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Further Characterization of Rat Liver Mitochondrial Fractions

LIPID COMPOSITION AND SYNTHESIS, AND PROTEIN PROFILES

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1. Heavy and light mitochondrial fractions obtained by differential centrifugation were further characterized with respect to their lipid composition and synthesis and protein profiles, as seen by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. 2. The light mitochondrial fraction was rich in total lipids, phospholipids and cholesterol. The cardiolipin content, however, was low. 3. Rates of [^{3}H]glycerol incorporation into phospholipids of heavy mitochondria and microsomal fractions were almost identical. On the other hand, incorporation into the individual phospholipids in light mitochondria was about 4–6 times higher. Incorporation into cardiolipin of light mitochondria was about 10-fold higher than in the heavy mitochondria. 4. Analysis of protein profiles by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis showed that the pattern obtained for the light mitochondria was similar to that for heavy mitochondria. However, the light fraction was relatively poor in high-molecular-weight proteins and rich in low-molecular-weight proteins. The microsomal protein profile was altogether different. 5. The significance of these findings is discussed in relation to mitochondrial biogenesis.

Earlier studies from this laboratory had indicated that rat liver mitochondrial population is heterogeneous and could be resolved by differential centrifugation into heavy, light and fluffy fractions, having definable properties which could be greatly altered according to the thyroid status of the rat (Katyare *et al.*, 1970; Satav *et al.*, 1973). The three mitochondrial fractions appeared to represent discrete entities in terms of their enzymic properties, chemical make-up and protein-synthetic ability *in vivo* and *in vitro*. These studies revealed that the light mitochondrial fraction, which had a high synthetic activity and turnover rate, represented transitional forms in the process of development into stable heavy mitochondrial structures, and the fluffy fraction appeared to be a mixture containing degenerating mitochondria (Katyare *et al.*, 1970; Satav *et al.*, 1973).

In the present studies attempts have been made to bring out further the differences between the properties of the heavy and light mitochondrial fractions with respect to their lipid composition and ability to incorporate [^{3}H]glycerol *in vivo* into their individual phospholipids. Observations on protein profiles obtained by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis are also included. For comparison, parallel studies carried out with microsomal preparations are also detailed.

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Materials and Methods

Chemicals

Phospholipid standards, 2,5-diphenyloxazole (PPO), 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene (dimethyl-POPOP), bovine serum albumin, ovalbumin, rabbit muscle aldolase, sperm-whale myoglobin, horse heart cytochrome *c* type VI, sodium dodecyl sulphate and Coomassie Brilliant Blue R were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Trypsin inhibitor was from Worthington Biochemical Corp., Freehold, NJ, U.S.A., and silica gel G was from E. Merck A.-G., Darmstadt, Germany. Acrylamide, *NN'*-methylene bisacrylamide, and ammonium persulphate were purchased from Koch–Light Laboratories, Colnbrook, Bucks., U.K. All other chemicals were of analytical-reagent grade.

[^{3}H]Glycerol was obtained from the Isotope Division of this Research Centre and was further purified by t.l.c. on silica gel G, with butan-1-ol/water (9:1, v/v) as solvent (Randerath, 1964). The final product had a specific radioactivity of 69.7 mCi/mmol.

Preparation of mitochondrial fractions

Mitochondrial fractions were isolated from livers of rats weighing 160–180 g as described previously (Katyare *et al.*, 1970; Satav *et al.*, 1973) and washed

three times with the isolation medium (0.25M-sucrose containing 10mM-Tris/HCl, pH7.4, and 1mM-EDTA) at their sedimentation velocities, which were 6500*g* for 10min and 12000*g* for 10min for heavy and light mitochondrial fractions respectively. The residual mitochondria were removed by centrifugation at 18000*g* for 10min and were discarded. Mitochondria-free supernatant was centrifuged at 105000*g* for 1h to obtain microsomal fractions, which were washed once by suspension in the isolation medium and re-sedimenting.

