuki and Konno, 1975; Woode *et al.*, 1976b).

In thin sections, rotavirus particles have an electron-dense core (25–30 nm) surrounded by a moderately electron-dense capsid layer 50–70 nm in diameter (McNulty, 1979). Enveloped particles (diameter 70–90 nm) are also seen in cisternae of the endoplasmic reticulum (see Section IV.F), and the envelope has sometimes been mistaken for the outer capsid layer, but by fixing and embedding purified preparations of double- and single-shelled particles of simian rotavirus SA 11, Petrie *et al.* (1981) have clarified the situation. They showed that double-shelled particles appeared as evenly electron-dense, smooth-edged ovals with dense nucleoids, whereas single-shelled particles had a more granular appearance, with ragged edges and protruding threads. Enveloped particles were clearly larger (Petrie *et al.*, 1981).

The obvious subunit structure, especially visible in the inner capsid of rotaviruses, has led to a number of attempts to define the capsid structure and symmetry. Various interpretations have been suggested; all agree on icosahedral symmetry, but propose triangulation numbers $T = 3$, 4, 9, or 16 (Esparza and Gil, 1978; Kogasaka *et al.*, 1979; Palmer *et al.*, 1977; Stannard and Schoub, 1977). The problem is the old one of double-sided images produced by negative staining, as was shown by Woode *et al.* (1976b), who were able to make large ring-shaped "capsomers" appear and disappear simply by tilting their specimens on a goniometer stage in the electron microscope.

By freeze–drying and shadowing particles of bovine rotavirus, Roseto *et al.* (1979) were able to obtain clear single-sided images and showed that the structure of the inner capsid is a skewed one with $T = 13$ (Fig. 2). Although they calculated that this structure should have 132 capsomers, in fact if a model is built, it is found that a $T = 13$ structure cannot be divided into nonoverlapping hexamers but only described in terms of 260 trimeric subunits (Holmes, 1982). The basic trimers had already been

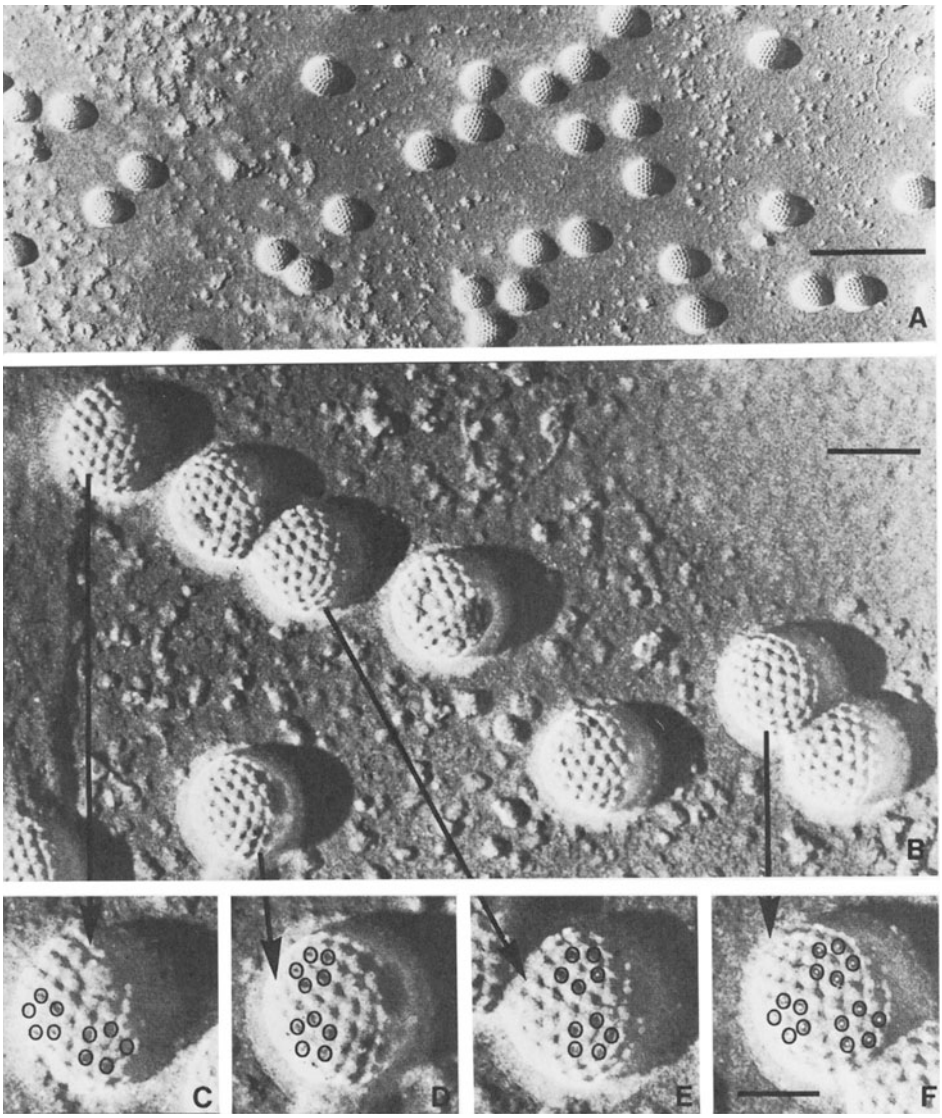

FIGURE 2. Freeze–dried single-shelled particles of bovine rotavirus, platinum-shadowed. (A) Lower-magnification field illustrating the golfball aspect. Scale bar: 200 nm. (B) High-magnification field showing the arrangement of surface subunits. Scale bar: 50 nm. (C–F) Four particles from (B) enlarged further, with sets of five hollows surrounding the five-fold axis hollows indicated. Note the skewed icosahedral arrangement. Scale bar: 25 nm. Kindly provided by A. Roseto, slightly modified from Roseto *et al.* (1979), and reproduced with the permission of Academic Press, Inc.

demonstrated by negative staining of disintegrating particles (Esparza and Gil, 1978; Martin *et al.*, 1975).

The outer capsid has generally been assumed to follow the same symmetry as the inner one, and this conclusion was supported by the freeze–drying study, which showed a smooth surface perforated by small holes, but no actual subunits could be seen (Roseto *et al.*, 1979).

Morphologically, the rotaviruses that lack the common inner-capsid antigen appear to be indistinguishable from conventional rotaviruses (Bridger *et al.*, 1982; Bohl *et al.*, 1982; McNulty *et al.*, 1981).

B. Physicochemical Properties

Centrifugation in cesium chloride density gradients separates complete (double-shelled) rotavirus particles (density 1.36 g/cm^3) from inner-capsid (single-shelled) particles (density 1.38 g/cm^3) (Newman *et al.*, 1975; Rodger *et al.*, 1975b; Tam *et al.*, 1976). Infectivity depends on the presence of the outer capsid (Bridger and Woode, 1976; Elias, 1977) and thus is lost when intact particles are converted to the single-shelled form by treatment with either of the calcium-chelating agents EDTA and ethyleneglycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA) (Cohen, 1977; Estes *et al.*, 1979b).

For bovine rotavirus in sucrose gradients, sedimentation coefficient estimates are 500 and 450–478 S (Newman *et al.*, 1975; Liebermann *et al.*, 1979). For human rotavirus particles first separated on the basis of density in cesium chloride, complete particles of density 1.36 g/cm^3 sedimented at 520–530 S, while single-shelled particles of density 1.38 g/cm^3 gave a value of 380–400 S (Tam *et al.*, 1976).

Rotavirus infectivity and particle integrity are resistant to fluorocarbon extraction and exposure to ether, chloroform, or deoxycholate (Fernelius *et al.*, 1972; Much and Zajac, 1972; Welch and Thompson, 1973; Tam *et al.*, 1976; Estes *et al.*, 1979b), but chloroform is reported to destroy the hemagglutinating ability of Nebraska bovine rotavirus, which is also a property of double-shelled (smooth) particles (Bishai *et al.*, 1978). Sodium dodecyl sulfate (SDS) at 0.1% inactivates simian rotavirus SA 11, but exposure to certain nonionic detergents actually enhances infectivity, probably by dispersing aggregates (Ward and Ashley, 1980).

Bovine rotavirus infectivity is stable within the pH range 3–9, but SA 11 rotavirus is slightly more acid-labile and is inactivated below pH 4 (Malherbe and Strickland-Cholmley, 1967; Welch and Thompson, 1973; Palmer *et al.*, 1977; Estes *et al.*, 1979b). All rotaviruses are stable at −70°C, and bovine and human samples have retained infectivity for months at 4 or even at 20°C, when stabilized by 1.5 mM $CaCl_2$ (Shirley *et al.*, 1981). Bovine and simian rotaviruses are relatively stable even at 45–50°C, losing 10–90% of infectivity per hour, but their stability varies with the diluent; for example, they are less stable in 1 M $MgCl_2$ or in

Tris- or phosphate-buffered saline than in maintenance medium or even water (Welch and Thompson, 1973; Estes *et al.*, 1979b). Murine rotavirus is reported to be much more labile, even at 4°C (Much and Zajac, 1972). The hemagglutinating ability of bovine rotavirus is lost very rapidly at 45°C, and both bovine and SA 11 hemagglutinins are destroyed by repeated freezing and thawing (Bishai *et al.*, 1978; Bastardo and Holmes, 1980).

Disinfectants active against rotaviruses include a chlorinated phenolic compound, cresols, an iodophore, and formalin (although 4–10% formaldehyde is required for rapid action), but both SA 11 and ovine rotaviruses are highly resistant to hypochlorite (Snodgrass and Herring, 1977; Tan and Schnagl, 1981). Ethanol, 95%, was the most effective disinfectant of all, and the hemagglutinins and particle integrity of bovine and simian rotaviruses are also destroyed by ethanol at 25 or 50% (vol./vol.) or methanol at 50% (Bishai *et al.*, 1978; Bastardo and Holmes, 1980; Tan and Schnagl, 1981).

C. Genomes

In early studies employing orcinol and diphenylamine reactions and thermal denaturation, it was shown that bovine and murine rotaviruses contained double-stranded RNA (dsRNA) (Welch, 1971; Much and Zajac, 1972; Welch and Thompson, 1973; Rodger *et al.*, 1975b), and by gel electrophoresis the bovine rotavirus genome was found to consist of 11 segments ranging in molecular weight from about 2.2 to 0.2×10^6 (Newman *et al.*, 1975; Rodger *et al.*, 1975b). Similar results were soon obtained for rotaviruses of human, ovine [or bovine ("O" agent)], simian, and porcine origins (Schnagl and Holmes, 1976; Kalica *et al.*, 1976; Todd and McNulty, 1976, 1977).

Although minor differences were evident in the electrophoretic band patterns, the grouping of four large segments, then two of medium size, a closely running triplet that could not always be resolved, and finally two small segments was immediately recognizable as a "rotavirus" pattern (Fig. 3) and was very easy to distinguish from those of reoviruses and orbiviruses (Schnagl and Holmes, 1976; Todd and McNulty, 1976). Many molecular-weight estimates have been published, but all have been obtained by electrophoresis of dsRNA and using reoviruses as standards. Since the last five segments or rotaviral RNA are all smaller than reovirus segment 10 and a completely denaturing system is probably essential for precise determinations, the absolute accuracy of the molecular-weight estimates is uncertain. As a representative set of values, those obtained for molecular weights of genome segments of bovine (Nebraska) rotavirus by Barnett *et al.* (1978) are 2.18, 1.73, 1.64, 1.48, 0.94, 0.77, 0.50, 0.50, 0.50, 0.29, and 0.22×10^6, giving a total molecular weight of 10.75×10^6. The most recent (unpublished) estimates obtained by comparing

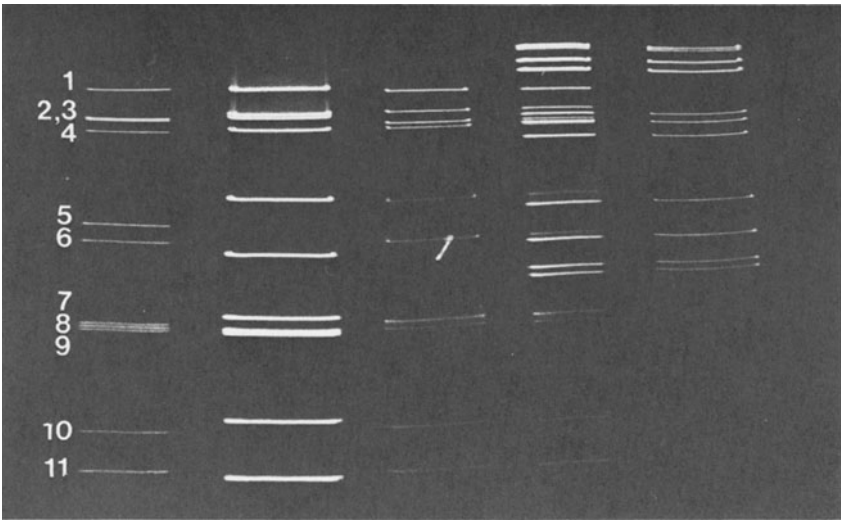

FIGURE 3. Electrophoretic fractionation patterns of the dsRNA genomes of human rotavirus (Hu/W. Australia/76) (A), bovine rotavirus (Bo/Australia/3/75) (B), simian rotavirus SA 11 (C), co-run of SA 11 plus reovirus 3 (D), and reovirus type 3 (Abney) (E) on a Laemmli 10% polyacrylamide gel. Courtesy of S.M. Rodger.

glyoxal-denatured RNA or cDNA copies with DNA standards suggest that the published molecular weights are about 10% too low (M.L. Dyall-Smith, A.A. Azad, and G.W. Both, A.R. Bellamy, J.E. Street and L.J. Siegman, personal communications). For the U.K. strain of bovine rotavirus, the current estimates of base pairs per segment are 3300, 2600, 2550, 2370, 1550, 1340, 1050, 1050, 1050, 760, 680 giving a total of 18,300 nucleotide pairs and a total molecular weight of 12.08×10^6 (M.L. Dyall-Smith, personal communication).

Since rotaviral serology has until recently remained technically difficult, gel electrophoresis of genome RNA was rapidly recognized as a very useful method for distinguishing among different isolates. Where segments of different strains had similar electrophoretic mobilities, it was necessary to co-run mixtures of the RNAs to establish small differences, and the change over to slab gels was a technical advance that greatly facilitated such comparisons. Segment-pattern differences among rotaviruses obtained from single species, e.g., human, bovine, or equine, were then established (Verly and Cohen, 1977; Kalica *et al.*, 1978b; Rodger and Holmes, 1979; Rodger *et al.*, 1980), and further applications of this approach ("electropherotyping") for epidemiological studies are discussed in Section VI.C.3.

The most noticeable deviations from the "average" rotaviral RNA electrophoretic patterns (see Fig. 4) have been reported for murine rotavirus, the segment 11 of which runs close to segment 10 (M. Smith and Tzipori, 1979); avian rotaviruses, with segment 5 migrating close to seg-

ment 4 and segments 10 and 11 co-running (Todd *et al.*, 1980); human rotaviruses, with segment 11 displaced, giving a "short" pattern (Dyall-Smith and Holmes, 1981a) see Sections V.C and VI.C.3); and finally the "pararotaviruses" of pigs, chickens, and humans, which are all quite distinct and show marked displacements of segment 5, 7, or 9 (Bohl *et al.*, 1982; Bridger *et al.*, 1982; McNulty *et al.*, 1981; S.M. Rodger, personal communication).

Comparison of rotaviral genomes by hybridization studies will undoubtedly be important in the future. In the first report of this kind, Matsuno and Nakajima (1982) showed that a Japanese human rotavirus isolate (strain TK20) was closely related to the human Wa strain isolated in the United States (see Sections III.B and VI.D), whereas Wa strain RNA had very little sequence homology with bovine (Nebraska) or simian (SA 11) strains. Using a Northern blot hybridization technique which makes it possible to look for different degrees of homology, Street *et al.* (1982) and Schroeder *et al.* (1982) studied a number of human and animal rotavirus strains and found a low order of sequence relatedness was general for corresponding segments, but hybridization under more stringent conditions showed considerable sequence diversity even among apparently similar human strains. The first actual sequence comparisons can be found in Appendix 1.

