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RESOLUTION OF FRAGMENTS OF PLASMA AND SARCOTUBULAR MEMBRANES IN HEART MUSCLE MICROSOMES

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SUMMARY

1. Microsomes from heart muscle yield two components by flotation in polyanion-containing sucrose gradients. Both components have ATPase activity, but only that in the heavier component is stimulated by calcium ions. Under the same conditions, microsomes from skeletal muscle yield only a single component and this has ATPase activity which is stimulated by Ca^{2+}.

2. The light component of heart muscle microsomes has a low content of esterase and NADH–cytochrome *c* reductase and a high level of non-specific nucleoside phosphate phosphohydrolase activities. In these respects, it resembles previously described plasma membrane preparations from liver and cultured fibroblasts. Non-specific nucleoside triphosphate phosphohydrolase activity is not affected by calcium ions or the sulphydryl reagent, mersalyl, but is inhibited by azide.

3. The heavy component of heart muscle microsomes contains almost all of the esterase and NADH–cytochrome *c* reductase. A Ca^{2+}-dependent ATPase is coincident with these activities. This ATPase resembles the sarcotubular ATPase of skeletal muscle with regard to optimum concentration of Ca^{2+}, sensitivity to mersalyl and insensitivity to azide, but differs in its specificity for ATP and a lower apparent affinity for ATP.

INTRODUCTION

In muscle, the sarcotubular system is attributed a central role in the contraction–relaxation cycle by regulating myoplasmic Ca^{2+} levels[1]. Microsomes prepared from muscle have been used extensively in studying the parameters of transport, but little information has been available concerning the heterogeneity of such microsome preparations and knowledge of a wider spectrum of activities is still meagre[2,3].

The present work has focused on the question of heterogeneity in heart muscle microsomes, where a reduced capacity for the uptake of Ca^{2+} and a high level of ATPase activity[4] were suggestive of a greater degree of heterogeneity than in skeletal muscle microsomes. This has been confirmed by finding that the ATPase activity of heart muscle microsomes distributes as two components on density gradients, one of which is stimulated by Ca^{2+} and the other not, whereas in skeletal muscle microsomes, only the Ca^{2+}-dependent ATPase was found. The Ca^{2+}-dependent ATPase of heart

Abbreviation: EGTA, Ethyleneglycol-bis-(β-aminoethyl ether) *N,N*-tetraacetic acid.

muscle has a number of properties that are similar to that of skeletal muscle, indicating that this component has a similar functional significance. The component not stimulated by Ca^{2+} resembles in several respects "light" microsome sub-fractions that have been recognised in some other tissues and identified as fragments of plasma membrane[5–7].

MATERIALS AND METHODS

Fractionation of heart muscle homogenates

Hearts were excised from mongrel dogs immediately after exsanguination under sodium pentobarbitone (25 mg/kg) anaesthesia. Ventricular muscle (40 g) was cut into small pieces with scissors and added to 180 ml ice-cold medium containing 0.25 M sucrose, 0.0025 M disodium ATP, 0.005 M $MgCl_2$ and 0.011 M triethanolamine. After brief (5 sec) Waring blender treatment, the pH of the resulting coarse dispersion was consistently 6.9–7.0. Further dispersion of the tissue fragments was accomplished with a teflon pestle and glass tube, which was kept immersed in ice–water. A "cytoplasmic extract" was prepared from the homogenate by centrifuging for 5000 × g·min and filtering the resulting supernatant through two layers of tissue paper. As shown in Fig. 1a, an efficient separation of microsomes (as esterase) from mitochondria (as cytochrome oxidase) can be obtained by differential centrifugation of supernatants prepared in the above manner. Substitution of 0.1 M KCl for sucrose made no significant difference (Fig. 1b). However, low yields of microsomes were sometimes obtained and this appeared to be due to precipitation during preparation of the cytoplasmic extract. Fig. 1c shows that precipitation of microsomes is readily brought about by warming the cytoplasmic extract to room temperature. Isolated microsomes invariably yielded a "fast-sedimenting" fraction, even when re-suspended at 0°. The influence of a number of reagents on aggregation of isolated microsomes, described in RESULTS, led to the

