Effects of Quaternary Ammonium Compounds with 0.1% Sodium Hydroxide on Swine Vesicular Disease Virus

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(Received 23 October 1996/Accepted 16 January 1997)

ABSTRACT. The effects of quaternary ammonium compounds (QACs) with sodium hydroxide on swine vesicular disease virus (SVDV), an enterovirus were studied. Didecyldimethylammonium chloride (DDAC) with 0.1% NaOH showed a stronger effect against SVDV than other QACs with 0.1% NaOH. The effect of DDAC with 0.1% NaOH was strong at 40ºC. DDAC was effective against SVDV at pH values around 11.0, but not in the distilled water control. The effect of DDAC with 0.1% NaOH was already observed at 1 min after mixing of the DDAC with SVDV. Observation under an electron microscopy revealed that the probable mechanism of inactivation of DDAC with 0.1% NaOH is as follows: The virus particles were partially destroyed by 0.1% NaOH. DDAC gathered these affected particles and formed a micelle, then SVDV lost its infectivity. From these results, QACs with 0.1% NaOH are considered to be very effective against SVDV representing enteroviruses. — KEY WORDS: disinfection, electron microscopy, 0.1% NaOH, quaternary ammonium compound, swine vesicular disease virus.

J. Vet. Med. Sci. 59(5): 323–328, 1997

Swine vesicular disease (SVD) is a contagious viral disease of swine indistinguishable in the field from foot-and-mouth disease (FMD) [10, 15]. The pathogen of SVD is in the enterovirus group of picornaviruses [10] and its properties link it to the enteroviruses [10, 15]. SVD virus (SVDV) is resistant to the chemicals and disinfectants commonly used against FMD virus [2, 5].

Quaternary ammonium compounds (QACs) are used widely as disinfectants against pathogens including viruses. However, the activities against the pathogens are mainly studied only in bacterial pathogens because the QACs are not effective against almost all viruses [6]. Noll and Younger [11] classified viruses into three groups as follows: group A, enveloped viruses (e.g. Herpesviridae, Paramyxoviridae, Orthomyxoviridae); group B, small (20–30 nm) non-enveloped viruses (e.g. Picornaviridae, Parvoviridae) and group C, other non-enveloped viruses (e.g. Adenoviridae, Reoviridae, Papovaviridae, Birnaviridae). The effect of QACs was found to be limited to the group A viruses [6, 9]. However, recently, inactivation of infectious bursal disease virus (IBDV) by QACs with 0.1% sodium hydroxide (NaOH) was reported [13]. IBDV belongs to the group C viruses and it is resistant to commonly used QACs.

Therefore, in this study, we examined the inactivation effect of QACs with 0.1% NaOH on SVDV representing enteroviruses (group B, small non-enveloped viruses) and reveal the mechanism of the inactivation of viruses by QACs by morphological examination.

MATERIALS AND METHODS

Cell culture and viruses: IBRS-2 cell cultures were used in this study. SVDV strain J1 was isolated in Japan from vesicles of affected pigs [14]. It was plaque cloned 3 times and passaged many times in IBRS-2 cells. SVDV strain H/3'76 was isolated in Japan from the stool of a clinically normal pig [8]. It also was plaque cloned 3 times and passaged many times in IBRS-2 cells. Both virus strains were inoculated onto confluent monolayers of IBRS-2 cells after 1 day of cultivation, at a multiplicity of infection of 1/10. Infectious fluid was centrifuged at 3,000 × g for 10 min and stored at −80°C until used as inoculum.

Virus titration: Virus titration was carried out by plaque formation in confluent monolayers of IBRS-2 cells grown in 30-mm-diameter 6-well plastic plates. Plaque formation was carried out according to the previous method [7]. The titrated viruses (0.1 m*l*) were inoculated into each well. Plaque-forming units (PFU) in log 10 per 0.1 m*l* of the material were determined from the dilution in which about 5 to 10 plaques had formed.

