The Effect of Ethylene Glycol and DMSO on Fusion of Isolated Sarcoplasmic Reticulum Membranes

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Fragmented sarcoplasmic reticulum (FSR) vesicles from rabbit skeletal muscle were suspended in 5–10% ethylene glycol (EG) or in 5, 10, or 25% dimethylsulfoxide (DMSO) and were pelleted onto flat aluminum foil disks. No vesicle fusion occurs with either 5 or 10% EG treatment and 2–2½ hours drying. After 4–5 hours drying, 5% EG-treated vesicles have established more areas of close contact and individual vesicles have begun to flatten when compared with the 10% EG vesicles. Overnight drying results in formation of extended sheets of fused membranes called double bilayers in both the 5 and 10% EG samples. In the 5% EG-treated vesicles, formation of large spaces in the pellet compresses the adjacent bilayer pairs.

Extensive vesicle fusion accompanies 4–5 hours drying of both 5 and 10% DMSO-treated samples. No fusion was observed in the 25% DMSO-treated sample after 4–5 hours drying and individual vesicles remained round as in the 2–2½ hour samples of all treatments. Overnight drying also causes extended sheets of bilayer pairs to form in the 5% DMSO-treated samples but, with 10 and 25% treated vesicles, destroys the double bilayers and only occasional dense regions of membrane whorls remain. Both EG and DMSO promote more rapid fusion of FSR vesicles than does glycerol but overnight drying after treatment with 10 or 25% DMSO destroys the fused membrane.

Introduction

Various fusogenic agents have been used to induce fusion of biological membranes. Lysolecithin promotes fusion of hen erythrocytes [1] but causes lysis and membrane disintegration within 6–8 hours after fusion. Lysolecithin has also been reported to solubilize approximately 75% of the lipids and 50% of the proteins in sarcoplasmic reticulum vesicles [2]. Counting the number of bi- or multinucleated hen erythrocytes was the method of detecting cell fusion in a survey of fusogenic agents [3] which included dimethylsulfoxide (DMSO), glycerol, sorbitol, mannitol, ethylene glycol, polyethylene glycol and sucrose. Although DMSO and glycerol-induced fusion of hen erythrocytes reportedly was calcium-dependent [3], glycerol-induced fusion of isolated fragmented sarcoplasmic reticulum (FSR) vesicles did not require added calcium [4]. DMSO causes membrane blisters to form in lymphocytes [5], a phenomenon also observed after treatment of fibroblasts with aleylamine [6]. Because fibroblast plasma membranes fuse in these blistered regions, DMSO has been presumed to cause membrane fusion by causing some type of rearrangement or disruption of the membrane structure.

The purpose of the experiments reported here was to compare the effects of DMSO and ethylene glycol on isolated FSR vesicles with those observed during glycerol treatment of the same system [4]. Of particular interest was the temporal sequence of fusion and the influence of various concentrations of DMSO and ethylene glycol on membrane fusion.

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Materials and Methods

FSR was prepared from rabbit muscle by the method of Hasselbach and Makinose [7]. Pellets were obtained by centrifugation of FSR vesicles through an 0.08 M KCl, 0.1 M PO_4, pH 7.15 solution as described previously [4] which contained, in addition, either 5 or 10% EG or 5, 10 or 25% DMSO. Both EG and DMSO were obtained from Merck (Darmstadt). Pellets were weighed immediately after formation, and after 2 – 2.5, 4 – 5 and 20 – 21 hours of drying in the cold. After weighing, pellets were fixed in Karnovsky's fixative [9], rinsed in Millonig's PO_4 buffer, postfixed in OSO_4, dehydrated in cold, graded acetones and embedded in Epon-Araldite [8]. Blocks were sectioned with an LKB Ultrotome III. Sections were doubly stained with 2% uranyl acetate in methanol followed by lead citrate and were examined at 80 KV in a Siemens Elmiskop 101 equipped with an anticontamination device.