Suitable methods for extraction of genome RNA from feces or crude rotaviral preparations have been described by Rodger and Holmes (1979), Clarke and McCrae (1981), Croxson and Bellamy (1981), and Theil *et al.* (1981). For gels to be stained with ethidium bromide, the sharpest resolution of segments is generally obtained by using the discontinuous buffer electrophoresis system of Laemmli (1970). A silver staining technique recently described by Herring *et al.* (1982) increases both the sensitivity of detection and the resolution of rotaviral genome segments and is highly recommended.

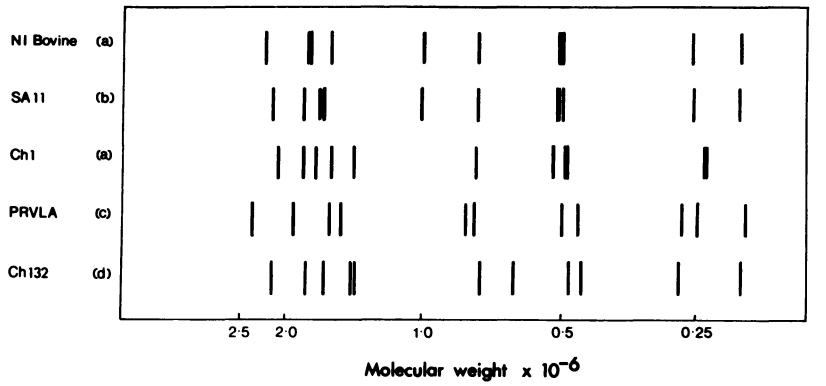

FIGURE 4. Diagrammatic representation of the migration of the dsRNA genome segments of various rotaviruses on polyacrylamide gels, based on data from Todd *et al.* (1980) (a), S.M. Rodger, personal communication (b), Bridger *et al.* (1982) (c), and McNulty *et al.* (1981) (d).

D. Capsid Proteins

Analyses of capsid proteins were first reported for bovine rotaviruses (Newman *et al.*, 1975; Rodger *et al.*, 1975b; Bridger and Woode, 1976; Matsuno and Mukoyama, 1979), but comparisons with human, simian, ovine, porcine, equine, murine, and lapine rotaviruses soon followed (Rodger *et al.*, 1977; Todd and McNulty, 1977; Thouless, 1979). Surprisingly, the major component of the outer capsid was shown to be glycosylated (Rodger *et al.*, 1977; Cohen *et al.*, 1979; Matsuno and Mukoyama, 1979).

Thouless (1979) introduced a simple and easily remembered terminology for the polypeptides indicating whether they belonged to the inner capsid (I) or outer capsid (O) or were non-structural (NS), but unfortunately further studies have led to one amendment (I_4 had to become O_1) and considerable disagreement about the total number of polypeptides that are structural. Since we are not yet concerned with nonstructural proteins, a full comparison of nomenclature is left for Section IV.D (see Table I), and the proteins will be designated here by the prefix "p" followed by numbers denoting their molecular weights ($\times 10^3$), using revised estimates for simian rotavirus SA 11 (Dyall-Smith and Holmes, 1981a). The molecular weights of corresponding proteins of different rotaviruses do vary, but few precise comparisons have been made (Thouless, 1979; Espejo *et al.*, 1980b; Dyall-Smith and Holmes, 1981b).

There is general agreement on one minor and two major proteins of the rotaviral inner capsid, p116, p96, and p42 (Espejo *et al.*, 1981; Estes *et al.*, 1981; Novo and Esparza, 1981), but Dyall-Smith and Holmes

TABLE I. Molecular-Weight Estimates and Nomenclature of Rotavirus Polypeptides Found in Infected Cells

SA 11[a]	SA 11[b]	Various[c]	UK bovine[d]	SA 11[e]
p113	125 VP1	I_1	VP1	VP1
p96	94 VP2	I_2	VP2	VP2
p91	—	I_{3a}	VP3	NCVP1
p84	88 VP3	I_{3b}	VP4	VP3
p57	53	(O_1)	VP5	NCVP2
p42	41 VP6	(I_4)	VP6	VP6
gp34	38 VP7a	(O_2)	VP7	VP7
	36 VP7	—	VP7c	—
p33	34	NS_1	VP8	NCVP3
p31	32	NS_2	VP9	NCVP4
p26	28 VP8	O_3	VP10	—
gp25	—	O_4	VP10c	NCVP5
	27 VP9	—	VP11, 11c	NCVP6
p21	20	NS_3	VP12	pNCVP5

[a] Dyall-Smith and Holmes (1981a). [b] Mason *et al.* (1980).
[c] Thouless (1979). [d] McCrae and Faulkner-Valle (1981).
[e] Arias *et al.* (1982).

(1981b) would add a minor protein p91 and McCrae and Faulkner-Valle (1981) include the bovine rotavirus protein corresponding to p91 and also the one corresponding to p33; this latter protein is believed to be nonstructural by all other workers. Estes *et al.* (1981) have shown that proteolytic breakdown products of p96 can be found in the region of p91 and below, but Dyall-Smith and Holmes (1981b) confirmed that their p91 is a primary gene product (see Section V.C) and not related to p96.

In the virus particle and in infected cells, p42 is found to be aggregated via disulfide bonds (Bastardo *et al.*, 1981). Novo and Esparza (1981) have shown that the corresponding p45 in bovine rotavirus is found in the form of trimers, and since it comprises about 80% of the inner capsid, it must be the main component of the visible morphological units.

Analysis following limited proteolysis of corresponding proteins of simian (SA 11) and human (Wa) rotaviruses showed marked similarities in the patterns of digestion of inner-capsid polypeptides (Dyall-Smith and Holmes, 1981b), but no studies on sequences have yet been reported.

With regard to the outer capsid, Espejo *et al.* (1981) and Estes *et al.* (1981) showed that the primary outer-shell polypeptide p84 (which they estimate as p88) is cleaved specifically by trypsin after the assembled virion has been released from its host cell, to form p62 and p28. This has clarified some apparent discrepancies; for example, in bovine (Nebraska) virus, Novo and Esparza (1981) found only the uncleaved p84 in their preparations. Dyall-Smith and Holmes (1981b) have confirmed that their p84 is related to p62, but were not able to detect the expected p28 cleavage product. In the nomenclature of Thouless (1979), this means that the assignment of I_4 to the inner capsid was incorrect, but it is in fact the precursor of p62, which she called O_1.

The major outer-shell component is glycoprotein 34 (gp34), which can occur in multiple forms (Dyall-Smith and Holmes, 1981b; Estes *et al.*, 1981). The apparent molecular weights and proportions of (usually) two versions vary from one rotavirus strain to the next, and probably even among different current stocks of SA 11 rotavirus, but two glycosylated bands are particularly evident in some reports on bovine rotaviruses (Cohen *et al.*, 1979; Novo and Esparza, 1981).

In addition to the cleavage product p28 mentioned above, Estes *et al.* (1981) reported another minor outer-capsid component, p27. Both Bastardo *et al.* (1981) and McCrae and Faulkner-Valle (1981) considered that p58 and gp25 could be capsid components, but most other investigators believe that they are nonstructural. It appears that we are at the limits of the techniques of purification and analysis now being employed.

E. Enzymes

Cohen (1977) showed that when bovine rotavirus particles were treated with the chelating agent EDTA or subjected to heat shock, the outer capsid dissociated and an RNA-dependent RNA polymerase asso-

ciated with the inner-capsid particles was activated. The activity was measured by incorporation of UTP into an acid-insoluble product and required the presence of all four ribonucleoside triphosphates. The optimum conditions for the reaction were pH 8, 50°C, and a concentration of Mg^{2+} around 8–10 mM. The product was sensitive to ribonuclease, showing that it was single-stranded RNA, but became ribonuclease-resistant following hybridization with an excess of genome RNA.

These findings were confirmed and extended by Hruska *et al.* (1978), who obtained similar results with another bovine rotavirus (Nebraska strain) and a number of human rotavirus samples, partially purified from stools.

Cohen *et al.* (1979) used the specific chelating agent EGTA to demonstrate that the concentration of free calcium ions was the crucial factor in activation of the polymerase, which occurred even in the presence of magnesium. By hybridization of [^{3}H]uridine-labeled products of the polymerase with unlabeled, denatured dsRNA from the virus, followed by ribonuclease treatment and polyacrylamide gel electrophoresis (PAGE), Cohen and Dobos (1979) proved that complete transcripts of all the genome segments were produced, but the *in vitro* reaction appeared to produce an excess of transcripts of the segments of low molecular weight.

RNA-dependent RNA polymerase in simian rotavirus SA 11 was activated similarly by Mason *et al.* (1980), but they found it advantageous to add bentonite to the reaction mixtures. Even so, the higher-molecular-weight transcripts that were demonstrated by gel electrophoresis following a 2-hr reaction at 40°C appeared to be degraded if the incubation was continued to 18 hr.

The porcine rotaviruslike agent that lacks the common group antigen was also converted to the single-capsid form when treated with EDTA, but no studies of polymerase in these particles have yet been reported (Bridger *et al.*, 1982).

A polyadenylate [poly(A)] polymerase activity was reported to be associated with complete, double-shelled human rotavirus particles purified from stools, but could not be detected in cell-culture-grown bovine rotavirus (Gorziglia and Esparza, 1981). The reaction was carried out under conditions established for poly(A) polymerase activity of reovirus (Stoltzfus *et al.*, 1974).

F. Antigenic Determinants

A common (group) antigen shared by bovine and human rotaviruses was originally detected by immunoelectron microscopy (IEM) and immunofluorescence (IF) (Flewett *et al.*, 1974a). It is also detectable by complement fixation (CF), immune adherence hemagglutination assay (IAHA), and enzyme-linked immunosorbent assays (ELISA), and has also

been found in rotaviruses of porcine, ovine, lapine, equine, murine, simian, and avian origin (Kapikian *et al.*, 1976a; Woode *et al.*, 1976b; Matsuno *et al.*, 1977b; Thouless *et al.*, 1977b; Scherrer and Bernard, 1977; Yolken *et al.*, 1977; McNulty *et al.*, 1979). The only rotaviruses that lack this antigen are the "pararotaviruses" from pigs, chickens, and humans (Bohl *et al.*, 1982; Bridger *et al.*, 1982, McNulty *et al.*, 1981; S. Rodger and R. Bishop, personal communication).

Studies by IEM on reactions of various rotaviruses with homologous and heterologous antirotaviral sera showed that the group antigen was located on the inner capsid and was thus masked in intact, double-shelled particles (Woode *et al.*, 1976b; Bridger, 1978). Peak CF reactivity was also associated with particles lacking the outer capsid when bovine rotavirus was purified in cesium chloride gradients (Fauvel *et al.*, 1978). In immunodiffusion reactions between crude (fecal) bovine and human rotavirus preparations and homologous and heterologous sera, electron microscopy of the precipitated "line of identity" by Mathan *et al.* (1977) showed antibody-cross-linked aggregates of intact and broken inner capsids and significant amounts of amorphous material interpreted as unassembled capsid subunits from infected cells.

Some discrimination between certain human and animal rotavirus strains on the basis of inner-capsid antigens has been achieved using selected hyperimmune guinea pig or gnotobiotic calf infection sera, by CF and more recently by ELISA or IAHA (Zissis and Lambert, 1978, 1980; Yolken *et al.*, 1978b; Zissis *et al.*, 1981; Kapikian *et al.*, 1981). This subgrouping is discussed in more detail in Section VI.D, but must be mentioned here because the work of Kapikian *et al.* (1981) and Kalica *et al.* (1981b) on a range of reassortants between a human and a bovine rotavirus showed that the subgroup specificity was always associated with genome segment 6 of either parental virus. Since it is known that this segment codes for the major inner-capsid polypeptide p42 or p45 (M.L. Smith *et al.*, 1980; McCrae and McCorquodale, 1982), this protein must carry the subgroup determinants. It also carries the common group determinant(s) because it is immunoprecipitated by the most commonly encountered class of antirotaviral monoclonal antibodies, which react with a range of rotaviruses (H. Greenberg, personal communication; S. Sonza and A. Breschkin, personal communication).

A greater degree of discrimination between rotavirus strains can be obtained using neutralization (N) or hemagglutination-inhibition tests, and the finding that immune sera could be specific by N test yet widely cross-reactive by IF, CF, or IAHA led to the conclusion that distinct antigens were involved (Woode *et al.*, 1976b; Matsuno *et al.*, 1977b; Thouless *et al.*, 1977b). This was confirmed when it was found that hemagglutination, like infectivity, was a property of intact, double-shelled virions, and that the neutralizing specificities of sera were reflected by their IEM reactions with complete but not single-shelled particles (Fauvel *et al.*, 1978; Bridger, 1978). Thus, it was clear that the antigen involved

in neutralization and hence in serotype specificity must be located in the outer capsid.

Serological analysis of human–bovine rotavirus reassortants and determination of the parental origin of their genome RNA segments by electrophoretic comparisons enabled Kalica *et al.* (1981b) to show that in bovine (UK) and human (Wa) rotaviruses, RNA segment 9 codes for the neutralization antigen. By testing antisera prepared by immunization of rabbits with individual polypeptides of simian rotavirus SA 11 that were separated by PAGE, Bastardo *et al.* (1981) demonstrated directly that the major outer-shell glycoprotein (gp34) elicits the best neutralizing response. The antiserum thus produced was type-specific. The antigenicity of gp34 was sensitive to reduction with mercaptoethanol, so the electrophoresis had to be carried out under nonreducing conditions, but it survived at least partial denaturation with SDS.

Monoclonal antibodies that neutralize SA 11 rotavirus have been prepared by A. Breschkin and S. Sonza (personal communication) and shown to immunoprecipitate gp34. Most are specific for SA 11, but some also neutralize other rotaviruses, so there must be both serotype-specific and some shared antigenic determinants on this glycoprotein. This observation may explain why many antisera show a degree of cross-reactivity in rotavirus N tests. Most of the neutralizing monoclonal antibodies also inhibit hemagglutination. One monoclonal antibody that neutralized SA 11 virus appears to react not with gp34 but with a polypeptide of somewhat lower molecular weight, which has not yet been identified. Bastardo *et al.* (1981) also obtained a lower but significant level of neutralization with a serum prepared against trace amounts of an unreduced protein identified as p26, which could be either a cleavage fragment of p84 or possibly the product of RNA segment 10 or 11. The suggestion that segment 11 might code for a minor neutralization antigen is supported by the finding of Matsuno *et al.* (1980) that a reassortant between SA 11 and bovine rotavirus that derived its major neutralization antigen and RNA segments 4 and 10 from SA 11 virus but segment 11 from the bovine parent showed an increased cross-reaction with anti-bovine rotavirus serum.

III. CELL SPECIFICITY AND CULTIVATION

A. Differentiated Nature of Host Cells

In infected animals, the main or usually the only sites of replication of rotaviruses are the columnar epithelial cells lining the villi of the small intestine. This has been shown by immunofluorescence (IF) staining for rotaviral antigens during histopathological studies of naturally or experimentally infected mice, calves, piglets, and lambs (Wilsnack *et al.*, 1969; Mebus *et al.*, 1971b; Hall *et al.*, 1976; Pearson and McNulty, 1977; Snod-

grass *et al.*, 1977a). Necessarily limited studies in humans by electron microscopy or IF suggest the same conclusion (Bishop *et al.*, 1973; Davidson *et al.*, 1975b). In mice and lambs, rotaviral infection of the colonic epithelium has also been found (Banfield *et al.*, 1968; Snodgrass *et al.*, 1977a).

