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Rotavirus Vaccines: an Overview

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INTRODUCTION

Rotavirus is the leading etiologic agent of severe diarrheal disease in infants and young children worldwide (73) (Fig. 1). In developed countries, rotavirus has been detected in 35 to 52% of infants and young children hospitalized with acute diarrhea (16, 31, 84). It has been estimated that in the United States, 3 million infants and young children develop rotavirus diarrhea annually, leading to 82,000 hospitalizations and up to 150 deaths per year (60, 68). In the developing world, rotaviruses are also the most frequently detected pathogen in children with severe gastroenteritis who are less than 2 years old (9, 15, 71). In developing countries, over 125 million cases of rotavirus diarrhea have been estimated to occur annually in children under the age of 5 years; 18 million of these cases are moderately severe, and 873,000 lead to death (69). Because of the significant morbidity and mortality associated with rotavirus diarrhea during the first 2 years of life, there is an urgent need to develop rotavirus vaccines targeted for use in early infancy. The goal of the vaccine is to prevent severe diarrheal illness which may lead to dehydration (72, 81).

EPIDEMIOLOGY

Rotaviruses are ubiquitous, infecting over 90% of humans by 3 years of age (73). In temperate climates, most episodes of rotavirus diarrhea occur during the colder months of the year (16, 17, 79), whereas in tropical countries, a seasonal pattern of illness is usually not observed (30). Rotavirus diarrhea occurs most frequently in 6- to 36-month-old children, followed by those under 6 months old, although the latter group experienced the highest frequency in a few studies (10, 79).

Rotaviruses are shed in large numbers in the feces during episodes of rotavirus diarrhea and are transmitted by the fecal-oral route. The possibility of respiratory transmission has been considered because of the rapid acquisition of serum antibody in the first 3 years of life regardless of hygiene conditions and the failure to document fecal-oral transmission in a few large outbreaks (30, 45, 77, 122); however, rotaviruses have been detected infrequently in respiratory secretions (126, 149, 150). Animal-to-human transmission has not been documented under natural conditions. However, human rotavirus strains that possess certain gene segments with a high degree of homology with animal rotavirus genes have been found (47, 73, 103, 104). This was probably due to genetic reassortment between human and animal rotaviruses. This occurrence does not appear to be of clinical or epidemiologic importance.

CLASSIFICATION AND STRUCTURE

Rotaviruses, which are classified as a genus in the family *Reoviridae*, are etiologic agents of diarrhea in humans and numerous animal species (147). The intact virion is 70 nm in diameter and is characterized by a distinctive double capsid (38, 73, 97) (Fig. 2). Within the inner capsid is a third layer that encompasses the core containing the virus genome. The ge-

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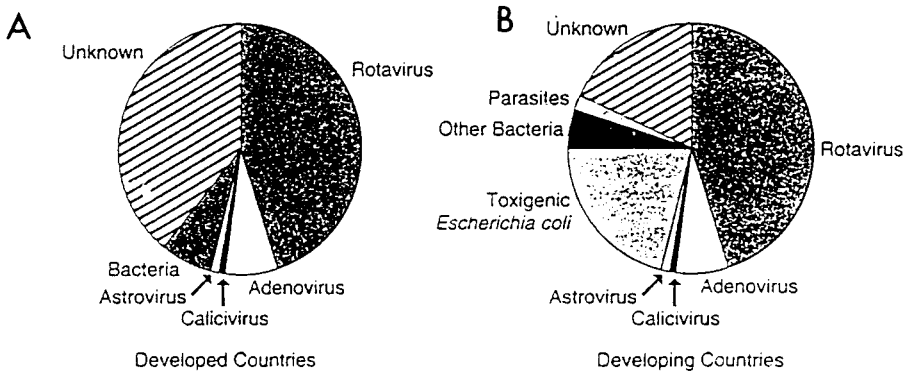

FIG. 1. An estimate of the role of etiological agents in severe diarrheal illnesses requiring hospitalization of infants and young children in developed countries (A) and in developing countries (B). Reprinted from reference 71 with permission of the publisher.

nome consists of 11 segments of double-stranded RNA that encode six structural and five nonstructural proteins. Core proteins VP1, VP2, and VP3 and inner capsid protein VP6 are encoded by gene segments 1, 2, 3, and 6, respectively; outer capsid protein VP4 is encoded by segment 4, and outer capsid protein VP7 is encoded by segment 7, 8, or 9 (Fig. 3).

Rotaviruses possess three important antigenic specificities, based on group, subgroup, and serotype (73, 97). Group specificity is determined predominantly by VP6; most epidemiologically significant rotaviruses of human and animal origin belong to group A, which is therefore the primary target of vaccine development. Group A rotaviruses are further classified by subgroup specificity, which is also mediated by VP6. The majority of strains belong to either subgroup I or II, although some isolates carry both subgroup I and II specificities and a few do not belong to either subgroup (61). Serotype specificity is determined by VP4 and VP7, both of which independently induce neutralizing antibodies (51, 66, 97, 107).

Although 10 of 14 VP7 serotypes (also designated G serotypes because VP7 is a glycoprotein) identified to date have been isolated from humans, only VP7 serotypes 1, 2, 3, and 4 appear to be of epidemiologic importance (61, 62, 73). At least seven VP4 serotypes, including two subtypes (also designated P serotypes because VP4 is protease sensitive), have been de-