Effect of QACs on SVDV with or without 0.1% NaOH: Three QAC-based commercial formulations were selected. The J1 and H/3'76 strains of SVDV were each mixed with equal volumes of didecyldimethylammonium chloride (DDAC) [1] diluted with distilled water to give final dilutions of 1/200, 1/400, 1/600, and 1/800 with or without NaOH at a concentration of 0.1%. The mixtures were incubated at room temperature for 30 min. The J1 strain was mixed with an equal volume of alkylbenzyl-dimethylammonium chloride (ABDAC) [3] or [mono-bis (trimethylammonium-methylene chloride)]-alkyl ($C_{9\text{-}15}$) toluene (MBTAMCAT) [4] diluted with distilled water to give the same final concentrations and NaOH content as DDAC. The experiment conditions were the same as for DDAC. Distilled water controls were used with or without NaOH at a concentration of 0.1%. Following incubation, the specimens were immediately diluted and titrated.

Effect of DDAC at different temperatures on the virus: The J1 strain was mixed with an equal volume of DDAC in distilled water to give dilutions of 1/200, 1/400, and 1/800 with or without 0.1% NaOH. The mixtures were incubated at 4°C, room temperature (about 20°C), or 40°C for 30 min.

A distilled water control containing 0.1% NaOH was used under the same conditions as for DDAC.

Effect of DDAC at various concentrations of NaOH on the virus: The J1 strain was mixed with an equal volume of DDAC in distilled water to give a dilution of 1/400 with NaOH at concentrations of 0%, 0.0125%, 0.025%, 0.1%, 0.2%, and 0.5%. The pH of each dilution was determined before and after mixing with the virus suspension. Distilled water controls contained the same concentrations of NaOH as DDAC.

Effect of time of incubation of DDAC with 0.1% NaOH on the virus: The J1 strain was mixed with an equal volume of DDAC at a dilution of 1/400 with 0.1% NaOH. The mixtures were incubated at room temperature for 1, 5, 10, 30, and 60 min. Distilled water containing 0.1% NaOH was used as a control under the same condition as DDAC. A DDAC control without NaOH was mixed with the virus and incubated for 60 min at room temperature.

Effect of triton X-100 on virus infectivity with or without 0.1% NaOH: The J1 strain was mixed with an equal volume of triton X-100 (0.276 M: adjusted molecular weight as DDAC) diluted with distilled water to give dilutions of 1/200, 1/400, 1/600, and 1/800 with or without 0.1% NaOH. The mixtures were incubated at room temperature for 30 min. Distilled water with or without 0.1% NaOH as a control was used. The effect of triton X-100 and DDAC with or without 0.1% NaOH on SVDV was also examined. The J1 strain was mixed with an equal volume of triton X-100 at a dilution of 1/400 with 0.1% NaOH, DDAC at a dilution of 1/400 with 0.1% NaOH, and triton X-100 and DDAC at a concentration of 1/200 each with or without 0.1% NaOH. Distilled water with or without 0.1% NaOH was used as a control under the same conditions as for the above compounds.

Electron microscopy: The J1 strain was propagated and purified for examination by electron microscopy. The virus was purified by the same procedure as before [7]. Briefly, the J1 strain was inoculated onto IBRS-2 cells, and the infectious fluid was harvested 24 hr after inoculation. The clarified infected fluid was centrifuged for 2 hr at 95,000 × g at 4°C with a 30% glycerol cushion. The pellet was suspended in TNE (0.01 M Tris-HCl pH 7.4, 0.1 M NaCl, 1 mM disodium EDTA) and banded by isopycnic centrifugation in CsCl (ρ=1.34 g/m*l*) for 16 hr at 150,000 × g at 7°C. The band of virus was collected and dialyzed against distilled water. The purified viruses were mixed with an equal volume of distilled water, 0.1% NaOH, a 1/400 dilution of DDAC, a 1/400 dilution of DDAC with 0.1% NaOH, a 1/400 dilution of triton X-100 with 0.1% NaOH, and a 1/200 dilution of DDAC and triton X-100 mixture with 0.1% NaOH and allowed to stand for 30 min at room temperature. One drop of each sample was mounted on a 400-mesh carbon-coated grid, and negatively stained with 2% phosphotungstic acid, pH 7.05, for 1 min. The samples were examined in a JEM-1200 EX electron microscope (JEOL Co., Tokyo, Japan).

RESULTS

Effects of QACs on SVDV with or without 0.1% NaOH: The effects of QACs on SVDV with or without 0.1% NaOH are summarized in Table 1. Both SVDV strains J1 and H/3'76 were inactivated by all dilutions of DDAC with 0.1% NaOH. However DDAC without 0.1% NaOH had no effect on either strain, even at high concentration. ABDAC and MBTAMCAT with 0.1% NaOH inactivated the J1 strain, but the inactivation rates were lower than those of DDAC. ABDAC and MBTAMCAT without 0.1% NaOH also had no effect on SVDV. The effects of three QACs with 0.1% NaOH were almost the same in 1/200 and 1/400 dilution, but its were lower in 1/600 and 1/800 dilution, respectively. The distilled water control with 0.1% NaOH combined with three QACs had little effect on SVDV.

