INFECTION AND IMMUNITY, May 1980, p. 344-349
0019-9567/80/05-0344/06$02.00/0

Vol. 28, No. 2

Passive Immunity in Calf Rotavirus Infections: Maternal Vaccination Increases and Prolongs Immunoglobulin G1 Antibody Secretion in Milk

DAVID R. SNODGRASS,[1]* KEVIN J. FAHEY,[1] PETER W. WELLS,[1] IRIS CAMPBELL,[1] AND ALEXANDER WHITELAW[2]

Animal Diseases Research Association, Moredun Institute, Edinburgh EH17 7JH, Scotland,[1] and Hill Farming Research Organisation, Penicuik, Midlothian, Scotland[2]

Ten heifers were inoculated on two occasions with an inactivated preparation of tissue culture-grown calf rotavirus, and a further ten heifers received a placebo vaccine. Serum anti-rotavirus antibody titers were significantly increased throughout pregnancy in the vaccinated group. After calving, the mean neutralizing antibody titer of colostral whey in control cows was 100, associated with immunoglobulins A and G1. No antibody was detected in the milk of these cows after the 4th day postpartum. The colostral whey from the vaccinated cows had a mean antibody titer of 20,452; 28 days after calving, the mean milk antibody titer was 320, associated mainly with immunoglobulin G1. Calves were challenged with a large oral inoculum of calf rotavirus at the 7th day of age. There was significant lengthening of the incubation and prepatent periods in calves born to vaccinated dams, but rotavirus-associated diarrhea of equal severity occurred in both groups. Evidence is presented which suggests that rotavirus antibody in milk can protect against a smaller challenge dose. Maternal immunization against rotavirus may be a practical proposition.

It has been shown experimentally that passively acquired antibodies from either serum or colostrum can protect young animals against diarrhea caused by rotavirus infections (2, 4, 12).

The practical exploitation of this passive protection is to stimulate the dam to produce in her colostrum and milk high titers of antibodies to rotavirus. Maternal vaccination has been used to protect piglets against transmissible gastroenteritis (1, 9), but has not been used in enteric virus infections of ruminants. In a preliminary experiment in sheep, titers of milk antibodies to rotavirus were successfully elevated for the first 10 days after lambing (14). The work reported in this paper aimed to repeat that experiment in cattle, to follow milk antibody for a longer period and in more detail, and to observe the effects of rotavirus infection on calves born to vaccinated and control dams.

MATERIALS AND METHODS

Animals. Ten Hereford × Friesian and ten Blue-Grey (Shorthorn × Galloway) 2-year-old heifers were allocated to two comparable groups by breed and preexisting serum neutralizing antibody titers to rotavirus. Ten heifers were vaccinated and ten received a placebo vaccine. All were mated naturally commencing 2 weeks after initial vaccination. They were revaccinated in a similar manner 7 months later, approximately 2 to 3 months before calving. Nine control and seven vaccinated heifers produced live calves at term.

Immediately after calving, cows and their calves were moved to separate clean accommodation. On the 7th day postpartum, each cow and her calf were again moved to separate housing, and the calves were infected orally with calf rotavirus. No contact was allowed between this postchallenge group and the animals at earlier stages of the experiment. A harness for total fecal collection was put on all male calves on the 7th day (seven bull calves born to control cows and three born to vaccinated cows).

Rotavirus. For vaccine preparation, tissue culture-adapted calf rotavirus was used (2). Virus was treated with trypsin (10 μg/ml) for 1 h at 37°C and then was inoculated onto bovine embryo kidney (BEK) cells with trypsin (10 μg/ml) included in the maintenance medium (13). Rotavirus at the sixth and ninth passage in our laboratory was used for the first and second vaccination, with titers prior to inactivation of $10^{4.8}$ and $10^{7.8}$ $TCID_{50}$ (50% tissue culture infective doses) per ml, respectively. The virus was inactivated by overnight incubation with 0.5% formaldehyde at 4°C. Equal volumes of calf rotavirus and incomplete Freund adjuvant (Difco Laboratories) were emulsified, and 2.0 ml of the emulsion was inoculated by deep intramuscular injection into the neck. The placebo vaccine was identically treated control BEK cultures.

