Biochimica et Biophysica Acta, 771 (1984) 109–118
Elsevier

BBA 72030

STEREOLOGICAL ANALYSIS OF TRANSVERSE TUBULES AND SARCOPLASMIC RETICULUM ISOLATED FROM NORMAL AND DYSTROPHIC SKELETAL MUSCLE

RONALD J. BASKIN and RICHARD KAWAMOTO *

Department of Zoology, University of California, Davis, CA 95616 (U.S.A.)

(Received October 5th, 1983)

Key words: Membrane vesicle; Sarcoplasmic reticulum; Transverse tubule; Muscular dystrophy; Freeze-fracture stereology; (Chicken skeletal muscle)

Vesicles isolated from the transverse tubules and sarcoplasmic reticulum of normal and dystrophic chicken skeletal muscle were analyzed for enzymatic activity and examined following freeze-fracture. A stereological procedure was used to determine particle density distributions on the resulting membrane fracture faces. The particle densities measured in this investigation were compared with those of an earlier study on intact muscle. Isolated sarcoplasmic reticulum vesicles showed a characteristically high P-face (cytoplasmic leaflet) particle density (5108 ± 169 particles/μm^2) and a low E-face (luminal leaflet) particle density (505 ± 57 particles/μm^2). Transverse tubule fractions showed a high E-face particle density (2346 ± 179 particles/μ^2) as well as a substantial P-face particle density (1019 ± 129 particles/μm^2). The high transverse tubule E-face particle density represents a characteristic morphological feature in the same way that the very high P-face particle density is characteristic of sarcoplasmic reticulum membranes. The major morphological alteration in dystrophic membranes was a shift in the E-face particle density distribution of isolated transverse tubules to a lower average particle density. (The E-face particle density of sarcoplasmic reticulum fractions showed no differences.)

In order to effect a biochemical analysis of normal and dystrophic membranes it is desirable to examine isolated muscle membrane fractions. Research involving isolated membrane fractions had lead to apparent differences in enzymatic activity and lipid composition between normal and dystrophic muscle membranes [1,2].

Crude microsomal fractions from normal and dystrophic chicken muscle are sufficiently heterogeneous that clear morphological differences between these fractions cannot be identified [3]. We were able to establish, however, that dystrophic microsomes, separated and purified on a sucrose-density gradient, showed a lower density of 8 nm freeze-etch particles on vesicle concave faces when compared to normal, purified microsomes [4]. A similar result was also obtained by other workers [5].

Malouf and Sommer [6] suggested that the proliferation of low particle density vesicles in the dystrophic preparations originated from enlarged transverse tubules that they had observed in dystrophic chicken muscle. In a stereological analysis of developing sarcotubular membranes [7] we determined the surface and volume densities of sarcoplasmic reticulum and transverse tubules in chicken pectoralis muscle fibers. In a subsequent paper we characterized the freeze-fracture (8 nm) particle density on each of these membranes [8]. We next completed a stereological analysis of normal and dystrophic muscle and provided further morphological evidence supporting the notion that

* Present address: Department of Pharmacology, University of Miami, Miami, FL 33101, U.S.A.

the transverse tubule system was enlarged in dystrophic chicken muscle [9]. Thus it is likely, that using earlier isolation methods, (including sucrose density gradient separation), 'sarcoplasmic reticulum' from dystrophic muscle contained more transverse tubule membranes than 'sarcoplasmic reticulum' isolated from normal muscle. Accordingly, some of the reported differences may simply be the result of different levels of transverse tubule contamination.

In response to this question we have elected to prepare relatively pure preparations of normal and dystrophic membranes. In order to do this, we have applied the isolation procedure of Caswell and his co-workers [10,11] to normal and dystrophic chicken muscle. We have then conducted a stereological analysis of the isolated fractions in order to assess their degree of homogeneity. Details of our isolation procedure as well as the results of enzymatic assays and lipid determinations will be reported elsewhere. In this paper we report the results of our ultrastructural analysis of transverse tubule and sarcoplasmic reticulum fractions from normal and dystrophic chicken muscle.

In particular we have focused attention on E-face (luminal leaflet) particle densities. Whereas the E-face particle density in intact muscle sarcoplasmic reticulum is much lower than P-face (cytoplasmic leaflet) particle density, this is not the case for intact transverse tubules [8] where E-face and P-face particle densities are similar. Thus, isolated, purified fractions of transverse tubules should show a greater correspondence between E-face and P-face particle densities. This has indeed been found to be the case as we show in the following work.

Materials and Methods

Isolation of sarcoplasmic reticulum vesicles

Isolation of microsomes and eventual sarcoplasmic reticulum was by a modification of procedures described by Caswell and co-workers [10,11] for rabbit skeletal muscle and was as follows.