Effects of DDAC with NaOH on SVDV under different conditions: Based on the results of the effects of QACs on SVDV, DDAC was used for the following other experiments as a representative QAC. The effects of DDAC with 0.1% NaOH at different temperatures on the virus are shown in Table 2. At 40°C incubation, all dilutions of DDAC with

Table 1. The effects of QACs with or without 0.1% NaOH on SVDV

QAC	Diluton	Presence of 0.1% NaOH	Virus titers: Strain J1	Virus titers: Strain H/3'76
DDAC	× 200	–	6.78	7.00
	× 400	–	6.78	7.26
	× 600	–	6.95	7.00
	× 800	–	6.90	7.00
	× 200	+	2.60	1.90
	× 400	+	2.60	2.00
	× 600	+	3.11	3.04
	× 800	+	3.11	3.60
Control		–	6.90	7.30
		+	6.95	5.70
ABDAC	× 200	–	7.00	
	× 400	–	7.00	
	× 600	–	7.00	
	× 800	–	7.00	
	× 200	+	2.90	
	× 400	+	4.30	
	× 600	+	4.40	
	× 800	+	4.60	
Control		–	7.15	
		+	6.00	
MBTAMCAT	× 200	–	7.08	
	× 400	–	7.20	
	× 600	–	7.28	
	× 800	–	7.30	
	× 200	+	3.70	
	× 400	+	3.60	
	× 600	+	4.78	
	× 800	+	4.90	
Control		–	7.32	
		+	6.60	

Virus titer is indicated as log10 PFU per 0.1 m*l* of the samples.

0.1% NaOH had a strong effect on SVDV strain J1, and viruses were not detected at high concentrations of the drug (1/200 and 1/400 dilutions). At 20°C (room temperature) incubation, the effect of DDAC was moderate, and it was strong at high concentrations (1/200 and 1/400 dilutions). At 4°C incubation, the effect of DDAC was weaker than at 40°C and 20°C, and there was no effect at the lowest concentration (1/800 dilution).

The effects of DDAC at various concentrations of NaOH on the virus are summarized in Table 3. When the DDAC contained NaOH even at low concentration, it inactivated the virus, and the effect was stronger at high concentration (0.1%, 0.2%, and 0.5%). However, the distilled water control had no effect at low concentrations of NaOH (0.0125% and 0.025%). A strong inactivating effect on the virus was also observed when the concentration of NaOH was 0.1% or higher. The effect on the virus was generally stronger in DDAC solution than in distilled water solution.

The effects of incubation time of DDAC with 0.1% NaOH on virus infectivity are shown in Table 4. A strong effect of DDAC with 0.1% NaOH on SVDV was already observed after 1 min of incubation. And the effect appeared slightly higher until 60 min of incubation, whereas, the complete effect of 0.1% NaOH on SVDV appeared after 30 min of incubation, and it was lower than that of DDAC.

Effects of triton X-100 with or without 0.1% NaOH on SVDV: The effects of triton X-100 with or without 0.1% NaOH on SVDV were compared with those of QACs. The effects of triton X-100 with 0.1% NaOH were slight at dilutions of 1/200, 1/400, and 1/600 (data not shown). The effects of the mixture of triton X-100 and DDAC with 0.1% NaOH on SVDV were smaller than those of DDAC alone. The effects of DDAC were inhibited by triton X-100 (Table 5).

Electron microscopy: Electron micrographs of virus particles affected by the compounds are shown in Figs. 1 and 2. Spherical particles about 28 nm in diameter were observed in many place (Fig. 1-a). These particles were destroyed by 0.1% NaOH (Fig. 1-b). The virus particles were gathered by a 1/400 dilution of DDAC (Fig. 1-c). Non-structural substances were observed in many places in the material treated by a 1/400 dilution of DDAC with 0.1% NaOH (Fig. 1-d). These substances were considered to be the micelles which were constructed from the viral particles destroyed by DDAC.