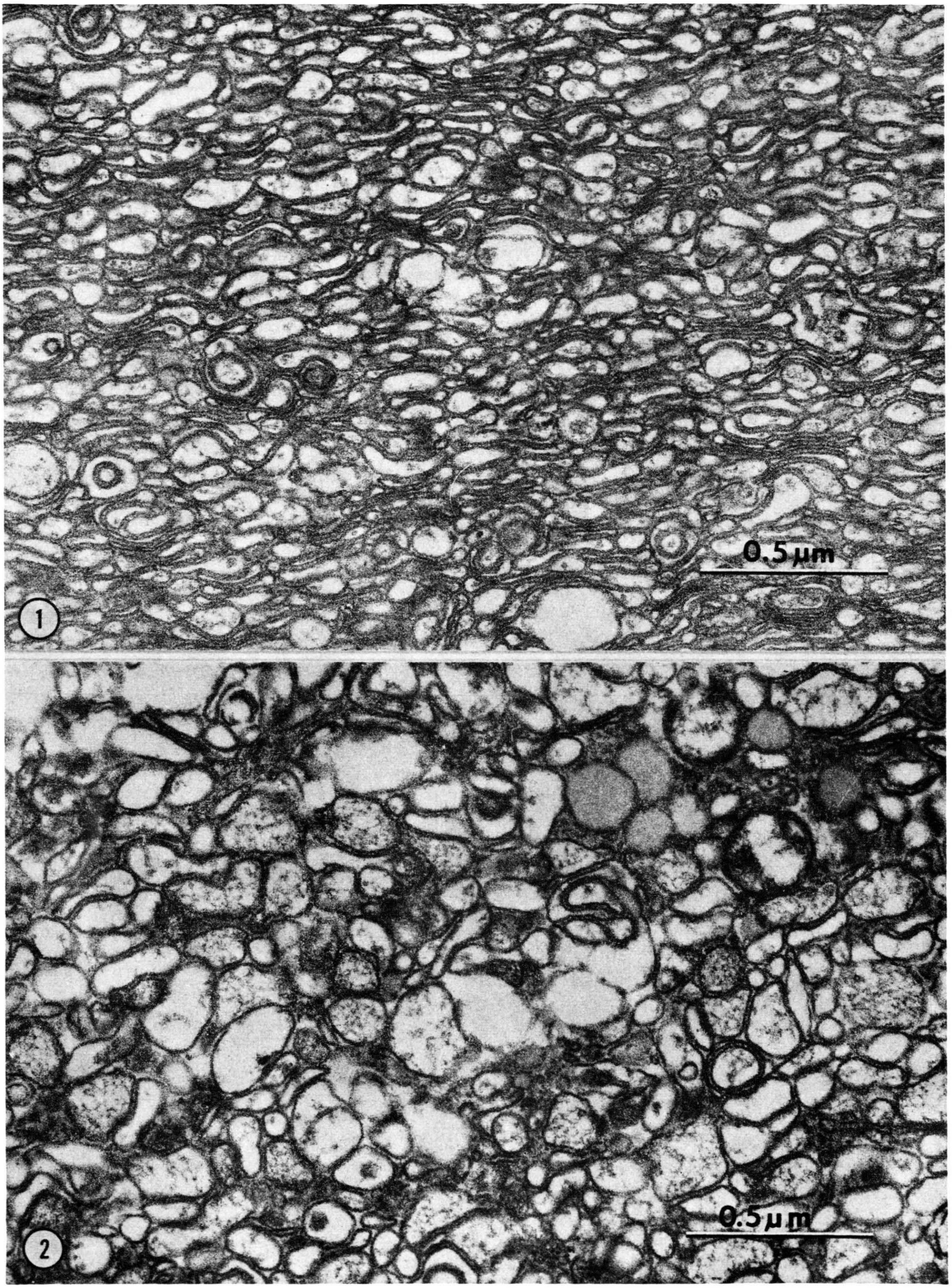

Fig. 1. Drying pelleted 5% EG-treated FSR vesicles for 4—5 hours results in many areas of close contact between individual flattened or partially flattened vesicles. X 60,000.

Fig. 2. Individual, round FSR vesicles very similar to those seen in the starting material are typical of 10% EG-treated FSR vesicles that have been pelleted and dried 4—5 hours. X 60,000.