Lysosomes were prepared by the procedure of Regab *et al.* (1967).

All operations were carried out at 0–4°C.

Microsomal and lysosomal contamination of the mitochondrial fractions was monitored in terms of the marker enzymes glucose 6-phosphatase (Recknagel & Lombardi, 1961) and acid phosphatase (Gianetto & de Duve, 1955) respectively. The percentage contamination was calculated by comparing specific activities of marker enzymes in mitochondrial fractions with those in purified microsomal and lysosomal fractions.

Incorporation studies

For radioactive experiments, rats received 25μCi of [^{3}H]glycerol (in 0.9% NaCl)/100g body weight intraperitoneally and were killed after 15, 30 or 60min.

Lipid analysis

After isolation, mitochondrial and microsomal fractions were immediately used for lipid extractions. Lipids were extracted with chloroform/methanol (2:1, v/v) as described by Folch *et al.* (1957). Total lipids were measured by Bragdon's (1951) dichromate method. Phospholipids, after acid digestion at 160–180°C, were determined by Bartlett's (1959) method. Phosphorus values were multiplied by a conversion factor of 25 to obtain values in terms of average weight of phospholipids. Cholesterol was determined by the method of Zlatkis *et al.* (1953). Individual phospholipids were separated by t.l.c. in chloroform/methanol/water (65:25:4, by vol.) (Wagner *et al.*, 1961). Coating of chromatography plates, chamber saturation and general conditions were as described by Stahl (1965); individual phospholipids were identified by co-chromatography of authentic samples on the same thin-layer plates and by their respective R_F values. Samples were spotted in duplicate, one set of samples being used for phospholipid phosphorus analysis and the other set for counting of radioactivity. Phospholipid spots on the developed plates were made visible by brief exposure to iodine vapour; zones were marked, and iodine was allowed to sublime off. Marked zones were scraped on to glazed paper and transferred to centrifuge tubes. Phospholipids were extracted from silica gel, initially with 3vol. of chloroform/methanol/water (65:25:4, by vol.) and subsequently with 2×3vol. of methanol to ensure complete extraction. Extracts from single spots were pooled and evaporated, and phospholipid phosphorus was determined as described above for total phospholipids. Recovery of the samples from chromatography plates was 85–90%.

For counting radioactivity, the eluted samples were taken directly into counting vials to which 10ml of scintillation fluid (0.3% of PPO and 0.01% of dimethyl-POPOP in toluene) was added after complete evaporation of the solvent, and radioactivity was counted in a Beckman LS 100 liquid-scintillation spectrometer. Counting efficiency for ^{3}H was 35%.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of mitochondrial proteins was carried out as described by Weber & Osborn (1969) with some modifications. Proteins were solubilized at a concentration of 2mg/ml in a solvent containing 10% (w/v) glycerol, 1% sodium dodecyl sulphate, 1% mercaptoethanol and 0.002% Bromophenol Blue in 0.01M-sodium phosphate buffer, pH7.0. Monomerization of the dissolved proteins was achieved by incubation in a boiling-water bath for 2min (Laemmli, 1970). The 7.5% (w/v) polyacrylamide gels (7.0cm long) were prepared in 0.02M-sodium phosphate buffer, pH7.0, containing 0.1% sodium dodecyl sulphate and were polymerized in the presence of the catalyst (0.07% ammonium persulphate) by incubation at 37°C for 1h. Between 100 and 150μg of dissolved proteins were applied per tube, and electrophoresis was carried out with a current of 8mA per tube until the tracking dye (Bromophenol Blue) had reached the bottom of the tube. After completion of the electrophoresis, the gels were fixed in 10% (w/v) trichloroacetic acid and stained for 5–6h in 1% Coomassie Brilliant Blue R made up in methanol/acetic acid/water (5:1:5, by vol.). The gels were destained by repeated changes of 7% acetic acid and scanned for densitometric traces in a Canalco model F microdensitometer coupled to a Serva/Riter II recorder. This scanner has an electronic area integrator from which the area of the individual peak and hence percentage distribution could be calculated.

