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Biochemical Studies on a Reovirus-Like Agent (Rotavirus) from Lambs

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Ten polypeptides were detected in double-capsid lamb rotavirus; four of these appeared to be associated with the outer capsid. Lamb rotavirus RNA, which consisted of 11 or 12 segments, differed from pig rotavirus RNA in the electrophoretic mobility of one of the genome segments.

Reo-like viruses or rotaviruses (3) have been associated with diarrhea in a wide variety of animal species (1). Biochemical investigations with calf (6, 7), pig (10), and human (8) rotaviruses have shown that the genome is double-stranded RNA consisting of 11 or 12 segments with molecular weights in the range 2.2×10^6 to 0.2×10^6. Calf and human rotaviruses contain two major and up to seven minor polypeptides (2, 6, 7). Although rotaviruses have also been isolated from lambs (4a, 9), these viruses have not yet been characterized biochemically. In the experiments reported in this paper, we analyzed the RNA and polypeptides of a rotavirus from lambs.

Lamb rotavirus was purified from the feces of colostrum-deprived lambs that had been experimentally infected with diarrheic feces from lambs with naturally occurring rotavirus infections (4a). Additional fecal material containing lamb rotavirus was kindly provided by D. R. Snodgrass of a Moredun Institute, Edinburgh, Scotland. Pig rotavirus was purified from the feces of hysterectomy-derived piglets that had been experimentally infected with pig rotavirus (M. S. McNulty, G. R. Pearson, J. B. McFerran, D. S. Collins, and G. M. Allan, Vet. Microbiol., in press). The Northern Ireland calf rotavirus isolate (4) was grown in Madin-Darby bovine kidney cells (American Type Culture Collection no. 22) as described by Todd and McNulty (10).

The lamb, pig, and calf rotaviruses were purified as described previously (10). Briefly, this involved fluorocarbon extraction, differential centrifugation, sedimentation through 40% (wt/vol) sucrose, treatment with DNase and pancreatic RNase, and equilibrium density gradient centrifugation in CsCl. In some purification procedures, nuclease treatments were omitted. Lamb rotavirus, which consisted of 72-nm double-capsid particles (Fig. 1a), banded in CsCl at a density of 1.35 g/ml, whereas the 60-nm single-capsid calf (Fig. 1b) and pig rotaviruses banded at 1.37 g/ml. Virus bands were removed with a Pasteur pipette and diluted with 0.02 M Tris-hydrochloride (pH 7.5; Tris buffer), and the virus was collected by centrifugation at 75,000 × *g* for 1 h. Virus pellets were suspended in Tris buffer prior to electron microscopic or biochemical examination.

The polypeptides present in purified lamb and calf rotavirus were analyzed by electrophoresis on cylindrical (6-cm) polyacrylamide gels as described elsewhere (5), except that solutions of 7.5% (wt/vol) acrylamide and 0.225% *N,N'*-methylenebisacrylamide were used. Gels were stained with Coomassie brilliant blue and scanned at 590 nm with a Unicam SP500 spectrophotometer fitted with a Gilford model 222 photometer and model 2410-S linear transport gel-scanning accessory. The molecular weights of the viral polypeptides were determined by comparing their electrophoretic mobilities relative to the tracker dye with those of reference proteins subjected to electrophoresis on parallel gels. The reference proteins were lactoperoxidase (molecular weight, 92,000), β-galactosidase (molecular weight, 130,000), bovine serum albumin (molecular weight, 67,000), immunoglobulin G (heavy chain and light chain; molecular weights, 55,000 and 23,000, respectively), and lactic dehydrogenase (molecular weight, 38,000).