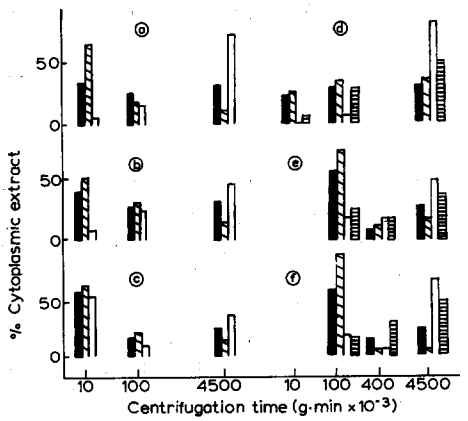

Fig. 1. Sedimentation profiles of heart muscle mitochondria and microsomes. Sedimentation analysis was carried out on the cytoplasmic extract obtained after removal of tissue debris. Homogenates were prepared under the following conditions: (a), (c) in 0.25 M sucrose; (b) in 0.1 M KCl; (d), (e) in 0.25 mM sucrose containing 0.1 % heparin; (f) in 0.25 M sucrose containing 0.5 % heparin. In (c), the cytoplasmic extract was warmed to room temperature. Filled bars, protein; bars with inclined hatching, cytochrome oxidase; empty bars, esterase; horizontally hatched bars, oligomycin-insensitive ATPase.

choice of heparin to control this property and ensure uniform yields of microsomes. When heparin was added to the homogenizing medium, the sedimentation rates of both mitochondria and microsomes were affected (Fig. 1d), necessitating a change in the schedule of centrifugation as shown in Figs. 1e and 1f. The scheme shown in Fig. 1e was employed in most experiments since it ensured a good yield of microsomes and economised in the use of heparin. The isolated microsomes were washed in 0.25 M sucrose or 0.6 M KCl, as indicated in the text.

Skeletal muscle microsomes

These were prepared from rabbit longissimus dorsi muscle as described by NAGAI *et al.*[8].

Density gradients

Stock 60 % sucrose used for preparation of gradients was treated with Dowex 50, filtered and the pH adjusted to 7.2 with triethanolamine. Linear gradients of 5.0 ml were collected in 20 fractions.

Assays

Esterase and antimycin-insensitive NADH–cytochrome *c* reductase were assayed by the methods of IMAI *et al.*[3]. The units used are μmoles β-naphthol acetate hydrolysed per h and nmoles cytochrome *c* reduced per h, respectively.

Cytochrome oxidase was measured by the method of COOPERSTEIN AND LAZAROW[9], except that a lower concentration of sodium dithionite (0.12 M) was used for reduction of cytochrome *c*. One unit of cytochrome oxidase corresponds to an increase in oxidised cytochrome *c* concentration of one order of magnitude per min.

Phosphohydrolase activity was assayed in 0.4 ml medium containing 0.002 M substrate, 0.005 M $MgCl_2$, 0.1 M KCl and 0.02 M triethanolamine, pH 7.2. After 10 min at 37°, 2.0 ml 0.625 M $HClO_4$ were added, the mixture centrifuged and the supernatant decanted into 0.5 ml Fiske–Subbarow reagent containing molybdate for estimation of phosphate[10]. Oligomycin-insensitive ATPase was assayed in the presence of oligomycin at 2 μg/ml and Ca^{2+}-dependent ATPase with the further addition of phosphoenolpyruvate (0.005 M) and pyruvate kinase (Sigma Type 1, 0.1 mg/ml). 1.0 μmole phosphate released per h was taken as the unit of activity.

Protein was estimated by the method of LOWRY *et al.*[11] except for fractions collected from sucrose gradients, when an ultraviolet light absorption method[12] was used.

Phospholipid was determined as total phosphorus, estimated by the method of BARTLETT[13] in a chloroform–methanol extract prepared by adding 2.0 ml chloroform–methanol (2:1, v/v) to 0.05–0.20 ml microsome suspension, followed by two washes, each with 0.15 ml 0.9 % NaCl.

