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Definition of Human Rotavirus Serotypes by Plaque Reduction Assay

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Twenty different human rotavirus reassortants were characterized serologically by a plaque reduction assay as belonging to one of three distinct serotypes. Fourteen were similar if not identical to our prototype Wa strain; two were like the prototype DS-1 strain, and four belonged to a third serotype for which a prototype has not yet been selected. Hyperimmune sera raised against the three serotypes were required to distinguish among them, since postinfection sera had lower titers and were more cross-reactive than hyperimmune sera. These results confirmed the ability of a qualitative cytopathic neutralization test to predict correctly the Wa or DS-1 serotype. A strain of rhesus rotavirus (MMU 18006) was identified as belonging to the newly defined third serotype. Finally, an attempt was made to correlate previously published serotype analysis by neutralization of fluorescent cell-forming units with the results determined by the plaque reduction neutralization assay.

The difficulty in cultivating human rotaviruses efficiently in vitro has impeded the definition of serotypes among these viruses by conventional neutralization assays. Others have described assays in which the neutralization of fluorescent focus formation was quantitated and in which human rotavirus replication was inhibited by hyperimmune or infection sera (1, 3, 6, 20, 23). Differences in titer of fourfold or greater in one study were considered sufficient to differentiate three human rotavirus serotypes (1). This same test had been used previously to distinguish between human, calf, piglet, foal, lamb, mouse, and rabbit rotaviruses by at least a fourfold difference in homologous and heterologous antiserum titers (21).

Subsequently the in vitro cultivation of a single human rotavirus strain, Wa, after multiple serial passage in gnotobiotic piglets enabled its comparison with various cultivatable animal rotaviruses by a conventional plaque reduction (PR) neutralization assay (25, 25a). Human rotavirus (Wa), bovine rotaviruses (NCDV and UK), simian rotavirus (SA-11), and porcine rotavirus (OSU) were distinguishable from each other by the PR assay; this provided evidence for distinct serotypes of rotavirus which did not cross species boundaries among those viruses studied (25a). High-titered hyperimmune antisera prepared in guinea pigs yielded clear-cut distinctions of 20-fold or greater, whereas one-way cross-reactions were observed in some cases with the use of postinfection sera.

Difficulty in growing human rotaviruses in cell culture has now been largely overcome. Recent studies from Japan have described the successful cultivation and characterization of six human rotavirus strains in MA104 roller tube cultures with the use of trypsin (17, 23). In our laboratory, previously noncultivatable human rotaviruses have been rescued by genetic reassortment with *ts* mutants of bovine rotavirus, UK strain (7). Initially, one such rescued virus, DS-1 strain, was compared with Wa and UK rotaviruses and found to be serologically distinct based on a 20-fold or greater difference between homologous and heterologous antibody titers. Thus Wa and DS-1 strains represent our human rotavirus prototypes which are distinct by the PR assay with hyperimmune antisera. A series of 33 additional rescued human rotaviruses from diverse geographical regions worldwide is described in the accompanying paper (8); 22 of the 33 viruses are similar to Wa or DS-1 strains as determined by a qualitative cytopathic effect neutralization assay. The classification of the first 20 of these rescued viruses into three distinct serotypes by the PR assay will be described in this report.

MATERIALS AND METHODS

Cell cultures. PR assays were carried out in a fetal rhesus monkey kidney cell line, MA104, kindly supplied by E. H. Bohl (Ohio Agricultural Research and Development Center, Wooster, Ohio) and propagated by MA Bioproducts, Walkersville, Md., or in our

laboratory (15a). Cells at passage 72 or less were grown in six-well plastic dishes (Costar) and were used 4 to 8 days later when confluent. Cells were grown in Eagle minimum essential medium with 8 to 9% fetal calf or calf serum and were incubated in humidified air plus 5% CO_2.

Viruses. Cultivatable human rotavirus Wa, bovine rotavirus UK (supplied by T. H. Flewett and G. N. Woode), and rhesus monkey rotavirus MMU 18006 (supplied by N. J. Schmidt) were used as reference strains (2, 18, 19). Twenty rescued human rotaviruses were used for serotypic characterization; their cultivation as well as that of 13 others is described in the accompanying paper (8). All viruses used were plaque purified in primary African green monkey kidney cells or MA104 cells, except for rhesus rotavirus MMU 18006, which was plaque purified in a rhesus monkey fetal kidney cell line and further purified by terminal dilution in primary cynomolgus monkey kidney cells by G. Stuker and co-workers (18).