The villous epithelium consists of a continuously differentiating population of cells. Cell division occurs in the crypts, and the cells mature as they move up the sides of the villi, elongating from a cuboidal to a columnar shape and developing a brush border with numerous new enzyme activities en route. Their lifetime as differentiated cells is limited to a few days, from the time they reach one-third or halfway along the villi until they fall off at the tips.

Both electron-microscopic and IF studies suggest that only the differentiated epithelial cells are susceptible to rotaviruses, since no particles or antigens are found in the proliferating cells in the crypts, but both appear in the enterocytes on the apical halves of the villi (Adams and Kraft, 1967; Mebus *et al.*, 1971b; Stair *et al.*, 1973; Snodgrass *et al.*, 1977a). Following the accelerated loss of infected cells from villous tips, the villi become covered with immature cuboidal epithelial cells that appear resistant to rotavirus infection, and Mebus *et al.* (1971b) suggested that this may limit the duration of the infection. The immature cells may lack receptors for the virus (Holmes *et al.*, 1976).

Even when grown in organ culture, pieces of small intestine do not retain their differentiated epithelial cells for long, and attempts to propagate rotaviruses in such organ cultures have met with only limited success (Rubenstein *et al.*, 1971; Wyatt *et al.*, 1974).

B. Cell-Culture Adaptation and the Role of Trypsin

Ironically, the first rotaviruses adapted to cell-culture growth were the simian rotavirus SA 11 isolated from a healthy monkey and the "O" agent from abattoir waste, long before they were known to have anything to do with diarrheal disease (Malherbe and Strickland-Cholmley, 1967). It was only after a great deal of patient and painstaking work that cell-culture adaptation of the Nebraska strain of bovine rotavirus was achieved (Mebus *et al.*, 1971a; Fernelius *et al.*, 1972) and other bovine strains were isolated in the United Kingdom, France, and Northern Ireland (Bridger and Woode, 1975; L'Haridon and Scherrer, 1977; McNulty *et al.*, 1976a). In each case, cultures of kidney epithelial cells were the most successful for rotavirus growth. Infected cells were monitored by IF staining, because cytopathic effects were variable.

There were many attempts to grow human rotaviruses in cell culture, but they proved to be more difficult. Banatvala *et al.* (1975) found that they could infect moderate numbers of cells if they centrifuged human rotavirus inocula onto cell monolayers, and this became the basis of a

successful method for diagnosis and serotyping of human rotaviruses (Bryden *et al.*, 1977; Thouless *et al.*, 1978), but on further passaging, the percentage of infected cells diminished rapidly, and very few particles were produced (Albrey and Murphy, 1976; Wyatt *et al.*, 1976a).

Although they grew bovine rotavirus satisfactorily, Welch and Twiehaus (1973) had little success with plaque formation, so Matsuno *et al.* (1977a) made a major advance when they were able to develop a plaque assay using the sensitive rhesus monkey kidney cell line MA-104 and both trypsin and DEAE–dextran in the overlay. Other workers soon found that bovine, porcine, and avian rotaviruses could be serially passaged in the presence of low concentrations of trypsin (Babiuk *et al.*, 1977; Theil *et al.*, 1977; Almeida *et al.*, 1978; McNulty *et al.*, 1979, 1981).

By ingenious experiments with trypsin inhibitors and the enzyme immobilized on agarose beads, Barnett *et al.* (1979) proved that trypsin was acting directly on the virus, not on the host cell. Various other proteases were found to have an enhancing effect on rotavirus growth, but Ramia and Sattar (1980) showed that with all except elastase and subtilisin, the effect was abolished by soybean trypsin inhibitor and was presumably due to traces of trypsin contaminating the other enzymes. It was also realized that the inhibitory effect of antibody-free fetal calf serum on rotavirus growth that had been noted in earlier studies (Welch and Twiehaus, 1973; Barnett *et al.*, 1975) was due to its trypsin-inhibitory capacity (Graham and Estes, 1980).

As discussed in Section II.D, the essential effect of trypsin is specific cleavage of one of the outer-capsid proteins (Espejo *et al.*, 1981; Estes *et al.*, 1981). Clark *et al.* (1981) showed that this cleavage does not affect the efficiency or rate of attachment of bovine rotavirus to cells, but activates a previously noninfectious fraction of particles by facilitating its uncoating in the cell. Trypsin enhances replication of even those rotaviruses that had been culture-adapted in its absence, such as simian rotavirus SA 11, but the effect is much more marked with cells in which it multiplies poorly, such as the Vero line of monkey kidney, than in MA-104 cells, which give quite good virus yields in the absence of trypsin (Graham and Estes, 1980). Presumably, cultures of cells like those of the MA-104 line contain sufficient protease of trypsinlike specificity to activate SA 11 virus, but not enough for many other rotaviruses. In the intestine, of course, trypsin would always be present, and probably bound to the surface of the enterocytes (Goldberg *et al.*, 1968).

For unknown reasons, human rotaviruses proved to be the most difficult to cultivate *in vitro*. The first success was obtained by Wyatt *et al.* (1980) with the Wa strain; after 11 passages through gnotobiotic piglets, the virus was trypsin-treated and could be serially passaged thereafter in primary African green monkey kidney (AGMK) cells. In the presence of trypsin Wa strain replicates moderately well also in MA-104 and CV-1 monkey kidney cell lines. Drozdov *et al.* (1979) have reported a similar isolation, without piglets.

Very recently, almost routine isolation of human rotaviruses of different serotypes has been achieved by a number of researchers in Japan (Sato *et al.*, 1981; Urasawa *et al.*, 1981; R. Kono, personal communication; T. Konno, personal communication). Trypsin treatment, MA-104 or CV-1 cells, and rolling of the cultures during the initial passages appear to be the critical factors, and several passages are needed before the isolate can be regarded as established. Isolation of a range of human rotaviruses will greatly facilitate identification of serotypes (see Section VI.D), and the aforementioned achievement is an excellent example of how scientists gradually build on earlier results.

C. Adaptation by Reassortment

The fact that viruses with segmented genomes are frequently capable of genetic reassortment during mixed infections led Greenberg *et al.* (1981) to try to rescue genes of uncultivable human rotaviruses by this means. The nonadapted human rotavirus strains Wa and DS-1, which could normally be passaged only once or twice in AGMK cells, and temperature-sensitive mutants of the cultivable bovine rotavirus (UK strain) were chosen. After mixed infection of the AGMK cells at 34°C, reassortants were selected from plaques produced at 39°C and/or in the presence of antiserum capable of neutralizing the bovine parent. A number of reassortants that grew well in cell culture but carried the neutralization antigens of either Wa or DS-1 strains were isolated. A more extensive series of rescues of 33 noncultivable human rotavirus strains including 12 which are serologically distinct from either Wa or DS-1 has been reported since (Greenberg *et al.*, 1982). Such reassortants have been important in the identification of antigenic polypeptides, as was discussed in Section II.F. Genome segment 4 of the bovine rotavirus appeared to be essential for cultivability under the conditions employed (Greenberg *et al.*, 1982).

D. Assay Systems

Because of the difficulty of conventional isolation of rotaviruses despite their importance in human and animal disease, a great deal of effort has been put into the development of alternatives to the usual infectivity assays, mainly for diagnostic purposes. Thus, it seemed opportune to briefly mention these methods at this point, but readers who are interested primarily in diagnostic methods should refer to reviews such as those by Ellens *et al.* (1978), Kapikian *et al.* (1979), and de Leeuw and Guinee (1981) for details and comparative evaluation of the available methods.

1. Methods That Do Not Rely on Infectivity

At first, rotavirus detection and quantitation depended almost entirely on electron microscopy using negative-staining methods. Preliminary concentration (and sometimes partial purification) has mostly involved differential centrifugation (Fernelius *et al.*, 1972; Flewett *et al.*, 1973, 1974b; Bishop *et al.*, 1974), but ammonium sulfate precipitation and concentration using dried polyacrylamide gel (lyphogel) also have their exponents (Caul *et al.*, 1978; Rogers *et al.*, 1981). Immunoelectron microscopy is not necessary for rotavirus recognition, but has often been employed in differential diagnosis of the various viruses involved in diarrheal disease (Kapikian *et al.*, 1974; Flewett and Boxall, 1976). The use of supporting films coated with specific antibodies may increase the sensitivity of rotavirus detection (Nicolaieff *et al.*, 1980; Obert *et al.*, 1981), but on the whole, electron-microscopic methods are not ideal for quantitation.

Precipitation methods include the fluorescent virus precipitin test of L.G. Foster *et al.* (1975), in which aggregates obtained after mixing virus suspensions with fluorescein-conjugated rotavirus antibodies are scored by UV microscopy, and (counter)immunoelectrophoresis, which is convenient but not very sensitive or quantifiable (Grauballe *et al.*, 1977; Middleton *et al.*, 1976; Spence *et al.*, 1977; Tufvesson and Johnsson, 1976).

Radioimmunoassays (RIAs) and enzyme-linked immunosorbent assays (ELISAs) are most popular for rotavirus antigen detection and quantitation, because they can be readily applied to large numbers of samples (Kalica *et al.*, 1977; Middleton *et al.*, 1977a; Cukor *et al.*, 1978; Yolken *et al.*, 1977; Scherrer and Bernard, 1977; Ellens *et al.*, 1978; Kapikian *et al.*, 1979; de Leeuw and Guinee, 1981). As a method for rotavirus detection, ELISA is about 100 times more sensitive than immunoelectrophoresis (Grauballe *et al.*, 1981b) and equivalent to RIA, if antisera of equivalent titers are used (Sarkkinen *et al.*, 1980)

Bradburne *et al.* (1979) used erythrocytes coupled to rotaviral antibodies as the detection system in an assay otherwise similar to an RIA or ELISA, which they christened the solid-phase aggregation of coated erythrocytes (SPACE) test. It offers speed and simplicity, but at the cost of lower sensitivity than ELISA. Another rapid test that looks very promising for field use also employs fixed erythrocytes coupled to purified rotaviral antibodies; it is the reverse passive hemagglutination test of Sanekata *et al.* (1979). Alternatively, rotaviral antibodies can be adsorbed on to protein A–carrying *Staphylococcus aureus* cells, and these are agglutinated by samples containing rotavirus antigen (Hebert *et al.*, 1981).

Finally, hemagglutination of human or sheep erythrocytes can be employed for assay of rotaviruses such as SA 11, but not all bovine and very few human rotavirus strains appear to hemagglutinate (Spence *et al.*, 1976, 1978; Inaba *et al.*, 1977; Kalica *et al.*, 1978a; Shinozaki, 1978).

As mentioned in Section II.F, hemagglutination is a property of particles with an intact outer capsid (Fauvel *et al.*, 1978).

2. Infectivity Assays

Assays of rotaviral infectivity in infant animals are possible, but rotaviruses are notorious for contaminating the environment and are extremely contagious, so susceptible animals have to be maintained in strict isolation. In her pioneering studies of murine rotavirus [epizootic diarrhea of infant mice (EDIM)], Kraft (1958) developed the use of filter-topped cages for her litters of mice, but although gnotobiotic calves and piglets have been used extensively in rotavirus research, they are too expensive for use in titrations.

Even with rotaviruses that have not been cell-culture-adapted, infectivity titers can be estimated by fluorescent focus assays. Monolayers of bovine kidney, LLC-MK2, or MA-104 cells are exposed to dilutions of the rotavirus to be assayed, with centrifugation during the adsorption period for human strains; then, after incubation for about 16 hr to complete the first cycle of replication, infected cells are stained and counted by immunofluorescence (Barnett *et al.*, 1975; Bryden *et al.*, 1977; Moosai *et al.*, 1979).

Plaque assays are generally the method of choice for infectivity titrations, since in quantal assays rotaviral cytopathic effects can be variable and difficult to read. Following the lead of Matsuno *et al.* (1977a), similar plaque assays for other rotaviruses have been developed (Ramia and Sattar, 1979; E.M. Smith *et al.*, 1979). Inclusion of trypsin in the overlay is usually essential, but plaquing of bovine rotavirus in AGMK cells not only without trypsin or DEAE–dextran but even with fetal calf serum in the overlay has been described (Kapikian *et al.*, 1979).

E. Growth Kinetics

Not many growth curves for rotaviruses have been published. Welch and Twiehaus (1973) infected primary bovine embryonic kidney cells with an estimated multiplicity of bovine rotavirus (Nebraska strain) of 10 ID_{50} per cell. An increase in titer was detected by 4 hr, and there was a steady rise in titer until 18 hr, with most of the virus cell-associated. Similar results were obtained for Nebraska bovine rotavirus by Kurogi *et al.* (1976) and Matsuno and Mukoyama (1979), for Northern Ireland bovine rotavirus by McNulty *et al.* (1977), and for simian rotavirus SA 11 in MA-104 cells by Estes *et al.* (1979a), but lower multiplicities of infection were used and the duration of a single cycle of growth was difficult to estimate.

The most convincing single-cycle growth curves so far published are for bovine rotaviruses: the Nebraska strain in Madin-Darby bovine kidney

cells and the UK strain of bovine rotavirus in the BSC-1 line of AGMK cells, infected at high multiplicity (20 plaque-forming units per cell) (Clark *et al.*, 1979; McCrae and Faulkner-Valle, 1981). Synthesis of new viral double-stranded RNA was detected by 3 hr postinfection, and maximum yields of progeny virus were obtained by 10–12 hr.

F. Effects on Host Cells

Cytopathic effects in rotavirus-infected cultures have been reported for SA 11 virus and the "O" agent by Malherbe and Strickland-Cholmley (1967) and Estes *et al.* (1979a) and for bovine rotavirus by Welch and Twiehaus (1973) and McNulty *et al.* (1977). The main visible changes are cytoplasmic vacuolization and the appearance of small, eosinophilic intracytoplasmic inclusions. A very characteristic feature of rotavirus-infected cultures that was noted by C.A. Mebus is "flagging" (Holmes, 1979). Infected cells tend to remain attached to the monolayer by a single process, so they wave about when the medium moves. Cytopathic effects are more notable in rolled than in stationary cultures, probably because there is more cell detachment, but replication may also be enhanced (Kurogi *et al.*, 1976; McNulty *et al.*, 1977).

At the biochemical level, a shutdown of host-cell protein synthesis is detected if the infecting multiplicity is sufficiently high (i.e., if a sufficient proportion of cells are infected) (Thouless, 1979). McCrae and Faulkner-Valle (1981) noted that this shutdown occurred by 4 hr postinfection, but found that the overall levels of DNA, RNA, and protein synthesis in BSC-1 cells infected with UK bovine rotavirus were not markedly affected. On the other hand, Carpio *et al.* (1981) reported margination of chromatin and a decreased synthesis of DNA, RNA, and proteins in BSC-1 cells infected with bovine rotavirus strain C486, with apparent breakdown of cellular DNA.

Welch and Twiehaus (1973) showed that interferon was produced by bovine embryonic kidney cells exposed to UV-inactivated bovine rotavirus, whereas unirradiated virus was a less effective inducer. La Bonnardiere *et al.* (1980) found bovine rotavirus a poor inducer, and only weakly susceptible to interferon. In MA-104 cells pretreated ("primed") with low concentrations of interferon, however, infectious simian rotavirus SA 11 can induce significant quantities of interferon (McKimm-Breschkin and Holmes, 1982), and the replication of SA 11 virus is sensitive to interferon if the infected cells are simultaneously exposed to UV-treated SA 11 particles. This suggests that the rotavirus normally fails to activate the antiviral enzymes produced in cells exposed to interferon, but if the enzymes are activated by UV-treated particles, they effectively block rotavirus multiplication. It thus seems possible that interferon may have a more significant role in limiting rotavirus replication *in vivo* than the *in vitro* experiments at first suggested, since in the intestine other

agents capable of inducing interferon and of activating the antiviral enzymes would be present at the same time as the rotavirus.