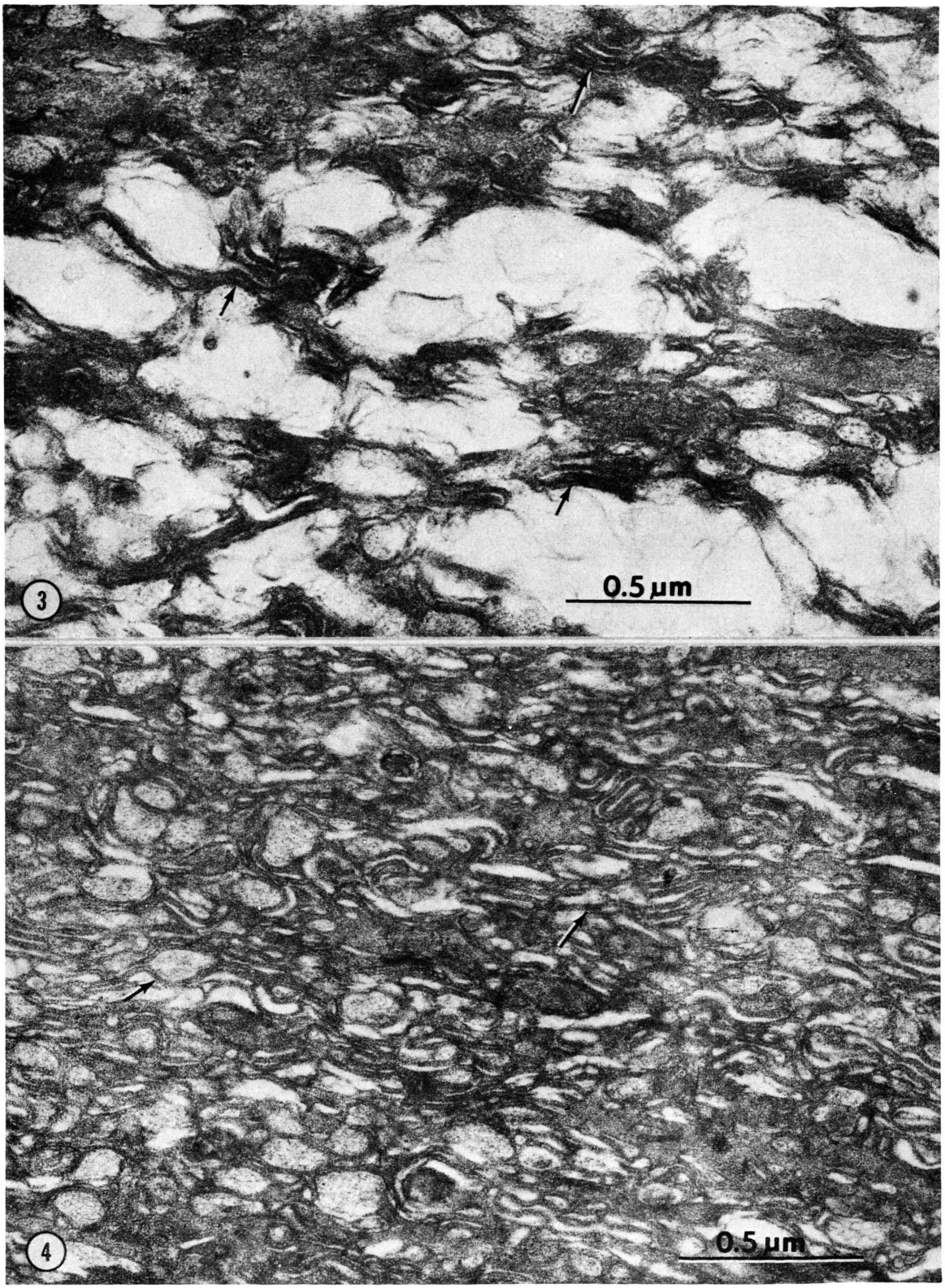

Fig. 3. Overnight drying of 5% EG-treated vesicles usually causes large spaces to form which displace the double bilayer into dense sheets of lamellae (arrows). Areas of extended, flattened double bilayers with no unusually large spaces are occasionally seen, but the appearance of the material shown here predominates. X 60,000.

Fig. 4. Pelleted 10% EG-treated FSR vesicles dried overnight are flattened and have moved closer together. Although some sheets of double bilayers are present (arrows), it is difficult to trace double bilayers over distances comparable to those observed in DMSO-treated preparations. X 60,000.