RNA was released from purified lamb or pig rotavirus by treatment of virus suspensions with an equal volume of a solution containing 6 M urea, 2% (wt/vol) sodium dodecyl sulfate, and 20% (wt/vol) sucrose at 37°C for 30 min. Samples (20 to 30 μl) of the mixture were subjected to electrophoresis on cylindrical (8-cm) polyacrylamide-agarose gels containing 2.5% (wt/vol) acrylamide (10). Gels were stained with ethidium bromide or methylene blue as

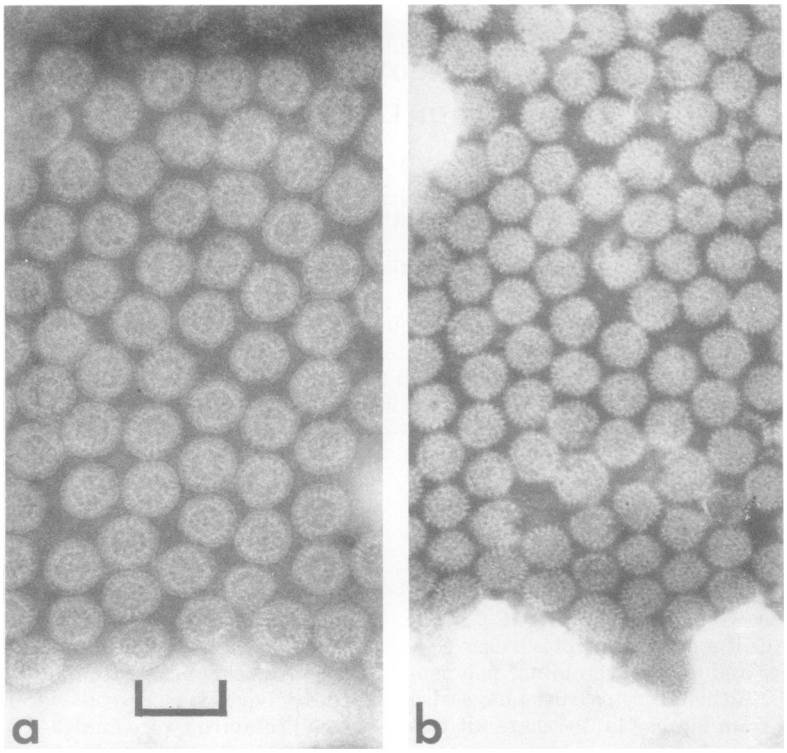

FIG. 1. *Electron micrographs of purified rotaviruses. Preparations of purified virus were mounted on carbon-coated Formvar grids and negatively stained with 4% sodium phosphotungstate, pH 7.1. (a) Double-capsid particles of lamb rotavirus. Bar represents 100 nm. (b) Single-capsid particles of calf rotavirus.*

described previously (10). Methylene blue-stained gels were scanned at 590 nm as described above. To determine the molar ratios of the RNA segments of lamb rotavirus, the areas under each peak of scanned electropherograms were measured. Molar ratios were then calculated by dividing the peak area by the estimated molecular weight of each RNA species.

After electrophoresis on 7.5% polyacrylamide gels, purified double-capsid lamb rotavirus was resolved into 10 polypeptide bands ranging from 128,000 to 30,000 in molecular weight (Fig. 2a). In numbering the lamb virus polypeptides, we adopted the system used by Rodger et al. (7) for calf and human rotavirus polypeptides. Band A was probably a contaminant, since it was found inconsistently. The molecular weights of the lamb rotavirus polypeptides are shown in Table 1. Our molecular weight estimates for the smaller polypeptides of the lamb virus were higher than those reported for the calf and human viruses (6, 7). Nevertheless, the polypeptide profile of double-capsid lamb rotavirus closely resembled that obtained for double-capsid calf virus (7). The major polypeptides of lamb, calf, and human rotaviruses were VP2 and VP6. Polypeptides equivalent to calf VP1 through VP8 were detected in the lamb virus, but no equivalent of calf VP9 was found in the lamb virus. In this respect, the lamb rotavirus was similar to the human virus (7). A further difference was that polypeptides equivalent to lamb VP6a and VP6b were not detected in either calf or human rotaviruses (7).