Isolation of crude microsomes. Chickens of line 03 (normal) and 433 (dystrophic), ranging from 6 to 8 weeks of age, were killed by cervical dislocation. Breast muscles comprised of pectoralis major and supracoracoideus were excised and placed in ice-cold buffer (5.0 mM histidine/0.25 M sucrose/0.5 mM EDTA, pH 7.4). The volume of buffer was adjusted to equal 10-times the tissue volume. The muscle was homogenized with a Waring blender set to high speed for 1.5 min. The pH of the homogenate was maintained neutral with 1 M NaOH.

The homogenate was first centrifuged at 8000 $\times g$ for 15 min. The supernatant was filtered through glass wool wrapped in cheese cloth. The suspension was centrifuged again at 8000 $\times g$ for 30 min. The resulting supernatant was centrifuged at 40000 $\times g$ for 45 min. The pellet was resuspended in 0.25 M sucrose and recentrifuged at 40000 $\times g$ for 45 min to obtain a crude microsomal preparation.

Purification of sarcoplasmic reticulum. The crude microsomal suspension was layered on a continuous sucrose density gradient (12.5% to 60% w/w, with 0.1% NaN_3, pH 7.4). Density gradient separation was achieved by centrifugation on a Beckman model L5-75 ultracentrifuge for approx. 17 h at 77000 $\times g$. Separation resulted in four distinct regions. Most of the material was collected in fraction LSR (light or longitudinal sarcoplasmic reticulum) and HSR (heavy sarcoplasmic reticulum). Fraction LSR oriented between 23% and 30% sucrose and fraction HSR was generally between 31% and 39% sucrose. The two fractions were easily identifiable as fraction LSR isolated as a diffuse band while HSR formed a firm band. Purified sarcoplasmic reticulum was subsequently extracted from the HSR fraction presumed to consist of terminal cisternae sarcoplasmic reticulum, both free and in association with transverse tubules.

Fraction HSR was resuspended in 0.6 M KCl and pelleted by centrifugation at 72000 $\times g$ for 60 min. The supernatant was removed and the pellet was resuspended in a small volume of 0.6 M KCl. The microsomes were pressure disrupted using an Aminco French Press set at 5000 lb/inch^2 to disrupt triadic junctions and membrane aggregates. The resulting suspension was layered over a continuous sucrose density gradient (12.5%–60% in 0.6 M KCl, 0.01% NaN_3, pH 7.4) and centrifuged approx. 17 h at 77000 $\times g$. The HSR after pressure disruption resulted in two distinct membrane fractions. Fraction 2 covered sucrose con-

centration range of 12%–21% and was considered to be T-tubule vesicles. Fraction 3 located between 23% and 34% sucrose and was considered to be the major sarcoplasmic reticulum fraction. A more dense fraction 4 was obtained (40%–44%). The lack of significant calcium transport activity suggested these might be damaged membrane fragments, and consequently, were not used in this study. Fraction 2 and 3 were collected and morphologically characterized.

Protein was determined by a modification of procedures described by Bradford [12] using a Bio-Rad protein dye reagent.

Electron microscopy

Membrane fractions were prepared for freeze-fracture and thin sectioning by methods that were discussed previously [7,8]. Replicas and thin sections were examined on a Hitashi HU11E electron microscope calibrated with a ruled grating.

The stereological method used for estimating relative membrane surface area in freeze-fracture preparations of subcellular fractions was that of Weibel et al. [13]. The sample was restricted to vesicle profiles without cast shadow. A discussion of sampling errors possible when using stereological methods was presented in an earlier publication [9].

Meausrements of ATPase activity

Basal and Ca^{2+}-sensitive ATPase activities were determined by an enzyme-coupled assay [14].

Results

Isolated triads

Purified preparations of isolated sarcoplasmic reticulum and transverse tubules are obtained by breaking isolated triads using a French press [10,11]. Thin sections of isolated triads are shown in Fig. 1. Triads isolated from normal chicken muscle (Fig. 1A) exhibit a characteristic appearance as described by Brunschwig et al. [15]. Both round and elongate vesicles are seen, the former being mostly terminal cisternae and the latter junctional portions of the transverse system. Many of the round vesicles contain a dark staining material.

In contrast to the triads isolated from normal chicken muscle, those isolated from dystrophic muscle show few elongate vesicles (Fig. 1B). The majority of the vesicles are found, many containing a dark staining material. In intact dystrophic muscle both the surface and the volume density of the transverse tubules are considerably greater than in normal muscle [19]. Thus, the round vesicles seen in the isolated preparation would represent portions of both terminal cisternae and junctional transverse tubules.