For determination of molecular weights, the gel system was calibrated by measuring the migration of several standard proteins under identical experimental conditions. The marker proteins used were bovine serum albumin (68000), ovalbumin (43000), rabbit muscle aldolase (40000), soya-bean trypsin inhibitor (23000), sperm-whale myoglobin (17200)

and horse heart cytochrome *c* (12000). A standard calibration curve of relative mobility was plotted against log(molecular weight) of each standard protein, and molecular weights of the unknown proteins were estimated. Replicate determinations indicated that maximum variation in the molecular-weight determination was of the order of ± 10%.

Protein was measured by the method of Lowry *et al.* (1951), with crystalline bovine serum albumin as a standard.

Results

Purity of mitochondrial fractions

The results in Table 1 show that, in agreement with the previously reported values (Satav *et al.*, 1973), the heavy and light mitochondrial fractions are contaminated with microsomal fraction to the extent of 2.8 and 5.1% respectively; the corresponding values for lysosomal contamination are 3.5 and 9.6% respectively.

In the present studies, microsomal and lysosomal contamination was determined by expressing the specific activities of the marker enzymes in the mitochondrial fractions as percentages of the specific activities of these enzymes in microsomal and lysosomal fractions. It is recognized that the best method for calculating the contamination would have been to record the recovery of the enzymes in various fractions with respect to the homogenate activity. We could not adopt this method because we had to sacrifice yield of mitochondrial fractions for the sake of purity (Satav *et al.*, 1973). However, it should be pointed out here that the method used in our studies can give rise to arbitrarily high contamination values if a poor recovery of marker enzyme is obtained in the fraction in which it is expected to be recovered. This may be the case with lysosomes from which, during purification of this fraction, some of the marker-enzyme activity may be lost. Nevertheless, the present results emphasize the fact that the two mitochondrial fractions consist mostly of mitochondria (Table 1) and are not artifacts of preparative procedures.

The fluffy fraction was not included in the present studies since it seemed to represent a mixed population containing degenerating mitochondria (Katyare *et al.*, 1970; Satav *et al.*, 1973).

Lipid analysis

Table 2 shows the lipid composition of heavy and light mitochondria and of microsomal fractions. Compared with the heavy mitochondria the light fraction is rich in total lipids (30% higher), phospholipids (25% higher) and cholesterol (54% higher). The individual phospholipid composition of the mitochondrial and microsomal fractions is shown in Table 3. The values obtained for heavy mitochondria are comparable with those reported by others (Colbeau *et al.*, 1971). The light mitochondrial fraction contains amounts of phosphatidylcholine phosphatidylethanolamine and phosphatidic acid

Table 1. *Microsomal and lysosomal contamination in the mitochondrial fractions*

Isolation of microsomal, lysosomal and mitochondrial fractions was as described in the text. Glucose 6-phosphatase activity was determined by the method of Recknagel & Lombardi (1961). Acid phosphatase activity was determined by the method of Gianetto & de Duve (1955). Results are given as means±S.E.M. of at least six independent experiments.

Fraction	Glucose 6-phosphatase (μg of P_i/20min per mg of protein)	Acid phosphatase (μg of P_i/10min per mg of protein)	Contamination (%) by	
			Microsomal fraction	Lysosomal fraction
Microsomal	78.54±2.54	—	—	—
Lysosomal	—	167.00±0.38	—	—
Heavy mitochondrial	2.22±0.17	5.85±0.38	2.83	3.50
Light mitochondrial	4.00±0.18	16.00±1.28	5.09	9.58

Table 2. *Lipid content of mitochondrial and microsomal fractions*

Lipids were extracted as described by Folch *et al.* (1957). Total lipids (Bragdon, 1951), phospholipid phosphorus (Bartlett, 1959) and cholesterol (Zlatkis *et al.*, 1953) were measured. Results are given as means±S.E.M. of at least eight independent experiments. *$P<0.01$, †$P<0.02$ and ‡$P<0.001$ compared with values for heavy mitochondria.