Electrophoresis of purified single-capsid calf rotavirus particles yielded only six polypeptide bands, two major and four minor (Fig. 2b), which corresponded in size and intensity to six of those present in the lamb virus. The pattern obtained for calf virus polypeptides was similar to that reported by Newman et al. (6) for single-capsid calf virus grown in tissue culture, but these authors did not detect a polypeptide equivalent to VP4. Coelectrophoresis experiments demonstrated that the calf virus polypeptides VP1, VP2, and VP6 comigrated with the corresponding lamb virus polypeptides (data not shown).

Since VP1, VP2, VP3, VP4, VP6, and VP6a were present in both double-capsid lamb and

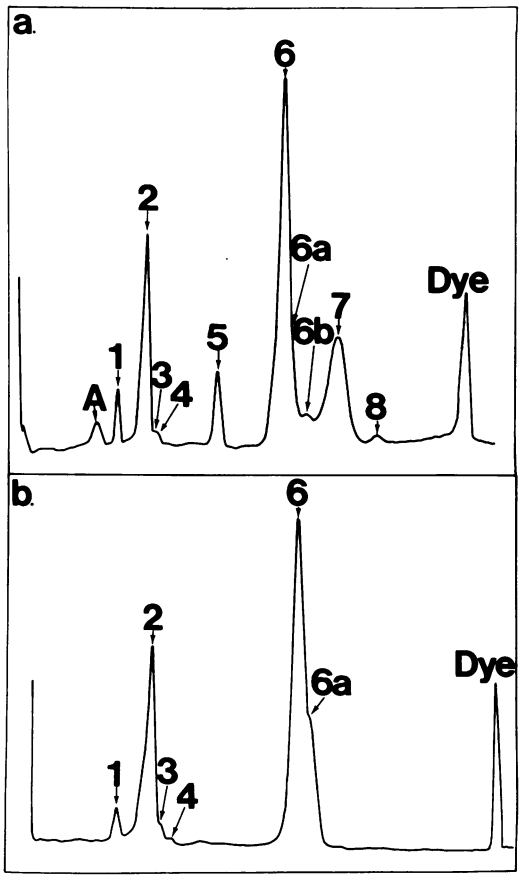

FIG. 2. *Extinction profiles obtained by scanning Coomassie brilliant blue-stained polyacrylamide gels containing the fractionated polypeptides of (a) double-capsid lamb rotavirus and (b) single-capsid calf rotavirus. The profiles start with the top of the gels on the left.*

single-capsid calf virus particles, it is likely that VP5, VP6b, VP7, and VP8 are the polypeptides that comprise the outer shell of lamb double-capsid particles. A similar conclusion was drawn by Rodger et al. (7), who reported that VP5, VP7, VP8, and VP9, and VP5, VP7, and VP8 were probably the outer capsid polypeptides of the calf and human viruses, respectively. The results of the present investigation are in partial agreement with those reported by Bridger and Woode (2), who found that only one polypeptide, equivalent to VP5, was associated with the outer capsid of calf virus preparations purified from infected cells.

Electrophoresis of lamb rotavirus RNA on 2.5% (wt/vol) acrylamide-agarose gels showed that the genome was segmented and migrated as nine bands (Fig. 3a). Band 7 was proportionately more intense in relation to its size, indicating the presence of more than one RNA species. Coelectrophoresis of the RNAs from the lamb and pig viruses revealed only one difference between the RNA segments. This difference occurred on band 5 where the lamb virus RNA segment migrated less rapidly than that of the pig virus (Fig. 3c and d). Apart from this, the lamb virus RNA segments were identical in size to those of the pig virus characterized in an earlier publication (10). Recently it was reported that the RNAs of calf and human rotaviruses, isolated in Australia, are also different with respect to the relative mobilities of segments 1, 4, and 5 (8). Furthermore, different isolates of human rotavirus were distinguished by differences in RNA segments 1, 2, and 5 (8).

The molecular weights of each of the lamb rotavirus RNA bands, resolved by electrophoresis, are listed in Table 1. The pig rotavirus RNA depicted in Fig. 3b contained an additional band which had the smallest electrophoretic mobility. This particular pig virus preparation had not been treated with DNase during purification. The additional band was not present in DNase-treated preparations and thus probably represents DNA.