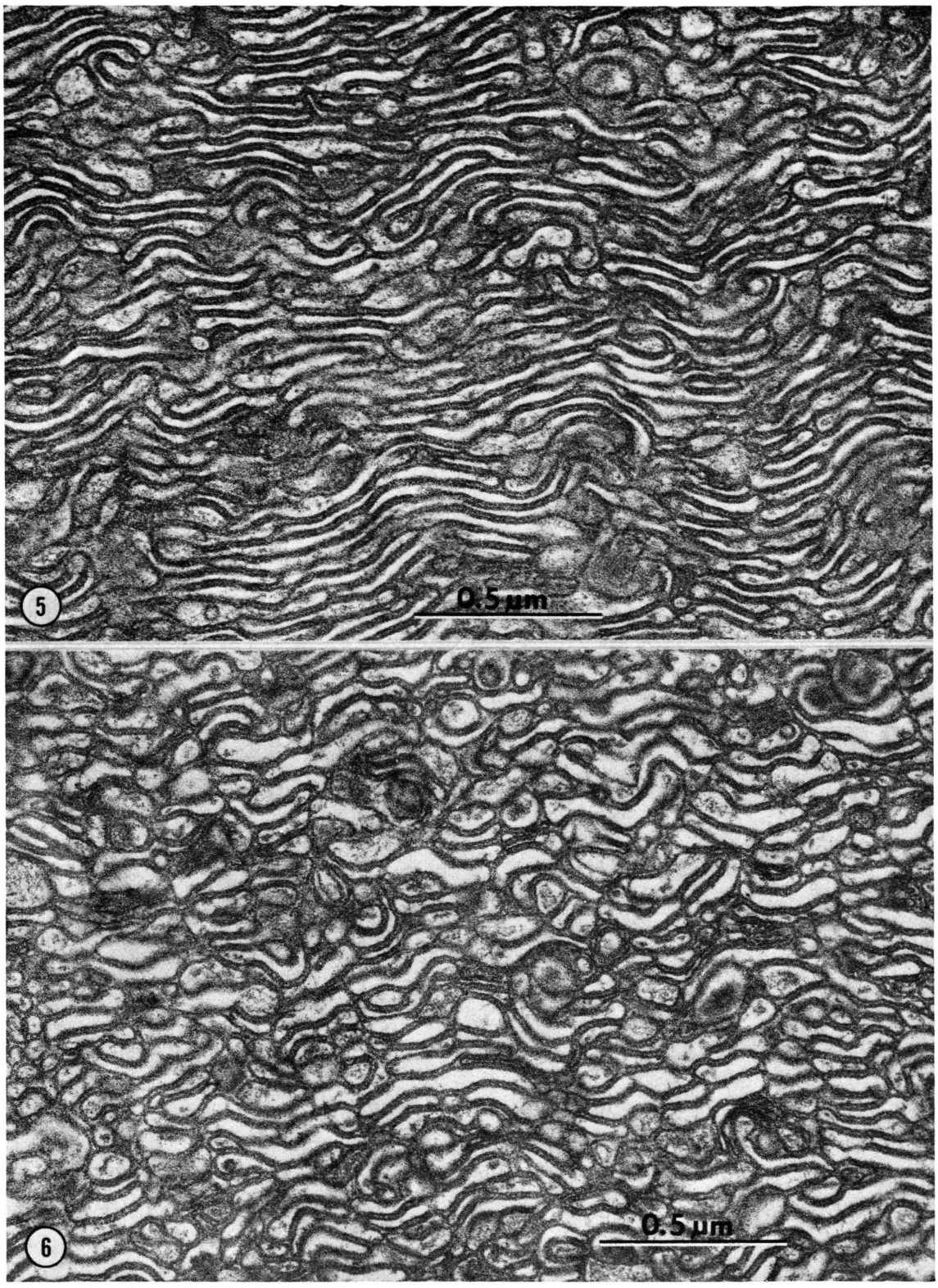

Fig. 5. Drying pelleted 5% DMSO-treated FSR vesicles for 4—5 hours causes extensive fusion as judged by the extended double bilayers. The electron opacity of the space between the two halves of the double bilayers is especially clear. X 60,000.