The challenge calf rotavirus was intestinal contents from the eighth gnotobiotic calf passage, obtained from J. C. Bridger, Institute for Research on Animal Diseases, Compton, England. Volumes of 2 ml of in-

testinal contents containing 10^{11} particles per g, diluted in 10 ml of phosphate-buffered saline, were used as an oral challenge in calves. The vaccine and the challenge rotavirus were both derived from the same origin (2). No virus other than rotavirus was detected by electron microscopic examination of this inoculum.

Observations. Each heifer was bled for serum at intervals throughout pregnancy, at parturition, and 28 days later. Colostrum and milk samples were collected on 1, 2, 3, 4, 6, 8, 10, 14, 21, and 28 days after calving, the first sample being obtained within 8 h of calving, and where possible before the calf had sucked. Samples of feces were collected. Total feces collected from the postchallenge bull calves were examined daily for total fecal output and were dried to constant weight for dry-matter estimation. All calves were examined clinically at least once a day.

Neutralization test. Serum samples, and whey from colostrum and milk samples, were tested for the presence of neutralizing antibody to tissue culture-adapted calf rotavirus on BEK cells or Vero cells grown in microtiter plates (11). Titers are expressed as the reciprocal of the highest dilution giving complete neutralization.

Feces examination. Fecal samples were examined for rotavirus by counterimmunoelectroosmophoresis (6). The antiserum used was prepared by inoculation of rabbits with calf rotavirus purified by centrifugation on cesium chloride density gradients.

Additional fecal samples were taken at least once from each calf while scouring and were examined by electron microscopy to detect rotavirus and other viruses. At the same time, the feces were examined bacteriologically. Three *Escherichia coli* isolates from each calf were grown in Minca medium (3) and tested for the presence of K99 antigen by slide agglutination.

Fractionation and analysis of whey. Equal volumes of whey from individual vaccinated cows on the 1st, 3rd, 6th, 14th, and 28th days after calving were pooled. Whey from control cows was similarly pooled on the 1st and 3rd days after calving. Two milliliters of pooled whey from day 1 or 5 ml of whey obtained at the other times was loaded onto a 2.6 by 82 cm column of S300 (Pharmacia Fine Chemicals, Inc.) and eluted with 0.1 M tris(hydroxymethyl)aminomethane-hydrochloride–1.0 M NaCl buffer (pH 8.0) at 16 ml/h. Every other 6-ml fraction was concentrated to 2 ml by dialysis against Carbowax PEG, 20 M (Union Carbide Corp.), and phosphate-buffered saline. The fractions were assayed for both virus neutralizing antibody and the class and relative concentration of immunoglobulin. Immunoglobulins were analyzed by single-radial immunodiffusion using monospecific rabbit anti-sheep immunoglobulin sera (10), which cross-reacted with the respective bovine immunoglobulins. In the absence of standard bovine immunoglobulin preparations, the results are calculated and displayed as the relative concentrations of each immunoglobulin in the fractions.

Fractions from the immunoglobulin G (IgG) region of the S300 whey fractionation from the control cows on day 3 and from the vaccinated cows on days 3 and 14 were pooled, concentrated, dialyzed, and loaded onto a 1.5 by 25 cm column of DE52 (Whatman, Inc.) and eluted with 0.01 M phosphate buffer, pH 7.6, and then 0.03 M NaCl–0.01 M phosphate buffer, pH 7.6, at 30 ml/h. The eluates from each step were pooled, concentrated, and assayed for antibody activity. The pools were analyzed for immunoglobulin class by immunoelectrophoresis using monospecific anti-sheep immunoglobulin sera.

