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The effect of lyophilization and dithiothreitol on vesicles of skeletal and cardiac muscle sarcoplasmic reticulum

The Ca^{2+} uptake and ATPase activity of fragmented sarcoplasmic reticulum are known to change with time after preparation; as the Ca^{2+} uptake declines, ATPase activity increases[1]. Cardiac and red skeletal muscle fragmented sarcoplasmic reticulum preparations are particularly labile. Some stabilization can be achieved by storing the vesicles in 45 % sucrose solution[2-4], and recently VAN DER KLOOT[5] has reported that the initial rate of Ca^{2+} uptake of lobster fragmented sarcoplasmic reticulum was more than one order of magnitude greater when vesicles were stored in 1,4-dithiothreitol[5].

We wish to report that after lyophilization of fragmented sarcoplasmic reticulum no loss of activity occurred even after 4 months of storage*. Rabbit fragmented sarcoplasmic reticulum was prepared by homogenizing muscle in a Waring blendor (90 sec) in 0.3 M sucrose, 10 mM Tris maleate, pH 7.0, and collected as the fraction sedimented between 8000 $\times$ *g* and 30000 $\times$ *g*. Before use the lyophilized fragmented sarcoplasmic reticulum was reconstituted in the original amount of cold distilled warte, homogenized and centrifuged at 8000 $\times$ *g* for 4 min. Comparison of freshly prepared white muscle fragmented sarcoplasmic reticulum and lyophilized 4-month-old fragmented sarcoplasmic reticulum (Table I) shows no marked difference in either Ca^{2+} uptake or ATPase activity.

In view of VAN DER KLOOT's[5] report and our unsuccessful attempts a few years ago to use dithiothreitol to prevent the loss of activity of cardiac fragmented sarcoplasmic reticulum we reinvestigated the effect of dithiothreitol on fragmented sarco-

TABLE I

COMPARISON OF Ca^{2+} UPTAKE AND ATPase ACTIVITY OF FRESH AND RECONSTITUTED LYOPHILIZED FRAGMENTED SARCOPLASMIC RETICULUM

Fragmented sarcoplasmic reticulum was prepared from the rabbit adductor magnus muscle. Conditions: 0.1 M KCl, 10 mM Tris maleate, pH 6.5, 5 mM $MgCl_2$, 0.075 mM $^{45}CaCl_2$, 1 mM ATP, 1 mM phosphoenolpyruvate, 0.2 mg pyruvate kinase per ml and 0.2 mg fragmented sarcoplasmic reticulum per ml, 22°. When oxalate (5 mM) was used the medium contained 20 mM Tris maleate, pH 7.0, 30 mM KCl, 5 mM $MgCl_2$, 4 mM ATP, 0.2 mM $^{45}CaCl_2$, 0.2 mM EGTA and 0.02 mg fragmented sarcoplasmic reticulum protein per ml. The Ca^{2+} uptake was measured as described earlier[6] using Millipore filters. Liberation of inorganic phosphate was measured at 0.5, 2.5, 5 and 10 min; the values given in the table represent the initial slope.

Fragmented sarcoplasmic reticulum	*Ca^{2+} uptake*				*ATPase activity; initial rate (μmoles/mg per min)*	
	No oxalate		*Oxalate*			
	Initial rate (μmoles/mg per min)	*Total uptake (μmole/mg)*	*Initial rate (μmoles/mg per min)*	*Total uptake (μmoles/mg)*	*No oxalate*	*Oxalate*
Fresh	1.80	0.30	2.48	6.00	0.30	1.65
Lyophilized	1.75	0.28	2.45	6.20	0.36	1.60

* After completion of this work we came across a statement in a paper by BAIRD AND PERRY[8] to the effect that "freeze-dried granule suspensions retained activity (to inhibit myofibrillar ATPase) after storage for several weeks".

plasmic reticulum. There was no increase in the rate or in the total amount of Ca^{2+} uptake by rabbit white muscle fragmented sarcoplasmic reticulum. In the case of the more labile and less active red muscle fragmented sarcoplasmic reticulum, the use of dithiothreitol increased the activity by 15–30 %, the best value being 0.3 μmole/mg per min for initial uptake rate and 1.8 μmoles/mg protein for total uptake during 15 min. Lyophilization was also effective in maintaining the activity of the extremely labile cardiac fragmented sarcoplasmic reticulum (Table II). The loss of Ca^{2+} uptake ability of the cardiac fragmented sarcoplasmic reticulum is evident within 1 day (*cf.* ref. 4). Dithiothreitol had neither an immediate effect on Ca^{2+} uptake of cardiac fragmented sarcoplasmic reticulum, nor did it affect the stability during storage.

