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Comparative Study on the Mechanisms of Rotavirus Inactivation by Sodium Dodecyl Sulfate and Ethylenediaminetetraacetate

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This report describes a comparative study on the effects of the anionic detergent sodium dodecyl sulfate and the chelating agent ethylenediaminetetraacetate on purified rotavirus SA-11 particles. Both chemicals readily inactivated rotavirus at quite low concentrations and under very mild conditions. In addition, both agents modified the viral capsid and prevented the adsorption of inactivated virions to cells. Capsid damage by ethylenediaminetetraacetate caused a shift in the densities of rotavirions from about 1.35 to about 1.37 g/ml and a reduction in their sedimentation coefficients. Sodium dodecyl sulfate, on the other hand, did not detectably alter either of these physical properties of rotavirions. Both agents caused some alteration of the isoelectric points of the virions. Finally, analysis of rotavirus proteins showed that ethylenediaminetetraacetate caused the loss of two protein peaks from the electrophoretic pattern of virions but sodium dodecyl sulfate caused the loss of only one of these same protein peaks.

The ability of viruses to survive in the environment depends on the responses of the viruses to a number of environmental factors. One typical environment for enteric viruses is wastewater sludge. In an accompanying report (21), it is shown that rotavirus, an important enteric pathogen belonging to the Reoviridae, is destabilized by ionic detergents which are present in sludge. When the effects of individual ionic detergents on rotavirus were tested, it was found that this virus is particularly sensitive to several of these detergents. Included in the group is the anionic detergent sodium dodecyl sulfate (SDS).

It is also shown in previous reports (19-21) that slight modifications in the structures of detergents and the environment in which they are placed can greatly alter their effects on rotavirus and other enteric viruses. One approach to understanding why detergents have such different effects on viruses is to determine what effects they have on the structures of viruses. Knowledge concerning the state of a virus particle after inactivation by a detergent should provide a basis for understanding the mechanism of inactivation by that detergent. This study was carried out to gain this knowledge for the inactivation of rotavirus SA-11 by SDS. Because other investigators have performed similar studies on rotavirus with the chelating agent ethylenediaminetetraacetate (EDTA) (3, 6), a simultaneous study was performed with this compound for purposes of comparison.

MATERIALS AND METHODS

Cells and virus. CV-1 cells and simian rotavirus strain SA-11 were used for all experiments described in this paper. The production of virus and the infectivity analysis by plaque assay were carried out as described in the accompanying report (21).

Production and purification of radioactively labeled rotavirus. All rotavirus experiments described below were carried out with purified, radioactively labeled viruses. Two types of purified virus samples were prepared. Viruses labeled in their ribonucleic acid were grown in [^{3}H]uridine, and those tagged in their protein were grown in ^{14}C-labeled protein hydrolysate (Schwarz mixture). For virus production, confluent monolayers of CV-1 cells (mycoplasma-free) were washed three times with Eagle minimal essential medium (MEM) without serum and then infected with rotavirus SA-11 (multiplicity of infection, 10). After a 30-min adsorption at 37°C, the cells were overlaid with either MEM without serum (preparation to be labeled with [^{3}H]uridine) or MEM containing 5% of the normal concentration of amino acids, also without serum (preparation to be tagged with ^{14}C-labeled amino acids). After an additional 4 h of incubation at 37°C, 20 μCi of [^{3}H]uridine per ml of standard culture medium or 4 μCi of ^{14}C-labeled protein hydrolysate per ml of low-amino-acid medium was added, and incubation was continued for an additional 18 h. The total lysates, including cells and medium, were harvested and centrifuged in an SW27 rotor at 24,000 rpm for 1.5 h at 4°C. The pellets were suspended in 6 ml of reticulocyte standard buffer (RSB) [0.01 M NaCl, 0.01 M tris(hydroxymethyl)aminomethane, pH 8, 0.003 M $MgCl_2$], blended for 2 min at 4°C with an equal volume of Genetron, and centrifuged at 3,000