Non-structural substances shaped like destroyed viral particles were observed in many places in the material treated by a 1/400 dilution of triton X-100 with 0.1% NaOH (Fig. 2-a). These substances were observed also in the material treated by a 1/200 dilution of triton X-100 and DDAC with 0.1% NaOH. In this material, the substances which were observed in samples treated with DDAC with 0.1% NaOH were also observed, but there were fewer of them (Fig. 2-b). The substances shaped like destroyed viral particles were considered to be the micelles which were constructed from viral particles destroyed by triton X-100.

Table 2. Effects of temperature of DDAC with 0.1% NaOH on SVDV

Dilution	Virus titer at temperature 4°C	20°C	40°C
× 200	3.48	2.85	1>
× 400	3.70	2.78	1>
× 800	7.30	3.78	2.11
Distilled water	7.38	6.48	4.48

The virus titer is indicated as log10 PFU per 0.1 m*l* of the sample. SVDV strain J1 was used.

Table 3. Effects of pH of DDAC at various concentrations of sodium hydroxide on SVDV

Material	NaOH (%)	pH[a)]	Virus titer[b)]
1/400 DDAC	0	7.32 (8.28)	7.30
	0.0125	8.27 (11.34)	6.95
	0.025	8.89 (11.68)	6.95
	0.1	10.85 (12.26)	2.00
	0.2	11.88 (12.53)	1>
	0.5	12.49 (12.86)	1>
Distilled water	0	7.32 (7.47)	7.30
	0.0125	8.14 (11.36)	7.26
	0.025	8.85 (11.69)	7.32
	0.1	10.78 (12.26)	6.30
	0.2	1.86 (12.50)	3.00
	0.5	12.49 (12.84)	2.30

a) The numbers indicate the pH of mixed samples. The pH of the original material is shown in parentheses.
b) Virus titer is indicated as log10 PFU per m*l* of the sample. SVDV strain J1 was used.

Table 4. Effects of time of incubation of DDAC with 0.1% NaOH on SVDV

Material	Time (minute)	Virus titer
0.1% NaOH	1	7.00
	5	7.11
	10	7.04
	30	5.00
	60	4.78
1/400 DDAC with 0.1% NaOH	1	2.90
	5	2.70
	10	2.11
	30	1.48
	60	1.00
1/400 DDAC	60	7.08

Virus titer is indicated as log10 PFU per 0.1 m*l* of the sample. SVDV strain J1 was used.

DISCUSSION

QACs are very common disinfectants, and are widely used in the medical field and animal hygiene because they have low toxicity and are non-corrosive to metals [6]. However, QACs have a limited spectrum of activity and are ineffective against most viruses, mycobacteria and bacterial spores [6]. Among viruses, QACs are effective only against

Table 5. Effects of triton X-100 and DDAC with 0.1% NaOH on SVDV

Material	Presence of 0.1% NaOH	Virus titer
1/400 Triton X-100	+	4.15
1/400 DDAC	+	1.48
1/200 Triton X-100 with 1/200 DDAC	+	3.23
	–	6.95
Distilled water	–	7.00
	+	4.28

Virus titer is indicated as log10 PFU per 0.1 m*l* of the sample. SVDV strain J1 was used.

enveloped viruses (group A) [6, 9].

SVDV belongs to the enteroviruses (group B, small non-enveloped virus) [11] and are normally resistant to QACs [2]. However, our results showed that the QACs plus a small amount of NaOH inactivated SVDV, indicating that the activity of the QACs was enhanced by 0.1% NaOH. The mechanism of the effect of the QACs (especially DDAC) on SVDV was considered to be as follows. The viral particles of SVD are considered to be solid enough to prevent the effect of QACs alone (Fig. 1-C), whereas they were considered to become fragile and partially destroyed in alkaline condition (high pH) (Fig. 1-b). Although the effect of alkali alone in low concentration of NaOH was weak, the viral particles in alkali became very sensitive to the effect of QACs, as shown in Table 4. The effect of alkali on SVDV appeared very slowly, but that of DDAC with 0.1% NaOH appeared immediately. The partially destroyed viral particles formed micelles in response to DDAC (Fig. 1-d), and the infectivity of viral particles was considered to be lost by this micelle formation. This phenomenon also occurred in the case of triton X-100. But the effect of triton X-100 with 0.1% NaOH was smaller than that of DDAC. The cause of this was considered to be the difference of the ability of micelle formation between two detergents. The shape of micelles formed by triton X-100 (Fig. 2-a) was almost the same as that of the viral particles destroyed by alkali (Fig. 1-b), while those destroyed by DDAC seemed to be of a uniform sturdy shape (Fig. 2-c).