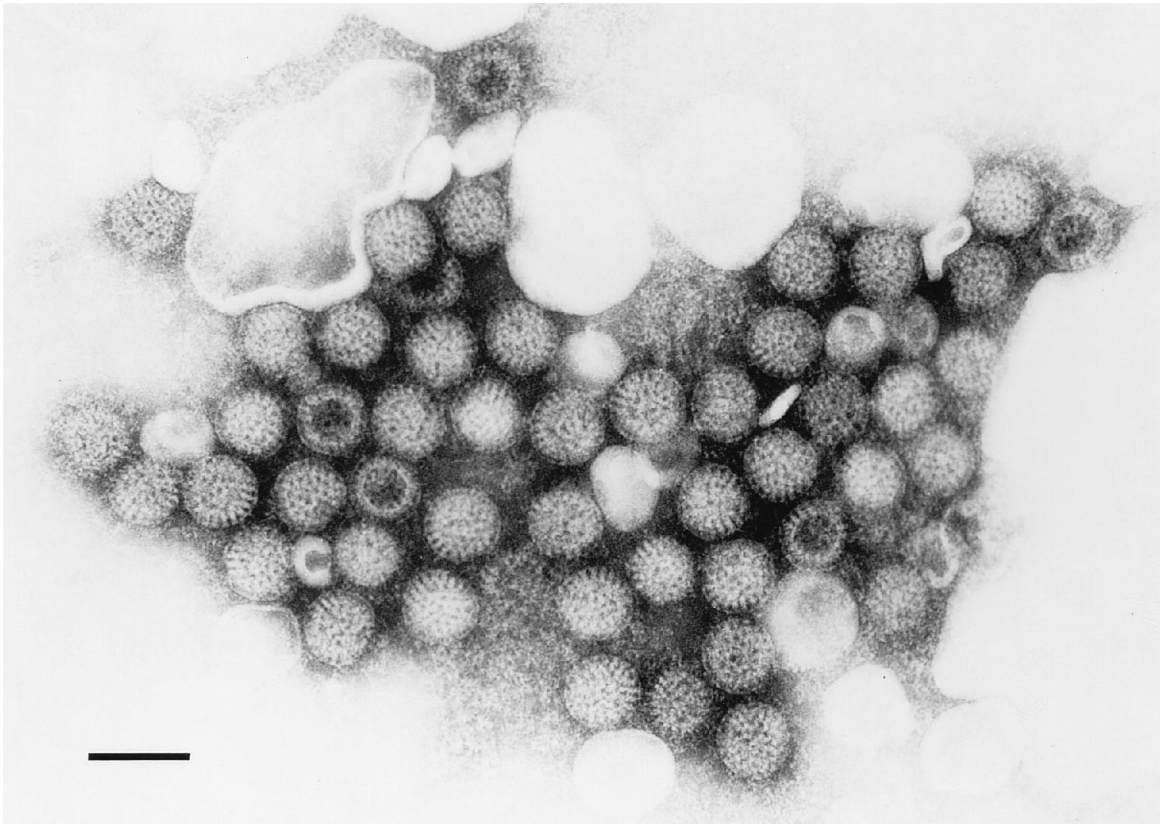

FIG. 2. Rotavirus particles demonstrated by immune electron microscopy in a stool filtrate obtained from a child with acute gastroenteritis. Reprinted from reference 79 with permission of the publisher.

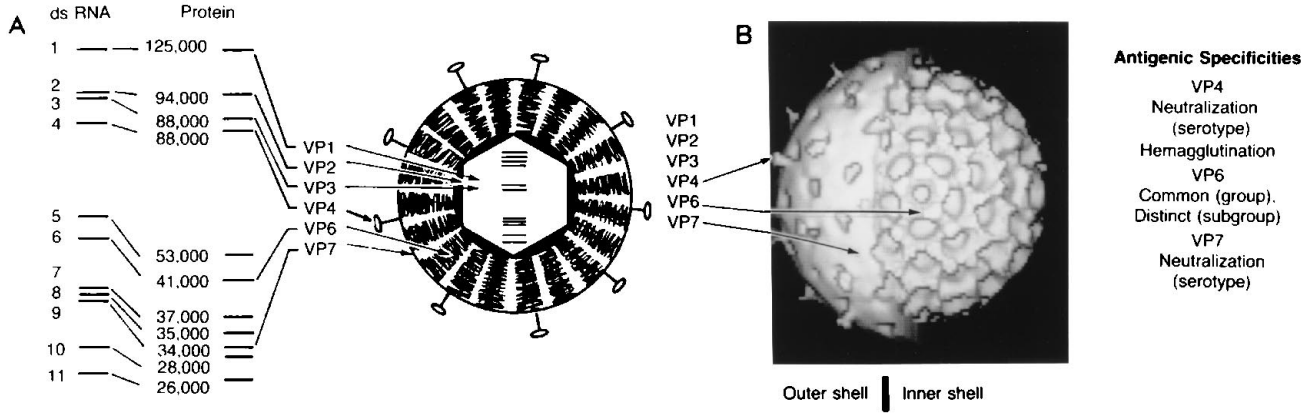

FIG. 3. (A) Schematic representation of the rotavirus double-shelled particle. Reprinted from reference 74 with permission of the publisher. (B) Surface representations of the three-dimensional structures of a double-shelled particle (on the left half) and a particle (on the right half) in which most, if not all, of the outer shell and a small portion of the inner shell mass have been removed. Reprinted from reference 115 with permission of the publisher.

scribed among human rotaviruses (62). The majority of epidemiologically important human strains of G 1, 3, and 4 specificity belong to P serotype 1A and those of G 2 specificity belong to P serotype 1B (51, 61). Although many animal strains share G 1, 2, 3, or 4 specificity with human strains, no animal strains bear P 1A or 1B specificity (61, 62).

IMMUNITY

The immunologic correlates of protection against rotavirus diarrhea are not well understood (11, 73, 95). Studies of the natural history of disease and volunteers challenged with wild-type human rotavirus suggest that serum or fecal antibody may be associated with but does not consistently confer protection from infection or disease (7, 14, 20, 29, 82, 94, 145). Adults, who usually have detectable antibody, frequently experience reinfections, which are usually asymptomatic (83). Likewise, children infected as neonates had significantly fewer episodes of severe rotavirus-associated diarrhea over the ensuing 3 years than did their uninfected counterparts, although the incidence of rotavirus infection was similar in the two groups (12). It is of interest that neonatal infections in general tend to be asymptomatic or associated only with mild diarrhea. Factors contributing to this phenomenon may include maternally acquired antibody, breast feeding, host factors, and characteristics peculiar to neonatal rotavirus strains (23, 40, 50, 114).

The role of locally produced intestinal antibody in resistance to illness or infection has not been clearly established, but antibody passively administered via the alimentary tract has been shown to alter susceptibility to disease. In studies of newborn calves, lambs, and mice, the presence of colostrum- or serum-derived rotavirus antibody in the gut lumen at the time of challenge conferred protection against disease whereas circulating antibody did not (18, 106, 129). A more recent study in gnotobiotic piglets has demonstrated a quantitative relationship between oral doses of virus-neutralizing antibody and protection from rotavirus infection or disease (128). Passive protection has also been demonstrated in humans in various settings (5, 32, 37). For example, in a study of low-birth-weight neonates, gamma globulin administered orally during the first week of life was associated with delayed viral excretion and milder illness when rotavirus infection occurred (5). Oral administration of preparations that contain rotavirus antibodies for treatment of chronic rotavirus illness in immunodeficient children has proved to be effective (88, 127). Treatment of acute gastroenteritis in normal children with rotavirus antibody-containing preparations by the oral route has yielded variable results (36, 55, 58).