rpm for 5 min to separate phases. The interphase and Genetron phase were reextracted with 1 volume of RSB as before, and the aqueous phases were combined. This material (about 12 ml) was layered onto 28 ml of 10% glycerol in RSB and centrifuged in an SW27 rotor at 24,000 rpm for 1 h at 4°C. The pellet and bottom 3 ml were mixed with 1 ml of RSB, sonicated for 2 min, and layered onto a 20 to 40% glycerol gradient in RSB. After centrifugation in an SW27 rotor at 24,000 rpm for 1 h at 4°C, fractions were collected, and those fractions containing the peaks of radioactivity near the middle of the gradients were combined. The virus was then concentrated by centrifugation and dialyzed against RSB. This material (about 3 ml) was layered onto preformed CsCl gradients (1.32 to 1.42 g/ml) and centrifuged at 24,000 rpm for 6 h at 4°C in an SW27.1 rotor. Fractions were collected from the bottoms of the tubes, and 20-μl samples were assayed for total radioactivity. These results, along with the densities of representative fractions, are plotted in Fig. 1. Particles with densities of about 1.37 and 1.35 g/ml were found, but infectivity was associated only with the less dense material. The remainders of fractions 13 through 15 and 18 and 19 from each gradient were separately combined, dialyzed against RSB, and stored at 4°C.

Inactivation of rotavirus with SDS and EDTA. Purified rotavirus particles with a density of 1.35 g/ml were diluted 10-fold into either 0.1% SDS or 0.01 M EDTA, both in RSB. The samples in SDS were incubated at 21°C for 60 min, and the samples in EDTA were incubated at 37°C for 20 min. After treatment, samples were placed in ice and analyzed immediately.

Analysis of rotavirus particles by CsCl gradient centrifugation. Treated samples of [^{3}H]uridine-labeled rotavirus were sonicated for 2 min at 4°C, layered onto 4.8-ml preformed linear gradients of CsCl (1.30 to 1.42 g/ml), and centrifuged for 2 h at 27,000 rpm in an SW50.1 rotor. Fractions were collected from the bottoms of the tubes and analyzed for CsCl concentration by refractometry. Portions of each sample were then precipitated with 5% trichloroacetic acid and assayed for recoverable radioactivity.

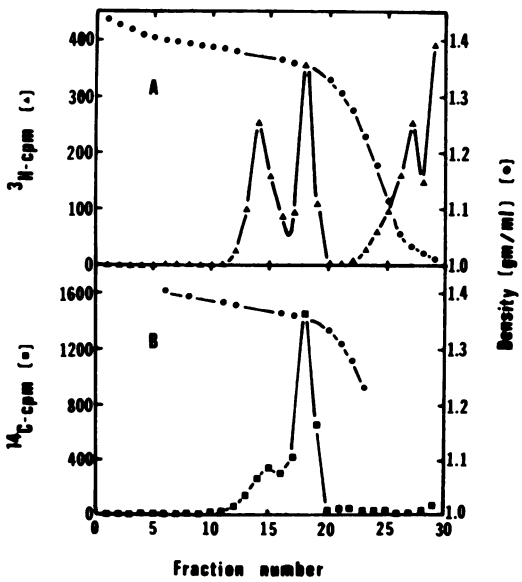

FIG. 1. *CsCl gradient centrifugation of rotaviruses tagged with either [^{3}H]uridine (A) or ^{14}C-labeled protein hydrolysate (B) as the final purification procedure.*

Glycerol gradient centrifugation of rotavirus particles. Treated samples of [^{3}H]uridine-labeled rotavirus (density, either 1.35 or 1.37 g/ml) were mixed with a small volume of ^{14}C-labeled virus (density, 1.35 g/ml), sonicated at 4°C, and layered onto 4.8-ml 20 to 40% glycerol gradients in RSB. After centrifugation in an SW50.1 rotor at 28,000 rpm for 1 h at 4°C, fractions were collected and assayed for total ^{14}C and ^{3}H counts per minute.

Adsorption of inactivated rotaviruses to CV-1 cells. [^{3}H]uridine-labeled rotaviruses were treated with 0.1% SDS or 10 mM EDTA in the manner described above and diluted 50-fold with MEM. Control samples of virus were mixed directly with SDS or EDTA in MEM at the diluted concentrations (0.002% SDS and 0.2 mM EDTA). These control samples were included to test the effects of very low concentrations of SDS and EDTA on the binding properties of infectious viruses. Confluent monolayers of CV-1 cells in 60-mm plastic petri dishes were washed three times with MEM without serum, and 0.2 ml of virus sample was added per plate (multiplicity of infection, 1). Adsorption was at 37°C for 60 min. Unabsorbed viruses were removed by three washes with MEM, the cells were scraped from the plates, and each sample was frozen in 2 ml of water. The acid-precipitable radioactivity in each sample was then measured. The results were corrected for loss of radioactivity due to quenching.