RESULTS

Effect of vaccination on serum antibody. Vaccination significantly raised the serum neutralizing antibody titers of the heifers from a geometric mean titer of 63 on the day of vaccination to a peak titer of 11,910 by 1 month after initial vaccination (Fig. 1). These titers waned throughout pregnancy and were increased by revaccination to a mean titer of 3,929. Immediately prior to parturition, at 9 months after vaccination, the mean titers in the vaccinated and control group were 3,929 and 74, respectively.

Effect of vaccination on milk antibody. Whey prepared from the first colostrum sample from control cows had a geometric mean neutralizing antibody titer of 100 (Fig. 2). No anti-rotavirus antibody was detected in whey from control cows after the 4th day of milking. First colostrum whey from vaccinated cows had a mean antibody titer of 20,452, which by 28 days after calving had declined to 320.

Analysis of colostral and milk antibody. Fractionation of whey obtained from control cows on the day of calving showed the peak antibody activity in fractions containing mainly IgA and IgG1, the titer decreasing in fractions containing IgG1 alone (Fig. 3). In vaccinated cows the titer of antibody in fractions of day 1

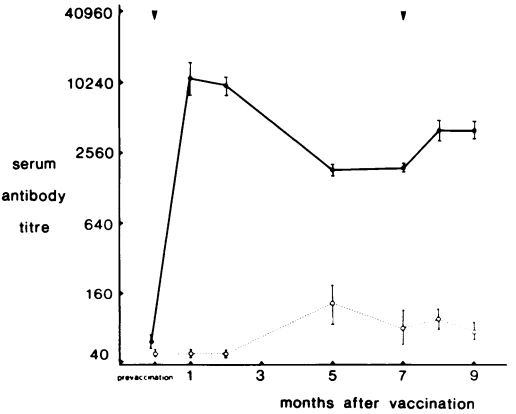

FIG. 1. *Serum neutralizing antibody titers (mean ± standard error) of rotavirus-vaccinated heifers (solid line) and placebo-vaccinated heifers (dotted line). Arrows indicate times of vaccination.*

whey containing IgA and IgG1 was substantially increased, although the peak antibody activity appeared in later fractions, some of which contained only IgG1 (Fig. 4). IgG2 was not detected in any of the fractions.

By 14 to 28 days after calving, the antibody activity in the milk from vaccinated cows was mainly in the IgG1 region of the elution profile. This was very pronounced by day 28, at which time antibody activity was almost exclusively associated with IgG1 (Fig. 5).

Fractionation of the IgG region from the S300 columns by anion-exchange chromatography confirmed the very low titers of IgG2 antibody in whey obtained 3 and 14 days after calving and demonstrated that activity was in the fraction containing IgG1 (Table 1).

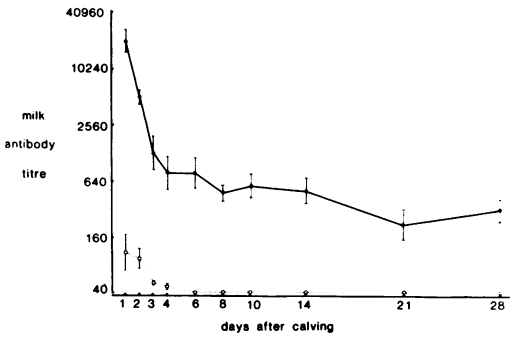

FIG. 2. *Neutralizing antibody titers (mean ± standard error) in whey from colostrum and milk of vaccinated (solid line) and control (dotted line) heifers after calving.*

Effect of vaccination on rotavirus infection in calves. After challenge, all nine calves born to control cows were observed to develop diarrhea, after a mean incubation period of 3.0 days (Table 2). Five of these calves became dull and clinically dehydrated, one requiring oral fluid replacement therapy. Most calves scoured for 3 to 5 days, and all recovered and subsequently thrived. Five of the seven calves born to vaccinated cows also developed diarrhea, after a mean incubation period of 4.8 days. Four of these five became dull, and the severity of reaction was indistinguishable from that of the controls.