Fig. 6. Although the flattening of vesicles and formation of double bilayers is less complete in 10% DMSO-treated vesicles dried for 4—5 hours than that seen in Fig. 5, all vesicles have established contact regions which are very similar to the double bilayers observed in the 5% DMSO-treated sample. X 60,000.

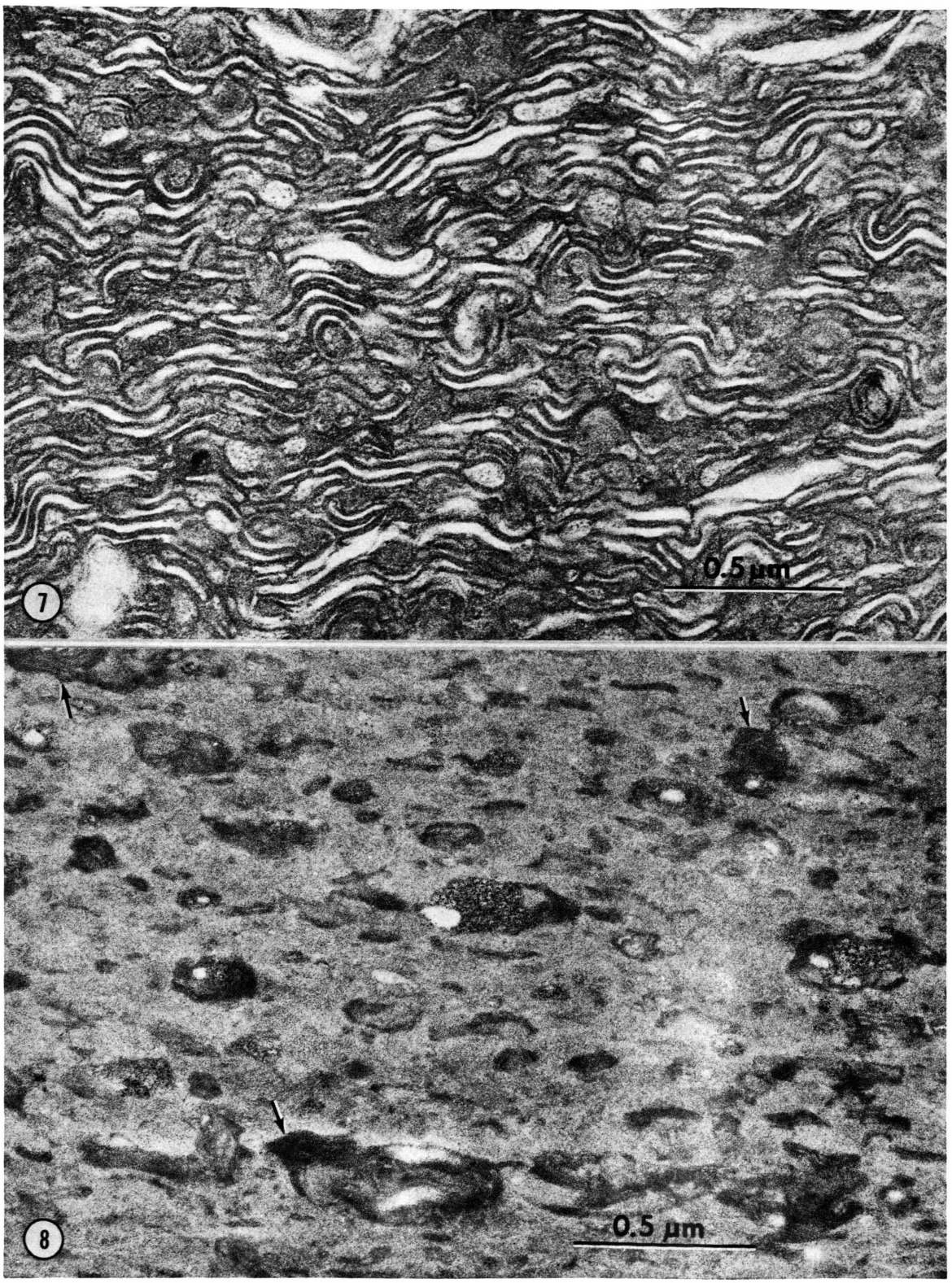

Fig. 7. Overnight drying of 5% DMSO-treated vesicles causes very little change when compared to the 4—5 hours sample (*c. f.* Fig. 5). Formation of small spaces between bilayer pairs the main difference. X 60,000.

