COMPARATIVE ULTRASTRUCTURE AND CALCIUM TRANSPORT IN HEART AND SKELETAL MUSCLE MICROSOMES

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INTRODUCTION

A number of previous investigations have provided measurements of calcium uptake in microsomal fractions from mammalian skeletal and heart muscle (1–7). On a comparative basis, there are strong indications that the amount and rate of calcium uptake are substantially lower in the heart preparations. The relatively low values of calcium uptake rates in isolated cardiac "reticulum" have been discussed by Weber (3) who noted that these values are too low to account for measured contraction and relaxation rates in heart muscle. However, Katz and Repke (8, 9) devised a modified preparation procedure which includes fractionation on a sucrose density gradient. These workers obtained a fraction which showed four to six times the previously reported values for heart microsome calcium uptake.

These results may be partially understood in terms of the comparative ultrastructure of skeletal and heart muscle. Sacroplasmic reticulum is less extensive in mammalian heart muscle than in skeletal muscle (10–13). The transverse tubular system, on the other hand, is larger and far more extensive than in skeletal muscle. All of the preparation methods presently used probably result in the isolation of vesicles from both the T system and the sarcoplasmic reticulum. If these two systems differed in their capacity for calcium accumulation, then the rate of calcium uptake in a preparation consisting of the two components would be dependent on the relative amounts of the components. On the basis of the known structure of heart muscle, it would be expected that microsomal preparations from heart would contain a greater amount of T system fragments than similar preparations obtained from skeletal muscle. Therefore, heart preparations would show a lower amount of calcium taken up per milligram of protein. It is also possible that preparative procedures which work reasonably well for skeletal muscle produce major contamination when applied to heart muscle. Mitochondrial fragments have been shown to be one major source of contamination (14). However, they do not contribute significantly to calcium uptake (2). In an attempt to clarify these points, we have undertaken a comparative biochemical and ultrastructural study of heart and skeletal microsomes.

METHODS

Isolation of Microsomes and Measurement of Calcium Transport

Fragmented sarcoplasmic reticulum was obtained from rabbit skeletal muscle by the method of Martonosi (15), except that Tricine (10 mM) was used as a buffer. Microsomes were obtained from rabbit hearts by the method of Fanburg and Gergely (2). Measurements of calcium uptake also were performed in the manner described by Fanburg and Gergely, but with Tricine as a buffer. All reactions were started with the addition of microsomes. Microsomal protein was measured by a Folin method with bovine serum albumin as a standard.

ATPase activity was expressed as the amount of inorganic phosphate liberated, as measured by the method of Fiske and SubbaRow (16), following termination of the reaction with 10% trichloracetic acid.

Preparations of both heart and skeletal microsomes were fractionated on a sucrose density gradient according to the method of Katz and Repke (8, 9).

Negative Staining and Freeze-Etch Microscopy

Microsomal suspensions were prepared for viewing with a Hitachi HU 11E electron microscope by nega-

tive stain and freeze-etch techniques as described in our previous investigation (17).

RESULTS

Calcium Uptake by Heart and Skeletal Muscle Microsomes

Values for calcium uptake by heart and skeletal muscle microsomes are shown in Table I. Our results confirm those of previous investigators, in that skeletal muscle microsome preparations accumulate over five times more calcium than heart muscle preparations. However, heart microsomes can be purified on sucrose density gradients. Purified preparations can accumulate as much as 2 μmoles calcium/mg protein, an uptake comparable to values obtained for skeletal muscle microsomes.

TABLE I

Calcium Accumulation by Heart and Skeletal Muscle Microsomes

μmoles Ca/mg protein	Accumulation time	Muscle type	Preparation age
	min		
0.98	10	Rabbit heart	Fresh-layer I
1.87	10	Rabbit heart	Fresh-layer I
2.02	10	Rabbit heart	Fresh-layer I
0.59	10	Rabbit heart	Fresh
0.46	10	Rabbit heart	Fresh
0.40	10	Rabbit heart	Fresh
0.28	10	Rabbit heart	Fresh
3.6	10	Rabbit skeletal	Fresh
3.4	10	Rabbit skeletal	2 days
1.5	10	Rabbit skeletal	Fresh
0.9	10	Rabbit skeletal	Fresh
0.8	10	Rabbit skeletal	3 days

The reaction mixture contained 5 mM Tricine, 0.1 M KCl, 0.25 M sucrose, 0.12 mM $^{45}CaCl_2$, 5 mM $MgCl_2$, 5 mM potassium oxalate, 5 mM ATP, pH = 6.8. Microsomal protein concentration varied between 0.01 mg/ml and 0.10 mg/ml. (Sucrose was not present in the skeletal muscle experiments.) Layer I was obtained by the method of Katz and Repke (8, 9). Temperature was 22°C.