Antisera. Hyperimmune antisera prepared in guinea pigs against the viruses were used in PR assays. All viruses were partially purified through a sucrose gradient, with the exception of rhesus rotavirus, which was not purified; three sequential doses of virus mixed in Freund complete adjuvant, Freund incomplete adjuvant, and in phosphate-buffered saline, respectively, were administered intramuscularly at approximate 3-week intervals, except in the case of rhesus rotavirus, which was mixed with Freund incomplete adjuvant and administered intramuscularly on two occasions at a 3-week interval (7; Kalica et al., unpublished data). These antisera against Wa, DS-1, and UK rotaviruses were also used at a single dilution for preliminary evaluation of reassortants by the qualitative cytopathic effect neutralization assay (8). Hyperimmune antiserum prepared in rabbits against three human rotavirus serotypes characterized in Birmingham, England, were supplied by T. H. Flewett. In addition, selected postinfection sera were used in the PR assay. Serum was obtained 21 days after experimental infection of a gnotobiotic piglet (no. 4) with human rotavirus Wa administered as a 2% human stool filtrate (25). Sera from gnotobiotic calves, 77-12 and 77-29, were collected 21 days after experimental infection with human rotavirus strains M and DS-1, respectively (10, 15). Finally, postinfection serum from an 8-month-old rhesus monkey (B480) was collected 35 days after experimental infection with rhesus rotavirus MMU 18006; preinfection serum from the same animal did not contain antibody to this rhesus rotavirus at a 1:20 dilution when tested by the PR assay.

PR neutralization assay. The PR assay was performed with techniques similar to those of Matsuno et al. (13). Briefly, cells were washed three times with L-15 or Eagle minimum essential medium containing 0.5% gelatin but no serum. Equal volumes of virus suspension and trypsin (20 μg/ml) were mixed and incubated for at least 1 h at 37°C before further processing. The mixture was then diluted to yield approximately 30 PFU per culture well and mixed with equal volumes of serial fourfold serum dilutions or control fluids; 1 ml of each mixture was added to a well of a six-well tissue culture dish. Sera were tested either in duplicate cultures or in at least two separate tests for confirmation. After incubation for 1 h at 37°C, the plates were washed once, and an Eagle minimum essential medium–0.9% agarose overlay containing 0.5 μg of trypsin per ml, 100 μg of DEAE-dextran per ml, and antibiotics was added. After 3 to 5 days, depending on the growth characteristics of the rotavirus, a second overlay containing neutral red (0.067 mg/ml) was added. The test then was usually read within 2 days when an optimal number (approximately 10 to 50) of clearly discernible plaques had developed in the control wells. Antibody titers were expressed as a calculated dilution of a serum which yielded a 60% reduction in plaque count (16).

Interpretation. A 20-fold or greater reciprocal difference in antibody titer was taken to indicate a significant antigenic difference between two viruses. Two rotaviruses were considered to be distinct serotypes only when both viruses and their homologous antisera were available and could be tested simultaneously to demonstrate such a difference.

RESULTS

Identification of three human rotavirus serotypes among rescued rotaviruses. Twenty different rotavirus strains, each represented by one or two reassortant rotaviruses ($n = 35$) with human neutralization specificity, were tested initially in the PR assay with hyperimmune anti-Wa, -DS-1, and -UK antisera. Antiserum to the bovine parent rotavirus was included to confirm successful selection against bovine neutralization specificity. None of the 35 reassortant rotaviruses was neutralized to high titer by the bovine rotavirus antiserum. In contrast, of the 20 different strains, Wa antiserum neutralized 14 reassorted strains to high titer, and DS-1 antiserum neutralized 2 reassorted strains to moderately high titer. Four reassortants were not neutralized or were neutralized to low titer by Wa and DS-1 antisera. These results confirmed the predicted classification of these strains by the cytopathic effect neutralization assay (8). Results are shown in Table 1.