Isoelectric focusing of rotaviruses. Treated [^{3}H]uridine-labeled rotaviruses (0.01 ml) were diluted to 0.2 ml with RSB, sonicated at 4°C for 2 min, and mixed with 0.12 ml of 40% (wt/vol) sucrose in 1% ampholine (pH 3.5 to 10). The remainder of the procedure was carried out by the method of Korant and Lonberg-Holm (8), as described previously (18).

SDS-polyacrylamide gel electrophoresis of rotavirus proteins. Treated samples of rotaviruses (0.6 ml) tagged with ^{14}C-labeled amino acids were diluted to 5 ml with RSB and centrifuged in an SW50.1 rotor at 45,000 rpm for 1 h at 4°C to concentrate the virus and separate virus and subviral particles from capsid proteins dissociated during treatment. The pellet was dried, suspended in 40 ml of water, and mixed with 15 ml of a solution containing 10% SDS, 0.25 M tris(hydroxymethyl)aminomethane (pH 7), 25% 2-mercaptoethanol, and 0.25% bromophenol blue. The remainder of the electrophoretic procedure was carried out as described previously (22), with the following exceptions. The separation gel (10 by 0.6 cm) contained 8% acrylamide and 0.2% bisacrylamide. After electrophoresis, the gels were frozen, sliced, dissolved in H_2O_2, and counted in 10 ml of toluene-based scintillation fluid containing 1 ml of solubilizer (Nuclear-Chicago Corp., Des Plaines, Ill.)

RESULTS AND DISCUSSION

Infectious rotaviruses from a number of species have been shown by several investigators (1, 2, 4, 5, 11, 12, 14) to have densities of 1.35 to 1.36 g/ml. It is well established that these particles have a double protein shell (10). Rotavirus particles with densities of 1.37 to 1.38 g/ml are also routinely isolated from infected cells and from the feces of infected animals and humans. These particles lack the outer protein shell and, in addition, are apparently noninfectious. In this study, we also obtained purified rotavirus particles with densities of about 1.35 and 1.37 g/ml from SA-11-infected CV-1 cells (Fig. 1). In agreement with the results of other investigators, only the particles with densities of 1.35 g/ml were found to be infectious.

It has also been reported that lower-density rotavirus particles can be readily converted to higher-density particles by treatment with EDTA (3, 6). This treatment causes the activation of a virion-associated ribonucleic acid polymerase and the apparent removal of the outer shell proteins and loss of infectivity. It has been shown previously that SDS causes loss of poliovirus infectivity by disruption of virion proteins (20). Therefore, it was anticipated that the protein capsid might also be the rotavirus component affected by SDS that caused the loss of rotavirus infectivity. Therefore, in the study reported here, the effects of SDS and EDTA on the state of rotavirus particles were studied simultaneously.

It was shown in the accompanying report (21) that treatment of a diluted lysate of rotavirus with 0.1% SDS for 60 min at 21°C caused a reduction in infectivity of more than three orders of magnitude. A similar result was found when the purified 1.35-g/ml rotavirus particles obtained after CsCl gradient centrifugation were treated under the same conditions. Likewise, treatment of these purified virions with 10 mM EDTA for 20 min at 37°C caused their infectivities to be reduced by more than two orders of magnitude (data not shown). These treatment conditions were used during the remainder of this study.

CsCl gradient analysis of rotaviruses after SDS and EDTA treatments. As mentioned above, it has been reported that treatment of rotavirus particles having densities of 1.35 to 1.36 g/ml with EDTA causes their conversion to a density of 1.37 to 1.38 g/ml (3, 6). Therefore, the first physical measurement of the effect of SDS on 1.35-g/ml SA-11 particles was to determine their density after treatment. SDS was found to cause no observable increase in the density of these 1.35-g/ml particles (Fig. 2). In contrast, EDTA caused the expected increase in the density of these particles to 1.374 g/ml.