Negative Staining

In order to compare ultrastructural aspects of heart microsomes with those which have previously been described in skeletal muscle microsomes, phosphotungstate negative stains of several preparations of each were examined. There were a number of differences in the ultrastructural composition of the two types of preparations:

(*a*) Heart microsomes contained many more mitochondrial fragments. These composed about one-third of the total number of vesicles in a typical preparation and could easily be distinguished by the 80–90 A particles adhering to their surface (Fig. 1).

(*b*) In general, vesicles not mitochondrial in origin were significantly larger in heart microsomes (0.24 μ, range 0.06–0.4 μ) than in skeletal microsomes (0.12 μ, range 0.03–0.24 μ).

(*c*) The particulate fringe which is readily apparent on skeletal muscle microsomes (Fig. 2, *inset*) was absent from the larger vesicles in heart microsomes, and could be seen only with difficulty on some of the smaller vesicles (Fig. 1, *inset*).

In an initial attempt to separate the ultrastructural entities described above, differential centrifugation was carried out on heart microsomes, as described by Katz and Repke (8, 9). Three bands were obtained and a sample of the solution in each band was negatively stained with phosphotungstate. The top band, which showed the greatest calcium uptake ability (see Table I), consisted of vesicles and small mitochondrial fragments. The middle layer contained a greater number of large mitochondrial fragments. The lowest band, which had no measurable calcium uptake, appeared as a mass of fibrous debris in which vesicular fragments had become enmeshed.

A second method for comparing ultrastructural aspects of calcium transport in the two preparations was to use a "marker" which differentiated between vesicles which accumulated calcium and those which did not. The marker used was calcium oxalate nodules and crystals which appeared within vesicles following incubation in calcium transport media (17–19). Both heart and skeletal muscle microsomes were allowed to take up calcium for 15 min and were negatively stained. These results are shown in Figs. 3–5. Vesicles containing nodules were present in both preparations. However, there was a significant difference in the proportion of vesicles with calcium in the two microsomal species. About 7% of heart microsomes had nodules (range 5–10%, three preparations, 400 counted) whereas 20% of skeletal microsomes had nodules (range 15–25%, three preparations, 300 counted). Nodules could not be found in larger