Antibodies to a variety of rotavirus antigens develop during infection; neutralizing antibodies to both major neutralizing proteins, VP4 and VP7, have been shown to induce homotypic protection in animal studies (38, 65, 107). However, heterotypic immunity has also been demonstrated in several animal models (13, 148, 152). Whether the serum-neutralizing antibody response in infants following primary natural rotavirus infection is predominantly homotypic or also heterotypic needs further study, because investigations have yielded variable results (19, 20, 26, 48, 116, 151). Cell-mediated immunity appears to be involved in protection against rotavirus gastroenteritis in mice (36, 108), but its importance in humans is unknown.

ROTAVIRUS VACCINE CANDIDATES

Vaccine Strategies

The initial development of rotavirus vaccines was based on Edward Jenner's approach to smallpox vaccination, which involved the use of an antigenically related, live virus derived from a nonhuman host. The fact that human and animal rotavirus strains had a common group antigen and that heterologous protection had been demonstrated in animal models supported this approach (148, 151). Specifically, gnotobiotic calves that had been immunized with bovine rotavirus strain NCDV in utero did not become ill when challenged at birth with a virulent human rotavirus strain, whereas most control calves developed diarrhea (148). A later study showed that gnotobiotic piglets infected with NCDV had a significant reduction in shedding upon subsequent challenge with various human rotavirus strains (152).

Indeed, the bovine strain NCDV was the first rotavirus vaccine candidate to be tested for efficacy in humans, followed by rhesus rotavirus (RRV) and then by another bovine strain, WC3. Studies involving these vaccine candidates are described below. The development of other vaccine candidates, such as human-animal rotavirus reassortants and human rotavirus strains, is also discussed. The oral route of vaccine administration has been used exclusively in human trials because of early animal studies suggesting the importance of local intestinal immunity (129).

TABLE 1. Efficacy trials of bovine rotavirus vaccine RIT4237

Study no.	Study site (reference)	Age (mo.) at vaccination	No. of doses[a]	No. of children given:		Efficacy (%) against rotavirus diarrhea		Predominant VP7 serotype
				Vaccine	Placebo	Any	Severe	
1	Finland (134)	8–11	1	86	92	50	88	NA[b]
2	Finland (132)	6–12	2	168	160	58	82	1
3	Finland (121, 130, 139)	0 (5–7 days)	1	123	121	0	100	1
4	Finland (140)	0, 7	2	124	128	43	89	1
5	Finland (121, 130)	0 (5–7 days)	1	123	122	0	23	1
6	Arizona (125)	2–5	1	106[c]	107	0	0	1
7	Gambia (57)	2–6	3	170	83	33	NA	NA
8	Rwanda (34)	3–8	1	122	123	0	0	NA
9	Peru (86)	2–18	1	96	100	0	0–63[d,e]	1
		2–18	2	96		0	15–59[d,e]	1
		2–18	3	99		40[d]	58–75[d,e]	1

[a] $10^{7.8}$ to $10^{8.3}$ $TCID_{50}$ per dose.
[b] NA, not available.
[c] A third group of 108 infants received RRV vaccine (10^4 PFU per dose), also without efficacy.
[d] Efficacy against "rotavirus-only" diarrhea, i.e., no other enteropathogens were isolated.
[e] Range indicates efficacy against various individual indicators of severity.

Bovine Rotavirus Vaccine Strain RIT4237

Derivation. The bovine rotavirus vaccine strain RIT4237 was derived from the Lincoln isolate of bovine rotavirus NCDV (99), which bears G6 and P6 serotype specificity; thus, neither of its major neutralization proteins, VP7 or VP4, is shared with the epidemiologically important human rotavirus strains, which are predominantly serotype G 1, 2, 3, or 4 and P 1A or 1B (61). The vaccine strain RIT4237 was cloned after 147 passages in fetal bovine kidney (FBK) cells, and after 149 passages, the virus was grown in *Cercopithecus* monkey kidney cells up to passage level 154 (33). Vaccine lots were lyophilized.

Safety, immunogenicity, and efficacy. In an initial study in Finland, the vaccine at a dose of $10^{8.1}$ 50% tissue culture infective doses ($TCID_{50}$) was shown to be safe and immunogenic in seronegative infants and young children, although neutralizing-antibody responses in these individuals were predominantly homotypic (133). Dose range studies in 4- to 6-month-old infants in Finland showed that a dose of $10^{8.3}$ $TCID_{50}$ was significantly more immunogenic than a dose of $10^{6.3}$ $TCID_{50}$, inducing an increase in the level of neutralizing antibody against the vaccine strain in 77 and 33% of the children, respectively (138). The difference in immunogenicity between a dose of $10^{8.3}$ $TCID_{50}$ and $10^{7.2}$ $TCID_{50}$ was not significant. This vaccine strain did not multiply effectively in the human intestine; it was detected in the stools of 21% of vaccinated children only after amplification in cell culture (135). The high passage level of RIT4237 was probably not necessary for its attenuation in humans in that low-passage NCDV also has been shown to be well tolerated (136).

Efficacy studies of RIT4237, which have used a dose of $10^{7.8}$ to $10^{8.3}$ $TCID_{50}$, have shown consistently that the vaccine is safe and is not significantly associated with any side effects; however, protective efficacy rates have been highly variable, ranging from 0 to 58% against rotavirus diarrhea in general and from 0 to 100% against severe rotavirus diarrhea (Table 1) (34, 57, 86, 121, 125, 130, 132, 134, 139, 140). In the first efficacy trial, Finnish infants 8 to 11 months of age received one dose of vaccine or placebo before the expected rotavirus season and were observed for two successive seasons (134). Serum antibody responses to vaccination were detected by immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA) in 50% of seronegative and 40% of seropositive vaccine recipients and 7 and 11% of placebo recipients who were seronegative or seropositive, respectively. The vaccine provided 50% protection against any rotavirus diarrhea and 88% protection against clinically significant rotavirus diarrhea. None of the rotavirus isolates was serotyped.