Effect of vaccination on virus excretion. No virus excretion was detected by counterimmunoelectroosmophoresis in any calf before challenge. Rotavirus excretion was detected after infection in all calves born to control cows, and in six of the seven born to vaccinated cows. The prepatent period to virus excretion in the vaccinated calves was 5.2 days compared with 2.0 days for the controls ($P < 0.01$) (Table 2). There was no difference between the two groups in duration of virus excretion. No virus other than rotavirus and no pathogenic bacteria or *E. coli* with K99 antigen were detected in any calf.

Effect of vaccination on feces measurement. The weight of feces produced by the bull calves increased from a normal level of less than 200 g daily to a mean maximum after infection

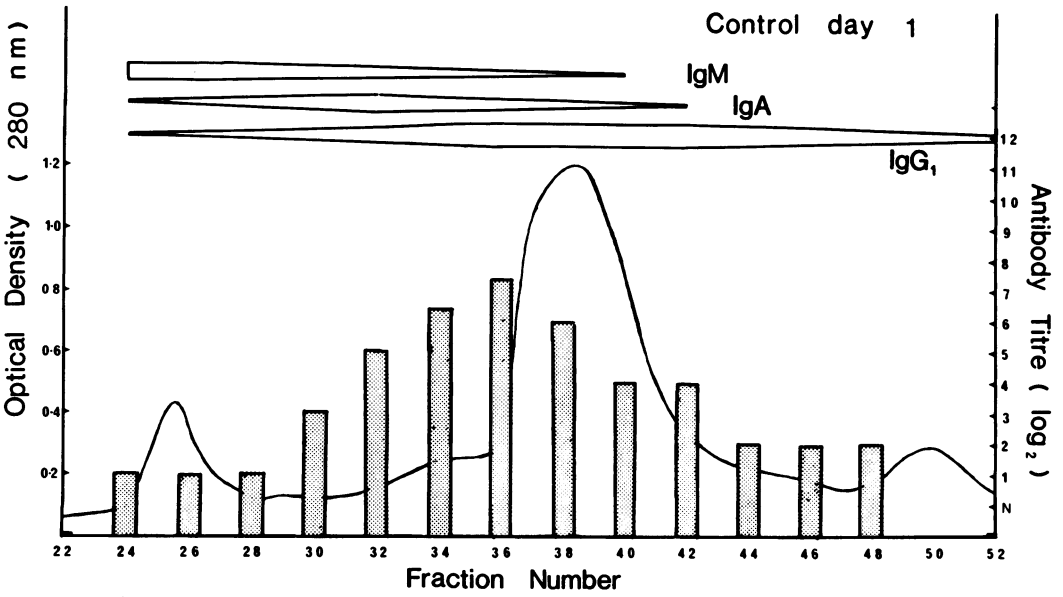

FIG. 3. *Fractionation on S300 of pooled whey from day 1 control cows. Optical density profile. Histograms: neutralizing antibody titer. Bars: relative immunoglobulin concentrations.*