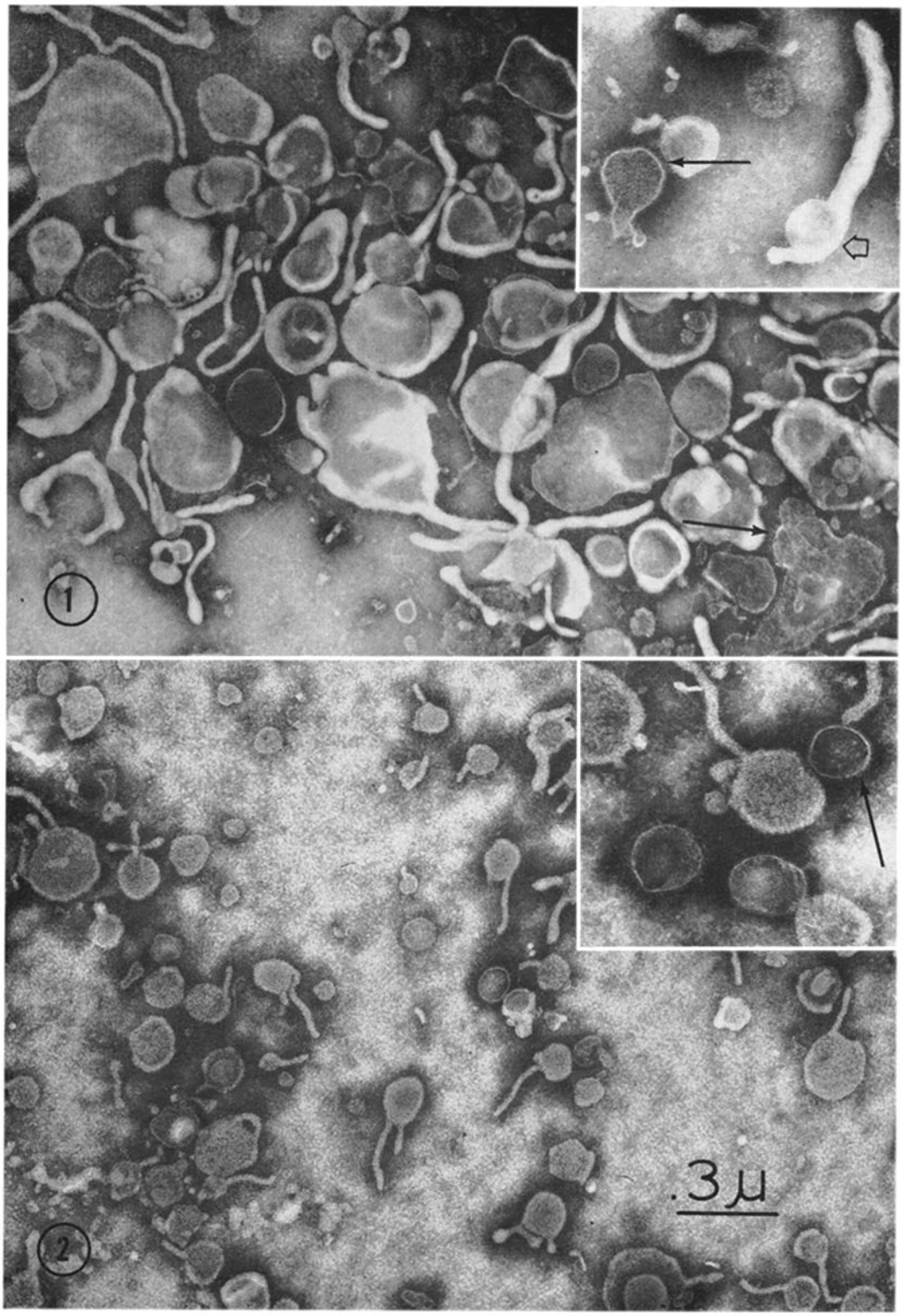
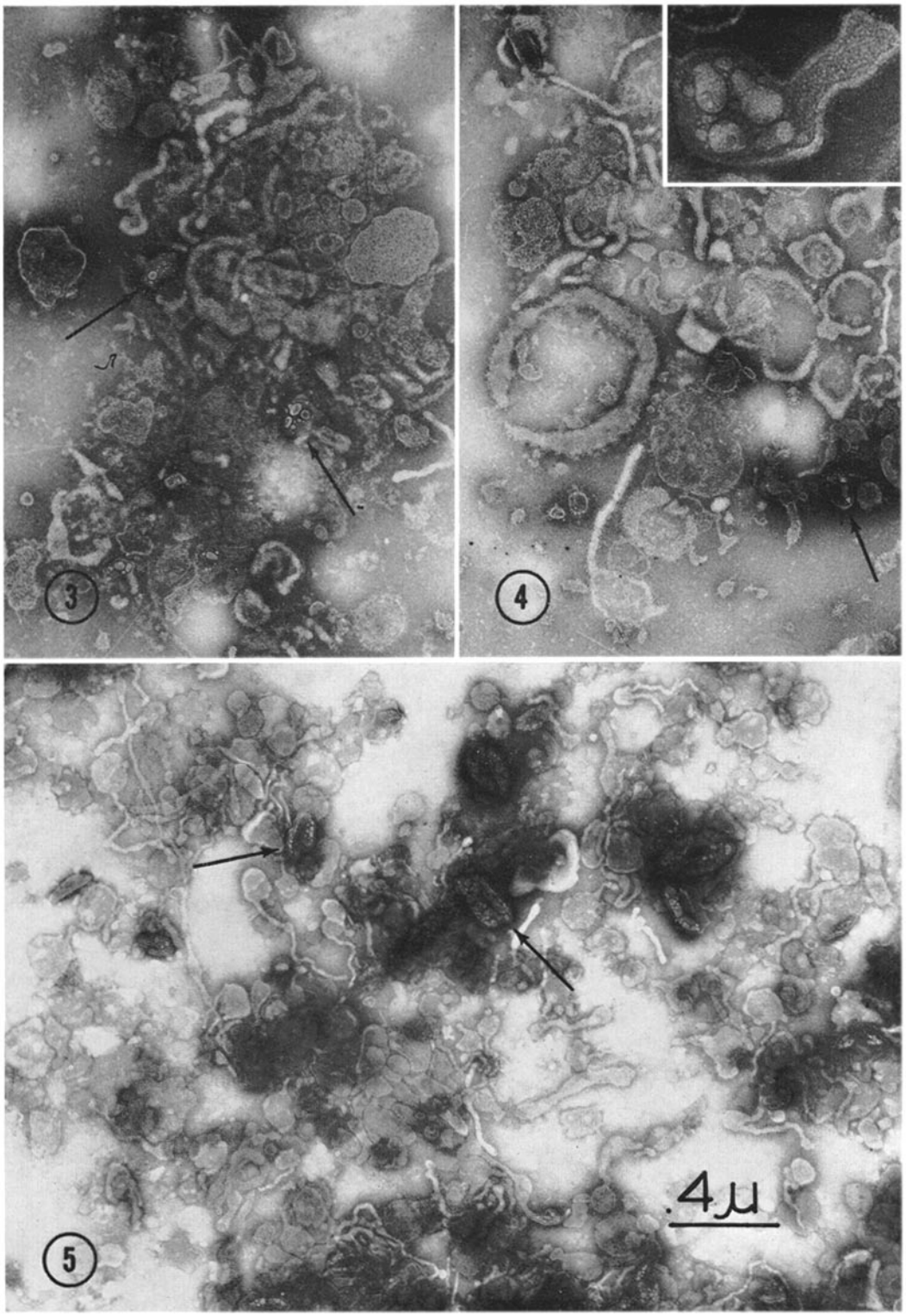