Fractions	Total lipids (μg/mg of protein)	Phospholipids (μg/mg of protein)	Cholesterol (μg/mg of protein)
Heavy mitochondrial	177.7±12.51	145.0±2.48	12.6±1.95
Light mitochondrial	241.0±14.00*	181.0±7.59‡	19.4±1.85†
Microsomal	324.6±13.86	296.0±21.00	29.1±1.65

Table 3. ***Phospholipid composition of mitochondrial and microsomal fractions***

Individual phospholipids were separated by t.l.c. (Wagner *et al.*, 1961) as described in the text and were identified by co-chromatography of authentic samples. Results are given as means ± S.E.M. of at least eight independent experiments. Values are expressed as percentage of the total phospholipids. Absolute values for total phospholipids in the three fractions are as given in Table 2. *$P<0.02$ and †$P<0.001$ compared with values for heavy mitochondria. NS, Not significant.

Phospholipids	Fractions		
	Heavy	Light	Microsomal
Lysophosphatides	2.5±0.35	1.4±0.27*	2.4±0.35
Sphingomyelin	3.67±0.27	6.8±0.12†	5.5±0.46
Phosphatidylcholine	47.5±1.78	44.5±1.50 NS	55.4±1.21
Phosphatidylethanolamine	35.7±1.70	37.0±1.04 NS	32.6±0.61
Cardiolipin	10.0±0.60	5.5±0.66†	2.2±0.33
Phosphatidic acid	1.8±0.08	1.8±0.21 NS	2.2±0.19

comparable with heavy mitochondria, a significantly low amount of cardiolipin (a phospholipid typical of mitochondria) and higher amount of sphingomyelin (Table 3). The lower content of lysophosphatides in this fraction could be related to low phospholipase activity. This possibility, however, has not been explored in the present studies.

In these experiments the lipid composition was determined by the method of Folch *et al.* (1957) for extraction, which might have resulted in poor recoveries of acidic phospholipids. However, we assumed that this would not seriously affect the relative pattern of phospholipid composition of the two mitochondrial fractions.

Incorporation studies

Since phosphatidylcholine and phosphatidylethanolamine are the major phospholipid components of mitochondria (Table 3), and cardiolipin is a phospholipid typical of mitochondria (Fleischer *et al.*, 1967; Getz *et al.*, 1968), the incorporation *in vivo* of [^{3}H]glycerol into these phospholipid components of heavy and light mitochondria and microsomal fractions was investigated and results are shown in Figs. 1–3. The heavy mitochondria and microsomal fractions have more or less similar incorporation patterns for phosphatidylcholine (Fig. 1) and phosphatidylethanolamine (Fig. 2). Incorporation of [^{3}H]glycerol into cardiolipin of heavy mitochondria was comparatively low (Fig. 3). Up to 15min, there was a slight initial lag in the incorporation rate in general, after which the rate of incorporation showed an increase, reaching a plateau by 60min. However, no attempt was made to measure incorporation of [^{3}H]glycerol into microsomal cardiolipin, since this phospholipid is known to be synthesized exclusively by mitochondria (Davidson & Stanacev, 1971; Hostetler *et al.*, 1971).