Isolated transverse tubules

Isolated transverse tubule fractions exhibit a striking feature. E-face particle density is quite

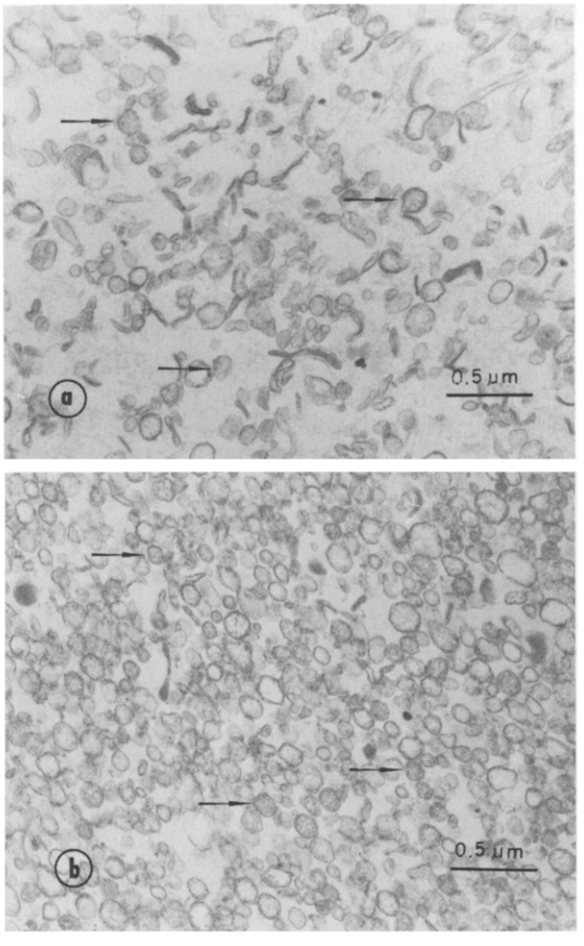

Fig. 1. Thin sections of isolated triad fractions. (a) Normal chicken pectoralis muscle. (b) Dystrophic muscle. Arrows indicate vesicles, containing a dark staining material, that are presumably derived from terminal cisternae. The dystrophic fraction exhibits mostly round vesicles. Magnification: ×21 600.

high (Figs. 2, 3; Table I). In transverse tubule fractions isolated from heavy sarcoplasmic, the E-face particle density is greater than that of the P-face. The appearance of regions of particle aggregates is not uncommon (Fig. 2). P-face particle density is also characteristic of isolated transverse tubules, being considerably less than that for isolated sarcoplasmic reticulum P-face. The fraction of origin of most P-face vesicles is thus immediately apparent.

The above features of transverse tubule fractions isolated from normal membranes are also seen in transverse tubule fractions from dystrophic membranes, although to a different extent. The high E-face particle density is also seen in dystrophic membranes (Fig. 3), however, it is significantly lower (Table I). As in the case of normal membranes the P-face particle densities in dystrophic transverse tubules are characteristically lower than the P-face particle densities for sarcoplasmic reticulum. They also show a lower average value than normal, however, the differences are not statistically significant.

TABLE I

PARTICLE DENSITIES OF ISOLATED TRANSVERSE TUBULES AND SARCOPLASMIC RETICULUM FROM NORMAL AND DYSTROPHIC MUSCLE

Figures presented are means ± S.E., sample size in parenthesis.

	Particle density (number/μm^2)	
	Normal muscle	Dystrophic muscle
P-face		
Sarcoplasmic reticulum	5108 ± 169(54)	4573 ± 308(30)
Transverse tubules	1019 ± 129(28)	765 ± 163(25)
E-face		
Sarcoplasmic reticulum	505 ± 57(50)	627 ± 104(22)
Transverse tubules	2346 ± 179(46)	1581 ± 224(29)

Although there are differences in average particle densities between normal and dystrophic