IV. REPLICATION

A. Adsorption

Whether the high degree of host-cell specificity of rotaviruses depends only on specificity of adsorption or on subsequent intracellular events does not appear to have been investigated, but the former is generally assumed. On the basis of the apparent development of rotavirus sensitivity during differentiation of intestinal epithelial cells (Section III.A) and the reported decrease in susceptibility of mice to murine rotavirus (EDIM) at about the time of weaning, Holmes *et al.* (1976) suggested that the cell-membrane receptor for rotaviruses might be lactase. Lactase activity is found on the kidney cells in which rotaviruses are often propagated, but other brush-border components may have a similar distribution.

Treatment of human group O and sheep erythrocytes with neuraminidase rendered them inagglutinable by simian rotavirus SA 11 (Bastardo and Holmes, 1980), and similar treatment of MA-104 cells makes them refractory to SA 11 virus infection (G. Raghu, personal communication), so in each case the presence of sialic acids on the receptors must be essential.

By electron microscopy, adsorption of rotavirus particles to host-cell plasma membranes has been demonstrated *in vitro* (Petrie *et al.*, 1981) and *in vivo* (Coelho *et al.*, 1981). In the latter case, murine rotavirus particles were mostly associated with the tips of microvilli, but some were situated between the microvilli, and endocytosis probably occurs at the base of the microvilli.

B. Penetration and Uncoating

Although rotavirus particles have been seen in lysosomelike bodies within infected cells (Lecatsas, 1972; Petrie *et al.*, 1981), uptake under single-cycle conditions has not yet been studied. On the other hand, as Cohen *et al.* (1979) point out, their findings on "uncoating" or disassembly of the rotaviral outer capsid at low calcium ion concentrations are very relevant to the penetration step in rotaviral infection. As soon as the incoming particle left the extracellular zone, in which the calcium concentration is relatively high, and passed through the plasma or vesicular membrane of the cell into the cytoplasmic compartment, where the Ca^{2+} concentration is maintained at less than 1 μM (Carafoli and Crompton, 1978), its outer capsid would be dissociated and its endogen-

ous RNA polymerase would be activated. Clark *et al.* (1981) presented evidence that the process of infection by particles with outer-capsid protein p84 that has not been cleaved by trypsin is probably blocked at this point.

C. Transcription

Following uncoating of the incoming rotavirus particle, transcription would presumably begin immediately in the cytoplasm. Some properties of the RNA-dependent RNA polymerase (Cohen, 1977) have already been mentioned in Section II.E. The transcripts do not self-hybridize, so they are transcribed fom only one strand of the genomic double-stranded RNA (dsRNA) (Cohen, 1977), but their behavior during electrophoresis following hybridization with excess genomic RNA shows that they are full-length copies of each of the 11 segments (Cohen and Dobos, 1979; Bernstein and Hruska, 1981). *In vitro,* there seems to be a selective degradation of the higher-molecular-weight transcripts during long-term incubations even in the presence of bentonite (Cohen and Dobos, 1979; Mason *et al.*, 1980), and the same appears to be true in SA 11 virus–infected MA-104 cells (Dyall-Smith and Holmes, 1981a; J.L. McKimm-Breschkin, personal communication). The transcripts do not appear to contain poly(A) tracts, but function as messenger RNA, directing synthesis of viral polypeptides in cell-free translation systems (Cohen and Dobos, 1979; Mason *et al.*, 1980).

D. Biosynthesis of Polypeptides

If cells are infected at a sufficiently high multiplicity to cause a rapid shutdown of cellular protein synthesis, newly synthesized rotaviral proteins can be detected by 4 hr postinfection (McCrae and Faulkner-Valle, 1981). At least 12 virus-specific polypeptides can be identified; molecular-weight estimates and the relationship among the systems of nomenclature used by different research groups are shown in Table I. In addition to the structural proteins discussed in Section II.D., there are between one and six nonstructural proteins, with most workers agreeing on four. These are the primary gene product p57, which was originally confused with the outer-capsid tryptic-cleavage product of p84 (Thouless, 1979; Smith *et al.*, 1980), p33 and p31, and gp25. McCrae and Faulkner-Valle (1981), Arias *et al.* (1982), and Ericson *et al.* (1982) have shown that the smallest polypeptide, p21, or NS_3 of Thouless (1979), is in fact the precursor of gp25. This glycoprotein is doubly unusual in that detectable amounts of its precursor are generally seen in infected cells in the absence of tunicamycin treatment (Thouless, 1979; Dyall-Smith and Holmes,

1981a; Arias *et al.*, 1982), and no other example of a nonstructural glycoprotein comes to mind.

Multiple forms of the structural glycoprotein gp34 can be found in infected cells, but the unglycosylated precursor is seen only in cells treated with tunicamycin (McCrae and Faulkner-Valle, 1981; Dyall-Smith and Holmes, 1981b; Arias *et al.*, 1982; Ericson *et al.*, 1982). The carbohydrate portions of both gp34 and gp25 can be hydrolyzed by endoglycosidase H, which indicates that they are simple mannose-rich oligosaccharides rather than more complex Golgi-processed ones (Arias *et al.*, 1982).

Ultrastructural studies suggest that both transcription and biosynthesis of viral polypeptides must occur within or around the moderately electron-dense, granular or finely fibrillar areas designated "viroplasm" or viral inclusions. These resemble the corresponding "virus factories" in reovirus- or orbivirus-infected cells and have been observed in cells infected with murine, simian, human, bovine, ovine, and porcine rotaviruses (Banfield *et al.*, 1968; Lecatsas, 1972; Holmes *et al.*, 1975; McNulty *et al.*, 1976c; Snodgrass *et al.*, 1977a; Saif *et al.*, 1978; Pearson and McNulty, 1979). Their protein composition is unknown, but no doubt includes some of the nonstructural proteins.

The tubules mentioned in Section II.A have also been seen within infected cells, where they appear as thin-walled structures 50–60 nm in diameter and may run from the cytoplasm into the nucleus. They are commonly associated with murine and porcine rotaviruses and have once been reported in human rotavirus–infected cells (Banfield *et al.*, 1968; Saif *et al.*, 1978; Pearson and McNulty, 1979; Suzuki and Konno, 1975), but are not produced by simian rotavirus SA 11. Their antigenic relationship to inner capsids (Holmes *et al.*, 1975; Kimura, 1981) suggests that they are probably composed of structural protein(s), but no purification or analysis has been published. Similar tubules are common in orbivirus-infected cells (Murphy *et al.*, 1971). Huismans and Els (1979) have shown that the orbivirus-associated tubules consist of a nonstructural protein, even though they appear to be precipitated by antisera prepared against purified particles. Thus, the rotavirus tubules could also turn out to be nonstructural.

Laminar structures that were described by Lecatsas (1972) as "membranous elements" and by Altenburg *et al.* (1980) as "tubular structures" are found in cells infected with SA 11 rotavirus. They occur both in nuclei and in cytoplasm, but differ from the tubules mentioned above. They are 15–20 nm thick, and fine cross-striations can be seen at high magnification (F.A. Murphy, personal communication). They closely resemble the cross-striated filaments found in cells infected with Colorado tick fever virus (Murphy *et al.*, 1968). With some antisera, they can be detected by immunofluorescence, and appear to be ribbon-shaped and up to 5 μm long (M. Dyall-Smith, personal communication). Neither their function

nor their composition is known, but they are probably paracrystalline aggregates of a nonstructural protein.

E. Viral RNA Synthesis

Biochemical studies on bovine rotavirus–infected cells have shown that newly synthesized genomic dsRNA can be detected by 2–3 hr postinfection, and all the dsRNA segments appear to be made at the same time (McCrae and Faulkner-Valle, 1981). It is not known whether the second strands are synthesized on plus-strand templates or whether the process occurs in viral precursor particles as has been shown for reoviruses (Joklik, 1974).

In studies of rotavirus morphogenesis, electron-dense (after staining) cores appear within particles while they are still associated with the viroplasm matrix (Banfield *et al.*, 1968; Holmes *et al.*, 1975; Pearson and McNulty, 1979) or during budding into rough endoplasmic reticulum (RER) vesicles (Fig. 5). Large numbers of "coreless" particles were produced in the porcine kidney PK-15 cell line infected with a high multiplicity of lamb rotavirus that was not adapted to serial passage in PK-15 cells (McNulty *et al.*, 1978). It would be very interesting to know whether such particles, the cores of which fail to stain during processing for thin-

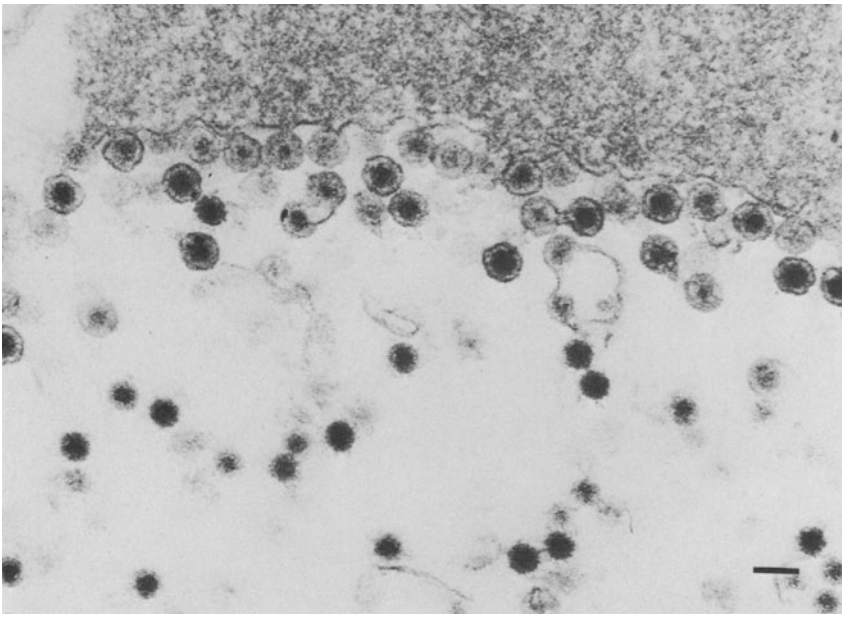

FIGURE 5. Simian rotavirus SA 11 in CV-1 cells, showing viroplasm and particles budding into the lumen of the RER. Note the increase in staining of the cores during budding. Scale bar: 100 nm. Kindly provided by F.A. Murphy and A.K. Harrison.

section electron microscopy contain single-stranded RNA, or any RNA at all, but no sequel to this study has appeared. As the authors suggested, the coreless particles could be defective interfering particles produced as a result of high-multiplicity passages.

F. Assembly

Electron-microscopic studies on the morphogenesis of rotavirus particles provide the necessary background for a discussion of the assembly stage of replication, but for an understanding of the process, we also need to consider recent biochemical findings. In the following account, I have cited published experimental results wherever possible, but parts of the hypothesis, which is based on discussions between the author and M.L. Dyall-Smith, have yet to be proven.

The most distinctive feature of rotavirus morphogenesis, in which rotaviruses differ from reoviruses or other genera in the family, is the budding process whereby particles accumulate in vesicles of the RER. This has been noted by almost all workers who have studied rotavirus-infected cells, and when the plane of the section is favorable, it can be seen that the particles bud directly from the edge of the viroplasm through adjacent areas of RER membrane (Adams and Kraft, 1967; Banfield *et al.*, 1968; Lecatsas, 1972; McNulty *et al.*, 1976c; Saif *et al.*, 1978; Pearson and McNulty, 1979; McNulty, 1979; Altenburg *et al.*, 1980). It has also been remarked that most of the particles in RER vesicles are unenveloped, but as illustrated in Fig. 6 (Holmes *et al.*, 1975), enveloped particles are usually located near the RER membrane. By the criteria of Petrie *et al.* (1981), most of the unenveloped particles in such vesicles are double-shelled. At present, it is technically impossible to determine whether the particles within the envelopes are single- or double-shelled, but we believe them to be single-shelled because of the considerations discussed below.

If the idea of automatic uncoating of rotaviruses in the low-calcium environment of the cytoplasm (Section IV.B) is accepted, it is hard to imagine how the outer capsids of progeny particles could assemble under the same conditions, even if single-shelled particles and the outer-capsid polypeptides were all present near the viroplasm. On the other hand, from what is known of the distribution of calcium in various kinds of cells, the Ca^{2+} concentration within the endoplasmic reticulum is more likely to be sufficiently high for complete particle assembly (Carafoli and Crompton, 1978). The problem then becomes one of transfer of single-shelled particles and the outer-capsid components to the lumen of the endoplasmic reticulum.

We suggest that the observed budding process indicates how the inner capsid is transferred, and predict that the outer-capsid polypeptides, or at least the major structural glycoprotein (gp34 in the case of SA 11 virus)

and possibly the second viral glycoprotein gp25, will be synthesized on membrane-bound ribosomes and secreted directly into the RER. A preliminary study of bovine rotavirus–infected cells employing immunoperoxidase staining (Chasey, 1980) directly supports this suggestion, since the reaction indicating viral antigens occurred not only on budding particles but also along the RER membrane nearby. Arias *et al.* (1982) have shown by endoglycosidase H digestion that the glycoproteins of SA 11 virus seem to contain only high-mannose oligosaccharide residues, which suggests that the glycosylation occurs at the RER membrane and that further processing (Robbins *et al.*, 1977) does not occur; they also suggest that maturation of particles occurs in the lumen of the endoplasmic reticulum, and Korolev *et al.* (1981) came to the same conclusion on morphological grounds.

One problem still remains: following budding, the inner capsid within what we shall now call the pseudoenvelope would still be separated from the outer-capsid components and Ca^{2+} ions in the lumen of the RER, so the pseudoenvelope must be removed. Pseudoenvelope removal depends on a glycosylated product synthesized in infected cells during rotavirus multiplication, and it is tempting to assume that it is

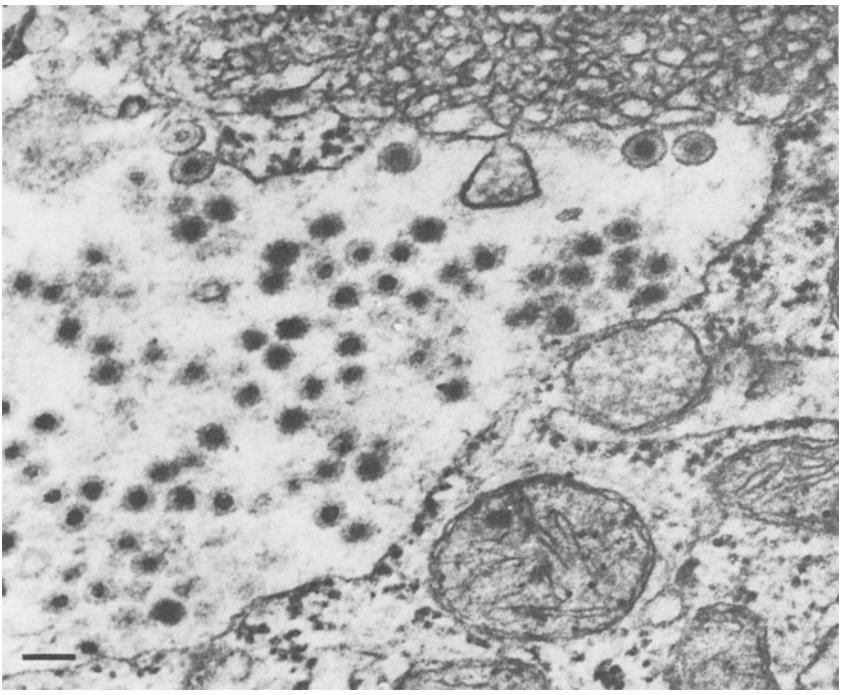

FIGURE 6. Portion of the cytoplasm of a human rotavirus–infected duodenal epithelial cell showing a mass of convoluted smooth membrane beside a cisterna of the RER, which contains enveloped and unenveloped virus particles and amorphous material. Scale bar: 100 nm. From Holmes *et al.* (1975), by permission of the American Society for Microbiology.