RESULTS

Aggregation of mitochondria and microsomes and effects on density gradient behaviour

When heart muscle microsomes were suspended in 0.25 M sucrose or 0.1 M KCl, extensive precipitation occurred if the suspension was centrifuged for $6 \cdot 10^3 \times g \cdot$min,

which is wel below the time required to sediment microsomes from a cytoplasmic, extract (Fig. 1). The use of a tight-fitting homogeniser increased, rather than decreased, the amount of fast-sedimenting material. Shaking such suspensions at room temperature resulted in the appearance of visible particles. This type of behaviour has been observed by others with skeletal muscle microsomes and has been referred to as aggregation[14–16]. Aggregation was counteracted by polyanions and oleate, but not by EDTA, as can be seen in Table I. These data show that the fast-sedimenting fraction contains a higher proportion of the total protein than of esterase activity, suggesting the involvement of an extraneous protein in aggregation. Analyses for protein and phospholipid confirmed a higher protein content of the aggregated material. The fast-sedimenting fraction, obtained by centrifuging for $6 \cdot 10^3 \times g \cdot \text{min}$, contained 4.2 mg protein per mg phospholipid, compared with 1.9 mg protein per mg phospholipid in the slowly-sedimenting fraction ($4.5 \cdot 10^6 \times g \cdot \text{min}$). Similar behaviour was found in salt solutions as well as in sucrose and addition of heparin to sucrose suspensions, although it reduced the amount of fast-sedimenting fraction (Table I), did not lower the protein content of the recovered microsomes. Since the amount of protein in the fast-sedimenting fraction appears to be higher than can be reasonably accounted for as adsorption to the membranes, it is possible that the extraneous protein exists in a readily sedimentable form such as filaments of actin or myosin, both of which can be prepared in filamentous form[17].

TABLE I

AGGREGATION OF MICROSOME SUSPENSIONS

Microsomes (prepared as in Fig. 1a) were re-suspended in 0.25 M sucrose–0.005 M triethanolamine buffer (pH 7.2) at 0.2 mg/ml and 0°, with the additions shown in the table. The suspensions were then centrifuged for $6 \cdot 10^3 \times g \cdot \text{min}$ to sediment aggregated material.

Addition	*Fraction of microsomes precipitated (% total)*	
	Esterase	*Protein*
None	29	40
Heparin (0.5 %)	10	17
Dextran sulphate (0.5 %)	11	14
Polythylene sulphonate (0.5 %)	20	26
Potassium oleate (0.005 M)	—*	15
Potassium oleate (0.005 M) + heparin (0.5 %)	—	8
EDTA (0.001 M)	27	45

* Activity was inhibited by oleate.

Aggregation also had marked effects on density gradient behaviour. Sedimentation of microsomes through discontinuous sucrose gradients produced a sharp pattern of bands located at each interface. All bands contained both esterase and cytochrome oxidase activity. There was a visible increase in granularity of the bands in the heavier layers, indicative of aggregation. When continuous sucrose gradients were used, there was a striking difference in behaviour according to whether the microsomes were sedimented or floated through the gradient (Figs. 2a and 2d). The sedimentation results were obtained in a much shorter centrifugation time than in the case of flota-

tion. The difference in behaviour could therefore be due to a slow dissociation of membrane aggregates. Heparin promoted such dissociation and was most effective when present throughout the gradient (Figs. 2b and 2c), suggesting that its primary role is to stabilise large aggregates of membrane and protein in a configuration which facilitates their separation. One possibility is that the vesicles have to escape through