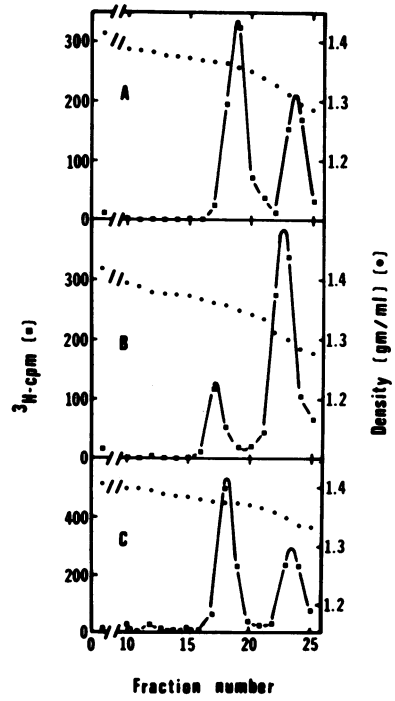

FIG. 2. *Analysis by CsCl gradient centrifugation of the effects of SDS and EDTA on the density of 1.35-g/ml rotavirus particles labeled with [^{3}H]uridine. (A) Untreated virions. (B) SDS-treated virions. (C) EDTA-treated virions.*

Many of the purified rotavirus particles shown in Fig. 2 were found at the top of the gradient, even without treatment with SDS or EDTA. This indicates that their density was no longer 1.35 g/ml but 1.34 g/ml or less. Treatment with SDS but not EDTA caused a significantly greater number of the 1.35-g/ml particles to shift to this lower density.

The presence of lower-density particles in rotavirus preparations has been reported by other investigators (14, 15). The results of these workers suggest that this lower-density material may be empty protein capsids (both double and single shelled) or particles associated with less dense debris. Because the rotavirus particles used in this study were purified extensively, it is highly unlikely that this type of debris was present. In addition, because these particles were labeled in their ribonucleic acid, the radioactively labeled low-density material could not be just empty capsids. It is most likely that some of the purified 1.35-g/ml particles were altered in some manner during storage at 4°C. This alteration, in turn, may have caused the density of the particles to be modified either before or during treatment

with high concentrations of CsCl, an effect that could be enhanced by pretreatment of these viruses with SDS.

Analysis of rotaviruses by glycerol gradient centrifugation. The states of rotavirus particles after SDS and EDTA treatments were examined by an analysis of the sedimentation rates of these particles in glycerol gradients. For this study both 1.35- and 1.37-g/ml purified rotavirus particles labeled with [^{3}H]uridine were treated and analyzed. Untreated ^{14}C-labeled viruses having a density of 1.35 g/ml were used as markers. SDS had no effect on the sedimentation velocity of 1.35-g/ml particles, but EDTA caused these particles to sediment at a reduced rate (Fig. 3). The rate of sedimentation of the EDTA-treated virions was not detectably different from that of untreated 1.37-g/ml rotavirus particles. This result shows that EDTA, but not SDS, modifies the sedimentation coefficients of 1.35-g/ml rotavirus particles.

Effects of SDS and EDTA on the adsorptive properties and isoelectric points of rotaviruses. The results presented above indicate that rotavirus inactivation by SDS has little effect on the densities or sedimentation coefficients of the viral particles. Therefore, we used other methods to detect alterations in the capsid components of the virions.

The initial step in the infection of a cell by a virus is the adsorption of the virus to receptors on the surface of the cell. Factors that prevent viral adsorption normally result in loss of infectivity. The portion of a nonmembranous virus involved in adsorption to cellular receptors is its capsid. Therefore, alterations in the capsid could result in a loss of viral adsorptive ability and, consequently, a loss of infectivity.

Treatment of rotavirus with SDS caused complete loss of ability to adsorb to CV-1 cells (Table 1). EDTA treatment also caused nearly complete loss of adsorptive ability. Therefore, it is clear that both treatments cause the viral capsids to be altered in some fashion which prevents the virions from attaching to cells. This result indicates that capsid damage could be responsible for the loss of infectivity in both cases.