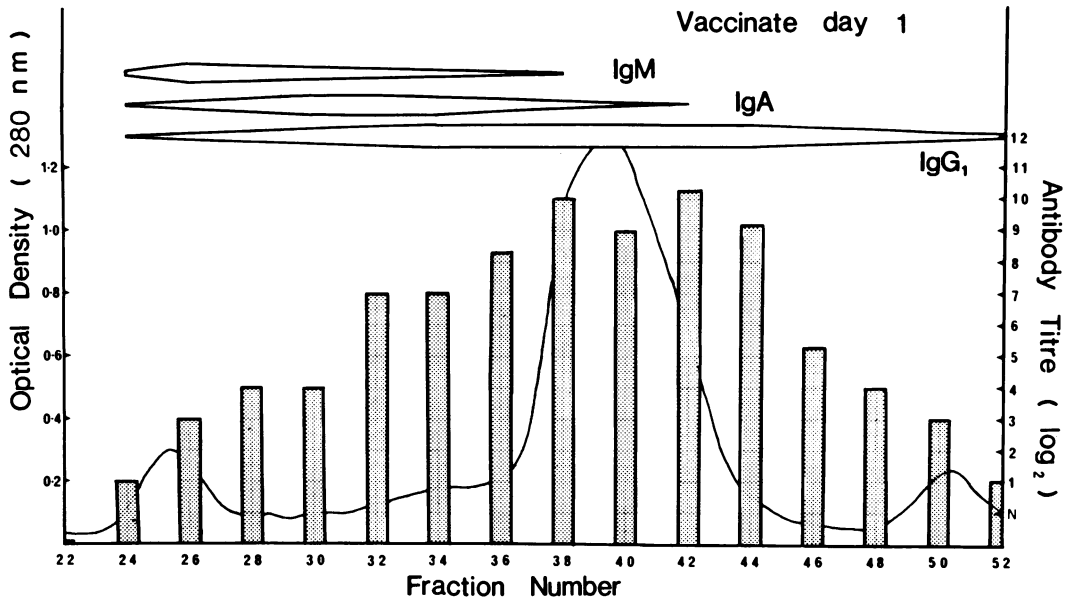

FIG. 4. *Fractionation on S300 of pooled whey from day 1 vaccinated cows. Optical density profile. Histograms: neutralizing antibody titer. Bars: relative immunoglobulin concentrations.*

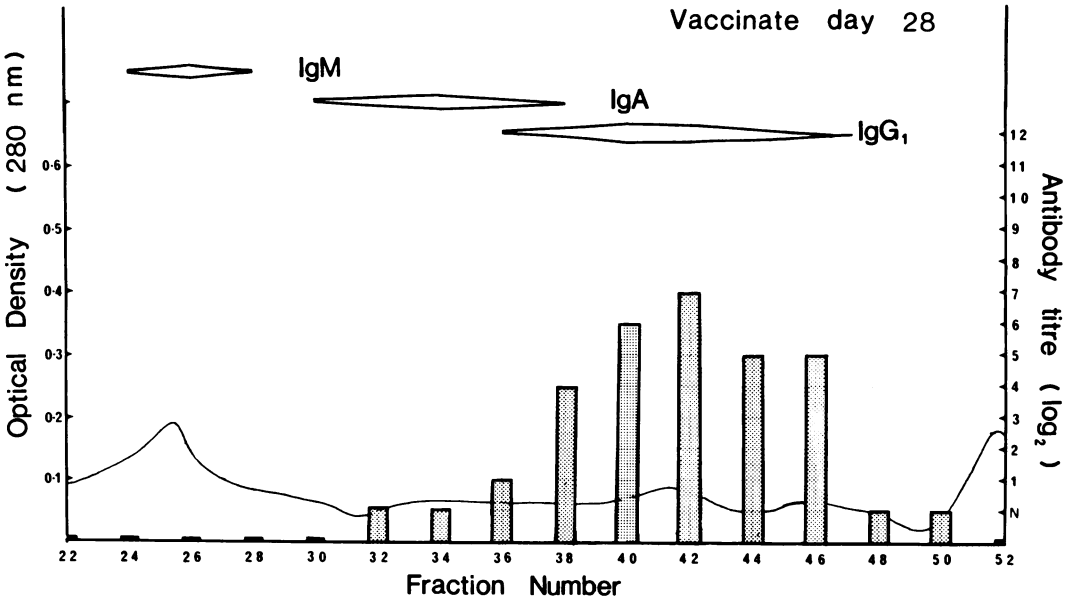

FIG. 5. *Fractionation on S300 of pooled whey from day 28 vaccinated cows. Optical density profile. Histograms: neutralizing antibody titer. Bars: relative immunoglobulin concentrations.*

of 1,888 g in the control calves and 1,868 g in the vaccinated calves (Table 2). The dry-matter content of the feces before infection was greater than 20%, and this fell to a mean minimum of 12% in both groups. There were significant delays in the increase in fecal weight and decrease in dry matter (1.8 days) in the vaccinated calves compared with the controls ($P < 0.01$).