Hyperimmune antisera generated against human rotavirus M, derived from the feces of an experimentally infected calf, and against three human rotavirus reassortants, B, 14, and 15, were used in multiple PR assays to compare these unclassified human rotavirus reassortants with Wa and DS-1 prototype strains (Table 1). In the same PR assay, Wa, DS-1, and M strains were shown to be distinct and thus could be characterized as three separate rotavirus serotypes (Table 1). Strains M, B, 14, and 15 were related in at least a one-way fashion, a finding which enables them to be grouped into the third distinct serotype. Hyperimmune M antiserum recognized to high titer only the homologous virus, and thus M could not be considered a broadly reactive prototype strain. None of the other three strains (B, 14, or 15) was more broadly reactive than the others, and therefore designation of a prototype for this serotype awaits further characterization.

TABLE 1. Comparison of 22 human rotaviruses and simian rotavirus by PR assay with hyperimmune sera

Rotavirus	Reciprocal of titer against antiserum from guinea pigs hyperimmunized with indicated rotavirus							
	Wa	DS-1	77-12 (M), (no. 5)	77-1955 (B)	WALK no. 57 (14)	YIP no. 43 (15)	MMU 18006 (simian, rhesus)	Bovine NCDV[a]
Wa	**≥81,920**[b]	<80[b]	3,290[b]	<80	<80	396	<80	<80
77-25 (W)[c]	4,578	<20	—[d]	—	—	—	—	<20
77-11 (G)[c]	75,723	72	—	—	—	—	—	<80
75-82 (D)[c]	≥20,480	<20	—	—	—	—	—	<20
77-28 (Fh) (K)[c]	72,745	<80	—	—	—	—	—	<80
6546 (E)[c]	≥20,480	64	—	—	—	—	—	31
7300 (F)[c]	59,862	<80	—	—	—	—	—	<80
078-41 (C)[c]	6,468	<20	—	—	—	—	—	<20
04/62 (d)[c]	≥20,480	<20	—	—	—	—	—	<20
017 (I)[c]	≥20,480	62	—	—	—	—	—	45
053 (J)[c]	≥81,920	23	—	—	—	—	—	<20
NAHL no. 39 (13)[c]	58,804	<80	—	—	—	—	—	<80
CMH no. 53 (16)[c]	49,724	<80	—	—	—	—	—	<80
YSH (11)[c]	≥81,920	87	—	—	—	—	—	259
YCS (12)[c]	≥20,480	190	—	—	—	—	—	<20
DS-1[c]	267 (<80)[b,e]	**4,474 (31,273)**[b,f]	<80[b]	211	2,847	6,540	206	95
AS (no. 3)[c]	140	2,508	—	—	—	—	—	<80
008A (no. 1)[c]	75	3,594	—	—	—	—	—	<20
77-12 (M) (no. 5)[c]	<80[b]	<80[b]	**≥81,920**[b,g]	7,117	≥81,920	≥81,920	≥81,920	81
77-1955 (B)[c]	<20	<20	96	**10,650**	≥81,920	≥81,920	9,363	<20
WALK no. 57 (14)[c]	202	40	<80	37,468	**≥81,920**	≥81,920	15,996	23
YIP no. 43 (15)[c]	35	<20	353	20,281	≥81,920	**≥81,920**	≥81,920	<20
MMU18006 (simian, rhesus)	192	<80	281	6,082	73,555	≥81,920	**≥81,920**	—

[a] Titer against bovine rotavirus (UK strain) is ≥1:81,920.
[b] Values from the same PR assay.
[c] Human-bovine reassortant virus.
[d] —, Not tested.
[e] Numbers within parentheses are values from another test.
[f] Titer of another hyperimmune antiserum identified later.
[g] Homologous values are in boldface type.

It was of further and particular interest that rhesus rotavirus (MMU 18006) could be classified into the third serotype since it could not be distinguished from human rotavirus strains B, 14, or 15 by the use of hyperimmune antisera in the PR assay (Table 1). This strain of rhesus rotavirus is serologically similar, if not identical, to SA-11 virus (18). In confirmatory studies performed in our laboratory, hyperimmune antiserum to SA-11 neutralizes SA-11 and rhesus rotaviruses to respective titers of 1:53,784 and 1:4,524, an approximate 12-fold difference in titer. Hyperimmune antiserum to the rhesus rotavirus neutralizes SA-11 and rhesus viruses to respective titers of 1:158,204 and 1:201,340.

Of these 20 strains tested by PR assay, Wa-like viruses were recovered from Guatemala, Egypt, Hong Kong, Malaysia, and the United States. DS-1-like viruses were identified from Venezuela and the United States. The other recovered strains belonging to the third serotype were from Hong Kong, Japan, and the United States.