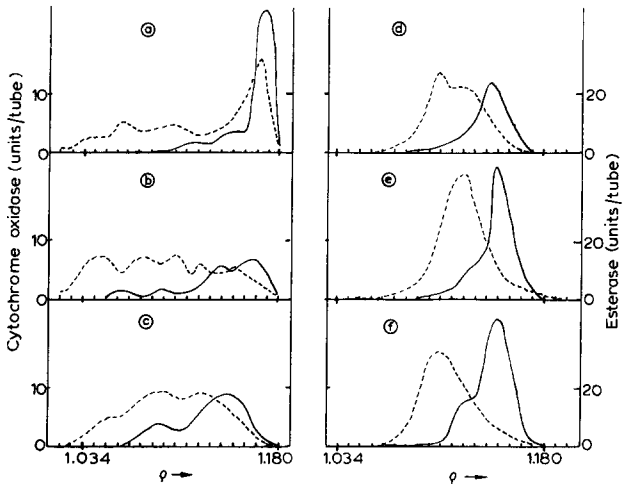

Fig. 2. Density gradient analysis of heart muscle microsomes. Linear gradients were prepared from 8.5% and 40% sucrose. Microsomes were prepared as in Fig. 1 (d) and washed in 0.25 M sucrose–0.001 M triethanolamine–HCl, pH 7.2. In (a), (b) and (c), 0.5 ml microsome supension in 0.25 M sucrose was layered on top of the gradients and these were centrifuged for $1.2 \cdot 10^6 \times g \cdot$min. In (d), (e) and (f), gradients were formed on top of 0.5 ml microsomes suspended in 60% sucrose and centrifuged for $1.4 \cdot 10^8 \times g \cdot$min. Heparin was added as follows: (a) and (d), none; (b) 0.5% heparin in microsome suspension only; (c) and (e), 0.5% heparin in suspension and gradient; (f) 2.0% heparin in suspension and gradient. ———, cytochrome oxidase; – – – –, esterase.

the interstices of a fibrous network of filaments. However, there may also be other proteins present which bind directly to the membranes, affecting their density. Figs. 2d and 2e show that heparin can influence apparent density even under conditions where the markers resolve as substantially separate peaks.

Resolution of Ca^{2+}-dependent and Ca^{2+}-independent ATPases in heart muscle microsomes

The most salient enzymological feature of muscle microsomes is a Ca^{2+}-activated ATPase. The relation of this ATPase to transport of Ca^{2+} has been extensively studied in skeletal muscle[18]. Although heart muscle microsomes are known to accumulate Ca^{2+} under similar conditions, they do so less efficiently[4], so that not all of the ATPase activity may be coupled to Ca^{2+} transport. This difference between heart and skeletal muscle is demonstrated in Fig. 4b, which compares the response to Ca^{2+} of the ATPases of heart and skeletal muscle microsomes in the presence of oligomycin to eliminate the mitochondrial ATPase. Ethyleneglycol-bis-(β-aminoethyl ether) *N,N*-tetracetic acid (EGTA) is present initially in excess of trace amounts of calcium known to contaminate commercial ATP preparations[19]. It can be seen that the "basic" activity in the absence of free Ca^{2+} is much higher in heart muscle than in skeletal muscle and stimu-

lation is considerably less in the case of heart muscle. Both of these features are consistent with the occurrence of ATPase(s) in addition to that coupled to the transport of Ca^{2+}.

The distribution of ATPase acitivity on gradients is illustrated in Fig. 3. In conjunction with this assay, efforts were made to further reduce effects of protein association. One way of doing this, was to discard the fast-sedimenting fraction of washed

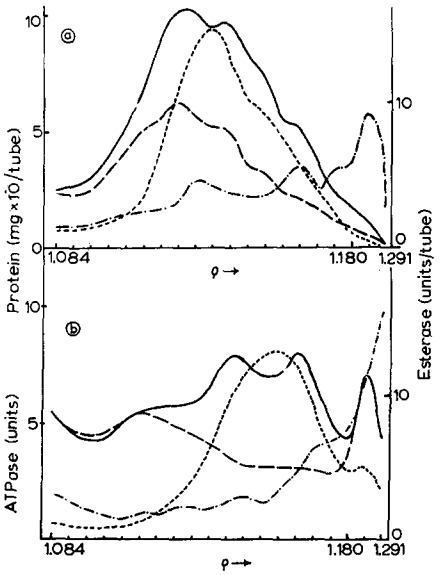

Fig. 3. Flotation analysis of heart muscle microsomal ATPases. Linear gradients were prepared from 20% and 40% sucrose, each containing 0.5% heparin. (a) slowly sedimenting microsomes obtained after removing material sedimenting at $6 \cdot 10^4 \times g \cdot$min; (b) unwashed microsomes. ———, oligomycin-insensitive ATPase; ——, oligomycin-insensitive ATPase in the presence of 0.2 mM EGTA; – – – –, esterase; —·—, protein.

