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Lipid and Protein Organization in Vesicular Stomatitis and Sindbis Viruses

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SUMMARY

Vesicular stomatitis virus was disrupted by 0·1 % SDS into RNA, lipid and the individual virus polypeptides. If the virus was first treated with 0·4 % formaldehyde, the particles retained their bullet shape on treatment with 0·1 % SDS, although they were extensively penetrated by phosphotungstic acid. Radiochemical analysis using virus labelled with [^{14}C]-amino acids or [^{14}C]-choline or [^{3}H]-glucosamine indicated that about 82 % of the glycoprotein and about 96 % of the protein was retained in the formaldehyde pre-treated particles, which were then treated with SDS, although more than 90 % of the lipid was lost. With Sinbis virus also the overall structure of the particles was retained when the virus which had been pre-treated with formaldehyde was treated with 0·1 % SDS. With this virus, about 60 % of the glycoprotein was retained in the SDS-treated formalinized particles despite the loss of all the lipid. These results suggest that the surface projections are located in close proximity to the core protein.

The question of whether the surface projections of enveloped viruses form a bridge across the lipid bi-layer to interact directly with the core is of importance in considering the maturation of the virus particles at the cell surface. From evidence obtained by the use of electron spin labels, Landsberger *et al.* (1971) concluded that the spikes of influenza virus are probably not involved in determining the organization of the lipid bi-layer. Similarly, Harrison *et al.* (1971) concluded from X-ray diffraction studies that protein probably does not form a bridge across the lipid bi-layer of Sindbis virus particles.

Some years ago it was observed that vesicular stomatitis virus particles which had been treated with phospholipase C appeared to retain their surface projections, although a large proportion of the lipid had been removed and the particles were penetrated by phosphotungstic acid (Cartwright, Smale & Brown, 1969). Retention of the surface projections was also indicated by serological tests, and from the evidence that the treated particles produced as much neutralizing antibody in guinea pigs as the untreated virus particles. Further evidence has now been obtained to support the idea that the surface projections are attached to the core of the particles.

Virus (Indiana serotype) was grown in BHK 21 cells in the presence of [^{14}C]-amino acids (Chlorella protein hydrolysate) or [^{3}H]-glucosamine or [^{14}C]-choline to label the protein, surface projections or lipid, respectively. Another preparation was labelled in the RNA and phospholipid by growing the virus in the presence of [^{32}P]-orthophosphate. The preparations were purified by precipitating with 60 % saturated ammonium sulphate, sedimenting at 20000 rev/min for 1 h and then centrifuging at 20000 rev/min for 2 h in a 15 to 45 % sucrose gradient, using the Spinco SW25.1 rotor. The absence of significant amounts of labelled cellular protein and glycoprotein constituents was demonstrated by polyacrylamide gel electrophoresis.

Mixing with 0·1 % SDS completely disrupted the structure of the virus (Fig. 1 *a*, *b*). How-