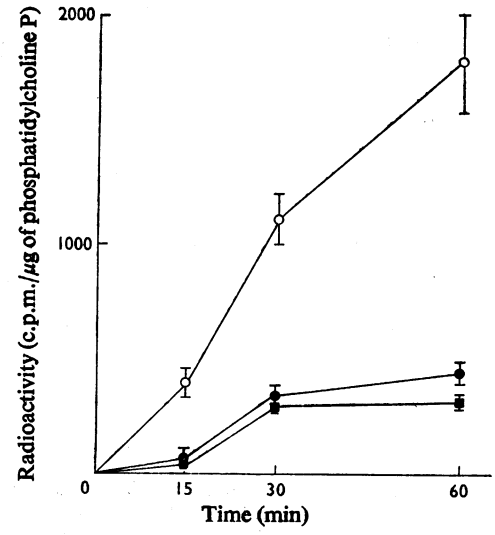
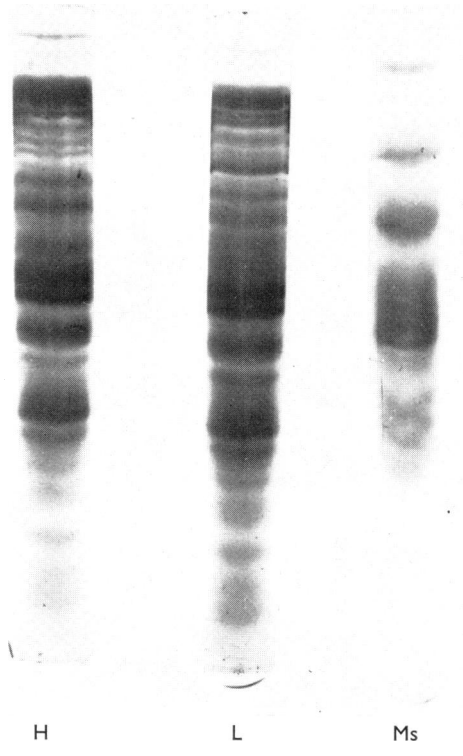


Fig. 1. *Incorporation in vivo of [^{3}H]glycerol into phosphatidylcholine fraction of heavy and light mitochondria and microsomal fractions*

Rats weighing 160–180g received 25μCi of [^{3}H]glycerol/100g body weight in 0.9% NaCl intraperitoneally and were killed after 15, 30 and 60min. Isolation of mitochondrial and microsomal fractions, extraction of total lipids and separation of phospholipid components by t.l.c. were carried out as described in the text. Radioactivity was counted in a Beckman LS 100 liquid-scintillation spectrometer. Results are given as means±S.E.M. of at least five independent experiments. ●, Heavy mitochondria; ○, light mitochondria; ■, microsomal fraction. Differences between the heavy mitochondrial and the microsomal fractions were not significant.

The light mitochondria exhibited a somewhat different incorporation pattern. The rate of [^{3}H]-glycerol incorporation into phosphatidylcholine and



EXPLANATION OF PLATE 1

Typical sodium dodecyl sulphate/polyacrylamide-gel pattern obtained for heavy (H) and light (L) mitochondrial and microsomal (Ms) proteins

Mitochondrial proteins were solubilized in sodium dodecyl sulphate and depolymerized as described in the text. Solubilized proteins (100–150 μg) were applied to 7.5% gels (7 cm long) and electrophoresis was carried out for 3.5 h with a current of 8 mA/tube. The proteins were stained with Coomassie Brilliant Blue R. Other details are as described in the text. The results are typical of six independent experiments.

(*Facing p.* 218)

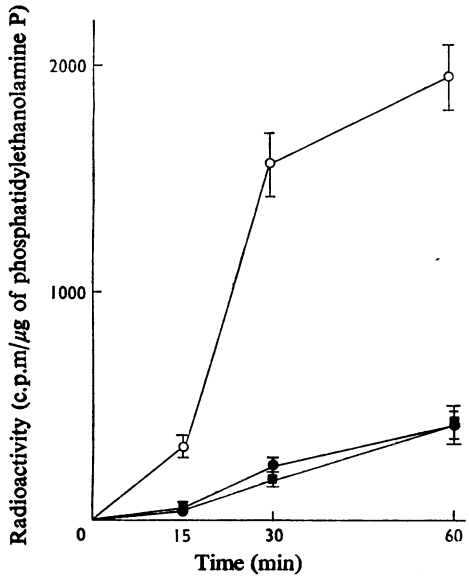


Fig. 2. *Incorporation in vivo of [^{3}H]glycerol into phosphatidylethanolamine from heavy and light mitochondria and microsomal fractions*

Other details are as described in Fig. 1 and in the text. Results are given as means±S.E.M. of at least five independent experiments. Differences between the heavy mitochondrial and the microsomal fractions were not significant.