microsomes (Fig. 3a); another was to omit washing altogether in view of the evidence that it promotes aggregation (Fig. 3b). In the case of unwashed microsomes, there is a distinct separation of two ATPases, the lighter component being insensitive to EGTA, while the heavier component is inhibited. Since the distribution of ultraviolet light absorbing material and of esterase activity was similar in both washed and unwashed microsomes, the lighter component may be more susceptible to protein binding or entrapment. The low level of esterase in this component reinforces the impression of two types of membrane fragment. Fig. 4a shows that the heavy component responds to Ca^{2+} in a manner qualitatively similar to that of skeletal muscle microsomes, stimulation being followed by inhibition at higher levels of Ca^{2+}. The response of the isolated heavy component is enhanced about 5-fold compared with unfractionated heart muscle microsomes, but is still considerably less than obtained with skeletal muscle.

Further comparison of light and heavy components of heart muscle microsomes

Effect of substrate concentration on ATPases

As shown in Fig. 5, the light and heavy ATPases of heart muscle responded in quite a different manner to increasing levels of ATP. In contrast to the normal satura-

tion behaviour shown by the light component, activity of the transport ATPase continued to increase up to 5 mM ATP. The ATPase of skeletal muscle showed the same property as the heavy component of heart muscle but differs with respect to a higher rate of hydrolysis at low ATP concentrations, indicative of a higher affinity of the skeletal muscle enzyme for ATP. The results point to the likelihood that ATP has some activating effect on the enzyme, in addition to being the substrate.

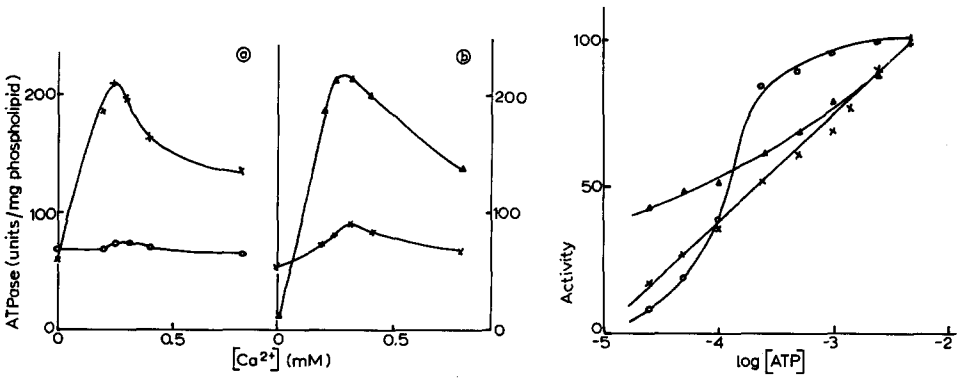

Fig. 4. Ca^{2+} dependence of ATPases in heart and skeletal muscle microsomes. Oligomycin-insensitive ATPase activity was assayed in the presence of 0.2 mM EGTA. (a) ○—○, light; ×—×, heavy components of heart muscle microsomes, separated as in Fig. 3b; (b) ×—×, unfractionated heart muscle microsomes washed in 0.6 M KCl. △—△, skeletal muscle microsomes.

Fig. 5. Effect of substrate concentration on microsomal ATPases. All measurements were carried out in the presence of oligomycin, phosphoenolpyruvate (5 mM) and pyruvate kinase. Mg^{2+} concentration was adjusted to 8 mM. ○—○, light component of heart muscle microsomes; ×—×, the heavy component, after subtraction of EGTA-insensitive hydrolysis; △—△, skeletal muscle microsomes.

Studies carried out by MAKINOSE[20] have shown that low levels of ADP inhibit the transport ATPase and simultaneously stimulate an exchange of ADP with ATP. This is also accompanied by a decrease in membrane-bound phosphoprotein. MAKINOSE[20] has postulated that higher concentrations of ADP form a relatively stable complex with the phosphoprotein intermediate, inhibiting both exchange and hydrolysis. Product inhibition is not sufficient to account for this effect of ATP on ATPase activity and moreover, ATPase activity has been measured in the presence of phosphoenolpyruvate and pyruvate kinase to ensure the rephosphorylation of ADP in the present experiments. However, the activating effect of ATP might be explained if ADP remains bound to the phosphorylation site as it is formed and if its displacement is brought about by ATP. This property emphasises the similarity of the calcium transport systems in heart and skeletal muscle and further suggests that a reason for the lower transport efficiency of heart muscle microsomes may be a more effective competition of ADP with ATP for the transport site in heart muscle.