Fig. 8. Pelleted 10% DMSO-treated FSR vesicles collapse completely after overnight drying. Dense lamellae patches (arrows) are the only recognizable remnants of membranes seen after this or after 25% DMSO treatment. X 60,000.

Results

After 2 – 2.5 hours of drying (not shown here), FSR vesicles treated with either 5 or 10% EG appear to be unchanged from the starting material and still contain individual round vesicles. Four to five hours of drying usually results in 60 – 70% weight loss from the pellet and, in the case of 5% EG treatment, results in many regions of close contact between individual vesicles (Fig. 1 *). This appearance is caused by individual vesicles moving closer together and becoming somewhat flattened. It should be noted that the integrity of individual vesicles has been maintained even though the limited areas of contact between vesicles strongly resemble the double bilayers observed after glycerol treatment [4]. With 10% EG treatment (Fig. 2), only very limited areas of contact between vesicles have been established; vesicles remain round and are changed little from the 2 – 2.5 hour sampling time. Overnight drying of the 5% EG treated vesicles causes the formation of compressed sheets of double bilayers with large empty spaces between the sheets (Fig. 3). These spaces are much smaller in the 10% EG-treated pellet (Fig. 4). Although limited areas of the 5% EG pellet closely resemble the 10% EG pellet with extensive formation of double bilayers separated by small empty spaces, the preservation of the double bilayer integrity is better in the 10% EG samples. These results are summarized in Table I.

Drying 5, 10 or 25% DMSO treated FSR vesicles for 2 – 2.5 h (not shown here) does not result in vesicle fusion. Extensive fusion of FSR vesicles occurs, however, after 4 – 5 hours of drying both with 5% (Fig. 5) and 10% (Fig. 6) DMSO. Because one can trace a given double bilayer for distances greater than one μm, it is very highly probable that fusion of FSR vesicles, which typically have diameters of 0.06 – 0.2 μm, would be required to form double bilayers of such length. Individual round vesicles, identical to those seen in the 2 – 2.5 hour dried, 5 and 10% DMSO-treated samples still are the only form seen in the 25% DMSO-treated sample after 4 – 5 hours of drying. Because the 25% DMSO-treated samples lose water somewhat more slowly during the first part of the drying process, the range of water loss (Table II) is greater for 2 – 2.5 and 4 – 5 hours drying than that noted in the EG samples (Table I).

Table I. Effect of ethylene glycol (EG) and drying on the appearance of isolated sarcoplasmic reticulum.

Drying time	Water loss	Initial concentration of EG 5%	10%
2 – 2.5 h	30 – 40%	individual round vesicles	individual round vesicles
4 – 5 h	60 – 70%	individual flattened vesicles in close contact	individual round vesicles
20 – 21 h	70 – 80%	compressed sheets of double bilayers and spaces	sheets of double bilayers and individual flattened vesicles in close contact

Overnight drying of DMSO-treated pellets results in large differences in morphology depending on the concentration of DMSO used. Five percent DMSO-treated vesicles remain as double bilayers (Fig. 7) which differ little from their appearance after four hours of drying. Treatment with 10% DMSO followed by overnight drying causes destruction of the bilayer structure (Fig. 8) and leaves only scattered dense patches of membrane whorls (arrows). Identical results (not shown here) are obtained after treatment with 25% DMSO and overnight drying. These findings are summarized in Table II.

Discussion

The double bilayers referred to in the Results section are the limiting membranes of more than

Table II. Effect of dimethylsulfoxide (DMSO) and drying on the appearance of isolated sarcoplasmic reticulum.