The next study, involving Finnish infants 6 to 12 months of age, was similar in design, except that two doses of vaccine or placebo were given 1 month apart (132). Antibody rises measured by an IgG ELISA were detected in 53% of seronegative and 23% of seropositive vaccine recipients and none of the placebo recipients. Protective efficacy was 58% against any rotavirus diarrhea and 82% against clinically significant rotavirus diarrhea. Of the rotavirus isolates that were classified, the majority were VP7 serotype 1. In this study, detectable serum antibody responses to vaccination appeared to correlate with better protection, although vaccinees without responses also derived some protection.

Subsequently, three studies were conducted with Finnish newborn infants (121, 139, 140). All infants received a dose of vaccine or placebo within a week of birth, and in one study, a booster was given at 7 months of age. In this latter study, serologic responses were detected by an IgM ELISA and homotypic neutralization assay in 36 and 45% of infants, respectively, after the first dose. After the second dose, 68% of vaccine recipients and 15% of placebo recipients had detectable rotavirus antibody by IgG ELISA (140). In these neonate studies, protective efficacy against any rotavirus diarrhea ranged from 0 to 43% and protection against severe rotavirus diarrhea ranged from 23 to 100% (130). Rotavirus strains isolated from patients with diarrhea were predominantly VP7 serotype 1.

No efficacy was demonstrated after one dose of RIT4237 vaccine in 2- to 5-month-old Native American infants in Arizona (125). This study also failed to show efficacy for one dose of RRV vaccine. Serologic responses by IgA ELISA were detected in 47% of RIT4237 vaccinees, 63% of RRV vaccinees, and 17% of placebo recipients. The rotavirus strains that were typed were predominantly VP7 serotype 1.

In The Gambia, 10-week-old infants were given three doses of vaccine or placebo at 1-month intervals, concurrently with oral polio vaccine; a third group of infants received three doses of rotavirus vaccine and inactivated poliovirus vaccine (57). All participating infants had received one or more doses of rota-

virus vaccine at least 1 month before the onset of the rotavirus season. A homotypic neutralizing-antibody response was detected in 45% of rotavirus vaccine recipients and 22% of placebo recipients. Serologic responses to rotavirus vaccination did not differ between the groups that received concurrent oral or inactivated poliovirus vaccine. The protective efficacy against any rotavirus diarrhea was 33%; the vaccine did not appear to afford greater protection against more severe cases, in contrast to the Finnish studies. None of the rotavirus isolates was serotyped, but the majority had a "short" RNA pattern by gel electrophoresis, suggesting that they were predominantly VP7 serotype 2. In Rwanda, one dose of vaccine in 3- to 8-month-old infants provided no protection. Serologic responses to vaccination were virtually nil in that antibody rises by IgG ELISA were found in 25% of vaccinees and 20% of placebo recipients.

In Peru, an efficacy trial comparing one, two, and three doses of vaccine was conducted with 2- to 18-month-old children; 77% of subjects were ≥6 months of age at the time of the first vaccination (86). Serologic responses measured by complement fixation assay were detected in 14 to 25% of subjects in the three vaccine groups and 6% of placebo recipients. Of a small subset of sera that were tested by neutralization assay with the vaccine strain, antibody rises were detected in 33% of the recipients of one dose of vaccine and 50% of the recipients of two or three doses. Three doses of vaccine had an efficacy of 40% in protecting against cases of rotavirus diarrhea in which no other enteropathogens were identified. Three doses provided between 58 and 75% protection against more severe cases of this "rotavirus-only" diarrhea. The majority of rotavirus isolates that were serotyped belonged to VP7 serotype 1 (52%) or serotype 2 (36%). Protective efficacy against serotype 1 was demonstrated, with a trend toward protection against serotype 2 in the three-dose group.

Thus, although RIT4237 vaccine did not protect against subsequent infection with human rotavirus, some degree of efficacy, especially against more severe cases of rotavirus diarrhea, was demonstrated in several trials (121, 132, 134, 140). The reason for the variable efficacy of RIT4237 is not known, although age at the time of vaccination, timing of vaccination with respect to the onset of the rotavirus season, and differences in vaccine immunogenicity among the trials may have been contributing factors.

The Finnish trials suggested that protective efficacy was greater in infants who were vaccinated or received a booster dose at ≥6 months of age and in close proximity to the onset of the rotavirus outbreak (121, 130). A decreased vaccine take has been proposed for the low or absent efficacy in the studies conducted in developing countries, although it is difficult to compare the immunogenicity among the efficacy studies because uniform assays to detect antibody were not used. It has also been suggested that higher levels of maternally acquired antibodies to rotavirus and frequent presence of enteroviruses in infants in developing countries may decrease the immunogenicity of the vaccine. Although concurrent administration of oral poliovirus vaccine has been shown to decrease the serum antibody response to RIT4237 vaccine in some studies (49, 143), this was not the case in the Gambian efficacy study, nor did the rotavirus vaccine interfere with seroconversion to poliovirus types 1 to 3 (57). Whether breast feeding may interfere with vaccine take has also been examined, but the serologic response rate has been only slightly lower in infants fed with breast milk than in those fed with formula prior to RIT4237 vaccination (153). The differences in efficacy cannot be explained on the basis of serotype specificity, in that VP7 serotype 1 was the predominant serotype identified in most of the efficacy studies.

Bovine Rotavirus Vaccine Strain WC3

Derivation. Bovine rotavirus vaccine strain WC3 was isolated from the diarrheal stool of a newborn calf in Pennsylvania. It was passaged once in African green monkey kidney (AGMK) cells and three times in CV1 cells, plaque purified twice in MA104 cells, and then serially passaged in CV1 cells up to passage level 12 (27). The vaccine used in early studies was made from this 12th passage level in CV1 cells, whereas subsequent vaccine lots were prepared by performing the last three passages of virus in tertiary rhesus monkey kidney cells before lyophilization (8). Strain WC3 belongs to VP7 serotype 6, as does bovine rotavirus NCDV; however, they are related only by a one-way cross in that anti-NCDV hyperimmune serum neutralizes WC3 to high titer but the converse is not the case (27). The VP4 specificity by neutralization of WC3 has not been reported, but with regard to genotype, it appears to be similar to UK virus and differs from NCDV (53). The vaccine was administered at a dose of 10^7 to $10^{7.5}$ PFU in the efficacy studies.

Safety, immunogenicity, and efficacy. This vaccine was shown to be safe, nonreactogenic, and immunogenic in infants 5 to 11 months of age (27). Serum neutralizing-antibody responses to the vaccine strain were detected in 95% of infants, whereas only 9.5% had a response to human rotavirus Wa, a VP7 serotype 1 strain. Vaccine virus shedding was detected after inoculation of stool specimens onto cell cultures in only 30% of infants.