Nucleotide specificity

Data in Table II compare the light and heavy components of heart muscle microsomes with regard to hydrolytic activities towards a range of nucleoside phosphates. The light component showed a greater activity and a wider range of activity than the heavy component. The latter is relatively specific for ATP, but the light com-

TABLE II

SUBSTRATE SPECIFICITY OF MICROSOMAL ATPASES

Gradient analysis was carried out as in Fig. 3b. "Light component" consisted of the first 4 fractions at the light end and "heavy component" consisted of 4 fractions surrounding and including the esterase peak.

Substrate	*Rate of hydrolysis (μmoles P_i per mg phospholipid per h)*		
	"Light component"	*"Heavy component"*	*Light / Heavy*
AMP	25	7	3.6
IMP	21	7	3.0
UMP	51	10	5.1
GMP	18	5	3.6
UDP	204	31	6.6
GDP	173	31	5.6
ATP	177	112	1.6
UTP	248	50	5.0
ITP	190	60	3.2
GTP	223	76	2.9
CTP	287	54	5.3

ponent has a high activity towards nucleoside diphosphates as well as triphosphates and among these, it is least active towards ATP and most active towards CTP.

Fig. 6 provides a further assessment of nucleotide specificity. In this case, ATPase activity was measured in the absence of oligomycin. Consequently there are three areas of ATPase activity corresponding to the mitochondrial ATPase and the heavy and light microsomal ATPases. The peak of GTPase activity is located in the light

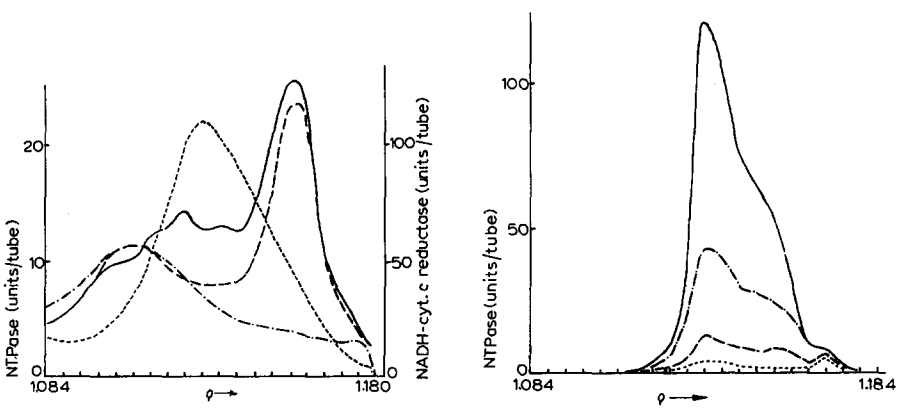

Fig. 6. Flotation analysis of ATPase and GTPase activities in heart muscle microsomes. Flotation of microsomes prepared as in Fig. 1e was carried out in a gradient containing 2 % heparin. ———, ATPase (oligomycin absent); – – – –, ATPase (with addition of 0.2 mM EGTA); —·—, GTPase; --------, antimycin-insensitive NADH–cytochrome *c* reductase.