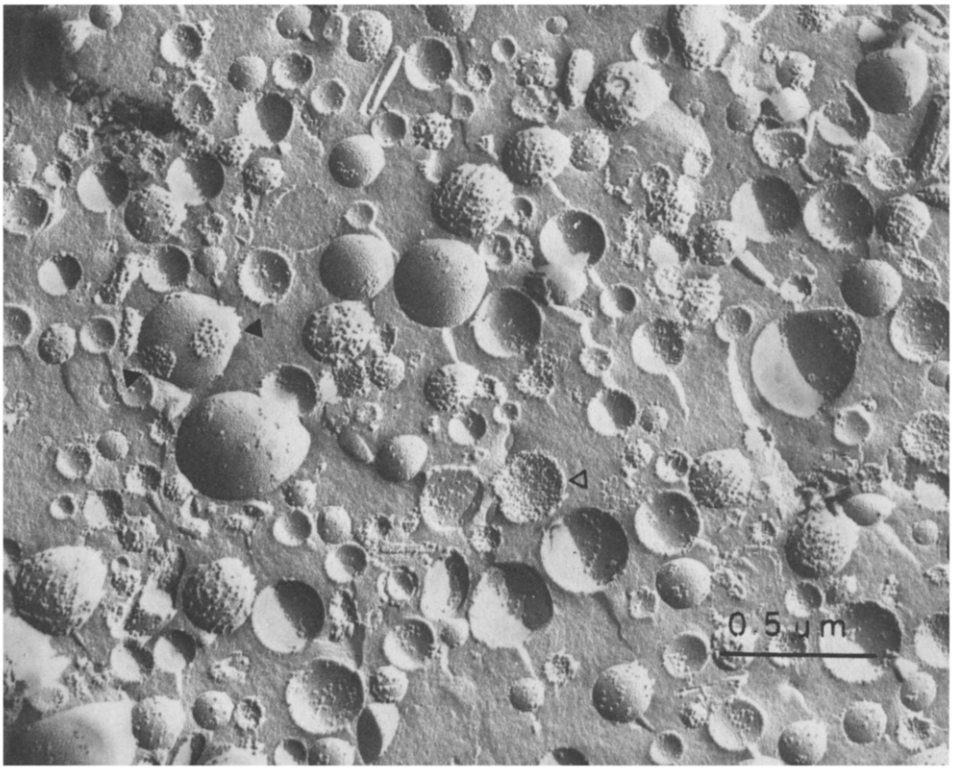

Fig. 2. Freeze-fracture of transverse tubule fraction from normal chicken pectoralis muscle. The fraction is characterized by particulate E-face vesicles. Particle clumps are observed on some vesicles (solid arrow). While most P-faces have few particles an occasional vesicle shows a high particle density characteristic of sarcoplasmic reticulum membrane (open arrow). Magnification: ×40000.