Extinction profiles obtained by scanning methylene blue-stained gels were used in the calculation of the molar ratios of the nine RNA bands. The data (Table 1) indicate that all bands except number 7 were present in equimolar amounts, whereas band 7 was comprised of three or four RNA species of similar molecular

TABLE 1. *Polypeptide and RNA composition of lamb rotavirus*

Polypeptide		RNA		
No.[a]	Mol wt	Band no.[b]	Mol wt × 10^{-6}	Molar ratio
1[c]	128,000	1	2.1	1.1
2[c]	108,000	2	1.7	1.0
3[c]	99,000	3	1.6	1.0
4[c]	96,000	4	1.5	0.9
5	72,000	5	0.9	1.0
6[c]	50,000	6	0.8	0.9
6a[c]	48,000	7	0.5	3.7
6b	45,000	8	0.3	1.0
7	37,000	9	0.3	1.1
8	30,000			

[a] The virus polypeptides, fractionated on 7.5% polyacrylamide gels, were numbered by the system of Rodger et al. (7) as shown in Fig. 2.

[b] RNA bands were fractionated on 2.5% polyacrylamide-agarose gels and numbered as in Fig. 3.

[c] Corresponding polypeptides were also detected in single-capsid calf rotavirus preparations.

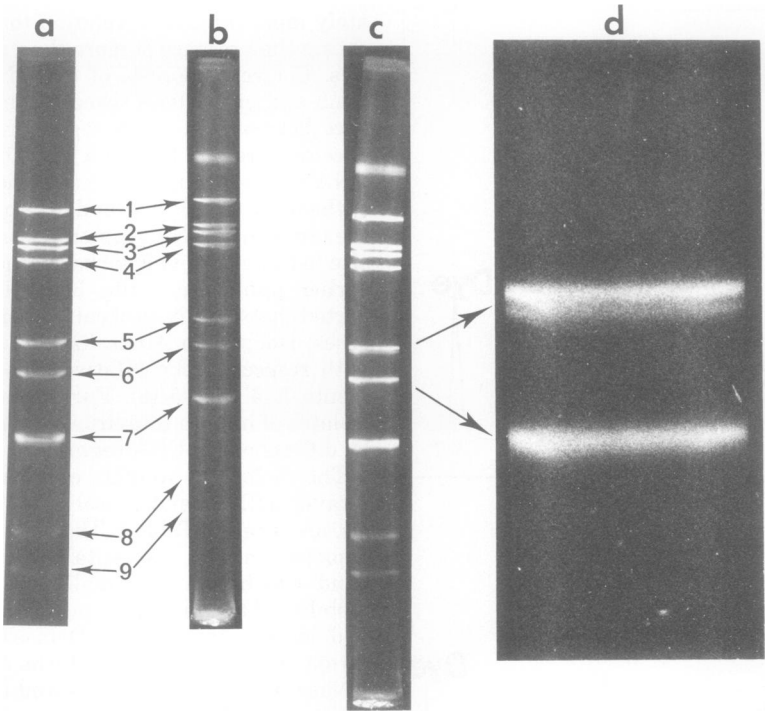

FIG. 3. *Electrophoresis of RNA on 2.5% polyacrylamide-agarose gels. Migration was from top to bottom. Ethidium bromide-stained RNA was visualized under a UV lamp (10). (a) Lamb rotavirus RNA. (b) Pig rotavirus RNA. (c) Lamb and pig rotavirus RNAs subjected to coelectrophoresis. (d) Magnification to show differences in the band 5 mobilities detected between the lamb and pig rotavirus RNAs.*

weight. Thus, the RNA of lamb rotavirus was composed of 11 or 12 segments with a total molecular weight of approximately 11×10^6.

The values for the molar ratios of each of the nine lamb rotavirus RNA bands were similar to those we obtained for the pig and calf viruses (10). It has not yet been demonstrated conclusively whether RNA band 7 is composed of three or four segments (6–8, 10); our results suggest that there are four rather than three segments comprising band 7, whereas other reports (7, 8) suggest the converse is true.

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