FIGURE 1 Rabbit heart vesicles negatively stained with 2% phosphotungstate. Mitochondrial fragments (arrow) are commonly observed. × 50,000. *Inset* shows vesicles with (solid arrow) and without (open arrow) particulate fringes. × 100,000.

FIGURE 2 Rabbit skeletal muscle vesicles negatively stained with 2% phosphotungstate. × 50,000. The *inset* shows vesicles with particulate fringes (arrow). × 100,000.



FIGURES 3 and 4 Rabbit heart vesicles following a 15 min incubation in a medium containing calcium and oxalate. Some vesicles have nodular deposits which presumably represent calcium oxalate precipitates (arrows). × 40,000. *Inset*, × 160,000.

FIGURE 5 Rabbit skeletal muscle vesicles following a 15 min incubation in a medium containing calcium and oxalate. Vesicles with nodular deposits (arrows) are more numerous than in heart muscle preparations (compare Figs. 3 and 4). × 40,000.

vesicles or in mitochondrial fragments of the heart preparations. Furthermore, heart microsomal vesicles which had nodules typically were fringed (see inset, Fig. 4).

Freeze-Etch Microscopy

In order to check the size distribution of vesicles as indicated by the negative staining procedures and to compare the general freeze-etch image of heart microsomes with that of skeletal microsomes, freeze-etching was carried out on several preparations of each tissue. Fig. 6 shows a freeze-etch field of skeletal microsomes. As noted in our previous study (17), the vesicles are spherical and the fractured surfaces are covered with particles ranging from 80 to 90 A in diameter. Some of the vesicles also present smooth surfaces. The diameters of the vesicles range from 0.04 to 0.15 μ, with an average of 0.09 μ.

Heart microsomes present a different freeze-etch image (Fig. 7). They are generally larger, with an average diameter of 0.13 μ (range 0.05–0.25 μ) and do not have the heavy concentration of particles that is seen on many of the skeletal microsomes.