Alterations in the capsid of rotavirus by SDS and EDTA were also studied by isoelectric focusing of treated virions. SDS caused some modification of the isoelectric points of 1.35-g/ml rotavirus particles, but the effect was not large (Fig. 4). EDTA caused a somewhat larger shift in these isoelectric points. The isoelectric points of EDTA-treated 1.35-g/ml particles were essentially identical to those of untreated 1.37-g/ml particles. This result supports the previous suggestion that both EDTA and SDS affect the

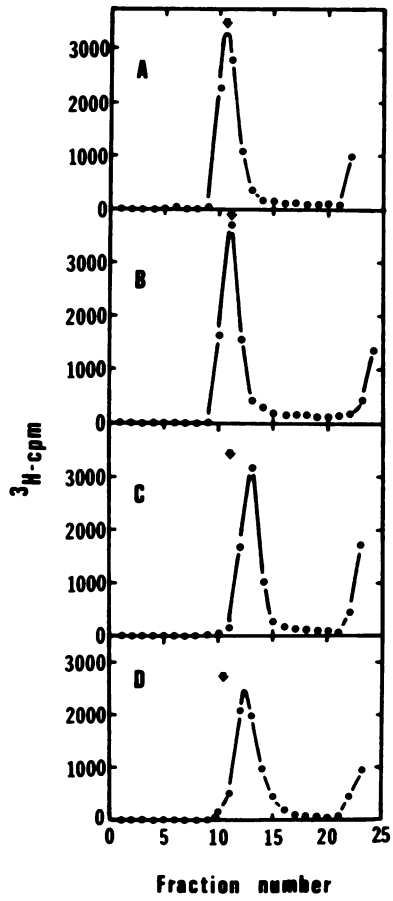

FIG. 3. *Analysis by glycerol gradient centrifugation of the effects of SDS and EDTA on the sedimentation rates of 1.35- and 1.37-g/ml rotavirus particles labeled with [^{3}H]uridine. (A) Untreated 1.35-g/ml particles. (B) SDS-treated 1.35-g/ml particles. (C) EDTA-treated 1.35-g/ml particles. (D) Untreated 1.37-g/ml particles. Arrows mark the positions of untreated, ^{14}C-labeled rotavirus particles mixed with the treated samples just before centrifugation. The direction of sedimentation was from right to left.*

capsids of rotavirus particles, but the effect of SDS is not as great as that of EDTA.

Analysis of proteins in rotaviruses treated with SDS and EDTA by gel electrophoresis. A final determination of the effects of SDS on infectious rotavirus particles was made through an analysis of the proteins in treated virions by SDS-polyacrylamide gel electrophoresis. A number of studies concerning the proteins in the inner and outer shells of rotavirus particles have been reported (1, 7, 9, 12–17). In general, these studies indicate that rotaviruses from several different species contain about four to six proteins in their inner shells and three to

TABLE 1. *Effect of SDS and EDTA treatments on the ability of [^{3}H]uridine-labeled rotaviruses to adsorb to CV-1 cells*[a]

Sample	Amt added (cpm)	Amt bound (cpm)	% Bound	% Of control
Untreated	705	104	14.8	
SDS treated	744	0	0.0	0.0
SDS control	755	63	8.3	
EDTA treated	715	7	1.0	8.3
EDTA control	680	82	12.1	

[a] [^{3}H]uridine-labeled rotaviruses were treated either with 0.1% SDS for 60 min at 21°C or with 0.01 M EDTA for 20 min at 37°C and diluted 50-fold. These samples, along with control samples of the infectious virus at the same dilution in either SDS (0.002%) or EDTA (0.2 mM), were added to confluent monolayers of CV-1 cells. The amounts of radioactivity added and bound were determined, and the percentages bound were calculated. All samples were run in triplicate.

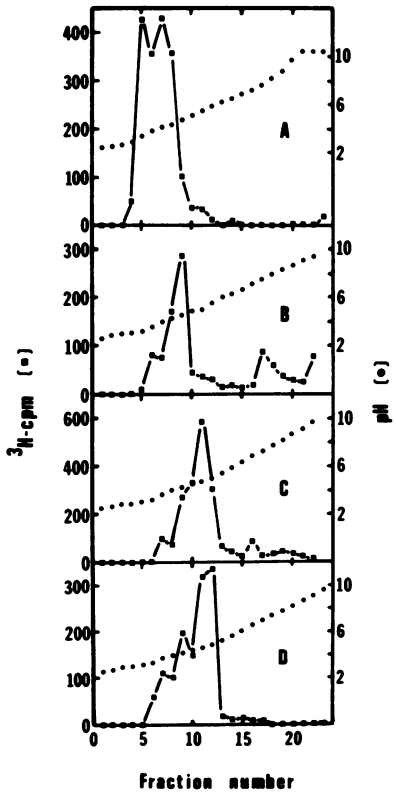

FIG. 4. *Isoelectric focusing of SDS- and EDTA-treated rotaviruses labeled with [^{3}H]uridine. (A) Untreated 1.35-g/ml particles. (B) SDS-treated 1.35-g/ml particles. (C) EDTA-treated 1.35-g/ml particles. (D) Untreated 1.37-g/ml particles.*

four proteins in their outer shells. There is some disparity in the results regarding the amounts of these proteins and their molecular weights.