Cross-reactivity among rotavirus serotypes as studied by PR assay with postinfection sera. Postinfection sera from gnotobiotic animals and a conventional rhesus monkey experimentally infected with human rotavirus Wa, DS-1, M, or rhesus rotavirus were tested in PR assays against homologous and heterologous viruses (Table 2). Only DS-1 and M strains were serologically distinct since 20 antibody units of the homologous antiserum failed to recognize the heterologous virus. By the same criteria, M strain and rhesus rotavirus were similar if not identical. Wa strain had a one-way serological relationship with DS-1, M, and rhesus rotavirus; a one-way relationship was also seen between DS-1 and rhesus rotavirus.

Relationship of Wa, DS-1, and M strains to

TABLE 2. Comparison of human rotaviruses (strains Wa, DS-1, and M) and simian rotavirus (rhesus MMU 18006) by PR assay with postinfection sera

Rotavirus	Reciprocal of titer against antiserum from animals (species) experimentally infected with indicated rotavirus[a]			
	Human Wa (porcine)[b]	Human DS-1 (bovine)[b]	Human M (bovine)[b]	Rhesus MMU 18006 (simian)[c]
Wa	**2,894 (2,334)**[d]	294 (281)[d]	34[d]	1,698
DS-1	<20 (43)[d]	**108 (284)**[d]	<20[d]	165
M	165[d]	<20[d]	**919**[d] **(555)**[e]	199[e]
Rhesus (MMU 18006)	72	24	187[e]	**3,374 (644)**[e]

[a] Homologous values are in boldface type. Numbers within parentheses are values from other tests. Values are from the same PR assay except where indicated.
[b] Gnotobiotic animal.
[c] Conventional animal.
[d] Assay comparing Wa, DS-1, and M.
[e] Assay comparing M and rhesus MMU 18006.

serotypes described previously. Hyperimmune rabbit antisera prepared against three different proposed serotypes determined by inhibition of fluorescent cell-forming units were made available to our laboratory (1). These sera were tested in the PR assay for a one-way relationship to Wa, DS-1, and M serotypes. As shown in Table 3, Wa was neutralized to high titer by antiserum no. 281 prepared against the Flewett type I virus, and M virus was neutralized to high titer by antiserum no. 285 against the Flewett type III virus. DS-1 virus was not neutralized to a high titer by any of the three antisera.

DISCUSSION

The availability of a large number of readily cultivatable human rotavirus reassortants has enabled comparison of rotavirus strains and classification into serotypes based on the PR assay. Twenty-fold or greater differences in titer were required in both directions before serotypes were established; thus the definition of serotype is consistent with that established for members of the family picornaviridae including enteroviruses and rhinoviruses (4, 5, 12). The first 20 strains studied could be assigned to three distinct serotypes; this indicates, in a preliminary way, limited antigenic diversity, a finding which should facilitate the development of immunoprophylaxis. It should be noted, however, that the use of bovine antiserum in genetic reassortment techniques might have selected against human rotaviruses which may share antigenic relatedness with bovine rotavirus, thus excluding recognition of such an additional serotype(s). It will be essential to examine the biological significance of this antigenic diversity through epidemiological studies and by cross-protection studies in experimental animals. The degree of protection, if any, afforded by infection with homotypic and heterotypic rotaviruses must be determined. A previous study in two adult volunteers indicated that resistance against diarrheal disease caused by homologous virus was present 19 months later (Kapikian et al., submitted for publication).

TABLE 3. Relationship between proposed serotypes published previously and human rotaviruses Wa, DS-1, and M

Human rotavirus	Reciprocal of titer against antiserum from rabbits hyperimmunized with indicated human rotavirus representing proposed serotypes published previously (1)		
	281 (I)[a]	146 (II)[a]	285 (III)[a]
Wa	≥81,920	132	1,280
DS-1[b]	476	1,351	936
M[b]	84	412	26,302

[a] Serotype designation within parentheses is from reference 1.
[b] Human-bovine rotavirus reassortant.