DISCUSSION

This investigation has established the following points:

(*a*) Heart microsomes are far more heterogeneous than skeletal microsomes in terms of membranous species. Calcium transporting membranes are present, but these numerically compose only 5–10% of the total vesicular components. Large numbers of contaminants are present in the form of mitochondrial fragments, fibrous protein, and an unknown membraneous species which probably represents remnants of the transverse tubule system in heart muscle.

(*b*) Purification procedures probably succeed as a result of removing larger mitochondrial fragments and fibrous protein. Since purified preparations can accumulate nearly as much calcium as skeletal preparations, and since they probably contain remnants of the T system, the presence of this component is not the major cause of decreased calcium uptake in the unpurified heart preparations.

(*c*) Nonmitochondrial vesicles in heart microsome preparations are larger in diameter than veiscles from skeletal muscle. Furthermore, the particulate fringe commonly seen on skeletal microsomes is generally absent from larger vesicles in heart microsomes, although fringes could occasionally be found on smaller vesicles.

Origin of Muscle Microsomes

Due to the extensive and well-developed sarcoplasmic reticulum that is present in skeletal muscle, it is generally assumed that the majority of vesicles which appear in the fragmented preparations originate from this component (1–7). In mammalian heart muscle, however, this is not the case. The most prominent membrane components within these cells are the tubules of the T system (10–13). It is likely, therefore, that the majority of vesicles in heart microsomal preparations are derived from the T system. Several lines of evidence support the possibility that large amounts of T system membranes are present in heart microsomes:

(*a*) The T system tubules in heart are larger than the membranous components of skeletal muscle. These range from 0.10 to 0.25 μ in diameter (11), whereas skeletal sarcoplasmic reticulum ranges from 0.05 to 0.08 μ (10). It would be expected that larger microsomal vesicles would be formed during the homogenization, and in fact, heart microsomes do have a population of vesicles up to twice the size found in skeletal microsomes.

(*b*) Ikemoto, Sreter, and Gergely, (19) as well as other investigators, (17, 20, 21) have provided evidence that the majority of skeletal muscle microsomes are surrounded by a particulate fringe. Since the majority of nonmitochondrial vesicles in heart muscle microsomes lack such a fringe, it is probable that these vesicles arise from a membranous system other than that of the sarcoplasmic reticulum.

(*c*) Finally, only small, fringed vesicles in heart preparations showed any ultrastructural evidence of calcium uptake in the form of calcium oxalate nodules. This fact again suggests that the larger, nonfringed vesicles, which did not contain nodules, represent remnants of T system tubules and are incapable of transporting calcium after isolation.

Calcium Uptake

A decreased calcium uptake capacity of heart microsomes when compared with skeletal muscle microsomes has been established by a number of previous studies (Table II). This finding is confirmed by the present investigation. In general, the heart microsomes show from 25 to 50% of the cal-