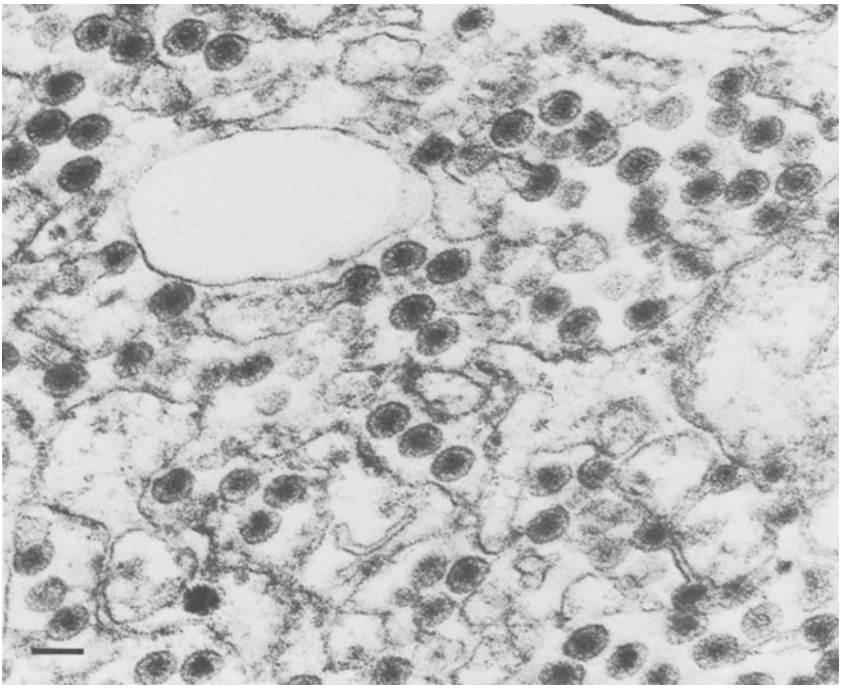

FIGURE 7. SA 11 rotavirus morphogenesis in tunicamycin-treated MA-104 cell. Note that all the particles in the endoplasmic reticulum vesicles retain their "pseudoenvelopes." Scale bar: 100 nm. Kindly provided by M.L. Dyall-Smith.

one or another of the rotavirus glycoproteins; at any rate, as Fig. 7 shows, if the infected cells are tunicamycin-treated, the pseudoenvelopes remain around all the particles within RER vesicles (M.L. Dyall-Smith, personal communication), and following lysis of the cells, only single-capsid particles are found. That tunicamycin-treated cells fail to produce complete double-shelled particles (but not the mechanism of the effect) has been noted independently by N. Ikegami, M. Petric, and R.T. Espejo (personal communications).

As the micrographs (Fig. 6 and 7) show, the RER vesicles in which rotaviral particles are found also contain considerable amounts of amorphous electron-dense material that could be viral outer-capsid components, and the eventual fate of the psuedoenvelope membranes may be to form the masses of convoluted smooth membrane (Fig. 6) that are another previously unexplained feature of rotavirus morphogenesis. They adjoin or even protrude into rotavirus-containing vesicles (Saif *et al.*, 1978) and could be produced by a membrane-fusion or -destabilization process.

In the normal (i.e., not tunicamycin-treated) rotavirus-infected cell, it is thus postulated that inner capsids bud into RER vesicles, where their

pseudoenvelopes are removed, so that the outer-capsid components, which have been secreted separately through the RER membrane, can assemble onto them. Thus complete double-shelled particles are formed free in the lumen of the RER where the calcium ion concentration is sufficient to allow the assembly to occur.

V. GENETICS

A. Isolation of Temperature-Sensitive Mutants

Temperature-sensitive (*ts*) mutants of the UK strain of bovine rotavirus have been isolated by Greenberg *et al.* (1981) following growth of the virus in AGMK cells in the presence of 200 or 400 μg per ml of 5-azacytidine. Clones were selected that grew and produced cytopathic effect at 34°C, but not at 39°C. Mutants were assigned to one of four recombination groups (A–D) on the basis of experiments in which pairs of mutants were grown together in AGMK or CV-1 cells for 36 hr at 34°C; yields were then assayed at 39°C and compared with the yields from similar cultures infected singly with each mutant. Recombination indices ranged from about 20 to greater than 1000. In all, seven mutants were obtained, with efficiencies of plating (EOPs) (39°C/34°C) between 0.002 and less than 0.00006. No biochemical characterization of the *ts* lesions in these mutants has yet been reported, but they have been used most successfully in the production of reassortment viruses, which are discussed in Section V.B.

Faulkner-Valle *et al.* (1982) have also isolated a number of *ts* mutants of UK bovine rotavirus which could be classified into five recombination groups. Representatives of two of the groups were unable to synthesize dsRNA or polypeptides at 39.5°C, but the others appeared to synthesize both RNA and polypeptides at the restrictive temperature. Ramig (1982) has reported the isolation of ten stable *ts* mutants of simian rotavirus SA 11 following mutagenesis with nitrous acid or hydroxylamine. They also fell into five recombination groups but the nature of the *ts* lesions has not yet been investigated.

M.E. Begin and S.M. Rodger (personal communication) have also isolated *ts* mutants of the Northern Ireland strain of bovine rotavirus and of simian rotavirus SA 11 following treatment of inocula with nitrous acid (to 10% survival) or growth in the presence of 200 μg/ml 5-azacytidine, in which case yields were about 1% of the normal. Both mutagens gave similar results; frequencies of isolation of *ts* mutants were low, only about 0.1% of clones tested had EOPs (39°C/34°C) of less than 10^{-3}, and were thus candidates for biochemical characterization. Four recombination groups have been identified, and for this purpose interstrain recombination appeared to be as efficient as intrastrain recombination (C.P.

Hum, personal communication). Studies of *ts* functions are currently in progress.

B. Genome Reassortment

By coinfection of MA-104 cells with bovine rotavirus (Lincoln strain) and UV-irradiated SA 11 virus, followed 24 hr later by plaque selection under an overlay containing anti-bovine rotavirus serum, Matsuno *et al.* (1980) obtained the first rotavirus reassortant clone. Electrophoretic comparisons of its double-stranded RNA (dsRNA) genome segments with those of the parental viruses showed that the reassortant had derived segments 4, 5, and 10 from SA 11 and segments 1, 2, 3, 6, and 11 from the bovine rotavirus, but it was not possible to determine the origin of segments 7, 8, and 9. By neutralization and hemagglutination-inhibition tests, the reassortant resembled SA 11 virus.

Greenberg *et al.* (1981, 1982) were able to obtain cultivable (i.e., cell-culture-adapted) reassortants that behaved in neutralization tests like human rotavirus strains, by mixed infection of AGMK cells with *ts* mutants of cultivable bovine rotavirus and noncultivable human rotaviruses. Following growth at 34°C, reassortants were selected by plaquing at 39°C under bovine rotavirus antiserum. The human strains thus "rescued" were Wa and DS-1, which represent different serotypes, so the reassortants have been most useful in studies of human rotavirus serotyping. These and additional reassortants produced by similar methods were employed in determining the genes of human and bovine rotaviruses that code for neutralization and subgroup antigens by Kalica *et al.* (1981b). This study was mentioned in Section II.F and is further discussed in Section V.C.

C. Gene Coding Assignments

Following the method of *in vitro* translocation of separated, denatured dsRNA segments that had been applied to reovirus by McCrae and Joklik (1978), M.L. Smith *et al.* (1980) identified the polypeptides encoded by dsRNA segments 1–6 of SA 11 rotavirus, and later the coding assignments of segments 10 and 11 were added (Dyall-Smith and Holmes, 1981a). Assignments for the remaining three segments have recently been completed (P. Kantharidis and M.L. Dyall-Smith, personal communication; G.W. Both and A.R. Bellamy, personal communication). The correlation between SA 11 genome segments and proteins is illustrated in Figure 8.

Arias *et al.* (1982) tackled the same problem using similar techniques, except that *in vitro* protein synthesis was carried out with a rabbit re-

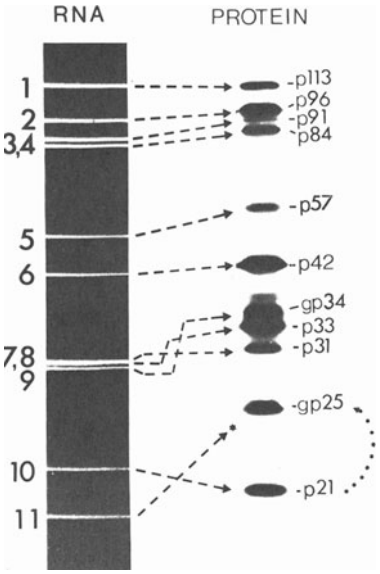

FIGURE 8. Genome coding assignments of simian rotavirus SA 11, showing the relationships between dsRNA segments as separated by electrophoresis in polyacrylamide gel and polypeptides detected in SA 11–infected cell cultures by [^{35}S]methionine labeling at 7.5 hr postinfection, gel electrophoresis, and fluorography. Kindly provided by M.L. Dyall-Smith.

ticulocyte lysate instead of a wheat germ preparation. There was complete agreement regarding the assignments for segments 1, 2, 5, 6, and 10, and although partial separation of segments 7–9 was achieved, it remained impossible to assign their three protein products individually. Arias *et al.* (1982) considered that the outer capsid protein that is cleaved by trypsin (Espejo *et al.*, 1981) (see Section III.B) was the product of genome segment 3, whereas it clearly appeared to be produced by segment 4 in the earlier study (M.L. Smith *et al.*, 1980). This disagreement may arise from a strain difference that has arisen during passaging of the SA 11 rotavirus stocks. Although they originally came from the same source, minor differences in the electrophoretic mobilities of certain dsRNA segments and of gp34 do seem to have evolved.

Although Arias *et al.* (1982) confirmed the finding of Dyall-Smith and Holmes (1981a) that segment 10 of SA 11 codes for what they respectively called pNCVP5 and NS_3, they further proved that it is the precursor of the glycoprotein gp25 (NCVP5 or O_4). Thus, the conclusion of Dyall-Smith and Holmes (1981a) that the segment 11 product was converted to gp25 must be incorrect. Instead, it must be a separate protein (molecular weight 24,000) that was seen as an *in vitro* translation product but was not detected in infected cells by Dyall-Smith and Holmes (1981a). For unknown reasons, Arias *et al.* (1982) were unable to translate segment 11 *in vitro*, so for SA 11 virus some uncertainties remain, but the situation is clearer for bovine rotavirus.

By translation of denatured dsRNA segments of the UK strain of bovine rotavirus, McCrae and McCorquodale (1982) showed that segments 1–6 coded for the proteins VP1–6 (see Table I for equivalents), as

in SA 11 virus. In bovine as in the simian rotavirus, segment 10 codes for the precursor (VP12) of the glycoprotein VP10, and in this case the product of segment 11, VP11, was detected in infected cells. In the bovine rotavirus also, segments 7, 8, and 9 were very difficult to resolve, but McCrae and McCorquodale (1982) consider that they encode VP8, the precursor of VP7, and VP9, respectively.

A peculiarity of the RNA electrophoretic patterns of human rotaviruses is that the smallest segments 10 and 11 of some strains appear to move more slowly than those of others that resemble simian, bovine, and other mammalian rotaviruses (Espejo *et al.*, 1979; Kalica *et al.*, 1981a). Segment 11 of strains with "short" electrophoretic patterns lines up approximately with segment 10 of strains with "long" patterns, and in fact both code for the same kind of protein, corresponding to p21 of SA 11 virus (Dyall-Smith and Holmes, 1981a). Similarly, segment 10 of a strain with a "short" pattern is analogous to segment 11 of a "long" pattern, and it is the considerable variation in mobility of this segment that gives rise to the different patterns.

As mentioned in Section II.F, studies of the neutralization type specificities and of the parental origins of genome segments of a series of reassortants between human (Wa) and bovine (UK) rotaviruses enabled Kalica *et al.* (1981b) to identify human rotavirus RNA segment 9 as the carrier of neutralization specificity for this human strain. Since in SA 11 rotavirus neutralizing monoclonal antibodies react with the outer-capsid glycoprotein gp34 (A. Breschkin and S. Sonza, personal communication), it is reasonable to assume that in the human (Wa) rotavirus, RNA segment 9 thus codes for the corresponding glycoprotein, gp35 (Dyall-Smith and Holmes, 1981a). In the case of the human rotavirus strain DS-1, it appears to be segment 8 that encodes the neutralization antigen (H. Greenberg *et al.*, personal communication), so it is apparent that the relative positions of RNA segments 7–9 can vary among rotavirus strains. Corresponding dsRNA segments in different rotavirus strains can now be identified by Northern blot hybridization carried out under conditions of low stringency, using DNA copies of segments 7–9 of UK bovine rotavirus cloned in the plasmid PBR 322 (M.L. Dyall-Smith, A.A. Azad and I.H. Holmes, personal communication).

VI. BIOLOGY OF ROTAVIRUSES

A. Diseases and Pathogenesis

Rotaviruses typically cause acute diarrheal disease in infants or the young of many kinds of animals (For review see Flewett, 1977; Flewett and Woode, 1978; McNulty, 1978). The first interspecies transmission of a rotavirus was probably achieved by Light and Hodes (1949) in the course

of an investigation of severe infantile gastroenteritis in Baltimore in 1941–1942.

"Scours" (e.g., white scours, milk scours, etc.) is an acute infectious diarrheal disease that very commonly affects calves and piglets and sometimes foals and intensively-reared lambs. Rotaviruses appear to be the most common causative agents, although similar symptoms are also produced by coronaviruses and certain bacteria, and the most severe disease may be caused by multiple infections (Tzipori *et al.*, 1981). In calves and pigs particularly, rotaviral enteritis causes severe economic losses both through deaths and through retardation of weight gain in animals that recover.

1. Epizootic Diarrhea of Infant Mice

Epizootic diarrhea of infant mice (EDIM) or murine rotavirus infection was described by Cheever and Mueller (1947) and Kraft (1958). The disease can be a major problem in mouse-breeding units, since it is highly contagious and hard to eradicate. The incubation period is about 2 days. The symptoms are yellow, watery diarrhea and dehydration in sucklings 3–16 days old. Susceptibility to symptomatic disease depends markedly on age (Kraft, 1957; Wolf *et al.*, 1981), and adult mice get asymptomatic infections. Since all individuals in the litter are affected, the task of cleaning up gets beyond the mother mouse, and the sucklings frequently end up stuck together by their dried excreta in rather bizarre rosettes. Losses can be high, although in endemically infected colonies, most infant mice recover within 4–5 days.

2. Calf Scours

Bovine rotaviral enteritis or calf scours has been described by Mebus *et al.* (1969), Mebus (1976), and Woode and Bridger (1975). The incubation period in experimental infections with large doses of virus can be as short as 13 hr. In order of appearance, the symptoms are depression, anorexia, and diarrhea with white or yellow feces for 6–12 hr. The calves may die as a result or dehydration or secondary bacterial infection, or recover in 3–4 days. Natural rotavirus infections appear to be most common in the 2nd week of life (Acres and Babiuk, 1978).

3. Piglet Enteritis

Rotaviral diarrhea in pigs has been reviewed recently by Bohl (1979). The symptoms resemble those caused by transmissible gastroenteritis of swine (a coronavirus), but are generally less severe, although fatal dehydration can occur in very young piglets. They include anorexia, diarrhea, and vomiting, after a 1 to 2-day incubation period (Lecce *et al.*, 1976; McNulty *et al.*, 1976d; Woode *et al.*, 1976a). Illness occurs in piglets

1–8 weeks of age, often soon after weaning, especially if it is early or abrupt (Lecce and King, 1978). Weight gains are severely depressed.