DISCUSSION

Vaccination of the heifers with an inactivated calf rotavirus preparation significantly increased

TABLE 1. *Antibody titers in DE52 fractions of bovine IgG*

Source	Original 7S pool	IgG2 pool	IgG1 pool
Control, day 3	32	4	64
Vaccinate, day 3	2,560	64	2,048
Vaccinate, day 14	256	16	256

TABLE 2. *Reaction of calves to rotavirus challenge*[a]

Parameter	Controls	Vaccinates	Significance
No. with diarrhea	9/9	5/7	NS
No. clinically dull	5/9	4/7	NS
Days virus detected	3.7 ± 0.5	3.0 ± 0.9	NS
Maximum fecal output (g/day)	1,888 ± 146	1,868 ± 96	NS
Minimum fecal dry matter (%)	11.8 ± 1.9	11.7 ± 1.2	NS
Onset of diarrhea (days)	3.0 ± 0.5	4.8 ± 0.7	$P < 0.01$
Onset of virus shedding (days)	2.0 ± 0.3	5.2 ± 0.9	$P < 0.01$

[a] The first five parameters relate to severity of reaction; the last two, to timing of reaction. Mean ± standard error. NS, Not significant.

serum antibody titers throughout pregnancy. There was also a marked effect on anti-rotavirus milk antibody levels during subsequent lactation, with high titers being detected in clostrum and milk throughout the 28-day observation period.

Although colostrum from control heifers contained anti-rotavirus antibody, which was largely IgA, no specific antibody was detected after the first 4 days of lactation. Porter (8) made similar observations on anti-*E. coli* antibody in cows' milk. IgA antibody was also present at similar or elevated levels in colostrum from the vaccinated heifers, but in addition their colostrum contained high titers of IgG1 anti-rotavirus antibody. From 2 days after calving, the anti-rotavirus antibody detected in the milk of the vaccinated heifers was predominantly IgG1, this antibody arising by selective transfer from serum (7). IgG2 was present in milk at very low concentrations and contained little antibody activity.

As rotavirus infections are endemic in cattle, most adult cows will have been naturally infected. The sustained antibody response in serum and milk of vaccinated cows may have been due, in part, to this primary gut exposure prior to systemic vaccination (1).

The effect of increased milk antibody on rotavirus diarrhea in the calves was to delay the establishment of infection, but not to reduce its ultimate severity. The lengthened lag phase suggests that the greater part of the inoculum was neutralized before it could infect the gut, but that sufficient viable virus survived to infect the calves subsequently. The lag and wide variability of rotavirus shedding by the vaccinates, as compared to the controls, was also noted. This is possibly due to the continuous ingestion of high levels of milk antibody by the calf of a vaccinated cow which neutralizes the virus resulting from the infection. Eventually, the amount of virus produced in the gut exceeds the amount of antibody ingested, which is then observed in the feces. Alternatively, the anorexia, which often accompanies diarrhea, reduces the uptake of milk antibody by the calf. Once this happens, there would be very little difference between calves suckling vaccinated or control cows. A high challenge dose of virus was chosen deliberately, as difficulties were expected in establishing rotavirus infections in conventional sucking calves (5). It is unlikely that calves under farm conditions will ingest at any one time a 2-g bolus of feces containing a high titer of rotavirus. Calves sucking vaccinated cows may have been protected against a smaller challenge of rotavirus.