As with RIT4237, efficacy studies of WC3 have shown highly variable rates of protection, although they have consistently shown the vaccine to be safe and well tolerated. In Pennsylvania, one dose of vaccine induced neutralizing-antibody responses to WC3 and Wa in 71 and 8%, respectively, of 3- to 12-month-old infants; shedding of vaccine virus was detected in 5% of infants. Although the vaccine did not prevent rotavirus infection, the protection rate was 76% against rotavirus diarrhea in general and 100% against more severe cases during a predominantly VP7 serotype 1 rotavirus season (24).

In a subsequent study in Ohio, 97% of infants 2 to 12 months of age developed a homotypic neutralizing antibody response after one dose of vaccine but only 7 to 9% developed heterotypic responses to human rotaviruses representing VP7 serotypes 1 to 4; 17% shed vaccine virus. However, no protection against any rotavirus diarrhea or the development of a more severe case was demonstrated during the ensuing predominantly VP7 serotype 1 rotavirus season (8). A third study in which two doses of WC3 were administered at a 1-month interval to 3-month-old infants in Bangui (Central African Republic) also failed to demonstrate efficacy against rotavirus diarrhea that was caused predominantly by VP7 serotype 1 (46). Neutralizing-antibody responses to the vaccine strain were detected in 61% of vaccine and 18% of placebo recipients.

As with RIT4237, a well-defined correlate of protection has not emerged, and therefore a clear explanation for the variable efficacy does not exist. Examination of the two efficacy trials in the United States provides no obvious clues for the disparate results. In both the Pennsylvania and Ohio trials, one dose of vaccine was administered to infants of similar ages. The vaccine induced primarily homotypic neutralizing-antibody responses in both sites and, if anything, was more immunogenic in the Ohio trial, in which it failed to protect against rotavirus

TABLE 2. Efficacy trials of RRV vaccine[a]

Trial no.	Study site (reference)	Age (mo) at Vaccination	No. of subjects given:		Efficacy (%) Against rotavirus diarrhea		Predominant VP7 serotype(s)
			Vaccine	Placebo	Any	Severe	
1	Maryland (118)	5–20	14	10	100[b]	NA[c]	NA
2	Sweden (52)	4–12	53	51	48	80	1
3	Venezuela (44, 112)	1–10	151	151	64	85	3
4	New York (21)	2–4	85	88	0	0	1
5	Arizona (125)	2–5	108[d]	107	0	0	1
6	Maryland (120)	2–11	63	49	29	~29	1
7	Finland (137)	2–5	100	100	38	67	1, 4
8	New York (93)	2–4	76[e]	73	66	NA	1

[a] One dose of 10^5 PFU in trials 1 and 2; one dose of 10^4 PFU in the remainder.
[b] Based on only three cases in the placebo group and none in the vaccine group.
[c] NA, not available.
[d] A third group of 106 infants received RIT4237 vaccine (10^8 $TCID_{50}$ dose), also without efficacy.
[e] A third group of 74 infants received human-RRV reassortant vaccine (D × RRV, VP7 serotype 1, 10^4 PFU dose), which had 77% efficacy.

diarrhea. Furthermore, VP7 serotype 1 strains predominated in both locations.

Rhesus Rotavirus Vaccine Strain MMU18006

Derivation. RRV vaccine strain MMU18006, which was isolated from the diarrheal stool of a rhesus monkey, was passaged nine times in primary or secondary monkey kidney cells and then seven times in semicontinuous diploid fetal rhesus lung (DBS-$FRhL_2$) cells (80). RRV belongs to VP7 serotype 3, one of the epidemiologically important human serotypes, but its VP4 (serotype 5B) is unrelated to that of human strains.

Safety, immunogenicity, and efficacy. A 10^4 PFU dose of vaccine was shown to be safe and immunogenic in the target population of 2- to 5-month-old infants, although in some studies, approximately one-third of infants developed a transient, low-grade fever 3 to 4 days after vaccination (1, 22, 75, 76, 78, 80, 113, 135, 146). At this dose, antibody responses were detected in 70% or more of infants and vaccine virus was isolated from stool by amplification in cell culture in approximately 59 to 75% of infants (90, 113). Neutralizing-antibody responses were predominantly homotypic in this young age group (54, 90).

A 10^5 PFU dose of RRV vaccine was shown to be unacceptably reactogenic in early studies in Finland and Sweden, where it was associated with a fever in 64 and 79% of infants, respectively (52, 135). These reactions occurred primarily in infants of 5 months of age or above, perhaps because of low levels of preexisting antibody to rotavirus in this age group (78, 119). In an early U.S. study, 50% of children 5 to 20 months of age who were given this dose of vaccine (and 15% of the controls) developed a fever 3 to 4 days following oral administration (89). Subsequent studies have shown that the higher dose is well tolerated in younger infants (110), possibly because of the presence of maternally derived humoral antibody to rotavirus (119). In contrast to the bovine rotavirus vaccine strains, which were usually given after a meal of breast milk or infant formula to neutralize stomach acidity, the RRV vaccine was administered after a meal of infant formula containing bicarbonate buffer in the majority of recipients.

Efficacy studies, most of which involved one 10^4 PFU dose of vaccine, have shown protection rates of 0 to 64% against rotavirus diarrhea (21, 44, 52, 112, 120, 125, 137) (Table 2). The 100% efficacy noted in the first trial in Maryland is not included in this range, because the small size of that trial made its findings suggestive only (118). Protective efficacy against moderate to severe cases of disease has been equally variable, ranging from 0 to 85%. A study in Venezuelan infants 1 to 10 months of age demonstrated the highest efficacy against rotavirus diarrhea. Antibody responses to the vaccine strain by tube neutralization assay were detected in 62% of vaccine recipients and 25% of placebo recipients. The vaccine was shown to induce homotypic protection, because strains isolated from patients with rotavirus diarrhea belonged predominantly to the same VP7 serotype as the vaccine (type 3). Heterotypic protection against VP7 serotype 1 was demonstrated in several trials (52, 93, 120, 137), usually with greater efficacy against more severe cases of rotavirus diarrhea; however, the RRV vaccine failed to provide any protection in two trials in which VP7 serotype 1 predominated (21, 125).