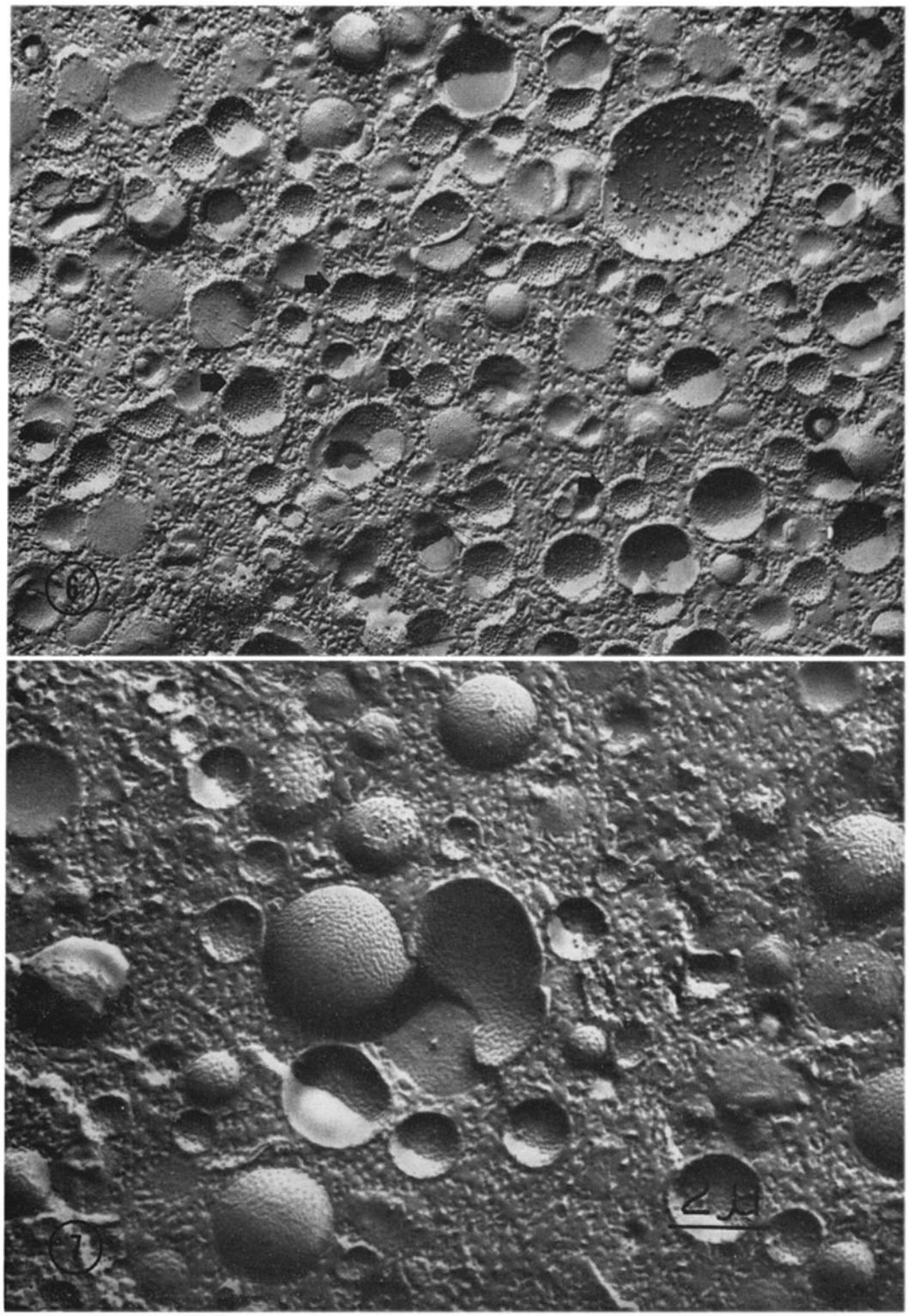


FIGURE 6 Freeze-etch image of rabbit skeletal muscle microsomes. Microsomal suspensions were centrifuged to a pellet in a medium containing 50% glycerol. The pellet was frozen and fractured as previously described (17). Vesicles appeared spherical, and some were covered with particles 80–90 A in diameter (solid arrows). × 75,000.

FIGURE 7 Freeze-etch image of rabbit heart muscle microsomes prepared as in Fig. 6. Note absence of vesicles with 80 A particles. × 75,000.

cium uptake activity found in skeletal muscle microsomes.

Comparisons of calcium uptake activity measured by different investigators must be considered with care since it is apparent that the preparation procedure affects the condition of the microsomes. A procedure which results in either fewer damaged vesicles or less extraneous protein will result in a preparation which has a greater calcium uptake activity. Nevertheless, present evidence indicates a higher calcium uptake ability in the skeletal microsome preparations than in the heart preparations. From the ultrastructural evidence presented above, we suggest that this finding is partially explained by the presence of large amounts of mitochondrial fragments and fibrous protein.

It is interesting to note that even in fresh skeletal muscle microsomes only a fourth of the vesicles show evidence of calcium accumulation in the form of calcium oxalate deposits. This suggests the possibility that heterogeneity also exists within skeletal muscle preparations. Possible, contributing factors could arise from membranes such as muscle cell membranes, outer membranes of mitochondia, and T system tubules. It is also possible that vesicles arising from tubular and cisternal components of sarcoplasmic reticulum have different capacities for calcium transport.