4. Disease in Other Domestic Animals and Birds

Equine rotaviruses are associated with diarrhea in foals that was previously attributed to hormonal changes in the mares that suckled them (Flewett *et al.*, 1975). A number of fatal infections of very valuable animals have been reported (Tzipori and Walker, 1978; Conner and Darlington, 1980). In lambs, symptomatic infections seem to occur only under conditions of intensive rearing under cover (McNulty *et al.*, 1976b; Snodgrass *et al.*, 1976), although antibodies in sheep are widespread in countries such as Australia where the disease has not been noticed. In chickens and turkeys, at least three serological types of rotaviruses have been associated with symptoms of diarrhea, poor or abnormal appetite, dehydration, and increased mortality (Jones *et al.*, 1979; McNulty *et al.*, 1979, 1980). The chicken pararotavirus (McNulty *et al.*, 1981) does not appear to cause severe disease.

5. Human Infantile Enteritis

a. Clinical Features

The clinical features of human rotaviral enteritis have been reported in considerable detail (Davidson *et al.*, 1975a; Flewett, 1977; Shepherd *et al.*, 1975; Hamilton *et al.*, 1976; Rodriguez *et al.*, 1977; Delage *et al.*, 1978). After an incubation period of 1–2 days, vomiting and then diarrhea occurs, and there is generally a mild fever (38–39°C). The diarrhea lasts for 4–5 days, and moderate dehydration is common. Severe illness is most common in the 6- to 18- month age group. Deaths due to rapid, severe dehydration occur even in centers where fluid–electrolyte therapy is available (Carlson *et al.*, 1978), and the disease is believed to be a major cause of infant mortality in many parts of the world (Kapikian *et al.*, 1980).

On the other hand, it must be remembered that the clinical descriptions have almost always been based on patients admitted to hospital, and the bulk of rotavirus infections in children are milder. Asymptomatic infections in neonates seem to be common (Cameron *et al.*, 1975; Madeley and Cosgrove, 1975; Murphy *et al.*, 1977; Chrystie *et al.*, 1978). An association of upper respiratory tract symptoms with rotaviral enteritis has been suggested (Carr *et al.*, 1976; Lewis *et al.*, 1979), but others have found an equally high incidence of respiratory symptoms in age-matched children with diarrhea due to other causes (Rodriguez *et al.*, 1977; Mäki, 1981). Like adenoviruses, rotaviruses may be a cause of intussusception in infants and young children (Konno *et al.*, 1978b), though this is still under investigation. Long-term diarrhea with rotavirus excretion for more

than 6 weeks and rotaviral antigens detectable in the serum has been reported in immunodeficient children (Saulsbury *et al.*, 1980). In many places, multiple infections with rotavirus and other enteric pathogens can be quite common (Evans *et al.*, 1977), but it is not yet known whether they are significantly more severe than single infections.

b. Pathogenesis and Pathology

Only limited investigations of the pathogenesis and pathology of rotavirus disease in humans have been possible, but many detailed studies of natural and experimental rotaviral infections in other animals have been reported, and all show very similar changes, so it is possible to generalize (McNulty, 1978; Mebus *et al.*, 1971b; Mebus and Newman, 1977; Pearson and McNulty, 1977; Snodgrass *et al.*, 1977a; Theil *et al.*, 1978). Rotaviruses infect epithelial cells lining the apical halves of villi of the small intestine (see Section III.A), causing vacuolation and premature shedding, so the villi are shortened and become covered with immature, cuboidal epithelial cells from the crypts, which appear to be insusceptible. The infection may progress from the upper to the lower small intestine. By immunofluorescence (IF), infection of epithelial cells in the colon has also been found in mice, humans, and lambs, but not in calves and only rarely in pigs (Banfield *et al.* 1968; Hamilton *et al.*, 1976; Snodgrass *et al.*, 1977a; Theil *et al.*, 1978). Histopathological changes in the lungs of pigs, lambs, and calves have also been noted, but no infected cells have been demonstrated outside the intestine (Mebus, 1976; McNulty, 1978).

Similar pathology was found in experimental infections of pigs with bovine rotavirus or human rotavirus and of calves with human rotavirus (Hall *et al.*, 1976; Davidson *et al.*, 1977; Mebus *et al.*, 1977). The immature epithelial cells that replace those destroyed by the virus have reduced levels of disaccharidases, including lactase, and glucose-coupled sodium transport is also impaired (Davidson *et al.*, 1977; Davidson and Barnes, 1979). Undigested lactose in milk exerts an osmotic effect, inflammation and bacterial degradation of the lactose in the large bowel compound this, and diarrhea is the end result (Flewett, 1977).

The villi return to normal within 3–4 weeks. Two stages in recovery were demonstrated by Crouch and Woode (1978) in a most interesting study in which porcine rotavirus was titrated and histopathology noted at five points along the small intestine of piglets, at various intervals after experimental infection. It was clear that after a peak of infectivity and of epithelial cells showing IF at about 20 hr postinfection, the infectivity titer dropped sharply from about 10^8 median tissue-culture infectious doses ($TCID_{50}$)/ml to about 10^5 $TCID_{50}$/ml, and the percentage of fluorescing cells dropped from 75 to 5% by about 40 hr postinfection. The lower levels were maintained until about 4 days postinfection, when the infectivity titer dropped to zero. It was suggested that the first phase of

recovery was nonimmune in nature, and likely to be due to the loss of susceptible cells and possibly the production of interferon. Final elimination of the virus in the second phase probably followed the production of local antibodies.

B. Geographic and Host Range

It was once interesting to record the countries from which rotaviruses had been reported, but now the lists are so long, for both human and bovine rotaviruses especially, that one can only say the distribution is worldwide. Even the most isolated human populations have antibodies as evidence of past infections, and occasional epidemics occur (S.O. Foster *et al.*, 1980; Linhares *et al.*, 1981).

Similarly, it appears likely that rotaviruses are to be found in all species of mammals surveyed and in chickens and turkeys, the only two avian species that have been studied. Curiously, although rotaviruses in mice were among the first to be found (Cheever and Mueller, 1947), they have not been reported in rats. Discoveries of rotaviruses in calves, pigs, lambs, and foals have been mentioned previously (Mebus *et al.*, 1969; Rodger *et al.*, 1975a; Lecce *et al.*, 1976; McNulty *et al.*, 1976b,d; Woode *et al.*, 1967a; Snodgrass *et al.*, 1976; Flewett *et al.*, 1975).

Rotaviruses appear to be common in rabbits (Bryden *et al.*, 1976; Petric *et al.*, 1978), but not in guinea pigs. They have been found in apes and monkeys (Ashley *et al.*, 1978; Soike *et al.*, 1980; Stuker *et al.*, 1980), cats (Chrystie *et al.*, 1979; Snodgrass *et al.*, 1979), dogs (Eugster and Sidwa, 1979), antelopes, deer, and other ruminants in a zoo (Reed *et al.*, 1976; Tzipori and Caple, 1976), and goats (Scott *et al.*, 1978). Finally, a variety of chicken and turkey rotaviruses have been found (Jones *et al.*, 1979; McNulty *et al.*, 1979).

A number of experimental, cross-species transmissions have been reported. No attempts to infect mice with rotaviruses from other species have been successful, but piglets are susceptible to bovine, equine, simian, and human strains (Woode and Bridger, 1975; Hall *et al.*, 1976; Woode *et al.*, 1976b; Tzipori and Williams, 1978; Bridger *et al.*, 1975; Middleton *et al.*, 1975; Torres-Medina *et al.*, 1976). Human rotaviruses have also been transmitted to calves (Light and Hodes, 1949; Mebus *et al.*, 1976), lambs (Snodgrass *et al.*, 1977b), monkeys (Wyatt *et al.*, 1976b), and dogs (Tzipori, 1976).

The latter transmission may be particularly significant from the human point of view, since the puppies appeared to be highly susceptible to the human virus, opportunities for virus exchange between dogs and children are obvious in almost all parts of the world, and a recent random sampling revealed a surprisingly high prevalence of rotavirus excretion by dogs (Roseto *et al.*, 1980). Naturally enough, familiarity with the epidemiology of influenza leads one to an interest in the possibility of zoon-

otic rotavirus infections of humans. Although no quantitative comparisons have yet been made of minimal infective doses or relative susceptibilities, the current general impression is that rotaviruses are moderately, though certainly not absolutely, host-specific in nature.

C. Epidemiology

1. Mode of Transmission

Since rotaviruses replicate in the cells lining the intestinal tract, feces are the source of subsequent infections, and they contain very large numbers of rotavirus particles. In humans, estimates of peak excretion soon after the onset of diarrhea range from about 10^9 to more than 10^{10} particles/g feces (Davidson *et al.*, 1975a; Chrystie *et al.*, 1978), and a sample of piglet feces contained at least 3×10^7 infectious doses/ml (Woode *et al.*, 1976a). In addition, the studies described in Section II.B showed that rotaviruses are rather stable, and their infectivity would be preserved in the feces of young animals because of the high calcium content (Shirley *et al.*, 1981). As a result, there is more than one possible mode of transmission, and it is difficult to determine which of them, if any, is most important overall.

Airborne spread of dust or droplets carrying virus was postulated by Kraft (1957) to explain the efficient spread of murine rotavirus from cage to cage within rooms despite all precautions to avoid cross-contamination. The addition of filter tops to the cages prevented the accidental transmission and confirmed her hypothesis (Kraft, 1958). Mebus *et al.* (1969) experimentally infected a calf with bovine rotavirus via an aerosol spray, but it was clear that at least part of the inoculum would be swallowed, and they later showed that there was no evidence of infection of the upper respiratory tract (see Section VI.A.5.b). Airborne spread of human rotaviruses in hospitals, perhaps associated with diaper-changing, has been suggested by Chrystie *et al.* (1975) and Campbell and Lang (1979). The high attack rate and speed of spread of a human rotavirus epidemic in the Truk Islands also led S.O. Foster *et al.* (1980) to consider airborne transmission, although they favored the idea of multiplication in the respiratory tract.

Circumstantial evidence of transmission of human rotavirus infections via water has been obtained by Lycke *et al.* (1978), Vollet *et al.* (1979), Linhares *et al.* (1981), Zamotin *et al.* (1981), and Sutmoller *et al.* (1982). In the case of an epidemic in a small Swedish town that affected over 3000 people of all ages, it appeared that the freshwater supply was contaminated with sewage effluent (Lycke *et al.*, 1978). It is known that infectious rotaviruses can survive for considerable periods in water (Hurst and Gerba, 1980), and they are highly resistant to chlorination (Snodgrass and Herring, 1977; Tan and Schnagl, 1981). They also seem to be adsorbed

less by soil than enteroviruses, and thus can circulate via groundwater and be found in wells (Keswick and Gerba, 1980; Murphy *et al.*, 1983).

Transfer of rotaviruses by direct contact with infected individuals or a contaminated environment is the idea favored by most workers dealing with domestic animals, and it also has its supporters among those investigating nosocomial infections in hospitals, where rotaviral enteritis is a considerable and costly problem (Middleton *et al.*, 1977b; Ryder *et al.*, 1977). In a study of diarrhea and rotavirus infection associated with differing regimens for postnatal care of newborn babies, Bishop *et al.* (1979) found that the incidence of diarrhea was far higher in babies in communal nurseries than in those "rooming in" with their mothers, and stated that the most important factors influencing incidence of diarrhea were proximity to other newborn babies and frequency of handling by unrelated adults. In the same vein, Lecce *et al.* (1978) described an artificial rearing regimen for piglets involving early weaning in which a buildup of rotavirus occurred repeatably over several weeks, resulting in earlier and more severe infections in the later arrivals.

The efficacy of increased hand-washing in reducing incidence of diarrhea in circumstances where rotavirus infections are common lends support to the direct-contact hypothesis (Ryder *et al.*, 1977; Koopman, 1978; Halvorsrud and Orstavik, 1980; Black *et al.*, 1981a).

2. Incidence of Infections

In general, symptomatic rotaviral infections occur mainly in neonatal or very young animals, but repeated infections occur at intervals throughout life, often without symptoms (McNulty, 1978; Holmes, 1979). In contrast to the situation in other animals, and for unknown reasons, human neonatal infections are frequently mild or asymptomatic (Cameron *et al.*, 1978; Chrystie *et al.*, 1978; Jesudoss *et al.*, 1979), whereas the most serious infections occur in the 6-month to 2-year age group (Davidson *et al.*, 1975a; Bryden *et al.*, 1975; Kapikian *et al.*, 1976b). The actual incidence of rotavirus gastroenteritis requiring hospitalization in a defined population in the United States was found to be 2–3/1000 children aged 0–2 years per year (Rodriguez *et al.*, 1980). In a prospective study in which a cohort of 104 infants and their families were followed for an average of 16 months, Gurwith *et al.* (1981) found that rotavirus was by far the most common enteropathogen. It was detected 82 times in 72 children. Altogether, 237 episodes of diarrhea were investigated in the 104 infants and 62 siblings.

Symptomatic infections are less common in older children, but epidemics occur in schoolchildren (Hara *et al.*, 1978). Adult diarrheal infections tend to be vastly underreported, but both symptomatic and asymptomatic rotavirus infections with quite high attack rates have been documented in parents, hospital personnel, and other adults in contact with pediatric patients (Kim *et al.*, 1977; Kapikian *et al.*, 1976b; von

Bonsdorff *et al.*, 1976; Rodriguez *et al.*, 1979; Wenman *et al.*, 1979), and epidemics have occurred even in the elderly (Cubitt and Holzel, 1980; Harvorsrud and Orstavik, 1980). Numerous diarrheal adult infections were a feature of epidemics in isolated populations (S.O. Foster *et al.*, 1980; Linhares *et al.*, 1981), and rotaviruses are a significant cause of diarrhea in adult travelers (Bolivar *et al.*, 1978; Vollet *et al.*, 1979; Ryder *et al.*, 1981; Sheridan *et al.*, 1981).

Seasonal and age factors are compounded in domestic animals with controlled breeding seasons, but in temperate climates there is a winter peak of rotavirus infections in both mice and humans (Cheever, 1956; Middleton *et al.*, 1974; Davidson *et al.*, 1975a; Kapikian *et al.*, 1976b; Konno *et al.*, 1978a). The reason for this is unknown, and in the tropics there is no consistent pattern; infections frequently occur throughout the year. Reported variations in incidence in dry and wet seasons are not consistent from country to country and may not be consistent in a single country from year to year (Hieber *et al.*, 1978; Maiya *et al.*, 1977; Soenarto *et al.*, 1981; V.I. Mathan, personal communication).

The level of immunity, especially passive immunity in young animals, will obviously affect the incidence of infection. This has been noticed in mice, where successive litters of the same mother show milder symptoms (Cheever, 1956), and in humans, where there is a reduced incidence of infections in breast-fed as opposed to bottle-fed babies, but discussion of this will be left for Section VI.E. Weanling diarrhea was recognized as a problem in humans long before rotaviruses were known (Gordon *et al.*, 1963), and in both humans and pigs, the shock of weaning and increased possibility of contamination of food may be as important as the lack of mother's milk in the observed increase of disease (Bishop *et al.*, 1979; Lecce and King, 1978).

3. Strain Variation

A further complication of rotavirus epidemiology, which is gradually being taken into account as techniques improve, is the existence of different serotypes of rotaviruses. Strains and serotypes of rotaviruses are discussed in the next section, but it is necessary to mention here publications on the epidemiology of human rotavirus subgroups and the use being made of double-stranded RNA (dsRNA) electrophoretic patterns in epidemiological studies. Since the distinction between rotavirus subgroups and serotypes was not drawn until recently (Kapikian *et al.*, 1981) and the methods available for serotype identification were formerly too difficult, the techniques applied in these studies [mainly enzyme-linked immunosorbent assay (ELISA)] were those that recognize subgroups. This must be kept in mind, since at the time the authors considered they were dealing with serotypes, and probably they frequently were, since there is at least a partial correlation between serotype and subgroup among human rotaviruses (see Section VI.D).