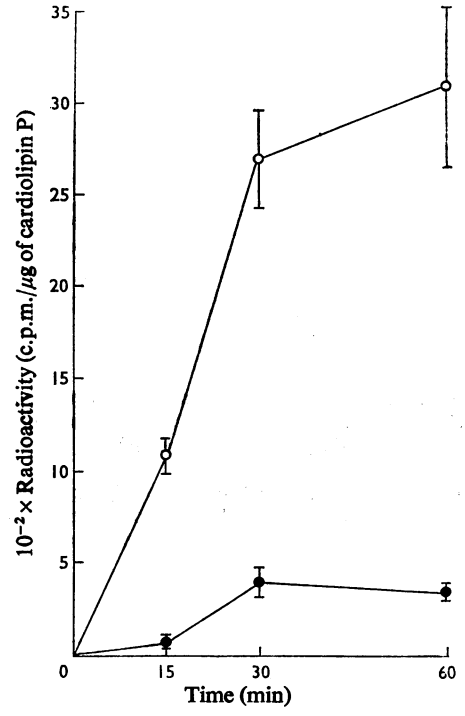


Fig. 3. *Incorporation in vivo of [^{3}H]glycerol into cardiolipin of heavy and light mitochondria*

Other details are as described for Fig. 1 and in the text. Results are given as means±S.E.M. of at least five independent experiments.

phosphatidylethanolamine of light mitochondria was about 4–6-fold higher than in the heavy mitochondrial and microsomal fractions (Figs. 1 and 2). For cardiolipin, there was a tenfold higher incorporation (Fig. 3).

In concomitant studies, the incorporation of [^{3}H]glycerol into phosphatidic acid was also examined, but the results were somewhat variable. This was probably due to the fact that the phosphatidic acid fraction, which migrates slightly below the solvent front, might have been contaminated with neutral lipids, which travel along with the solvent front in the system used for the separation of phospholipids. However, in spite of slight variations in the results, the incorporation into light-mitochondrial phosphatidic acid was found to be about 5–6 times higher than into heavy-mitochondrial phosphatidic acid.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

Typical protein patterns of heavy and light mitochondria and microsomal fractions obtained by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis are shown in Plate 1. The proteins of the two mitochondrial fractions resolved into approximately 20 distinct polypeptide bands, and those of the microsomal fraction resolved into 16 polypeptide bands. The light mitochondria showed similarities to as well as differences from the heavy mitochondria in their sodium dodecyl sulphate/polyacrylamide-gel-electrophoretic pattern. In particular, the light mitochondrial fraction appeared to be relatively poor in high-molecular-weight proteins and rich in low-molecular-weight proteins. Under these conditions, the gel pattern for the microsomal fraction was entirely different.

To bring out these differences more clearly, the polypeptides were characterized in terms of their molecular weights, and their percentage distribution was quantified. For calibration of molecular weights, electrophoresis was carried out under identical conditions with standard proteins of known molecular weight, as outlined in the Materials and Methods section. Relative mobilities of the standard proteins when plotted against log(molecular weight) gave a linear relationship (Fig. 4). By using this plot,

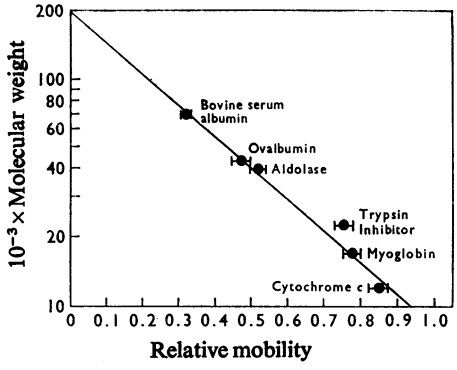


Fig. 4. *Relative mobilities of standard proteins separated on sodium dodecyl sulphate/polyacrylamide gel as a function of log (molecular weight)*

Details of sodium dodecyl sulphate/polyacrylamide-gel electrophoresis are given in Plate 1 and in the text. Each point represents mean±S.E.M. of four independent experiments.