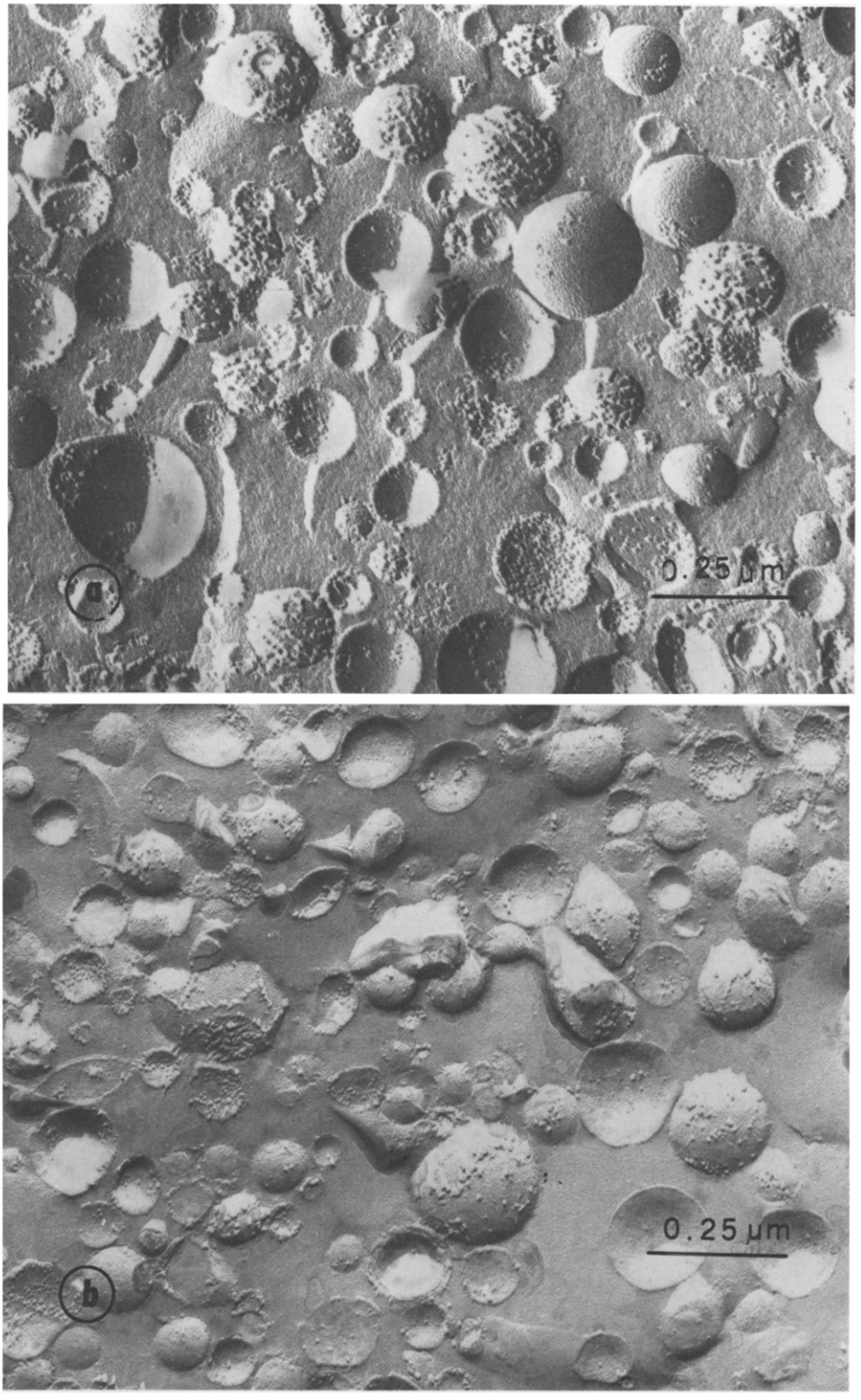

Fig. 3. Freeze-fracture of isolated transverse tubule fractions from normal (a) and dystrophic (b) muscle. Both fractions are characterized by particulate E-face vesicles. Magnification: ×80000.

TABLE II

ATPase ACTIVITIES OF ISOLATED FRACTIONS

Activities are presented in μmol per mg per h.

	Normal muscle		Dystrophic muscle	
	Mg^{2+}-ATPase	Ca^{2+}-ATPase	Mg^{2+}-ATPase	Ca^{2+}-ATPase
Sarcoplasmic reticulum	16.2 ± 3.60	107.4 ± 14.4	15.0 ± 3.60	92.4 ± 13.8
Transverse tubules	25.8 ± 3.60	7.20 ± 3.0	49.2 ± 10.2	15.0 ± 3.0

membrane transverse tubules, only the E-face differences are statistically significant. The relatively high E-face particle density, as contrasted with P-face particle density, is significant and appears to be characteristic of transverse tubules isolated and purified from heavy microsomes (triads). As such, E-face particle density is the most significant morphological feature of isolated transverse tubules and represents a membrane 'signature'.

Isolated sarcoplasmic reticulum

Sarcoplasmic reticulum membranes isolated from normal and dystrophic membranes (Fig. 4) also exhibit characteristic features. Most prominent is the high P-face particle density and the low E-face particle density (Table I). The slight differences in average values of particle density between normal and dystrophic membranes are not statistically significant. The high P-face particle density is seen only in the sarcoplasmic reticulum membranes and represents a unique morphological feature.

ATPase activities of isolated fractions

Mg^{2+}-ATPase and the Ca^{2+}-sensitive ATPase activities are characteristic of the isolated fractions (Table II). Mg^{2+}-ATPase activity is higher in transverse tubule fractions whereas Ca^{2+}-sensitive ATPase activity is considerably higher in isolated sarcoplasmic reticulum fractions. Dystrophic transverse tubules show a higher Mg^{2+}-ATPase activity than normal; the Ca^{2+}-sensitive ATPase activities are not significantly different. The high Ca^{2+}-sensitive ATPase activity in isolated sarcoplasmic reticulum fractions is a characteristic feature of their enzyme profile.

Frequency distribution of particle densities

Particle density distributions for normal and dystrophic membranes are shown in Tables II and IV. Particle densities of isolated transverse tubules are listed in Table III. In both E-face and P-face distributions a small shift to lower particle densities is characteristic of dystrophic membranes.

In the case of isolated sarcoplasmic reticulum membranes (Table IV), the P-face particle frequency distribution shows a slight shift to lower

TABLE III

PARTICLE DENSITY DISTRIBUTION OF ISOLATED TRANSVERSE TUBULE FRACTION

Particle density (number/μm^2) ($\times 10^{-3}$)	% total E-face		% total P-face	
	Normal	Dystrophic	Normal	Dystrophic
0–1	12	41	50	55
1–2	26	10	35	21
2–3	28	27	14	11
3–4	21	9	–	–
4–5	6	0	–	–

TABLE IV

PARTICLE DENSITY DISTRIBUTION OF ISOLATED SARCOPLASMIC RETICULUM FRACTION

Particle density (number/μm^2) ($\times 10^{-3}$)	% total E-face		% total P-face	
	Normal	Dystrophic	Normal	Dystrophic
0–1	85	50	0	0
1–2	15	40	0	4
2–3	–	–	2.5	10
3–4	–	–	20	35
4–5	–	–	20	20
5–6	–	–	26	8
6–7	–	–	16	7
7–8	–	–	7.5	10
8–9	–	–	3	3