We also compared the effectiveness of lyophilization and of dithiothreitol in

TABLE II

EFFECT OF DITHIOTHREITOL AND LYOPHILIZATION ON Ca^{2+} UPTAKE AND ATPase ACTIVITY OF CALF CARDIAC FRAGMENTED SARCOPLASMIC RETICULUM

Conditions as described in the legend to Table I. Dithiothreitol when used was added in 5 mM concentration. The Ca^{2+} uptake was determined at 30 min.

Fragmented sarcoplasmic reticulum	*Dithiothreitol*	*Ca^{2+} uptake (μmole/mg)*	*ATPase activity; initial rate (μmole/mg per min)*
Fresh	—	0.68	0.29
	+	0.74	0.30
1 day old	—	0.08	0.30
	+	0.13	0.26
Lyophilized			
5 days old	—	0.74	0.26
10 days old	—	0.74	0.28

TABLE III

EFFECT OF DITHIOTHREITOL AND LYOPHILIZATION ON Ca^{2+} UPTAKE OF LOBSTER FRAGMENTED SARCOPLASMIC RETICULUM

Conditions as described in the legend to Table I, except that in the presence of oxalate 0.01 mg of fragmented sarcoplasmic reticulum was used.

Fragmented sarcoplasmic reticulum	*No oxalate*			*Oxalate*	
	Dithiothreitol	*Initial rate (μmoles/mg per min)*	*Total uptake (μmole/mg)*	*Initial rate (μmoles/mg per min)*	*Total uptake (μmoles/mg)*
Fresh	—	0.98	0.12	4.6	7.0
	+	1.06	0.14	6.2	8.8
6 days old	—	—	—	0.2	0.6
	+	—	—	6.2	13.2
18 days old	—	0.00	0.00	0.01	0.05
	+	0.87	0.10	3.6	12.0
Lyophilized		1.01	0.11	6.0	13.6

lobster muscle fragmented sarcoplasmic reticulum (Table III). Dithiothreitol did prevent the loss of activity, in good agreement with VAN DER KLOOT[5]. However, no matter how soon after preparation the fragmented sarcoplasmic reticulum kept in the presence of dithiothreitol was tested, we were unable to obtain the high uptake rates reported earlier[5]. Lyophilized lobster fragmented sarcoplasmic reticulum was as active as fresh fragmented sarcoplasmic reticulum even after 18 days of storage.

Electron microscopic examination of negatively stained material shows that the structural integrity of the sarcoplasmic reticulum membrane was well preserved in reconstituted lyophilized preparations (Fig. 1). Most of the vesicles have a globular shape and are of a fairly uniform size, 0.15–0.3 μ in diameter; some have tails attached to them. The characteristic membrane surface, with its particles of about 40 Å diameter[6], is also well preserved. Thus, in view of both biochemical and ultrastructural findings, lyophilization appears to be useful for the storage of fragmented sarcoplasmic reticulum preparations.