Our electrophoretic pattern of the proteins from 1.35-g/ml particles (Fig. 5A) was quite similar to that found by Todd and McNulty with lamb rotavirus (17). Although these and other investigators resolved between 8 and 10 protein bands in their gel patterns, only 5 peaks were clearly resolved in our gel pattern. These major peaks are numbered 1 through 5, but it is assumed that they camouflage a number of minor peaks and, therefore, represent more than five viral proteins. Treatment with EDTA (Fig. 5B) caused the loss of almost all radioactivity from peaks 3 and 5. This finding agrees with the results of other investigators which suggest that rotavirus proteins of this approximate size and quantity are outer shell proteins. When 1.35-g/ml rotavirus particles were inactivated by SDS, only proteins banding in peak 3 were removed from the particles (Fig. 5C). This result confirms the suggestion that some capsid alteration occurs upon treatment of infectious rotavirus with SDS but this alteration is less extensive than that caused by EDTA.

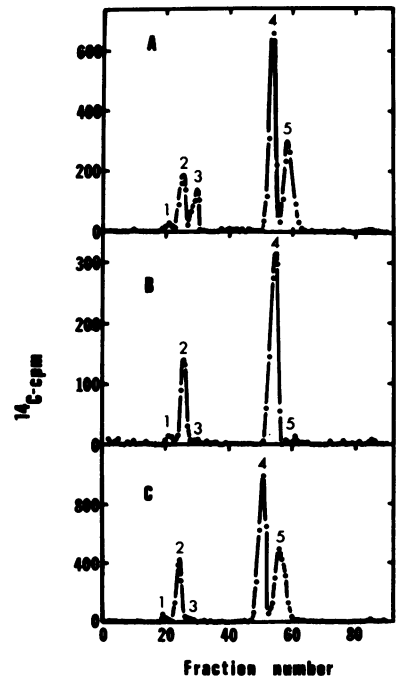

FIG. 5. *Electrophoretic analysis of ^{14}C-labeled proteins retained in rotavirus particles after EDTA and SDS treatments. (A) Untreated 1.35-g/ml particles. (B) EDTA-treated 1.35-g/ml particles. (C) SDS-treated 1.35-g/ml particles. The direction of electrophoresis was from left to right.*

The finding that proteins in peak 3 but not those in peak 5 are removed by SDS from rotavirus particles shows that only a portion of the proteins in the outer shell are removed by this detergent. This finding suggests that the protein(s) in peak 3 is located on the extreme outside of the viral particle and is, therefore, most easily removed. It is interesting that the loss of this protein did not detectably alter the density or sedimentation value of the rotavirus particles but that removal of the proteins in both peaks 3 and 5 by EDTA caused a significant change in both. The quantity of proteins in peak 3 is apparently about 43% of that in peak 5. On this basis alone, the loss of peak 3 protein should have partially altered the density and sedimentation properties of rotavirus. It is not clear why this was not observed.

The difference in the effects of EDTA and SDS on the outer shell proteins of rotavirus also leads to the suggestion that the chemical bondings through which the different outer shell proteins associate with other components of the virion are significantly different. Apparently, it is the properties of these bonds that can determine the susceptibility of the virus to a particular detergent or other chemical compound. It may be possible to understand certain properties of these bonds through further studies on the nature of the chemicals that can cause these bonds to be broken.

The results of this study suggest that the physical properties of rotavirus SA-11 are not significantly altered through inactivation by SDS and that some, if not most, outer shell proteins remain associated with the virions after this treatment. If the antigenicity of these virions is not changed appreciably and other rotavirus strains are affected in a similar fashion, inactivation by SDS may be an excellent method to prepare killed-virus vaccines of this virus.

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