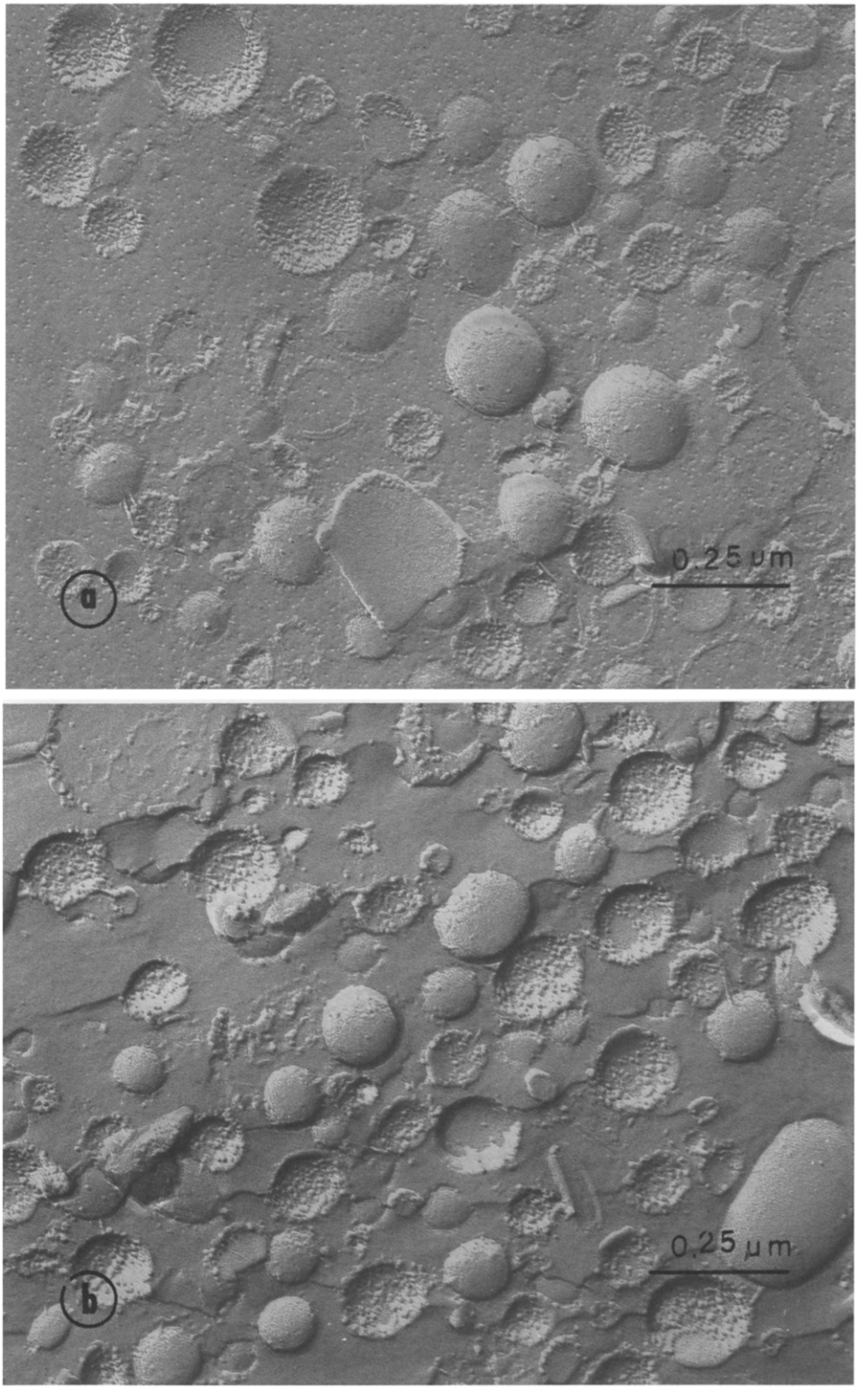

Fig. 4. Freeze-fracture of isolated sarcoplasmic reticulum fractions from normal (a) and dystrophic (b) muscle. P-face vesicles with a high particle density are seen in both fractions. E-face vesicles are almost devoid of particles. Magnification: ×80000.

particle density on the part of dystrophic membranes but the E-face distributions show the opposite result: namely a shift, on the part of the dystrophic membranes to a higher particle density.

Size of membrane particles

The average diameter of freeze-etch particles on the E-face of isolated T-tubules was determined and compared with the average diameter of P-face particles on isolated sarcoplasmic reticulum membranes. In each case, 100 particles from three separate preparations were measured. The measurements do not take into consideration the thickness of the metal shadow or the location of the particle on a concave or convex surface. The micrographs were oriented with the shadow direction on top and the particle diameter was measured at a right angle to the shadow direction. The average particle diameter for E-face, T-tubule particles was 11.2 ± 1.0 nm, while the average diameter of P-face, sarcoplasmic reticulum particles was 8.8 ± 0.9 nm.