The immunogenicity of the vaccine in the trials that did not demonstrate efficacy was similar to that seen in trials that did demonstrate efficacy. It is of particular interest to compare the results of two trials (Table 2, trials 4 and 8) conducted in different years by the same institution in Rochester, N.Y. VP7 serotype 1 strains predominated in both trials; however, the RRV vaccine failed to induce protection against rotavirus diarrhea in the first trial (21) but provided 66% protection against such illness in the second trial (93). Following vaccination, rises in levels of neutralizing antibody to the vaccine strain were detected in 67% of RRV vaccinees in trial 4 and 65% of RRV vaccinees in trial 8. However, neutralizing-antibody responses to human rotavirus Wa (VP7 serotype 1) were detected in 19% of RRV vaccine and 10% of placebo recipients in the trial that demonstrated efficacy (trial 8), whereas in the trial in which no efficacy was shown (trial 4), only 5% of the RRV vaccinees and none of the placebo group developed such a seroresponse. The difference in the number of responses to VP7 serotype 1 was statistically significant (93) and suggested that a serotype 1 strain had been circulating around the time of vaccination during the second trial (trial 8).

One possibility for the great variation in heterotypic protection observed in the rotavirus trials, in general, is that infants who had been primed by previous rotavirus infections developed a broadened response after vaccination, thereby inducing heterotypic immunity, whereas naive infants developed primarily homotypic responses and homotypic protection. This hypothesis was consistent with the finding that heterotypic protection was more likely to be seen in trials involving older infants. It also provided an explanation for the vaccine efficacy observed in young infants during the second trial in Rochester, in which priming with a VP7 serotype 1 strain appeared to have taken place around the time of vaccination.

The inability to reproducibly induce heterotypic protection after vaccination with any of the animal rotaviruses (bovine strains RIT4237 and WC3 and simian strain RRV) suggested that serotype-specific immunity against each of the epidemiologically important human rotaviruses might be necessary for protection in young infants. This led to the initiation of clinical evaluation of a modified Jennerian approach, in which human-animal rotavirus reassortants that expressed the VP7 protein of serotypes 1 to 4 were used as the immunogens as described below.

Human-Rhesus Rotavirus Reassortant Vaccines

Derivation. The human-rhesus rotavirus reassortant vaccines were developed with the goal of combining the specificity of the epidemiologically important VP7 serotypes with the attenuation phenotype of RRV (100, 102). Human-RRV reassortant strains were recovered after coinfection of AGMK cell cultures with the RRV vaccine strain and human rotavirus strain D, DS-1, or ST3. The D (VP7 serotype 1) and DS-1 (VP7 serotype 2) strains were initially detected in stools of children hospitalized with diarrhea and were then passaged in gnotobiotic calves, whereas ST3 (VP7 serotype 4) was derived from the stool of an asymptomatic newborn infant and was isolated in AGMK cells. Reassortant strains D × RRV (clone 6-1-1), DS-1 × RRV (clone 240-2-1), and ST3 × RRV (clone 39-2-1) were selected as vaccine strains because their VP7 gene, representing serotypes 1, 2, and 4, was derived from their human rotavirus parents but the remaining 10 genes were derived from RRV. These reassortant strains exhibited the VP7 neutralization specificity of their human rotavirus parents. Reassortment with a human rotavirus VP7 serotype 3 strain was not necessary because RRV belongs to this serotype. Each of these strains was plaque purified three times in AGMK cells, and vaccine lots were subsequently prepared in DBS-FRhL$_2$ cells, a semicontinuous diploid cell strain developed as a suitable vaccine substrate by the predecessor of the Office of Biologics of the U.S. Food and Drug Administration (144).

Safety, immunogenicity, and efficacy. Monovalent preparations of human-RRV reassortant vaccines have behaved similarly to the RRV vaccine in the target population of infants under 6 months old with regard to safety, reactogenicity, immunogenicity, and shedding of vaccine virus (42, 56, 93, 131, 142). Three studies comparing the efficacy of monovalent reassortant and RRV vaccines have been completed (87, 93, 131). In Rochester, N.Y., 223 infants 2 to 4 months of age received a 10^4 PFU dose of D × RRV or RRV vaccine or placebo (93). On days 4 and/or 5 after vaccination, fever (≥38°C) was detected in a significantly greater percentage of vaccinees (15 to 21%) than placebo recipients (2 to 8%). Neutralizing-antibody responses to the vaccine strain were detected in 65% of RRV and 54% of D × RRV vaccinees. Increases in neutralizing-antibody responses to human rotavirus Wa (VP7 serotype 1) developed in 19% of RRV, 52% of D × RRV, and 10% of placebo recipients. During the ensuing rotavirus season, in which VP7 serotype 1 strains predominated, the RRV and D × RRV vaccines provided 66 and 77% protection, respectively, against rotavirus diarrhea. A possible explanation for the heterotypic protection in the RRV vaccine group was discussed in the previous section. Preexisting serum antibody or serologic response to vaccination was not correlated with protection.

A second study which included two monovalent reassortants and a placebo was conducted in Finland (131); in this study, 359 infants 2 to 5 months of age received one dose of D × RRV (10^4 PFU), DS-1 × RRV (10^5 PFU), or placebo. Fever, mostly low grade, occurred in a significantly greater percentage of vaccinees (21 to 25%) than placebo recipients (4%) within 7 days after vaccination. Increases in antibody titers in serum were detected by IgA ELISA in 61% of D × RRV, 75% of DS-1 × RRV, and none of the placebo recipients. Neutralizing-antibody responses were not measured. During the ensuing season, protection against rotavirus diarrhea, which was caused predominantly by VP7 serotype 1 strains, was noted in 67% of D × RRV and 66% of DS-1 × RRV vaccinees. In contrast to the study in Rochester (93) and earlier studies of RRV vaccine in Sweden and Venezuela (52, 112), this study showed that efficacy was associated with the serologic response to vaccination. Protection rates for infants who had a serologic response after vaccination with D × RRV or DS-1 × RRV vaccine were 100 and 85%, respectively. The heterotypic protection of the DS-1 × RRV vaccine (VP7 serotype 2) against serotype 1 strains is unexplained.