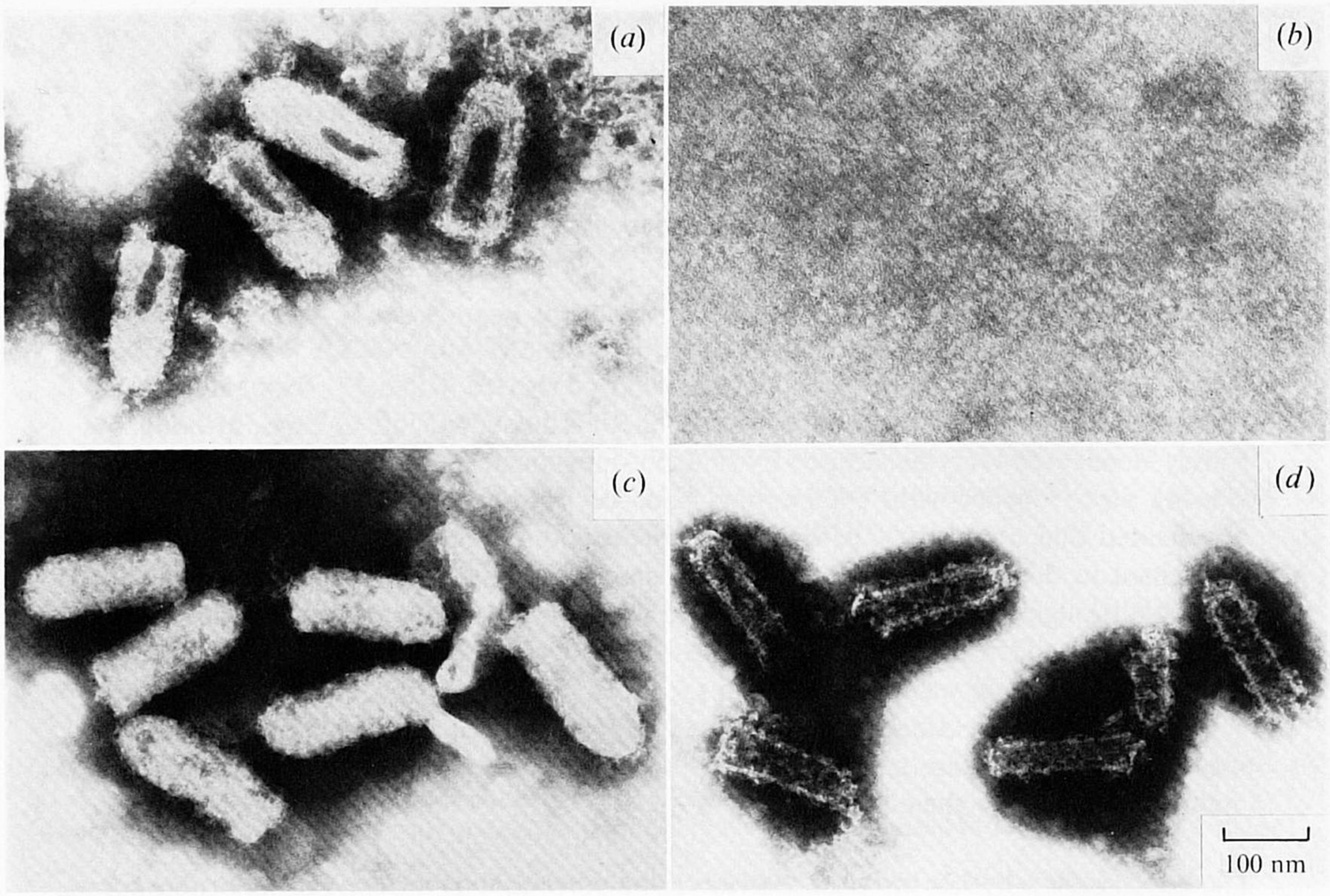

Fig. 1. Electron micrographs of vesicular stomatitis virus showing the effect of fixation with formaldehyde on the disruptive effect of SDS. (*a*) Unfixed virus; (*b*) unfixed virus in 0·1% SDS; (*c*) fixed virus; (*d*) fixed virus in 0·1 % SDS.

Table 1. *Radioactivity in the pellet fraction of vesicular stomatitis virus and fixed virus after treatment with* 0·1 % *SDS*

	% of radioactivity in pellet	
Label	Virus	Fixed virus
[^{14}C]-amino acid	< 1	96
[^{3}H]-glucosamine	< 1	82
[^{14}C]-choline	< 1	10
[^{32}P]-phosphate	4	20*

* 15% of ^{32}P counts were in RNA and phosphoprotein of this preparation.

ever, pre-treatment of the virus with 0·4% formaldehyde or 0·4% glutaraldehyde for 15 h at 20 °C prior to addition of the detergent prevented disruption of the structure (Fig. 1 *c*, *d*). The detergent-treated preparations were penetrated by phosphotungstic acid but a considerable proportion of the surface projections still appeared to be attached to the skeleton-like structure.

To provide a quantitative estimation of the extent of removal of the different constituents of the virus by the detergent, appropriately labelled preparations of the virus and fixed virus were treated with 0·1 % SDS, layered on to a 1 ml cushion of 15% sucrose and centrifuged for 1 h at 30000 rev/min. The radioactivity in the pellet fraction and the supernatant fraction, which consisted of the SDS-treated virus and sucrose layer, was then measured