Discussion

Stereological evidence for the identification of membrane function

In an earlier study [8], we determined the particle densities and frequency distributions of the intact sarcoplasmic reticulum and T-system of chicken pectoralis muscle. In the present investigation we have used an isolation technique that allows a separation of junctional transverse tubule membranes from junctional sarcoplasmic reticulum (terminal cisternae) membrane. Morphological evidence for this separation is given, first of all, by the absolute values and frequency distributions of P-face particle densities for the two fractions. The average value of the P-face particle density in the sarcoplasmic reticulum fraction (5108 particles/μm^2) is close to that found in intact muscle [8] sarcoplasmic reticulum (4800 particles/μm^2). Frequency distributions of particle density are also similar even to the extent that both show a slightly lower particle density (and shift in frequency distribution) for dystrophic membranes. In both intact and isolated sarcoplasmic reticulum membranes, dystrophic membranes show a frequency distribution to lower particle densities.

A second piece of morphological evidence is provided by an examination of E-face particle densities and frequency distributions. Intact sarcoplasmic reticulum has an E-face particle density less than 10% of the P-face value. Isolated sarcoplasmic reticulum membranes also show a low E-face particle density.

In contrast, intact transverse tubules show a similar (low) particle density on both the P-face and the E-face. In our isolated transverse tubule fraction we have found a similar situation except that the E-face particle density in the isolated junctional transverse tubule membranes is significantly higher than the P-face particle density. We believe that this E-face particle density is characteristic of junctional transverse tubule membranes. Some of the particles may be derived from the 'junctional feet' [16] joining the transverse tubules and the terminal cisternae.

A shift to lower E-face particle densities was seen in the particle frequency distributions of intact dystrophic transverse tubules when compared to the normal [8]. We find an analogous situation in isolated transverse tubule fractions. Dystrophic membranes show a shift to lower average transverse tubule E-face particle densities.

Junctional vs. non-junctional transverse tubules: membrane microheterogeneity

In our earlier study of intact membranes, we did not distinguish between junctional and non-junctional portions of the transverse tubules. Due to the nature of the isolation method used in the present study, we have isolated only junctional portions of the transverse system. This fact may be the reason why our E-face particle densities are higher than the P-face particle densities, both in normal and dystrophic muscle.

ATPase activity of membrane fractions

We have measured both Mg^{2+}-ATPase activity ('basal') and Ca^{2+}-ATPase activity in the membrane fractions isolated from normal and dystrophic muscle. The Ca^{2+}-ATPase activities are high in sarcoplasmic reticulum fractions from both normal and dystrophic muscle. In contrast, the Ca^{2+}-ATPase activity is quite low in both transverse tubule fractions. This contrasts with earlier reports [17,18] of a high Ca^{2+}-ATPase activity in

isolated transverse tubules, but is in agreement with recent reports [19]. Thus it appears that Ca^{2+}-ATPase activity is an enzymatic marker for sarcoplasmic reticulum membranes, the low levels found in transverse tubule preparations probably representing sarcoplasmic reticulum contamination.

The measurement of Mg^{2+}-ATPase activity also presents an interesting contrast. While our values of the Mg^{2+}-ATPase activity of normal and dystrophic sarcoplasmic reticulum are in the range of those reported by other workers [19], our Mg^{2+}-ATPase activity values for isolated transverse tubule fractions are considerably lower. They are in agreement, however, with values reported by Lau et al. [11]. All of the investigators reporting high transverse tubule Mg^{2+}-ATPase activities used an isolation procedure which selects non-junctional portions of the transverse tubule membrane, while we and others, using a technique that selects junctional portions of the membrane, report lower values. A likely explanation for the difference is that it is due to 'membrane microheterogeneity', that is, a lower percentage of junctional transverse tubule membrane protein has its origin in the Mg^{2+}-ATPase enzyme than in the case of non-junctional portions of the membrane. This is likely in view of the junctional proteins reported in these membranes [16]. (It is also possible that the junctional Mg^{2+}-ATPase enzyme has a different activity than the non-junctional enzyme, although this would appear an unlikely alternative.)

Appearance of isolated triad fractions in normal and dystrophic muscle

The appearance of triads isolated from normal chicken muscle is similar to that reported from other animals [15]. Dystrophic muscle triads, however, have a different appearance marked by the presence of mostly round vesicles. A possible explanation for this appearance is provided by the results of an earlier study [9]. In that investigation we showed that both the surface density and the volume density of the dystrophic transverse tubules were greatly increased. It is likely therefore, that many of the large, round vesicles observed in dystrophic triads are derived from this enlarged transverse tubule system. Indeed, the almost total absence of narrow, elongate vesicles in these preparations makes this explanation likely.

Differences between normal and dystrophic membranes

As was indicated earlier, two morphological differences characterize dystrophic muscle. The most prominent is the (already discussed) appearance of isolated triads. Second, the E-face particle density is significantly lower in the transverse tubule fraction but no significant difference is seen in the E-face particle density of isolated sarcoplasmic reticulum membranes.