In a third study, 800 Peruvian infants 2 to 4 months of age were given one 10^4 PFU dose of D × RRV, DS-1 × RRV, or RRV vaccine or placebo (87). Fever was noted more frequently in recipients of RRV vaccine than in the other vaccine or placebo recipients (13 versus 7 to 9%). Serologic responses were detected by IgA ELISA in 50% of RRV, 50% of D × RRV, and 70% of DS-1 × RRV recipients and 18% of placebo recipients. Preliminary analysis after 2 years of surveillance showed that only RRV vaccine provided efficacy, with 30% protection against any rotavirus illness, although most of the strains isolated from patients with diarrhea were the heterotypic VP7 serotype 1 or 2. Perhaps the final analysis of serotype-specific immune responses will provide a better understanding of these results.

After phase 1 studies with monovalent reassortants were completed, studies of multivalent preparations were begun. A quadrivalent vaccine containing 10^4 PFU of each of the three human-RRV reassortants representing VP7 serotypes 1, 2, and 4 and of RRV (VP7 serotype 3) was shown to be safe and well tolerated in young infants, inducing a similar rate of febrile reactions to that noted with 10^4 PFU of RRV or monovalent reassortant vaccines (41, 43, 81, 110, 111). Although over 70% of vaccinated infants developed a serologic response by IgA ELISA, increases in levels of neutralizing antibody to each of the four VP7 serotypes contained in the vaccine were detected in only 32 to 58% of infants and 17 to 39% of infants in two studies in Venezuela (43, 111).

Subsequent studies of dose ranges of 10^4 to 10^6 PFU showed that a three-dose regimen of quadrivalent vaccine containing 10^5 or 10^6 PFU of each component was perhaps optimal. The safety and reactogenicity profiles were similar to those of the RRV vaccine. VP7 serotype 1- to 4-specific responses were induced in approximately one-third to one-half of vaccinees (41, 81, 110). Three doses of vaccine (10^5 or 10^6 PFU) induced a significantly greater number of seroresponses to each of the four serotypes than did single dose of these vaccines (41). As noted in some earlier studies, the neutralizing-antibody responses to RRV were usually in excess of 70%, suggesting that VP4 was more immunogenic than VP7.

The initial efficacy studies performed with the quadrivalent vaccine used a preparation that contained 10^4 PFU of each component. In a multicenter trial in the United States, 903 infants 2 to 6 months of age received three doses of the quadrivalent vaccine, D × RRV vaccine (10^4 PFU, VP7 serotype 1), or placebo (123). The only difference in side effects between the vaccine and placebo groups was that mild fevers occurred more commonly on day 4 after the first dose of quadrivalent vaccine (7 versus 3% for the placebo group). Protective efficacy against rotavirus diarrhea during the first

season, in which 95% of the rotavirus isolates were VP7 serotype 1, was 65% for the monovalent serotype 1 reassortant vaccine and 63% for the quadrivalent vaccine. Protective efficacy during the second season, in which 30% of the isolates were non-serotype 1, was 10% for the monovalent vaccine and 48% for the quadrivalent vaccine, suggesting that broader protection was offered by the quadrivalent vaccine.

In an efficacy study in Peru, approximately 600 infants were given one or three doses of quadrivalent vaccine (10^4 PFU of each component) or placebo at 2, 3, and 4 months of age (85). The vaccine was safe, and serologic responses by IgA ELISA were detected in 59% of one-dose recipients, 75% of three-dose recipients, and 24% of placebo recipients. However, neutralizing-antibody responses were detected in only 16 to 36% of a subset of vaccinees and were similar in the one- and three-dose groups. Preliminary analysis suggests that protective efficacy against strains that were predominantly VP7 serotype 1 or 2 is limited. It will be interesting to see if the efficacy observed in the U.S. multicenter trial was associated with a higher percentage of VP7 serotype-specific responses to vaccination than was observed in the Peruvian trial. An efficacy trial with the 10^4 PFU dose of quadrivalent vaccine has also been conducted in Brazil, but the results are not yet available.

On the basis of the results of the dose-range studies, efficacy trials with a quadrivalent vaccine containing 10^5 PFU of each component were initiated in young infants in Burma, the United States, and Venezuela. Three doses of vaccine were administered in these studies, because the World Health Organization has recommended that if rotavirus vaccines become available, they ultimately must be administered on the same schedule used for oral poliovirus vaccines. The results of the Venezuelan trial will be particularly interesting in that this study has been designed to assess the efficacy of the vaccine in preventing dehydrating illness rather than rotavirus diarrhea in general.

The results of the U.S. trial with 10^5 PFU of each component of the quadrivalent vaccine have recently been reported (35). In a three-cell trial in 24 centers, the quadrivalent vaccine, a monovalent D × RRV (VP7 serotype 1) vaccine, or a placebo was given to 1,278 infants at approximately 2, 4, and 6 months of age. The vaccines were safe and well tolerated, with a fever of >38°C (axillary) being observed significantly more often on day 4 after the first dose of the quadrivalent vaccine when compared with the placebo (2.2 versus 0.2%). The relative efficacies of the quadrivalent and monovalent vaccines against rotavirus diarrhea were 49 and 54%, respectively, over a single rotavirus season. When illnesses were scored according to severity, the quadrivalent vaccine demonstrated 80% efficacy and the monovalent vaccine demonstrated 69% efficacy when compared with the placebo group.

Human-Bovine Rotavirus Reassortant Vaccines

Bovine rotavirus WC3 as donor strain. In human-bovine rotavirus reassortant WI79-9, the gene segment encoding the VP7 protein is derived from human rotavirus WI79, a VP7 serotype 1 strain, and the remainder of its genes are derived from bovine rotavirus strain WC3 (25). In an efficacy trial involving 77 U.S. infants 2 to 11 months of age, the reassortant was shown to be safe and not associated with any side effects. After two doses of approximately 10^7 PFU, neutralizing-antibody responses to WC3 and WI79 were detected in 97 and 22% of individuals, respectively, suggesting that the VP4 protein might be more immunogenic than the VP7 protein. However, 100% efficacy was observed against rotavirus diarrhea, which was caused by VP7 serotype 1 or 3 strains in this trial. Additional reassortants that contain the VP4 gene of the human rotavirus WI79 or both the VP4 and VP7 genes of WI79 against a WC3 background have also been generated (28). Trials with these reassortants are under way.