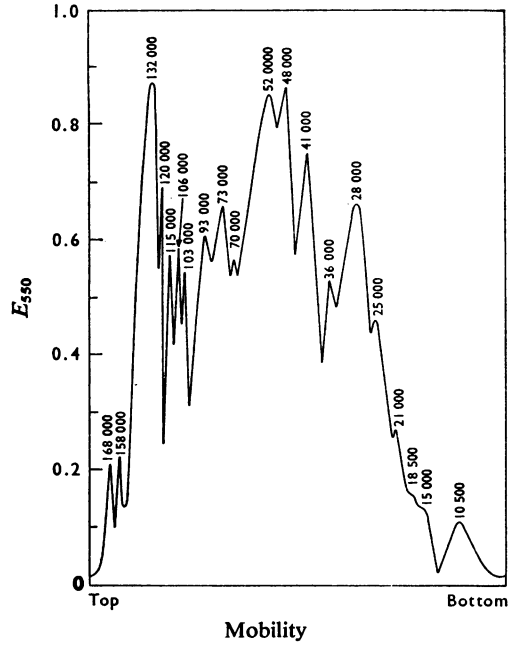


Fig. 5. *Densitometric trace (typical of six independent experiments) showing protein profile of heavy mitochondria*

Gels were scanned in a Canalco model F microdensitometer coupled to a Serva/Riter recorder II. Other details are as in the text and in Table 4.

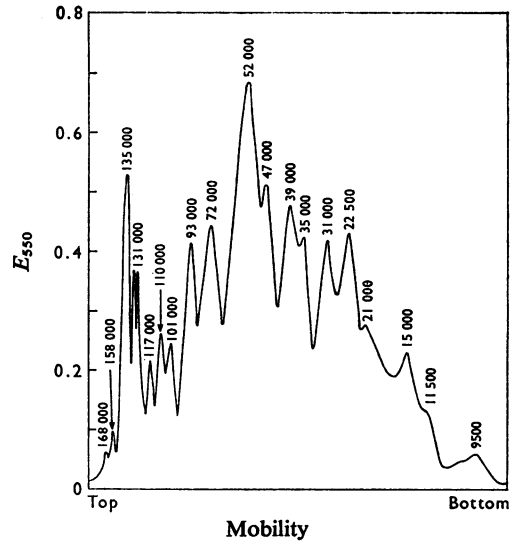


Fig. 6. *Densitometric trace (typical of six independent experiments) showing protein profile of light mitochondria*

Experimental details are as in Fig. 5 and Table 4.

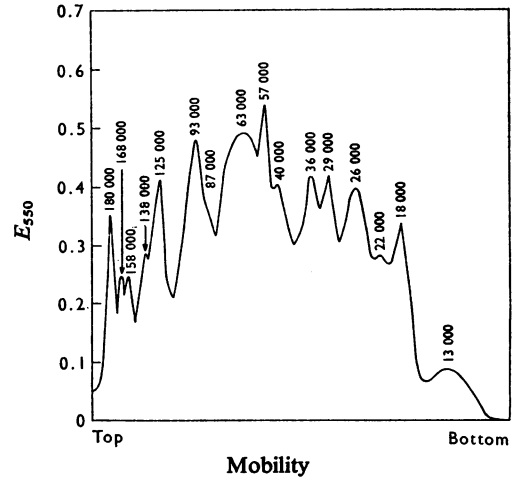


Fig. 7. *Densitometric trace (typical of six independent experiments) showing protein profile of microsomal fraction*

Experimental details are as in Fig. 5 and Table 5.

molecular weights of individual polypeptides were calculated from their respective relative mobilities.