Distinct serotypes were defined in PR assays with hyperimmune sera. Similar PR assays with postinfection sera yielded greater degrees of cross-reactivity than did those with hyperimmune sera, despite considerably lower homologous titers of the postinfection sera. It is of particular interest that sera gnotobiotic animals experimentally infected with Wa, DS-1, or M rotaviruses recognized the heterologous viruses in two cases. It was previously shown that primary rotavirus infection in infants leads to seroresponse to both Wa and DS-1 serotypes (25a). Further, in adult volunteers infected with a Wa-like rotavirus, strain D, antibody responses to DS-1 serotype were documented; in addition, the absence of antibody to DS-1 serotype correlated with susceptibility to diarrheal illness after oral administration of Wa-like rotavirus

(Kapikian et al., submitted for publication). It is possible, therefore, that naturally occurring antibody, such as that following infection, will be sufficiently cross-reactive to allow cross-immunization between human rotavirus serotypes or even between animal and human rotavirus serotypes.

It should be brought into perspective that two of the rescued strains, D and Fh, which are now classified as being Wa-like, had been studied extensively in a variety of ways. In addition to being administered to adult volunteers, the D strain has been given to gnotobiotic calves and piglets as well as to colostrum-deprived rhesus monkeys, and these animals developed infection and diarrheal illness (15, 22, 27). Pathogenesis of human rotavirus infection has been studied in calves after oral administration of the D strain (14). The Fh strain was previously cultivated in a limited fashion in roller tube cultures of primary human embryonic kidney cells (24). Further, the migration pattern of viral RNA from five of the rescued strains was previously known because of the availability of virus-containing feces from calves experimentally infected with these human strains (10). Thus these rescued Wa-like rotavirus reassortants represent strains about which information has been collected, and this information represents experience with what is now recognized as the most common human rotavirus serotype, designated Wa.

A distinct serotype that includes human and simian rotaviruses was identified. The significance of this finding with regard to possible transmission across species boundaries is unknown. It is possible that the monkey rotaviruses represent viruses of human origin transmitted to captive monkeys in contact with humans. Since rhesus rotavirus is of relatively low virulence and since it shares a close antigenic relationship with certain human rotaviruses, it may prove useful as a potential vaccine strain for humans, especially if other as yet unclassified reassortant human rotaviruses belong to this same serotype. It was shown previously that a bovine rotavirus administered in utero will protect gnotobiotic newborn calves against disease by heterologous Wa-like human rotavirus (26); in this case, the two viruses represented distinct serotypes. It is possible that a serologically related simian rotavirus might represent an even more effective immunogen (17a).

The classification of human rotaviruses into serotypes has been proposed, based on inhibition of fluorescent cell-forming units of rotaviruses that were not capable of sufficient replication in cell culture to permit PR assay; three and possibly four serotypes were described that met the criteria of fourfold or greater differences in titer (1, 6). The findings in this report confirm, extend, and solidify the existence of three distinct human rotavirus serotypes although it is not possible to correlate entirely our results with the previously published reports. In the one-way PR assay two serotypes (I and III), identified in England, appeared to be similar, if not identical, to two serotypes (Wa and M, respectively) identified in the United States. Serotype II from England and DS-1 from the current study did not appear to be related in a one-way PR assay. In a recent study from Japan, some serological relatedness to the Wa strain was reported for three isolates (17).

Rotaviruses have also been classified by using non-neutralization assays such as complement fixation, enzyme-linked immunosorbent assay, and immune adherence hemagglutination (11, 28–31). In these tests two, and in one case three, antigenically different subgroups or types have been identified. In a recent study of Wa virus in which gene-coding assignments were made, the antigenic determinant responsible for differences measured by these non-neutralization tests was coded for by gene number 6 (9). In contrast, gene number 9 of Wa virus coded for the protein responsible for the induction of neutralizing antibody (9). We suggested therefore that a subgroup classification be based on complement fixation, immune adherence hemagglutination, and enzyme-linked immunosorbent assay and that serotype classification be based on neutralization (11). Thus the Wa virus is serotype Wa, subgroup 2; DS-1 virus is serotype DS-1, subgroup 1; and M virus is serotype M, subgroup 2. Through future exchange of reagents and comparisons such as those made in this report, a systematic and logical classification scheme should result.