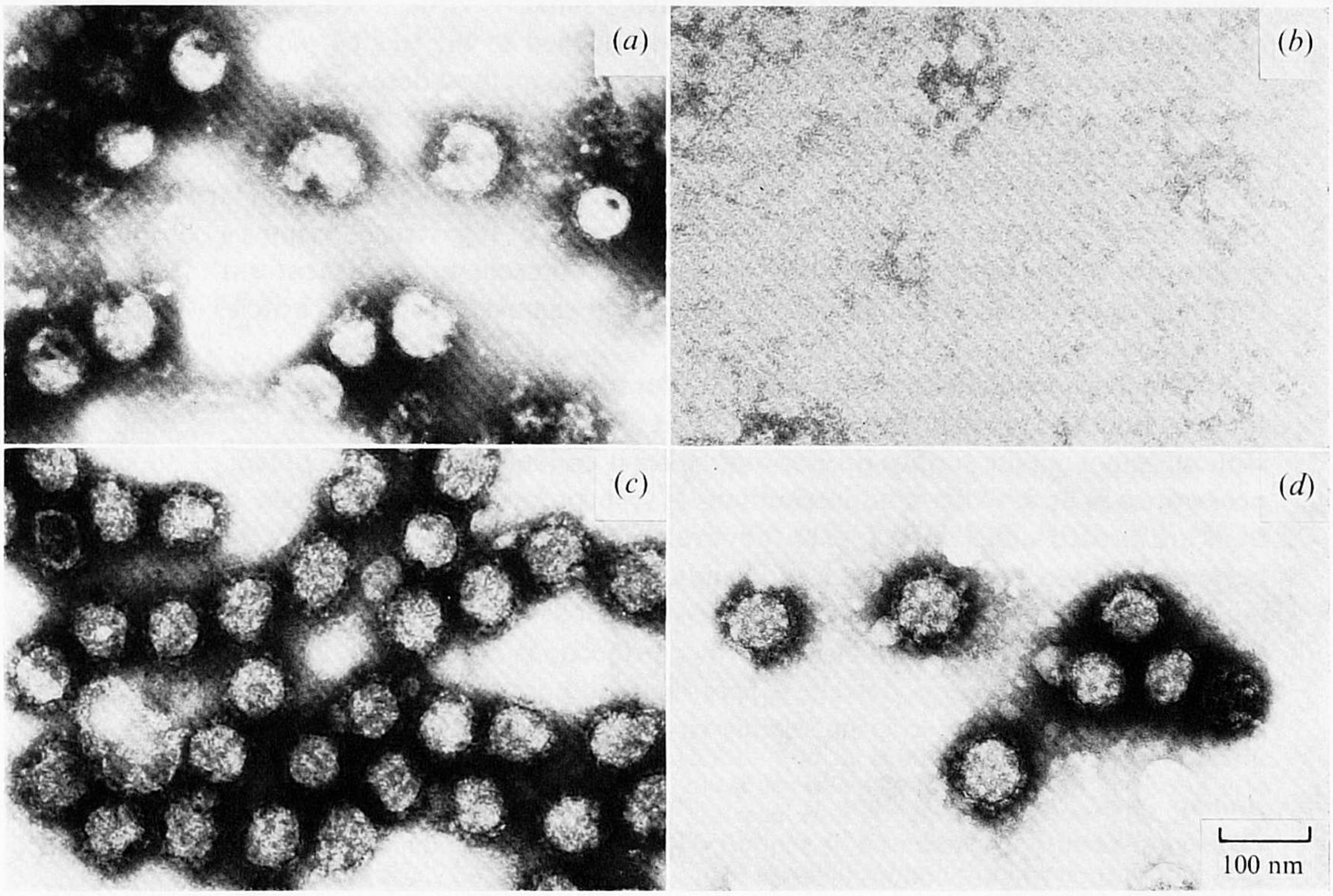

Fig. 2. Electron micrographs of Sindbis virus showing the effect of fixation with formaldehyde on the disruptive effect of SDS. (*a*) Unfixed virus; (*b*) unfixed virus in 0·1 % SDS; (*c*) fixed virus; (*d*) fixed virus in 0·1 % SDS.

Table 2. *Radioactivity in the pellet fraction of Sindbis virus and fixed virus after treatment with* 0·1 % *SDS*

	% of radioactivity in pellet	
Label	Virus	Fixed virus
[^{3}H]-glucosamine	0·9	60
[^{14}C]-choline	1·2	0·4

(Table 1). The distribution of radioactivity showed that most of the protein, glycoprotein, lipid and phospholipid was present in the supernatant fraction of disrupted virus. With the fixed virus preparations, however, 96 % of the protein and 82 % of the glycoprotein were present in the pelleted fraction, despite the presence of 90 % of the lipid and 95 % of the phospholipid in the supernatant fluid. These results indicate that most of the surface projections are retained in the fixed particles after treatment with SDS, despite the loss of the major part of the lipid. Moreover, more than 96 % of the total protein was present in the fixed particles, indicating that the matrix protein also was retained after SDS treatment.

Similar results were obtained with Sindbis virus. This virus was grown in BHK 21 cells in the presence of [^{3}H]-glucosamine or [^{14}C]-choline and purified by sedimenting through a 15 % sucrose cushion and then centrifuging at 35000 rev/min for 1 h in a 15 to 30 % sucrose gradient, using the Spinco SW 50 rotor. Mixing with 0·1 % SDS disrupted the virus particles (Fig. 2*a*, *b*) but pre-treatment of the virus with 0·8 % formaldehyde for 15 h at 20 °C pre-

vented disruption (Fig. 2*c*, *d*). As with vesicular stomatitis virus, a considerable proportion of the surface projections still appeared to be attached to the treated particles.

Examination of the radioactive preparations by the method described above for vesicular stomatitis virus showed that only traces of lipid were retained in the detergent-treated fixed particles but more than 60% of the glycoprotein was associated with the structures (Table 2). It is noteworthy in this connexion that Semliki Forest virus, which has a similar structure to Sindbis virus and also belongs to the alphavirus group of togaviruses, retains a considerable proportion of its haemagglutinating surface glycoprotein on treatment with Tween 80-tri(*n*-butyl)phosphate, although most of the lipid is removed from the particles (Mussgay *et al.* 1973).

Although formaldehyde reacts with unsaturated fatty acids and may thus form bonds between lipid and protein, the observation that SDS removes the lipid from the fixed particles without removing the surface projections makes it unlikely that the attachment of the surface projections is by protein–lipid interaction. It seems more probable that the surface projections are located sufficiently close to the core protein to allow formaldehyde to form a bridge between the two proteins. This would indicate that the surface projections pass through the lipid bi-layer. In the case of vesicular stomatitis virus, a model has been proposed by Cartwright *et al.* (1972) in which the surface projections are structurally related to the core proteins. The experiments described here lend support to that model and suggest that a similar relationship may exist in Sindbis virus.

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