Almost as soon as it had been shown in longitudinal studies that individual children frequently experienced more than one rotavirus episode within their first couple of years (Wyatt *et al.*, 1979b), it was found that sequential infections were often caused by viruses of different subgroups (Fonteyne *et al.*, 1978; Rodriguez *et al.*, 1978). Surveys conducted over 4 years in Washington, D.C., showed that although antibodies to viruses of subgroups 1 and 2 were acquired at equal rates, subgroup 2 rotaviruses were associated with 75% of the illnesses that required hospital treatment (Yolken *et al.*, 1978b; Brandt *et al.*, 1979). Thus, it was suggested that the subgroup 2 viruses were more virulent than those of subgroup 1, but the difference may not be so great as it appeared, since strains belonging to a third subgroup may have been included with subgroup 2 (Zissis *et al.*, 1981).

As mentioned in Section II.C, gel electrophoresis of genome RNA was rapidly recognized as a very useful method for distinguishing different rotavirus strains for epidemiological purposes. It is more discriminating than serology, and will remain invaluable for studies of the evolution and spread of new epidemic strains even now that serotyping is becoming easier, as has been found in studies of influenza (Hinshaw *et al.*, 1978).

The method was first applied with enthusiasm by Espejo *et al.* (1977, 1979, 1980a,c), and a number of similar studies have now been published (Kalica *et al.*, 1978b; Croxson and Bellamy, 1979; Rodger *et al.*, 1981; Lourenco *et al.*, 1981; Schnagl *et al.*, 1981). Figures 9 and 10 show the

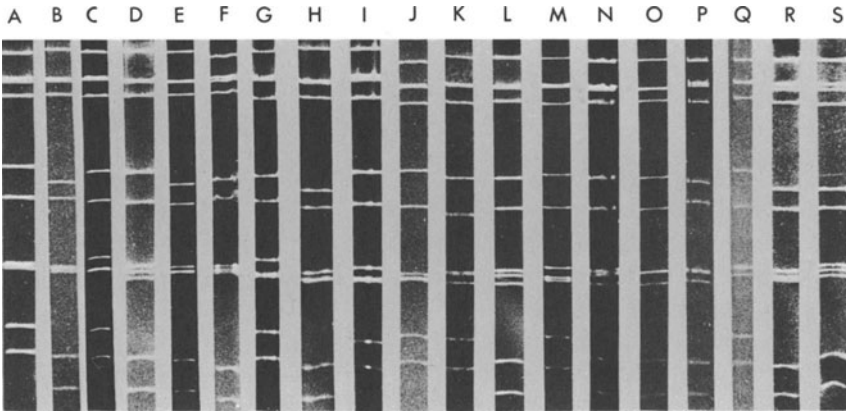

FIGURE 9. Human rotavirus "electropherotypes" as defined by differing dsRNA segment patterns produced by electrophoresis of rotaviral RNA extracted from a total of 335 fecal samples from children and neonates from various Melbourne hospitals over a 6-year period. Small differences cannot be judged by comparison with a composite picture such as this containing results from a number of gels, which is intended only to convey a general idea of the variations; samples producing similar patterns must be coelectrophoresed to establish identity or distinctness. From Rodger *et al.* (1981), by permission of the American Society for Microbiology.

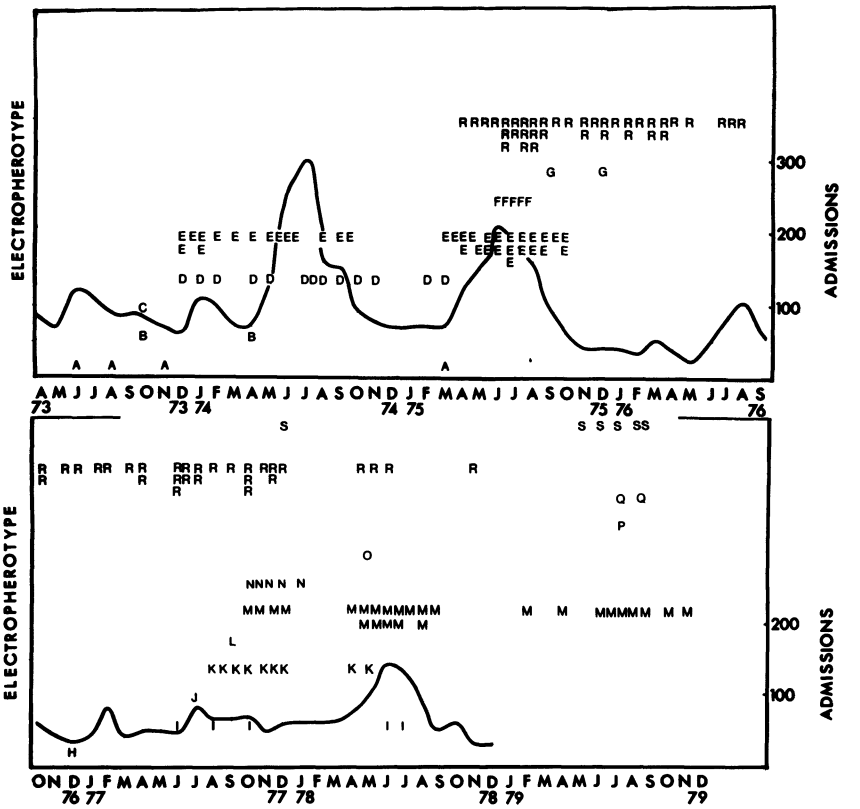

FIGURE 10. Occurrence of human rotavirus electropherotypes obtained from children in Melbourne hospitals between May 1973 and November 1979. (——) Total number of children admitted to these hopsitals with acute gastroenteritis (all causes). From Rodger *et al.* (1981), by permission of the American Society for Microbiology.

range of "electropherotypes" found in Melbourne, Australia, and their temporal distribution over 6 years (Rodger *et al.*, 1981). In the same study, it was noted, and subsequently confirmed in more detail by Kalica *et al.* (1981a), that rotaviruses with genomes that gave short electrophoretic patterns (such as those labeled A, C, G, I, J, K, M, and Q in Fig. 9) are found to belong to subgroup 1, while the long patterns correlate with subgroup 2 [or the putative subgroup 3 (G. Zissis and R. Bishop, personal communication)]. The correlation may not always hold because it is indirect and may be coincidental, since, as discussed in Sections II.F and V.C, the subgroup antigens depend on segment 6 rather than segment 11. Since so many fine variations of mobility occur among segments 7–9, it is not possible to read off serotype from the RNA electrophoretic pattern.

It is clear from Fig. 10 that a number of different rotaviruses can be present simultaneously in a large population [or even in a much smaller one, as was found by Schnagl *et al.* (1981)], that some types are much

more common in hospitalized patients than others, which were rarely seen, and that predominant types can persist through two or three annual epidemic periods.

D. Strains and Serotypes

1. Strains

The origins and nomenclature of the first cultivable rotavirus strains are listed in Table II. Now that cultivation is no longer a novelty, the number of true isolates is rapidly increasing, and a standardized system of nomenclature is urgently needed. For this reason, I have included cryptograms similar to those used to identify influenza isolates, as suggested by Rodger and Holmes (1979). It is not yet possible to incorporate neutralization serotype or subgroup designations, but these can readily be added in future as they are determined.

2. Serotypes

Shortly after it became possible to carry out infectivity assays on bovine rotaviruses, it was noted that some sera from humans and pigs,

TABLE II. Prototype Cultivable Rotaviruses

Origin	Strain designation	Cryptogram	Ref. No.
Simian (vervet)	SA 11	Si/S.Africa/H96/58	1
Bovine (or ovine)	"O" agent	Bo/S.Africa/9/65	1
Bovine	NCDV Lincoln	Bo/USA/23/69	2
	NCDV Cody	Bo/USA/2/67	2
	U.K.	Bo/England/27/73	3
	N.I.	Bo/N.Ireland/447/75	4
Porcine	OSU	Po/USA/ /75	5
Human	DS-1	Hu/USA/G621/76	6
	Wa	Hu/USA/G187/74	7
	M	Hu/USA/G529/76	8
Simian (rhesus)	RRV-2	Si/USA/2/79	9
Canine	K-9	Cn/USA/36/79	10
Feline	Taka	Fe/USA/11/80	11
Turkey	Ty1	Ty/N.Ireland/415/79	12
Chicken	Ch1	Ch/N.Ireland/363/78	12
Turkey	Ty3	Ty/N.Ireland/78/79	13
	132	Ty/N.Ireland/132/80	14

[a] References: (1) Malherbe and Strickland-Cholmley (1967); (2) Mebus *et al.* (1971a); (3) Bridger and Woode (1975); (4) McNulty *et al.* (1976a); (5) Theil *et al.* (1977); (6) Rodriguez *et al.* (1978); (7) Wyatt *et al.* (1980); (8) Kalica *et al.* (1978b); (9) Stuker *et al.* (1980); (10) Fulton *et al.* (1981); (11) Hoshino *et al.* (1981); (12) McNulty *et al.* (1979); (13) McNulty *et al.* (1980); (14) McNulty *et al.* (1981).

as well as bovine sera, contained neutralizing antibodies against them (Woode *et al.*, 1976b; Matsuno *et al.*, 1977a). The first series of cross-neutralization tests involving human, bovine, porcine, equine, ovine, murine, and lapine rotaviruses and corresponding convalescent antisera were performed by Thouless *et al.* (1977b). They were able to distinguish rotaviruses of different species origin, although the differences between neutralization titers of homologous and heterologous sera were often only 4- to 8-fold. Murine rotavirus was neutralized to a significant degree by almost all the sera, and it was conjectured that since only single-capsid particles were seen in highly infectious preparations, perhaps in the case of murine rotavirus such particles are infectious and can be neutralized by antibodies to the common inner-capsid antigens.

It has since been realized that in contrast to observations made on other viral genera, convalescent sera show a broader cross-reactivity by neutralization than hyperimmune sera prepared by repeated injections of particular rotaviruses into guinea pigs or rabbits, provided the animals do not have preexisting antibodies to other rotaviruses (Wyatt *et al.*, 1980; T.H. Flewett, personal communication; G.N. Woode, personal communication). Preexisting antibodies were noted in the rabbits immunized by Malherbe and Strickland-Cholmley (1967) against simian rotavirus SA 11 and the "O" agent. These sera showed a high degree of cross-neutralization, whereas similar sera prepared in rotavirus antibody–free rabbits by Lecatsas (1972) showed no cross-reaction at all. Similarly, Linhares *et al.* (1981) noted a boost of anti-type 3 antibodies in Tiriyo Indians following an outbreak of Birmingham type 1 human rotavirus (see below). As yet, these observations have not been followed up, but they need to be borne in mind by anybody undertaking the production of sera for specific identification of rotaviruses.

The original cultivable bovine rotaviruses are closely related by neutralization tests, and no information is available on porcine isolates, but two serotypes of turkey rotavirus have been identified (McNulty *et al.*, 1980).

Human rotavirus serotyping turned out to be more complex than was realized at first, and it was especially difficult since it has only recently become possible to grow stocks of prototype strains. By neutralization of fluorescent foci produced by nonadapted human rotavirus samples, Thouless *et al.* (1978) in Birmingham, England, were able to distinguish two or three serotypes, and they later confirmed the third and suggested that a fourth or even fifth may exist (Flewett *et al.*, 1978; Beards *et al.*, 1980).

Another approach was originated by Zissis and Lambert (1978), who found that hyperimmune guinea pig antisera prepared against some human rotaviruses reacted poorly against others by complement fixation (CF). Using sera prepared by Zissis against his "types" 1 and 2, Yolken *et al.* (1978b) were able to make the same discrimination by ELISA, and Zissis and Lambert (1980) devised their own ELISA system that provided

a slightly clearer distinction. Recently, Zissis *et al.* (1981) produced a third antiserum against a human rotavirus isolate that could not be typed by their previous sera, and have thus identified a third group of strains, some of which were previously considered to be "type" 2.

As mentioned earlier (Sections II.F and VI.C.3), experiments on a range of human and animal rotaviruses and on cultivable reassortants carrying human rotavirus serological specifities recently showed that the antigens detected by neutralization were distinct from those identified by ELISA or immune adherence hemagglutination assay (IAHA) (Kapikian *et al.*, 1981). Accordingly the "type" specificities distinguished by the current ELISA or IAHA tests will henceforth be defined as subgroups 1, 2, and 3, while the antigens distinguishable by neutralization will define the serotypes. It is too early to attempt a tabulation of strains according to this new nomenclature, since insufficient information has been published, but it appears that human rotavirus strains with the "Wa" or Birmingham No.1 serotype usually also have the subgroup 2 antigen, whereas the "DS-1" serotype (Birmingham 2 or 4) is associated with subgroup 1 antigen (Wyatt *et al.*, 1980; Greenberg *et al.*, 1981; Kapikian *et al.*, 1981; T.H. Flewett, personal communication). A third human serotype, "M" or Birmingham No. 3, is particularly interesting in that it seems to be closely related to simian rotaviruses SA 11 and RRV-2 and to some other rotaviruses of animal origin (Wyatt *et al.*, 1982; A.Z. Kapikian, T.H. Flewett, and G.N. Woode, personal communications). Whether it is associated in human strains with the subgroup 3 antigen of Zissis *et al.* (1981) is not yet known. At least one recent Japanese isolate of human rotavirus appears to differ from strain Wa by neutralization (Sato *et al.*, 1981).

Although research on the "pararotaviruses" is still in its early stages, it appears so far that they differ in both serotype and subgroup specificities from other rotaviruses, and no cross-protection was observed between the C or S strains of porcine pararotavirus and the classic porcine rotavirus OSU (Bohl *et al.*, 1982).

E. Immunity

It has been shown that in young animals, colostrally derived serum antibodies do not prevent rotavirus infections (Woode *et al.*, 1975; Snodgrass and Wells, 1976; McNulty, 1978). Similarly, in human neonates, transplacentally acquired (maternal) serum antibodies are not protective *per se*, and older children and adults can be susceptible to rotavirus infection despite the presence of serum antibodies that indicate past infections (Kapikian *et al.*, 1974; McLean and Holmes, 1981).

On the other hand, there is abundant evidence of protection against rotavirus diarrhea mediated by neutralizing antibodies in the lumen of the intestine. Maternal colostral antibodies have been shown to be pro-

tective in calves, lambs, piglets, and humans (Woode *et al.*, 1975; Snodgrass and Wells, 1976; Bohl, 1979; McLean and Holmes, 1981), and even heterologous colostral or serum antibodies added to the feed are effective, provided that they are capable of neutralizing the infecting rotavirus (Lecce *et al.*, 1976; Snodgrass and Wells, 1978; Bartz *et al.*, 1980).

In studies of immunity to rotaviruses, it is important to distinguish the immunoglobulin classes to which the antibodies belong, and this has been greatly facilitated by the development of ELISAs or radioimmunoassays for measuring anti-rotaviral immunoglobulin A (IgA), IgG, or IgM of humans, pigs, calves, or other animals (Yolken *et al.*, 1978a; Cukor *et al.*, 1979; Grauballe and Vestergaard, 1980; McLean *et al.*, 1980; Sheridan *et al.*, 1981; Corthier and Franz, 1981; Acres and Babiuk, 1978). In early bovine colostrum, antirotaviral antibodies are found in both IgA and IgG_1 fractions, but by 14–28 days after calving, they are predominantly of IgG_1type (Snodgrass *et al.*, 1980). In pigs, the total amount of antirotaviral IgG in colostrum is greater than that of IgA or IgM, but specific activity is highest in IgA, and in milk the antibody is mainly IgA (Corthier and Franz, 1981; Hess and Bachmann, 1981). In humans, only IgG antibody is transferred across the placenta, but almost all the rotavirus-specific immunoglobulin in colostrum and milk is secretory IgA (ScIgA) (Yolken *et al.*, 1978a; Cukor *et al.*, 1979; McLean and Holmes, 1980).