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LITERATURE CITED

1. **Beards, G. M., J. N. Pilford, M. E. Thouless, and T. H. Flewett.** 1980. Rotavirus serotypes by serum neutralization. J. Med. Virol. **5**:231–237.
2. **Bridger, J. C., and G. N. Woode.** 1975. Neonatal calf diarrhoea: Identification of a reovirus-like (rotavirus) agent in faeces by immunofluorescence and immune electron microscopy. Br. Vet. J. **131**:528–535.
3. **Bryden, A. S., H. A. Davies, M. E. Thouless, and T. H. Flewett.** 1977. Diagnosis of rotavirus infection by cell culture. J. Med. Microbiol. **10**:121–125.
4. **Committee on Enteroviruses.** 1962. Classification of human enteroviruses. Virology **16**:501–504.
5. **Committee on the ECHO Viruses.** 1955. Enteric cytopathogenic human orphan (ECHO) viruses. Science **122**:1187–1188.

6. **Flewett, T. H., M. E. Thouless, J. N. Pilford, A. S. Bryden, and J. Candeias.** 1978. More serotypes of human rotavirus. Lancet ii:632.
7. **Greenberg, H. B., A. R. Kalica, R. G. Wyatt, R. W. Jones, A. Z. Kapikian, and R. M. Chanock.** 1981. Rescue of non-cultivatable human rotavirus by gene reassortment during mixed infection with *ts* mutants of a cultivatable bovine rotavirus. Proc. Natl. Acad. Sci. U.S.A. 78:420–424.
8. **Greenberg, H. B., R. G. Wyatt, A. Z. Kapikian, A. R. Kalica, J. Flores, and R. Jones.** 1981. Rescue and serotypic characterization of noncultivable human rotavirus by gene reassortment. Infect. Immun. 37:104–109.
9. **Kalica, A. R., H. B. Greenberg, R. G. Wyatt, J. Flores, M. M. Sereno, A. Z. Kapikian, and R. M. Chanock.** 1981. Genes of human (strain Wa) and bovine (strain UK) rotaviruses that code for neutralization and subgroup antigens. Virology 112:385–390.
10. **Kalica, A. R., R. G. Wyatt, and A. Z. Kapikian.** 1978. Detection of differences among human and animal rotaviruses using analysis of viral RNA. J. Am. Vet. Med. Assoc. 173:531–537.
11. **Kapikian, A. Z., W. L. Cline, H. B. Greenberg, R. G. Wyatt, A. R. Kalica, C. E. Banks, H. D. James, Jr., J. Flores, and R. M. Chanock.** 1981. Antigenic characterization of human and animal rotaviruses by immune adherence hemagglutination assay (IAHA): evidence for distinctness of IAHA and neutralization antigens. Infect. Immun. 33:415–425.
12. **Kapikian, A. Z., R. M. Conant, V. V. Hamparian, R. M. Chanock, P. J. Chapple, E. C. Dick, J. D. Fenters, J. M. Gwaltney, Jr., D. Hamre, J. C. Holper, W. S. Jordan, Jr., E. H. Lennette, J. L. Melnick, W. J. Mogabgab, M. A. Mufson, C. A. Phillips, J. H. Schieble, and D. A. J. Tyrrell.** 1967. Rhinoviruses: a numbering system. Nature (London) 213:761–763.
13. **Matsuno, S., S. Inouye, and R. Kono.** 1977. Plaque assay of neonatal calf diarrhea virus and the neutralizing antibody in human sera. J. Clin. Microbiol. 5:1–4.
14. **Mebus, C. A., R. G. Wyatt, and A. Z. Kapikian.** 1977. Intestinal lesions induced in gnotobiotic calves by the virus of human infantile gastroenteritis. Vet. Pathol. 14:273–282.
15. **Mebus, C. A., R. G. Wyatt, R. L. Sharpee, M. M. Sereno, A. R. Kalica, A. Z. Kapikian, and M. J. Twiehaus.** 1976. Diarrhea in gnotobiotic calves caused by the reovirus-like agent of human infantile gastroenteritis. Infect. Immun. 14:471–474.
15a. **Myers, M. G., M. M. Vincent, S. A. Hensen, and N. M. Tauraso.** 1972. Problems in the laboratory isolation of simian hemorrhagic fever viruses and isolation of the agent responsible for the Sussex/69 epizootic. Appl. Microbiol. 24:62–69.
16. **Richman, D. D., B. R. Murphy, W. L. Cline, and D. W. Alling.** 1975. Determination of influenza virus neuraminidase inhibition titres. Bull. W.H.O. 52:233–234.