Direct evidence in support of this view comes from experiments with lamb rotavirus (K. J. Fahey and D. R. Snodgrass, unpublished data). Pooled milk taken from six control ewes 6 days after lambing was fed to two gnotobiotic lambs which, after rotavirus challenge, developed diarrhea and excreted rotavirus. By contrast, pooled milk taken from five vaccinated ewes (14) 6 days after lambing completely protected two gnotobiotic lambs against clinical and virological signs of rotavirus infection. This experiment demonstrated that the titer of antibodies present in milk obtained from vaccinated dams 6 days after parturition was sufficient to protect completely against a moderate challenge dose of rotavirus. This emphasizes the importance of using carefully titrated moderate challenge inocula in passive immunization experiments, where only a finite amount of antibody and hence protection can be present.

It is postulated that the technique of dam vaccination against rotavirus will stimulate IgG1 neutralizing antibodies in the circulation of the dam which are passively transferred to the milk for a substantial period after calving, and which may protect calves against natural infection under field conditions.

ACKNOWLEDGMENTS

We thank Janice Bridger for the gnotobiotic calf intestinal contents, A. Dawson for fractionating bovine wheys, and M. McLauchlan for the statistical analyses. Technical help was received from Margaret Gordon and help in handling the cattle, from G. Newlands.

LITERATURE CITED

1. **Bohl, E. H., and L. J. Saif.** 1975. Passive immunity in transmissible gastroenteritis of swine: immunoglobulin characteristics of antibodies in milk after inoculating virus by different routes. Infect. Immun. **11**:23–32.
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. **Guinee, P. A. M., W. H. Jansen, and C. M. Agterberg.** 1976. Detection of the K99 antigen by means of agglutination and immunoelectrophoresis in *Escherichia coli* isolates from calves and its correlation with enterotoxigenicity. Infect. Immun. **13**:1369–1377.
4. **Lecce, J. G., M. W. King, and R. Mock.** 1976. Reovirus-like agent associated with fatal diarrhea in neonatal pigs. Infect. Immun. **14**:816–825.
5. **Logan, E. F., G. R. Pearson, and M. S. McNulty.** 1979. Quantitative observations on experimental reo-like virus (rotavirus) infection in colostrum-deprived calves. Vet. Rec. **104**:206–209.
6. **Middleton, P. J., M. Petric, C. M. Hewitt, M. T. Szymanski, and J. S. Tam.** 1976. Counter-immunoelectro-osmophoresis for the detection of infantile gastroenteritis virus (orbi-group) antigen and antibody. J. Clin. Pathol. **29**:191–197.
7. **Newby, T. J., and J. Bourne.** 1977. The nature of the local immune system of the bovine mammary gland. J. Immunol. **118**:461–465.
8. **Porter, P.** 1972. Immunoglobulins in bovine mammary secretions. Quantitative changes in early lactation and absorption by the neonatal calf. Immunology **23**:225–238.
9. **Saif, L. J., and E. H. Bohl.** 1979. Passive immunity in transmissible gastroenteritis of swine: immunoglobulin classes of milk antibodies after oral-intranasal inoculation of sows with a live low cell culture-passaged virus. Am. J. Vet. Res. **40**:115–117.
10. **Smith, W. D., A. McL. Dawson, P. W. Wells, and C. Burrells.** 1975. Immunoglobulin concentrations in ovine body fluids. Res. Vet. Sci. **19**:189–194.
11. **Snodgrass, D. R., and J. A. Herring.** 1977. The action of disinfectants on lamb rotavirus. Vet. Rec. **101**:81.
12. **Snodgrass, D. R., and P. W. Wells.** 1978. Passive immunity in rotaviral infections. J. Am. Vet. Med. Assoc. **173**:565–568.
13. **Theil, K. W., E. H. Bohl, and L. J. Saif.** 1978. Techniques for rotaviral propagation. J. Am. Vet. Med. Assoc. **173**:548–551.
14. **Wells, P. W., D. R. Snodgrass, J. A. Herring, and A. M. Dawson.** 1978. Antibody titres to lamb rotavirus in colostrum and milk of vaccinated ewes. Vet. Rec. **103**: 46–48.