Both in animals and in man, the antibody titers in colostrum are maximal soon after parturition, but decrease sharply over the next few days (Woode *et al.*, 1975; Thouless *et al.*, 1977a; McLean and Holmes, 1980). Thus, rotavirus infections in suckling animals or humans tend to be postponed rather than completely prevented, although very often such infections under partial antibody protection are mild or asymptomatic; it is clear that the protective effect of colostrum depends on both its antibody titer and the volume ingested (Snodgrass and Wells, 1978; McNulty, 1978; Acres and Babiuk, 1978; McLean and Holmes, 1981). In humans, higher than average levels of colostral and milk antibodies against rotavirus almost certainly result from recent maternal infections, and boosts in lacteal antibody titers have been observed during lactation when mothers have experienced diarrheal episodes (Yolken *et al.*, 1978a; Cukor *et al.*, 1979; McLean and Holmes, 1981).

Although it has not yet been possible to carry out similar measurements in the rotavirus-infected human intestine, it is believed that earlier work on the effects of secretory antibodies on poliovirus infections of the nasopharynx are highly relevant. Ogra and Karzon (1969) found that preexisting poliovirus antibodies in nasal secretions had a quantitative rather than an all-or-none effect, so that in the presence of higher levels of secretory antibody, both the titers excreted and the duration of excretion of vaccine poliovirus were decreased. Almost certainly, a similar "dampening" of rotavirus production in the gut could result in asymptomatic infection instead of diarrheal illness.

Remarkably little is known about the development of active local immunity following human intestinal infection with rotaviruses. Even though the susceptibility of infants to serious illness extends up to about 2 years of age, whereas most animals are vulnerable only for a much shorter time, the ready availability of experimental animals has permitted much more work to be done on domestic livestock and laboratory animals, although unfortunately none of them is a particularly good model for humans (Shearman *et al.*, 1972; Husband and Watson, 1978).

It has been reported that no immune response was detectable by CF in neonates following rotavirus infections in the first week of life (Crewe and Murphy, 1980), but this may remain an open question until a more sensitive test for IgA is employed. Keller *et al.* (1969) demonstrated an intestinal IgA response to poliovirus in neonates, but they were somewhat older (1–2 weeks) when immunized. In four slightly older children and one adult following a family outbreak of rotavirus enteritis, Sonza and Holmes (1980) detected specific IgA, IgM, and IgG coproantibodies with peak titers between 10 and 30 days after onset of symptoms. The response was transient and coproantibodies were undetectable by about 2 months postinfection, but Grauballe *et al.* (1981a) have since claimed that they could detect IgA coproantibodies 4–7 months after rotavirus infections in a larger series of patients, so further work is required to settle this question also.

Grauballe *et al.* (1981a) suggested that the presence of antirotaviral ScIgA in serum was diagnostic of a recent rotavirus infection, but this would not apply in late pregnancy, when it is normally found in serum (McLean and Holmes, 1980). Nonsecretory IgA is more commonly present in adult sera, but diagnostic increases in antirotaviral IgA were demonstrated in a study of traveler's diarrhea (Sheridan *et al.*, 1981). Serum IgM responses are detectable in young children, but apparently not in adults (Konno *et al.*, 1975; Ørstavik and Haug, 1976; Ørstavik *et al.*, 1976; Sarkkinen *et al*, 1979; Sheridan *et al.*, 1981).

Practically no investigations have been reported on the specificity of antibody responses following sequential infections with different rotavirus serotypes. Similarly, nothing is known about possible cellular immune responses to rotavirus infections, but the observation by Saulsbury *et al.* (1980) that immunodeficient children who became chronic excretors of rotavirus could be successfully treated with milk containing specific antibodies suggests that these are the most important.

F. Prospects for Control

Current experience suggests that no foreseeable improvements in hygiene on farms, in homes, or even in hospitals are likely to control rotavirus infections completely, although this does not mean that the incidence of disease could not be lowered by changes in management

procedures and improvements in water supplies and general living standards. Encouragement of feeding of colostrum is obviously sensible, but in both animals and humans, such passive protection is effective for only a short time (Section VI.E). Consequently, most interest has been shown in the possibility of developing vaccines to stimulate local enteric immuhity (W.H.O. Scientific Working Group, 1980; Kapikian *et al.*, 1980).

A live, attenuated bovine vaccine was developed from the NCDV Lincoln rotavirus strain and has been widely used. Although reports on early trials of its efficacy were enthusiastic (Mebus *et al.*, 1973), trials conducted under different conditions have failed to show protection (Acres and Radostits, 1976; de Leeuw *et al.*, 1980). In the latter case, it was suggested that the vaccine was probably neutralized by colostral antibodies, and of course that problem is well known in relation to human poliovirus immunization.

At least under experimental conditions, the homologous vaccine approach does work, but results obtained with rotaviruses of heterologous origin are more variable. Woode *et al.* (1978) found that the NCDV vaccine protected three gnotobiotic calves against challenge with the more virulent UK bovine rotavirus, but cross-protection by foal or human rotaviruses was effective in only one of three calves tested with each. Wyatt *et al.* (1979a) showed that preimmunization (*in utero*) with bovine rotavirus protected gnotobiotic calves from symptomatic infection with human rotavirus, but Lecce and King (1979) found that bovine vaccine did not protect piglets against challenge with a field strain of porcine rotavirus. The idea of a heterologous animal rotavirus vaccine for human use has lost most of its appeal now that cell-culture growth of a range of human strains is possible (Section III.B).

Because of the current lack of a suitable experimental animal model for human rotavirus infections, the development and testing of attenuated strains is a major problem, even though recent advances in understanding of rotaviral genetics and antigenic structure resulting from basic virological research are most encouraging. Nevertheless, we are only at the beginning of the molecular genetic phase of rotavirus research, and there is a long way to go if the sophisticated strategies being explored for the production of a live influenza virus vaccine are to be followed (Chanock, 1981). We do not even know how many rotavirus serotypes are involved in human or animal infections.

An alternative possibility might be immunization of young animals or infants with wild-type rotaviruses under partial (lacteal) antibody protection. Experimentally, this approach has succeeded in piglets (Bridger and Brown, 1981), and it probably approximates the natural mode of acquisition of primary immunity in man and many other species, but its practical application would require very careful contol of the antibody level. If necessary, lacteal antibody levels could be increased by maternal immunization, as has been shown by the observation of natural human infections mentioned in Section VI.E and by experimental vaccinations of cattle (Snodgrass *et al.*, 1980). Note that for this purpose, parenteral

immunization with inactivated virus or perhaps single polypeptides produced by expression of cloned genes would be applicable, since parenteral immunization can boost SIgA responses provided the subject has previously experienced an immunizing intestinal infection (Svennerholm *et al.*, 1977; Snodgrass *et al.*, 1980).

At least in humans, control of dehydration and thereby of infant mortality due to diarrheal disease of any cause is seen as an achievable goal and is being pursued actively by the World Health Organization (W.H.O., 1979). A suitably formulated glucose–electrolyte solution for oral rehydration has been developed and shown to be effective, and the task has become one of organizing production so that the solution can be made widely available wherever it is needed and of educating mothers and primary-health workers in its use (W.H.O., 1976; Mahalanabis *et al.*, 1981).

VII. CONCLUDING REMARKS

The magnitude of the disease problems produced by rotaviruses in humans and domestic animals is so great that it is very difficult to obtain accurate statistics, but the best available estimates are striking (Acres and Babiuk, 1978; Kapikian *et al.*, 1980). It already appears that rotavirus is a major cause of diarrheal disease in most parts of the world, that human gastroenteritis associated with rotavirus is more severe than that caused by other agents, and that the estimated human death toll in Africa, Asia, and Latin America due to diarrheal diseases exceeds the total of all the other major infectious diseases put together (Kapikian *et al.*, 1980). Consequently, it has been suggested that to save lives, the concentration of effort should be on development of vaccines for enterotoxigenic *Escherichia coli* and rotaviruses (Black *et al.*, 1981b).

Currently, investigations on rotaviruses are popular because the potential of rotaviruses for producing disease is equaled by their potential as a field for new basic research with immediate practical applications. In this review, I have necessarily omitted all but the merest mention of a large body of work on seroepidemiology and on other agents that cause diarrheal disease, in order to cover as much as possible of the basic virology. I have attempted to draw attention to areas in which much more work is obviously needed, such as immunity, immunization strategies, definition and comparisons of serotypes, and genetics. New and improved methods are needed for studies of molecular epidemiology so that it can be determined whether genetic exchanges are occurring between rotavirus strains in different animal and human populations. Given the number of different rotaviruses that appear to exist, the frequency of rotavirus infections, and the fact that there does not seem to be a strict species barrier for rotaviruses, mixed infections that could lead to the creation and selection of new reassortant strains seem very probable. Not only are these interesting questions, but also they must be answered if control of rotaviral disease is to become a reality.

APPENDIX

Comparison of the first available sequences for a rotavirus gene, encoding a non-structural protein known as VP8 of UK bovine rotavirus and as p33 or NCVP3 of simian rotavirus SA 11 (cf. Table 1). The sequence of the coding strand of a cloned DNA copy of segment 7 of UK bovine

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                  SA11 SiRV Segment 8                                C                   46
                   UK BoRV Segment 7  5'-GGCTTTTAAAGCGTCTCAGTCGCCGTTTGAGCCTTGCGGTGTAGCC

                                                                     G            A      GT       A
ATG GCT GAG CTA GCT TGC TTT TGT TAT CCC CAT TTG GAG AAC GAT AGC TAT AAA TTT ATT CCG TTT AAC AAC TTG^121
Met Ala Glu Leu Ala Cys Phe Cys Tyr Pro His Leu Glu Asn Asp Ser Tyr Lys Phe Ile Pro Phe Asn Asn Leu_25
                                                                    Arg                 Ser

          G   T           A           G      A           A           T , C       G
GCT ATA AAA TGC ATG TTG ACG GCA AAA GTA GAC AGA AAA GAT CAG GAT AAA TTC TAT AAC TCA ATA ATT TAT GGT^196
Ala Ile Lys Cys Met Leu Thr Ala Lys Val Asp Arg Lys Asp Gln Asp Lys Phe Tyr Asn Ser Ile Ile Tyr Gly_50
                                            Lys

                                      T   C   C   G  G    T                                       A
ATT GCA CCA CCG CCA CAA TTC AAA AAA CGC TAT AAT ACA AAT GAC AAT TCA AGA GGA ATG AAC TAT GAA ACT TCG^271
Ile Ala Pro Pro Pro Gln Phe Lys Lys Arg Tyr Asn Thr Asn Asp Asn Ser Arg Gly Met Asn Tyr Glu Thr Ser_75
                                                    Ser

      T                  CG T                   C                         T                   A  G
ATG TTC AAT AAA GTG GCG GTA CTA ATT TGT GAA GCA TTG AAT TCA ATT AAA GTT ACA CAA TCT GAT GTT GCG AAT^346
Met Phe Asn Lys Val Ala Val Leu Ile Cys Glu Ala Leu Asn Ser Ile Lys Val Thr Gln Ser Asp Val Ala Asn_100
                        Ala                                                                     Ser

  A   T      A  A                 G     T A   G   C           A   A       G           G           A
GTG CTC TCA AGA GTA GTT TCT GTA AGA CAT CTG GAA AAT TTG GTG CTG AGG AGA GAA AAT CAT CAA GAC GTG CTT^421
Val Leu Ser Arg Val Val Ser Val Arg His Leu Glu Asn Leu Val Leu Arg Arg Glu Asn His Gln Asp Val Leu_125
            Lys Ile

  C   T   A       G T G T   C G           A C                     T               C   G   A
TTT CAC TCG AAA GAA CTA CTA TTA AAA TCA GTG TTA ATA GCT ATT GGT CAC TCA AAA GAA ATT GAA ACG ACT GCC^496
Phe His Ser Lys Glu Leu Leu Leu Lys Ser Val Leu Ile Ala Ile Gly His Ser Lys Glu Ile Glu Thr Thr Ala_150

              A   G                           A   T       A                   A   C C G       T
ACT GCT GAA GGG GGA GAA ATT GTT TTT CAA AAT GCT GCG TTT ACG ATG TGG AAA TTG ACG TAT TTA GAA CAC AAA^571
Thr Ala Glu Gly Gly Glu Ile Val Phe Gln Asn Ala Ala Phe Thr Met Trp Lys Leu Thr Tyr Leu Glu His Lys_175

C                                 T       A       A   A    C                                  G
TTA ATG CCA ATT TTG GAT CAA AAT TTC ATT GAG TAT AAG ATT ACA GTG AAT GAA GAT AAA CCA ATT TCA GAA TCA^646
Leu Met Pro Ile Leu Asp Gln Asn Phe Ile Glu Tyr Lys Ile Thr Val Asn Glu Asp Lys Pro Ile Ser Glu Ser_200
                                                            Leu

  C   C       G               A C     G       A   C   T   A       A       T
CAT GTG AAA GAA CTC ATT GCT GAG TTG CGA TGG CAG TAT AAC AAG TTT GCG GTA ATA ACA CAT GGT AAA GGT CAC^721
His Val Lys Glu Leu Ile Ala Glu Leu Arg Trp Gln Tyr Asn Lys Phe Ala Val Ile Thr His Gly Lys Gly His_225

              A   A                                               C                   C
TAC AGA GTT GTC AAG TAT TCA TCA GTT GCG AAT CAT GCA GAT AGA GTT TAT GCT ACT TTC AAG AGT AAT AAT AAA^796
Tyr Arg Val Val Lys Tyr Ser Ser Val Ala Asn His Ala Asp Arg Val Tyr Ala Thr Phe Lys Ser Asn Asn Lys_250

            A G A A           C A   T   T       A       A       G   T                   C
AAT GGA AAT GTA CTG GAA TTT AAT TTG CTA GAC CAA AGG ATA ATT TGG CAA AAC TGG TAT GCG TTT ACG TCT TCA^871
Asn Gly Asn Val Leu Glu Phe Asn Leu Leu Asp Gln Arg Ile Ile Trp Gln Asn Trp Tyr Ala Phe Thr Ser Ser_275
            Met Ile

              C               A               G       G                   G C         C       G
ATG AAA CAA GGT AAT ACT CTT GAC ATA TGT AAG AAA CTA CTC TTC CAG AAG ATG AAA AGA GAA AGT AAT CCA TTT^946
Met Lys Gln Gly Asn Thr Leu Asp Ile Cys Lys Lys Leu Leu Phe Gln Lys Met Lys Arg Glu Ser Asn Pro Phe_300
                            Glu

  G   G                                                                          A    GAA         A
AAA GAA CTG TCA ACT GAT AGA AAG ATG GAT GAA GTT TCT CAA ATA GGA ATT TAATTCGTTATCGGTTTGAAGGTGGTATGG^1028
Lys Gly Leu Ser Thr Asp Arg Lys Met Asp Glu Val Ser Gln Ile Gly Ile_317

 A   T
CAGAGCAAGAATAGAAAGCGCTTATGTGACC-3'^1059
```

rotavirus was determined by T.C. Elleman, P.A. Hoyne, M.L. Dyall-Smith and A.A. Azad and is shown in full, with the deduced amino acid sequence immediately below it. The corresponding sequence of segment 8 of simian rotavirus SA 11 was obtained by G.W. Both, A.R. Bellamy, J.E. Street and L.J. Siegman, who we thank for providing their data prior to publication. The SA11 sequence is identical except where base changes are indicated above the bovine rotavirus sequence, and where these have produced amino acid changes, these are indicated below the bovine amino acid sequence. A high degree of conservation of amino acid sequence is evident. The 121 nucleotide differences result in only 12 amino acid changes, i.e. over 85% of the mutational differences are silent, and the alternative amino acids are very similar in 11 of the 12 sites of variation.

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