Estimation of level of contamination of isolated fractions

As was pointed out by Lau et al. [20] vesicle morphology can be used to estimate the level of contamination of isolated fractions. Many workers [8,17] have shown that a high particle density on a vesicle P-face is characteristic of sarcoplasmic reticulum membranes and serves to identify vesicle origin. This study has shown that a second diagnostic feature is a high (1000–2000 particles/μm^2) E-face particle density, this being characteristic of junctional transverse tubule membranes. Low particle density P-face has also been considered diagnostic for transverse tubule membranes. The only remaining ambiguity concerns low particle density E-faces since these appear to originate from portions of both membrane fractions, especially in dystrophic animals.

Size of membrane particles

Apparent particle size differences between particles found in different membrane fractions must be interpreted with caution since particle size may be a function of preparation techniques including precise fracture conditions and other treatment of the membrane fractions. However, a number of investigators [21,22] have observed that transverse tubule particles appear to be larger than those found on sarcoplasmic reticulum membranes. This difference has been particularly noted in this investigation. Sarcoplasmic reticulum intramembrane particles average about 8–9 nm in diameter whereas transverse tubule particles, particularly those found on the E-face of vesicles (although including many on the P-face) have a diameter of 11–12 nm. It appears likely that some of these particles represent portions of the 'junctional feet'. (A precise analysis of particle size would involve considerations of the effect of metal

shadow and particle location on concave and convex fracture faces. Such an analysis was not done in the present study.)

Conclusions

Membrane fractions isolated from normal and dystrophic chicken muscle by the methods described in this investigation have been shown by stereological analysis and enzyme activities to be relatively pure fractions of sarcoplasmic reticulum and of transverse tubules. Each fraction has a characteristic particle density distribution which was shown to agree with those found in intact membranes. The major difference seen in purified dystrophic membranes was a shift to lower average particle densities in most fracture faces from both fractions. This investigation provides further support for the notion that membrane alterations are a basic feature of dystrophic muscle.

Acknowledgments

This study was supported, in part, by a grant from the Muscular Dystrophy Association, New York, New York to R.J.B.

References

1 Hanna, S., Kawamoto, R., McNamee, M. and Baskin, R.J. (1981) Biochim. Biophys. Acta 643, 41–54
2 Sumnicht, G. and Sabbadini, R. (1982) Arch. Biochem. Biophys. 215, 628–637
3 Baskin, R.J. (1970) Lab. Invest. 23, 581–589
4 Baskin, R.J. and Hanna, S. (1975) Physiologist 18, 132
5 Sabbadini, R., Scales, D. and Inesi, G. (1975) FEBS Lett. 54, 8–12
6 Malouf, N. and Sommer, J. (1976) Am. J. Pathol. 84, 299–316
7 Crowe, L.M. and Baskin, R.J. (1977) J. Ultrastruct. Res. 58, 10–21
8 Crowe, L.M. and Baskin, R.J. (1978) J. Ultrastruct. Res. 62, 147–154
9 Crowe, L.M. and Baskin, R.J. (1979) Am. J. Pathol. 95, 295–315
10 Caswell, A.H., Lau, Y.H. and Brunschwig, J.-P. (1976) Arch. Biochem. Biophys. 176, 417–430
11 Lau, Y.H., Caswell, A. and Brunschwig, J.-P. (1977) J. Biol. Chem. 252, 5565–5574
12 Bradford, M. (1976) Anal. Biochem. 72, 248–254
13 Weibel, E.R., Losa, G. and Bolender, R.P. (1976) J. Microsc. 107, 255–266
14 Warren, G., Toon, P., Birdsall, N., Lee, A. and Metcalfe, J. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 622–626
15 Brunschwig, J.-P., Brandt, N., Caswell, A. and Lukeman, D. (1982) J. Cell Biol. 93, 533–542
16 Cadwell, J. and Caswell, A.H. (1982) J. Cell Biol. 93, 543–550
17 Scales, D.J. and Sabbadini, R.A. (1979) J. Cell Biol. 83, 33–46
18 Rosemblatt, M., Hidalgo, C., Vergara, C. and Ikemoto, N. (1981) J. Biol. Chem. 256, 8140–8148
19 Sabbadini, R. and Okamoto, V. (1983) Arch. Biochem. Biophys. 223, 1–13
20 Lau, Y., Caswell, A., Brunschwig, J.-P., Baerwald, R. and Garcia, M. (1979) J. Biol. Chem. 254, 540–546
21 Baskin, R.J. (1974) J. Ultrastruct. Res. 49, 348–371
22 Franzini-Armstrong, C. (1980) Fed. Proc. 39, 2403–2409