Bovine rotavirus UK as donor strain. Reassortants whose VP7 gene is derived from human rotavirus strains D, DS-1, P, or ST3, which represent VP7 serotypes 1, 2, 3, and 4, respectively, and whose remaining genes are derived from bovine rotavirus strain UK have also been generated (100, 102). Phase 1 studies of these vaccine candidates are currently in progress. Other potential vaccine candidates include those whose VP4 gene is derived from a human rotavirus strain representing VP4 serotype 1A. Such a reassortant could be used in combination with reassortants that represent VP7 serotype 1 to 4 and might induce broadly cross-reactive antibody responses. Another reassortant, whose VP4 protein is derived from Wa (VP4 serotype 1A) and whose VP7 protein is derived from DS-1 (VP7 serotype 2) but whose remaining genes are derived from UK, has been characterized (64) and might potentially induce immunity against the four prevalent types of human rotaviruses.

Human Rotavirus Vaccine Strains

Strain M37, a VP7 serotype 1 human rotavirus, was isolated from a newborn infant in Venezuela, who was asymptomatically shedding rotavirus. This strain belongs to VP4 serotype 2, a serotype that includes many VP7 serotype 1, 2, 3, and 4 strains isolated from asymptomatic neonates (51). The impetus for using neonatal strains was that they appeared to be naturally attenuated and a natural-history study had shown that asymptomatically infected neonates subsequently had a reduced frequency and severity of rotavirus diarrhea (12).

Phase 1 studies in which 10^4 and 10^5 PFU doses of strain M37 were used showed that the vaccine was safe and moderately immunogenic (43, 101, 141); a febrile episode was detected in 18% of the vaccinees receiving the 10^5 PFU dose and in 6% of the controls during the week following oral administration (141). Neutralizing-antibody responses in young infants occurred more frequently to the M37 strain than to Wa virus, suggesting that the VP7 responses were primarily strain specific rather than VP7 serotype 1 specific (101). In a small efficacy study of 203 Finnish infants 2 to 6 months of age, the 10^4 PFU dose provided no protection against predominantly VP7 serotype 1 strains. A 10^5 PFU dose, evaluated in the same study, was more immunogenic, but further efficacy studies with this vaccine candidate have not been pursued. No studies with the neonatal strain that provided protection in the natural-history study noted above have been reported.

Other approaches to the development of human rotavirus vaccines include cold adaptation of human rotavirus strains or of reassortants between human rotaviruses. A VP7 serotype 1 human rotavirus that was adapted to grow at 26°C was described initially (96). More recently, cold-adapted reassortant viruses that belong to VP7 serotypes 1 and 2 and reassortants whose VP7 serotype 2 or 3 gene is derived from human rotavirus DS-1 or P, respectively, and whose remaining genes, including the gene encoding VP4 serotype 1A, are derived from human rotavirus Wa have been generated (63, 109). The rationale for using these strains as vaccine candidates is that their VP4 and VP7 neutralizing proteins are both of human rotavirus origin but these strains are likely to attenuated by virtue of cold adaptation.

Recombinant or Subunit Proteins

No vaccine candidates based on recombinant technology have been developed yet for human use. Attempts to express recombinant rotavirus proteins have focused on VP7 and VP4 because of their importance in inducing both passive and active immunity in animal models. VP7 or VP4 genes of simian, bovine, and human rotavirus strains have been expressed by using different bacterial and viral vectors (3, 4, 6, 39, 91, 92, 98, 105, 117, 124). Most rotavirus recombinants lack or have limited immunogenicity when inoculated into experimental animals, although baculovirus-expressed VP4 and certain vaccinia-rotavirus VP7 recombinants have induced protection in mice (2, 92).

CONCLUSIONS

Rotavirus vaccine development has focused on the delivery of live attenuated rotavirus strains by the oral route. The initial Jennerian approach involving bovine (RIT4237, WC3) or rhesus (RRV) rotavirus vaccine candidates showed that these vaccines were safe, well tolerated, and immunogenic; however, RRV was more reactogenic than the bovine strains, and in studies in which it was directly compared with RIT4237, it also was more immunogenic (125, 135). The rotavirus vaccines of animal origin were similar in that they induced highly variable rates of protection against rotavirus diarrhea caused by heterotypic strains. In those trials that demonstrated vaccine efficacy, there was generally greater protection against more severe cases of rotavirus diarrhea; rotavirus infection was not prevented by vaccination. It also appeared that vaccination was more likely to induce heterotypic protection in older infants or those primed by previous infection, perhaps because neutralizing-antibody responses in young infants are predominantly homotypic.

It is clear that the goal of a rotavirus vaccine is not to prevent rotavirus infection or mild illness but, rather, to prevent severe illness that can lead to dehydration in infants and young children in both developed and developing countries. This is a realistic goal, because following a naturally occurring rotavirus infection, reinfections are common. However, the consequences of reinfection are less severe than the initial infection (72). The strategies being pursued with live, orally administered attenuated vaccines are identical in both developed and developing countries.

The results of studies with the monovalent vaccine led to the concept that a multivalent vaccine that represented each of the four epidemiologically important VP7 serotypes might be necessary to induce protection in young infants, the target population for vaccination. Human-animal rotavirus reassortants whose gene encoding VP7 was derived from their human rotavirus parents but whose remaining genes were derived from the animal rotavirus parent were developed as vaccine candidates. The most extensive experience with a multivalent vaccine to date has been gained with the quadrivalent preparation containing RRV (VP7 serotype 3) and human-RRV reassortants of VP7 serotype 1, 2, and 4 specificity. Preliminary trial results demonstrated efficacy for this vaccine candidate in two multicenter U.S. studies, but the results from a study in Peru showed limited protection.

Human-bovine reassortant vaccines, including a candidate that contains the VP4 gene of a human rotavirus (VP4 serotype 1A), are also being studied. When the studies of the RRV-based quadrivalent vaccine and other vaccine candidates currently undergoing testing are fully analyzed, perhaps immunologic correlates of protection will emerge that will help in the future direction of vaccine development.

Other issues that must also be addressed are how to facilitate the administration of vaccine. Because of the acid lability of rotaviruses, vaccine has most often been given after oral ingestion of infant formula containing buffer. Microencapsulation of rotavirus vaccines might circumvent the need to buffer gastric acidity. The compatibility of rotavirus and oral poliovirus vaccines will have to be demonstrated, because these vaccines would ultimately be administered simultaneously as part of any routine immunization program. Limited studies have not shown interference between RRV-based rotavirus and poliovirus vaccines (59, 67, 70) but have shown a decreased response to bovine rotavirus strain RIT4237 when this vaccine was given simultaneously with poliovirus vaccine (49, 143).

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