Fig. 7. Flotation analysis of ATPase and GTPase activities in skeletal muscle microsomes. Conditions of gradient flotation were the same as in Fig. 6. ———, oligomycin-insensitive ATPase; – – – –, ATPase *plus* 0.2 mM EGTA; —·—, GTPase; --------, GTPase *plus* 0.2 mM EGTA.

component, but tailing of this peak could account for residual GTPase activity in the heavy component. Analysis of gradients for CDPase, ADPase and AMPase showed these activities to distribute in a similar way to GTPase. Fig. 6 also shows that NADH–cytochrome *c* reductase is another enzyme, which, like esterase, associates almost entirely with the heavy component. The distribution of these enzymes and those acting non-specifically upon nucleoside phosphates provides a clear distinction between the two microsome sub-fractions of heart muscle. One result of this distinction is the dissociation of non-specific nucleoside triphosphate phosphohydrolase activity from direct involvement in calcium transport. This conclusion is in marked contrast to skeletal muscle, where the hydrolysis of all NTP's is stimulated by calcium *via* a common phosphoprotein intermediate[21,22]. The behaviour of a preparation of skeletal muscle microsomes on density gradient flotation is shown in Fig. 7. ATPase and GTPase activities were both Ca^{2+}-dependent and coincident. This preparation of skeletal muscle microsomes has a remarkably high protein content (6.7 mg protein per mg phospholipid) so the possibility that aggregation effects may be more dominant in skeletal muscle microsomes than in those of heart muscle cannot be overlooked. On the other hand, the absence of nucleoside diphosphate phosphohydrolase activity towards CDP and GDP is consistent with the absence of a light component in skeletal muscle microsomes.

Effects of inhibitors

The light and heavy components of heart muscle microsomes responded in quite different ways to a number of inhibitors. Data in Table III compare the effects of the mercurial, mersalyl, on the ATPase and GTPase activities of the two components. Only the transport ATPase was affected and GTPase either in the light or heavy component was not inhibited. These observations confirm the specificity of transport for ATP in heart muscle. The absence of an effect of mersalyl on the hydrolysis of other nucleoside triphosphates, CTP, UTP and ITP, was confirmed using unfractionated heart muscle microsomes, thus establishing the differing significance of non-specific NTPase activity in heart and skeletal muscle microsomes. In skeletal muscle, mersalyl

TABLE III

SULPHYDRYL DEPENDENCE OF ATPASE AND GTPASE

Light and heavy components were isolated as in Table III. When present, mersalyl was added to microsomes prior to incubation. "Basic" ATPase is activity in presence of 0.2 mM EGTA. The proportion of "basic" to "Ca^{2+}-stimulated" activity in this preparation was 2:3.

Substrate	*Mersalyl (μmoles/mg protein)*	*Inhibition (%)*		
		Light component	*Heavy component*	
GTP	1.0	1	−2	
	5.0	−8	−6	
			"Basic"	*"Ca^{2+}-stimulated"*
ATP	1.0	3	22	96
	5.0	5	20	90

is known to inhibit all activities associated with Ca^{2+} transport, including hydrolysis of all NTP's [23].

Further differentiation between the light and heavy components was found with azide. In this case, calcium-dependent activity was not affected, confirming observations with skeletal muscle[18], but hydrolysis of both NTP's and NDP's by heart muscle microsomes was inhibited from 26% (CTP) to 64% (ADP) at 5 mM azide. In this respect, the NTPase activity of heart muscle microsomes bears a closer resemblance to that of liver microsomes[24] than it does to skeletal muscle.

DISCUSSION

The experience of previous investigators reveals special problems which hamper the isolation of subcellular components from muscle homogenates. Thus, a marked heterogeneity of mitochondria with regard to sedimentation rate is characteristic of a variety of muscles[25]. The so-called "heavy" mitochondria of heart muscle sediment in considerably shorter time than liver mitochondria. Also, it has often been noted that the "Ca^{2+}-sequestering granules" of muscle sediment over almost the complete range of centrifugal force employed in fractionating homogenates[26–28]. Results obtained in this study suggest that aggregation with muscle proteins is a factor which influences the sedimentation rate and apparent density of both mitochondria and microsomes in heart muscle. Only the presence of extraneous protein can account for the exceptionally high protein:phospholipid ratios of isolated microsomes. Values that have been reported for skeletal muscle microsomes are even higher than in heart muscle: MARTONOSI *et al.*[29] found 4–5 mg protein per mg phospholipid for skeletal muscle microsomes after washing with 0.6 M KCl. Skeletal muscle microsomes prepared in this laboratory according to the method of NAGAI *et al.*[8] were found to contain 6.7 mg protein per mg phospholipid. Both ATP[30] and heparin[31] which are included in the homogenising medium employed in this work, are known to cause dissociation of actomyosin. However, it is precisely these "plasticising" conditions which favour a high yield of microsomes. In any case, considerable release of myofibrillar proteins is known to accompany any method of homogenising muscle, since myosin is a recognised contaminant of microsome preparations[32,33]. It is possible that these and other proteins bind to microsomes in some manner so as to grossly alter "native" sedimentation and density characteristics.