17. **Sato, K., Y. Inaba, T. Shinozaki, R. Fujii, and M. Matumoto.** 1981. Isolation of human rotavirus in cell cultures. Arch. Virol. 69:155–160.
17a. **Schoub, B. D., G. Lecatsas, and O. W. Prozesky.** 1977. Antigenic relationship between human and simian rotaviruses. J. Med. Microbiol. 10:1–6.
18. **Stuker, G., L. S. Oshiro, and N. J. Schmidt.** 1980. Antigenic comparisons of two new rotaviruses from rhesus monkeys. J. Clin. Microbiol. 11:202–203.
19. **Stuker, G., L. S. Oshiro, N. J. Schmidt, C. A. Holmberg, J. H. Anderson, C. A. Glaser, and R. V. Hendrickson.** 1979. Virus detection in monkeys with diarrhea: the association of adenoviruses with diarrhea and the possible role of rotaviruses. Lab. Anim. Sci. 29:610–616.
20. **Thouless, M. E., A. S. Bryden, and T. H. Flewett.** 1978. Serotypes of human rotavirus. Lancet i:39.
21. **Thouless, M. E., A. S. Bryden, T. H. Flewett, G. N. Woode, J. C. Bridger, D. R. Snodgrass, and J. A. Herring.** 1977. Serological relationships between rotaviruses from different species as studied by complement fixation and neutralization. Arch. Virol. 53:287–294.
22. **Torres-Medina, A., R. G. Wyatt, C. A. Mebus, N. R. Underdahl, and A. Z. Kapikian.** 1976. Diarrhea in gnotobiotic piglets caused by the human reovirus-like agent of infantile gastroenteritis. J. Infect. Dis. 133:22–27.
23. **Urasawa, T., S. Urasawa, and K. Taniguchi.** 1981. Sequential passages of human rotavirus in MA104 cells. Microbiol. Immunol. 25:1025–1035.
24. **Wyatt, R. G., V. W. Gill, M. M. Sereno, A. R. Kalica, D. H. VanKirk, R. M. Chanock, and A. Z. Kapikian.** 1976. Probable *in vitro* cultivation of human reovirus-like agent of infantile diarrhoea. Lancet i:98–99.
25. **Wyatt, R. G., W. D. James, E. H. Bohl, K. W. Theil, L. J. Saif, A. R. Kalica, H. B. Greenberg, A. Z. Kapikian, and R. M. Chanock.** 1980. Human rotavirus type 2: cultivation *in vitro*. Science 207:189–191.
25a. **Wyatt, R. G., A. Z. Kapikian, H. B. Greenberg, A. R. Kalica, and R. M. Chanock.** 1982. Prospects for development of a vaccine against rotavirus diarrhea, p. 505–522. *In* T. Holme, J. Holmgren, M. H. Merson, and R. Mollby (ed.), Proceedings of the Conference on Acute Enteric Infections in Children. Elsevier/North Holland Biomedical Press, Amsterdam.
26. **Wyatt, R. G., C. A. Mebus, R. H. Yolken, A. R. Kalica, H. D. James, Jr., A. Z. Kapikian, and R. M. Chanock.** 1979. Rotaviral immunity in gnotobiotic calves: heterologous resistance to human virus induced by bovine virus. Science 203:548–550.
27. **Wyatt, R. G., D. L. Sly, W. T. London, A. E. Palmer, A. R. Kalica, D. H. VanKirk, R. M. Chanock, and A. Z. Kapikian.** 1976. Induction of diarrhea in colostrum-deprived newborn rhesus monkeys with the human reovirus-like agent of infantile gastroenteritis. Arch. Virol. 50:17–27.
28. **Yolken, R. H., R. G. Wyatt, G. Zissis, C. D. Brandt, W. J. Rodriguez, H. W. Kim, R. H. Parrott, H. B. Greenberg, A. Z. Kapikian, and R. M. Chanock.** 1978. Comparative epidemiology of human rotavirus types 1 and 2 as studied by enzyme-linked immunosorbent assay. N. Engl. J. Med. 299:1156–1161.
29. **Zissis, G., and J. P. Lambert.** 1978. Different serotypes of human rotaviruses. Lancet i:38–39.
30. **Zissis, G., and J. P. Lambert.** 1980. Enzyme-linked immunosorbent assays adapted for serotyping of human rotavirus strains. J. Clin. Microbiol. 11:1–5.
31. **Zissis, G., J. P. Lambert, J. G. Kapsenberg, G. Enders, and L. N. Mutanda.** 1981. Human rotavirus serotypes. Lancet i:944–945.