From these results and our previous study [13], QACs with 0.1% NaOH are considered to be very effective against the viruses belong to group B and C (small non-enveloped and other non-enveloped viruses) [11]. Therefore, we believe that the QACs are useful disinfectants against viral diseases, when they are used with 0.1% NaOH. An alkali such as 1% NaOH usualy used for disinfection is very dangerous for the human body, but NaOH at a 0.1% (final concentration of 0.05% in our experiments) concentration, is safe for the human body when used for alkalization of QACs [12]. In conclusion, QACs with 0.1% NaOH were effective against SVDV(enterovirus), further studies of QACs with 0.1% NaOH against other non-enveloped viruses reveal the usefulness of this disinfectant. And, we hope this method will be applied widely in the animal hygiene and medical field.

ACKNOWLEDGEMENTS. The authors thank Dr. Y. Kono for critical reading of the manuscript, and Dr. Y. Murakami for technical suggestions of an electron microscope.

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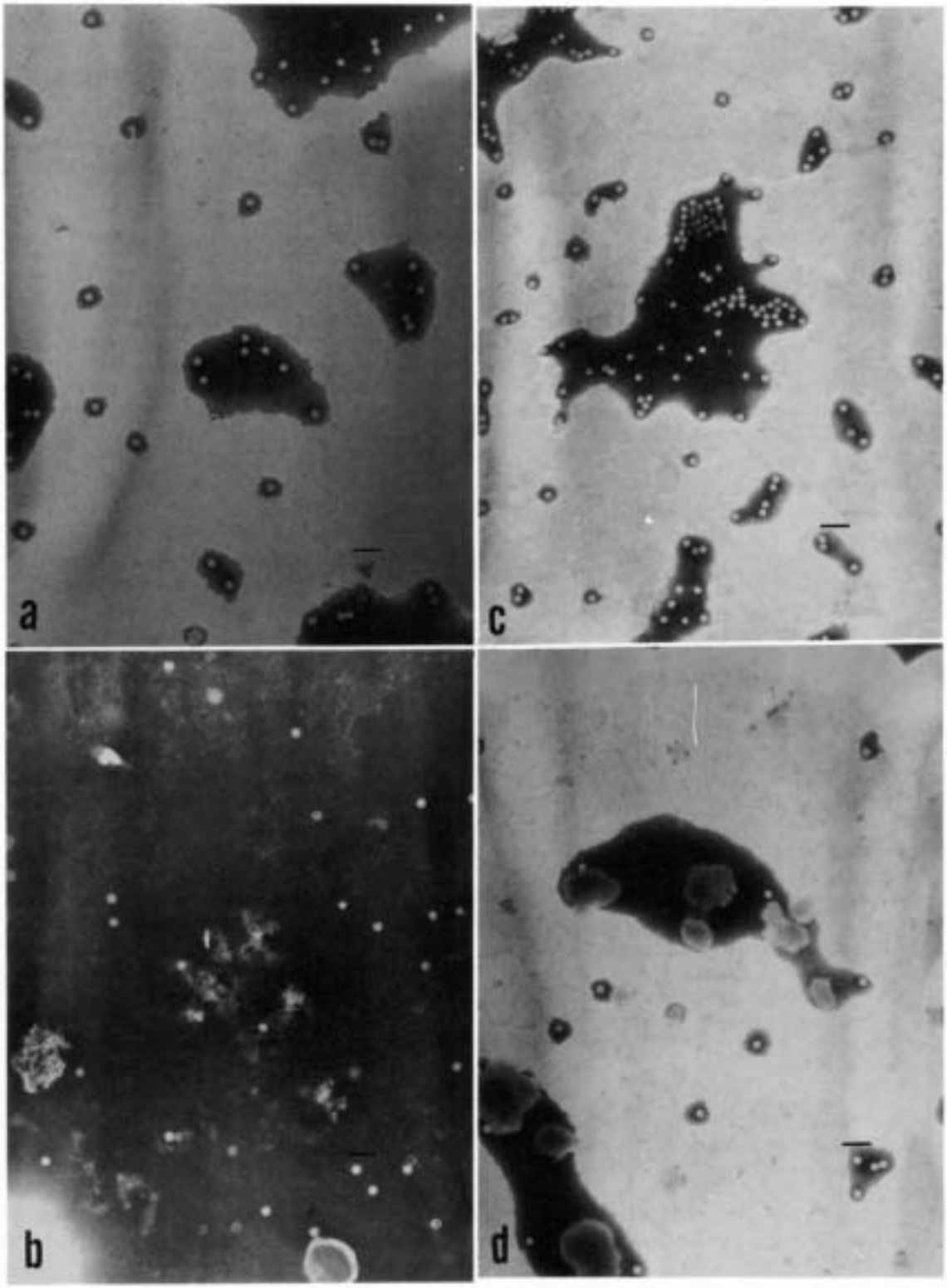