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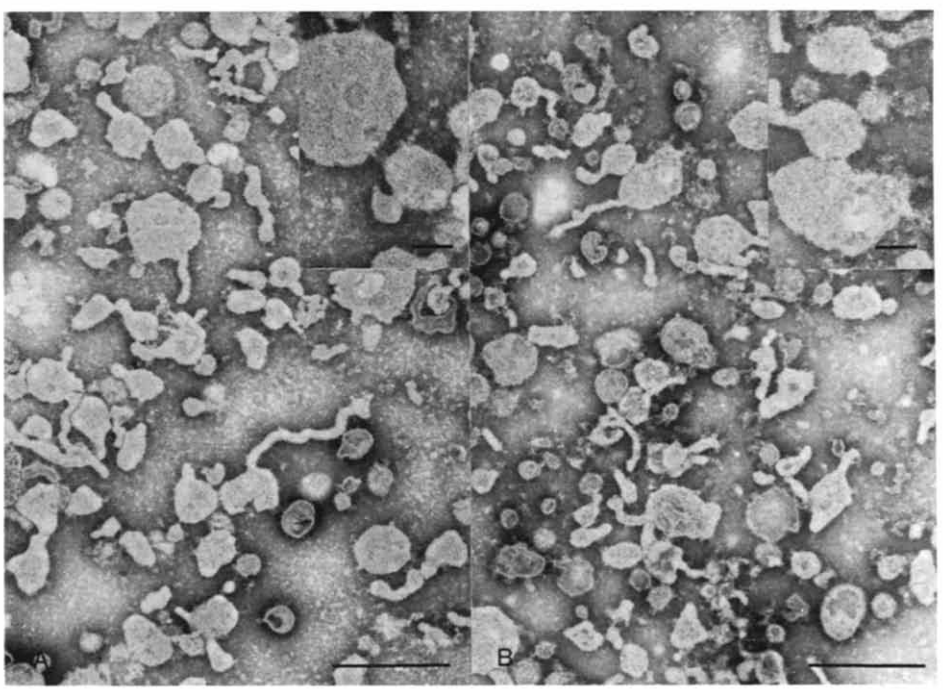

Fig. 1. Effect of lyophilization and storage on ultrastructure of rabbit white muscle fragmented sarcoplasmic reticulum. A. Fresh material. B. Lyophilized fragmented sarcoplasmic reticulum after storage for 4 months. Vesicles were negatively stained with 1% phosphotungstic acid (pH 7.0). Inserts show details at higher magnification. Note that integrity of the membrane structure is retained in the lyophilized fragmented sarcoplasmic reticulum. Markers indicate 0.5 μ and 0.1 μ for the main part of the figure and the insert, respectively.

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Department of Muscle Research, Retina Foundation, F. SRETER
Institute of Biological and Medical Sciences; N. IKEMOTO
Department of Neurology, Massachusetts General Hospital; J. GERGELY
and Departments of Neuropathology and Biological Chemistry,
Harvard Medical School, Boston, Mass. (U.S.A.)

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Carotenoid pigments and the stability of the cell membrane of *Sarcina lutea*

The carotenoid pigments of non-photosynthetic bacteria are located within the cell in association with the cell membrane[1–3], and have been shown to protect the cells against lethal photosensitization[4,5]. It has been suggested[6] that one of the ways that carotenoid pigments might function would be by stabilizing such damaged membranes. Indeed, SALTON AND ETHISHAM-UD-DIN[3] and SALTON AND FREER[7] found that occasional preparations of cells of the carotenoid-containing organisms *Micrococcus lysodeikticus* and *Sarcina lutea* whose membrane-associated carotenoids were completely depleted by growing the cells in the presence of diphenylamine lysed more readily, and membranes prepared from these cells appeared to be less stable. These observations and suggestions have prompted us to investigate whether carotenoid pigments play a role in stabilizing the cell membrane of *S. lutea*. In addition to diphenylamine-treated cells, we used a colorless mutant which accumulates the colorless precursors phytoene and phytofluene, the same compounds which accumulate in DPA-treated cells.

The organisms used in this study were a lysozyme-sensitive strain of *S. lutea* obtained from Dr. R. Y. Stanier, and a colorless mutant of this strain, mutant 2wm induced by exposure of the wild type to ultraviolet light. The cells were grown in nutrient broth (Difco) on a rotary shaker at 30°. For those experiments in which inhibition of production of colored pigment was desired, diphenylamine was added to the nutrient broth at the time of inoculation to give a final concentration of 0.1 mM. Diphenylamine was obtained from Distillation Products Industries, and lysozyme from Nutritional Biochemical Corp.