Freeze-Etch Image

Outside of providing a good estimate of vesicle diameters, the freeze-etch image of heart microsomes is not very instructive since the preparations are so heterogeneous. However, freeze-etching does have some value in terms of the comparative ultrastructure of heart and skeletal microsomes, particularly in regard to the 80–90 A particles which are found on the latter. There is naturally some question concerning the functional significance of the particulate structures, as they may simply be artifacts of freeze-etching. If this were true, one would expect them to be common to all microsomal vesicles. However, only a few of the heart microsomes have particles, and these are not as densely distributed as on skeletal microsomes. This strongly suggests that such particles are not common to all microsomal membranes and may in fact be related to the calcium transport system of skeletal microsomes.

TABLE II

Calcium Accumulation in Microsomal Preparation

Author	μmoles Ca/mg Protein	Muscle type
Fanburg, Finkel, and Martonosi (1)	0.66	Rabbit skeletal
	0.08	Calf heart
Chimoskey and Gergely (7)	2.6	Rabbit skeletal
	1.6	Dog heart
Weber, Herz, and Reiss (4)	0.8	Rabbit skeletal
	0.2	Dog heart
Baskin and Deamer	2.0*	Rabbit skeletal
	0.42*	Rabbit heart

* Average of four "fresh" preparations.

Physiological Significance

Weber (3) has pointed out that the available evidence indicates that the rate of calcium uptake measured in heart microsomes (in the presence of oxalate) is too slow to account for relaxation occurring during the cardiac cycle. This is further supported by the work of Katz and Repke (8, 9). A calcium uptake rate of about 3 μmoles/min for each milligram of microsomal protein for a heartbeat of 120/min is apparently required (3). The calcium uptake rates measured in isolated heart microsomes have a maximum value of about 0.3 μmoles/min for each milligram of microsomal protein. While this would appear to be much too low, two factors must be considered in this regard.

(*a*) Evidence provided by the work of Katz and Repke (8, 9) indicates that increasing the purity of microsomal preparations results in a greater amount and higher rate of calcium uptake.

(*b*) The present study clearly shows that only a small percentage of heart microsomes are capable of calcium transport, and that separation of the calcium-accumulating vesicles would obviously greatly increase the amount of calcium uptake.

(*c*) Van der Kloot (22) has reported that dithiothreitol can increase initial uptake rates in lobster muscle microsomes by a factor of 19. Thus, an optimal system for calcium uptake by microsomes may not have been achieved in past work.

It is apparent from the above considerations that the question has not been resolved. Kinetic studies on purified heart microsomal preparations which allow for the presence of noncalcium-accumulating vesicles are required. It is also apparent, however, that as purer preparations of heart sarcoplasmic

reticulum are obtained the measured values of calcium uptake increase, and it is likely that a "pure" preparation would show an amount and rate of calcium uptake that could account for contraction and relaxation of heart muscle.

SUMMARY

Heart muscle microsomes and skeletal muscle microsomes were examined on a comparative biochemical and ultrastructural basis.

(*a*) Heart microsomes had relatively low calcium-accumulating capacity when compared with skeletal microsomes. However, a three- to fourfold increment in calcium accumulation by heart microsomes could be obtained by purification on sucrose density gradients.

(*b*) Negative stains of heart microsomes show at least three distinct types of membranes: Smaller vesicles commonly had a particulate fringe (30–40 A diameter) on their surface. Larger vesicles which were not mitochondrial in origin typically had smooth surfaces. Obvious mitochondrial fragments with adhering 80–90 A particles were also commonly found.

(*c*) Freeze-etch microscopy of heart microsomes revealed textured surfaces. Smaller vesicles commonly had 80–90 A particles adhering to the fractured surface, but these were sparser and less well defined than in the highly particulate image obtained from skeletal microsomes.

(*d*) Negative staining of heart microsomes after calcium oxalate accumulation showed a small number (5–10%) of vesicles containing calcium oxalate nodules. Vesicles containing such nodules typically had particulate fringes.

It was concluded that heart microsome preparations are highly impure, and contain mitochondrial fragments, fibrous material, and remnants of the T system in addition to vesicles derived from the sarcoplasmic reticulum. The fact that smaller vesicles typically had 30–40 A particulate fringes following negative staining and contained calcium oxalate nodules, after calcium accumulation, suggests that these vesicles are remnants of sarcoplasmic reticulum in heart tissue. Larger vesicles lacking particulate fringes are probably derived from T system membranes.

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