Fig. 1. Electron micrographs of the effects of DDAC on SVDV. a) SVDV purified with an equal amount of distilled water, b) SVDV purified with an equal amount of 0.1% NaOH, c) SVDV purified with an equal amount of a 1/400 dilution of DDAC, d) SVDV purified with an equal amount of a 1/400 dilution of DDAC with 0.1% NaOH. Bar indicates 100 nm.

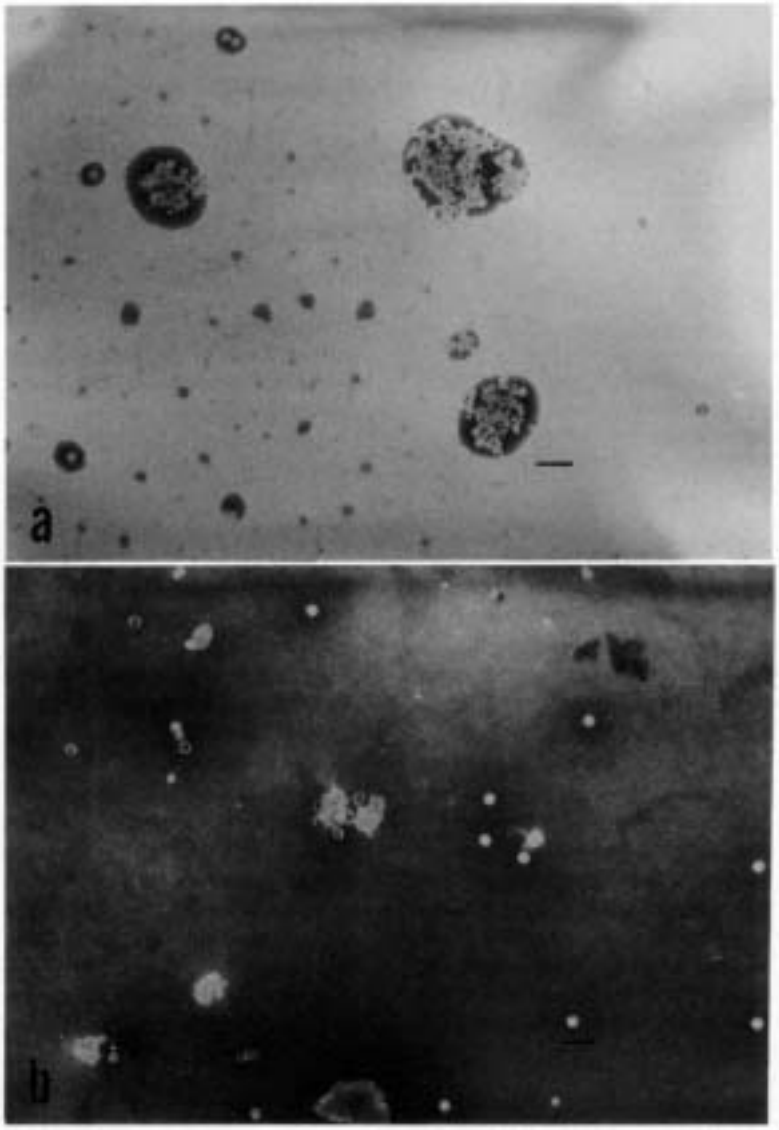

Fig. 2. Electron micrographs of the effects of triton X-100 and DDAC on SVDV. a) SVDV purified with an equal amount of a 1/400 dilution of triton X-100 with 0.1% NaOH, b) SVDV purified with an equal amount of a 1/200 dilution of triton X-100 and DDAC with 0.1% NaOH. Bar indicates 100 nm.