Drying time	Water loss	Initial concentration of DMSO 5%	10%	25%
2 – 2.5 h	25 – 40%	individual round vesicles	individual round vesicles	individual round vesicles
4 – 5 h	40 – 70%	sheets of double bilayers	individual flattened vesicles in close contact	individual round vesicles
20 – 21 h	70 – 80%	sheets of double bilayers	destroyed	destroyed

* Figs 1 – 8 see Plates on pages 708 a – d.

two FSR vesicles. The electron opaque space between the two halves of the double bilayer originates from the outer surface of the vesicles as shown by the Hg-phenyl azoferritin label [4]. Evidence that fusion of individual vesicles is responsible for the formation of these double bilayers is derived from a comparison of vesicle diameter with distances that double bilayers can be traced. If fusion had not occurred and individual vesicles had merely flattened and moved into close contact with other vesicles, the maximum distance that double bilayers could be traced would be half the typical vesicle circumference. Although the range of vesicle diameters is $0.06-0.2\ \mu$m, the majority of vesicles have diameters from $0.08-0.1\ \mu$m. The circumference of these vesicles is $0.25-0.31\ \mu$m. In preparations where fusion has occurred, double bilayers can be traced for distances much greater than $0.125-0.155\ \mu$m before one of the bilayers curves away from the other bilayer.

The steps in the vesicle fusion process in the presence of EG or DMSO appear to be identical to those observed in the presence of glycerol [4]. First, individual, round vesicles move closer together. Then partially flattened, or flattened vesicles establish limited areas of contact with adjacent vesicles. Finally, vesicles fuse and double bilayers form. The small radius of curvature at the margin of the flattened vesicles would be expected to perturb the bilayer structure and permit fusion sites to develop.

That EG and DMSO also promote fusion of isolated FSR vesicles can, in part, be explained by their hygroscopic nature. Although specific figures for water displacement by DMSO are not readily available, figures for ethylene glycol [10] indicate that it has approximately a 10% greater ability to displace water than does glycerol in the concentration range of $0.5-10\%$. This would account for the observation that EG-treated FSR vesicles either fuse (5% EG) or form extensive areas of close contact (10% EG) but that glycerol-treated FSR vesicles remain largely as separated, round vesicles after four hours of drying. This does not, however, explain why 5% EG-treated vesicles fuse before 10% EG-treated vesicles. Since the 10% EG solution should have twice the water displacing capacity of the 5% solution, one would expect the 10% to fuse first. A possible explanation is that the greater initial loss of water in the 10% EG-treated sample causes an initial rigidity which can later be overcome as the vesicles move still closer together. Based on pellet weight loss and initial concentration of EG, it is clear that the 5% treated pellet has, after overnight drying, a concentration of $18-20\%$ EG inside the pellet and that the 10% treated pellet has twice this amount. The apparent protecting affect of 10% EG on the structure of the bilayer pairs during overnight drying must outweigh the deleterious affect of greater water loss.

Fusion of FSR vesicles after 5 or 10% DMSO treatment and four hours of drying is essentially identical to that seen after overnight drying of glycerol-treated vesicles [4]. The finding that 25% DMSO followed by $4-5$ hours of drying results in no fusion or formation of areas of close contact could again be explained by an increase in rigidity of the membranes in response to the initial water loss. Overnight drying causes no detectable changes in the 5% DMSO-treated pellets but destroys the bilayer pairs in the 10 and 25% DMSO-treated pellets and leaves behind islands of multilayered debris. It is probable that, because 10% ethylene glycol-treated bilayer pairs survive overnight drying but that 10% or higher DMSO-treated bilayer pairs do not, either DMSO is more efficient in displacing water from this system or has some other more disruptive effect on membrane proteins. Since FSR membranes are about 65% protein [11] and since 80% of this protein is the ATPase molecule [12, 13], it is possible that DMSO is acting on the ATPase molecule or a nearby structural protein to cause this collapse. DMSO has been reported to either stimulate or inhibit a broad spectrum of hydrolytic enzymes [14].

The DMSO-promoted fusion of hen erythrocytes [3] is difficult to relate to the data presented here. Although 5 M DMSO (35%) was required to cause erythrocyte fusion in 5 min and no fusion occured at 3 M or lower concentration after 45 min incubation, it is possible that longer incubation times may have resulted in fusion. On the other hand, it is also possible that the composition and properties of the hen erythrocyte membrane are so different from those of the FSR that no comparisons are justifiable.

Data presented here show that both EG and DMSO promote fusion of isolated FSR vesicles. Less time is required for the lower levels of both reagents to cause fusion than for comparable levels of glycerol. Higher levels of DMSO accompanied

by overnight drying causes destruction of the fused membranes. No indication of membrane blistering was observed prior to fusion.

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