Depending upon the extent to which aggregation is controlled, microsomes from heart muscle separate into two distinct sub-fractions with widely differing functional characteristics. The density gradient results lead to the conclusion that NADH–cytochrome *c* reductase and esterase activities are localised in the component involved in calcium transport and that non-specific nucleoside phosphohydrolase activities are associated with some other membrane system(s). These differences between microsome subfractions of heart muscle are strikingly similar to differences that have been pointed out by EMMELOT *et al.*[5] between liver microsomes and plasma membranes prepared from the same tissue by a method which avoids vesicularization. These authors found that NADH–cytochrome *c* reductase and esterase are common to both plasma membranes and microsomes, but that their specific activities are several-fold higher in microsomes than in plasma membranes. Activities they found at higher level in plasma membranes were 5'-AMPase (EC 3.1.3.5), ADPase (EC 3.6.1.6) and (Na^+, K^+)-depen-

dent ATPase. The latter activity is associated with a Mg^{2+}-ATPase (EC 3.6.1.4), which later work[6] showed to be non-specific with regard to NTP's. From this, they concluded that liver microsomes contain elements of both plasma membrane and endoplasmic reticulum, with nucleoside phosphohydrolases concentrated in the former and esterase and NADH–cytochrome *c* reductase in the latter. Microsome sub-fractions with similar properties were obtained by PURDUE AND SNEIDER[7] who submitted a plasma membrane-enriched preparation from chicken fibroblasts to density gradient fractionation. They obtained a "light" fraction identified as plasma membrane, which had a low activity of NADH–cytochrome *c* reductase and a concentration of non-specific nucleoside phosphate phosphohydrolases compared with the "heavy" fraction. These results are quite comparable to those obtained with heart muscle microsomes. While in the above studies, plasma membranes were prepared by methods different from those used for preparation of microsomes, WATTIAUX-DE CONINCK AND WATTIAUX[34] have shown that it is possible to achieve separation of a plasma membrane marker (5'-AMPase) and a marker for endoplasmic reticulum (glucose-6-phosphatase) by flotation of liver microsomes through density gradients. No separation occurred when sedimentation procedures were used, showing that the problems encountered in the present work are not entirely unique to muscle. It is of further interest that nucleoside diphosphatase activity in liver microsomes resolved into two components, one specific for ADP, which moved with AMPase and the other non-specific, which moved with glucose-6-phosphatase. In heart muscle, both ADPase and NDPase (non-specific) were found in the light component. It can be concluded that the distribution of activities between light and heavy components of heart muscle microsomes shows an impressive similarity to the distribution of these activities between plasma membranes and endoplasmic reticulum in several other tissues.

In muscle, the plasma membrane forms the innermost part of a complex sheath or sarcolemma, which consists of several layers and includes collagen microfibers and mucoprotein in its composition[35]. The plasmalemma is characteristically invaginated in the form of finger-like projections, known as the transverse component of the sarcotubular system, or T-system. A longitudinal component (or L-system) has no connection with the T-system and is regarded as analogous to the endoplasmic reticulum of other cells[36]. It is not unreasonable to expect that elements of both the T-system and the L-system would be found in microsome preparations from muscle, but it is questionable whether the remainder of the plasma membrane would be freed from its more complex surface coat. In skeletal muscle, it is generally understood that the T-tubules end in a region known as a triad, where they are in apposition with terminals of the L-system. However, in heart muscle, it has recently been shown that the T-system is ramified to a far greater extent, much of it running parallel with the L-system, though never continuous with it[37]. Consequently, the T-system in myocardium could be expected to contribute much more to the microsome fraction than in the case of skeletal muscle. This circumstance, and the absence of a light component in skeletal muscle microsomes make it possible to suggest that the light component may consist of elements of the T-system of myocardium.

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