Figs. 5–7 show typical densitometric tracings of the sodium dodecyl sulphate/polyacrylamide gels for heavy and light mitochondria and microsomal

Table 4. *Distribution of peptides in heavy and light mitochondrial proteins*

The gels were scanned in a Canalco microdensitometer equipped with an electronic area integrator. Areas representing the protein peaks in a particular molecular-weight range are expressed as percentage of the total area. The results are means±S.E.M. of six independent experiments. *$P<0.001$ compared with values for heavy mitochondria. NS, Not significant.

	Mol.wt. range	Distribution (%) Heavy mitochondria	Light mitochondria
I	168000–100000	19.90±0.45	10.04±1.63*
II	93000– 70000	10.18±0.73	12.88±1.18 NS
III	52000– 28000	58.80±2.04	53.70±1.80 NS
IV	25000– 9500	11.02±1.75	23.92±2.17*

Table 5. *Distribution of peptides in microsomal proteins*

The gels were scanned in a Canalco microdensitometer equipped with an electronic area integrator. Areas representing the protein peaks in a particular molecular-weight range are expressed as percentage of the total area. Results are means±S.E.M. of five independent experiments. *$P<0.001$ compared with the corresponding values for heavy and light mitochondrial fractions (Table 4).

I	180000–125000	15.22±0.57
II	94000– 63000	31.96±0.66*
III	57000– 29000	28.55±0.99*
IV	26000– 13000	24.03±1.15

fractions respectively. Most of the protein peaks in the mol.wt. range 168000–48000 seem to be identical in heavy and light fractions, except that one peak at 70000 is absent from the light mitochondria. In the low mol.wt. range (25000–9500), light mitochondria seem to possess higher amounts of these proteins.

The microsomal protein profile was altogether different with respect to the number of peaks and their molecular weights (Fig. 7). Their proteins were resolved into 16 polypeptide bands, having mol.wts. in the range 180000–13000.

The percentage distribution of proteins in the two mitochondrial fractions is shown in Table 4. The results show quantitatively that the light mitochondria contain significantly lower amounts of high-mol.wt. proteins (10% compared with 20% in heavy mitochondria) and higher amounts of low-mol.wt. proteins (24% compared with 11% in heavy mitochondria). No changes are seen in intermediate-mol.wt. proteins (52000–28000) of heavy and light mitochondria.

The distribution of microsomal proteins (Table 5) differs from that of heavy and light mitochondrial proteins. The proteins in the mol.wt. range 57000–29000 are present at a significantly low amount in microsomal fractions (29% compared with 54–59% in heavy and light mitochondria). In addition, they contain higher amounts of proteins in the mol.wt. range 94000–63000 (32% compared with 10.2 and 12.9% in heavy and light mitochondria respectively).

Discussion

Studies on lipid content (Table 2) and phospholipid composition (Table 3) reveal that the light mitochondrial fraction has distinct differences from the heavy mitochondrial fraction. It is richer in total lipids, phospholipids and cholesterol and also contains significantly lower amounts of cardiolipin. These observations raise the question whether this could be a reflexion of the presence of non-mitochondrial membranes like plasma membrane or lysosomal membrane in this fraction. However, this possibility seems unlikely for two reasons. (1) Plasma membranes sediment at a much lower speed (Coleman *et al.*, 1967). Besides, any possible contamination with fragments of this membrane would be much less, in view of the fact that the liver tissue is homogenized under controlled conditions. (2) Lysosomal contamination in light mitochondria is only 9.5% (Table 1), and lysosomes are known to be poorer in total phospholipids (0.12mg/mg of protein) and have a higher proportion of cardiolipin (8%; Koenig, 1969). The lysosomal contamination will therefore not seriously affect the lipid composition of the light fraction. Microsomal contamination of the mitochondrial fractions is also minimal (Table 1). Besides, the actual values for microsomal contamination may be much less, in view of the observation that glucose 6-phosphatase may be a constitutive enzyme of the outer membranes of mitochondria (Brunner & Bygrave, 1969). The results (Tables 2 and 3) therefore truly represent lipid composition of the light mitochondria and are not artifacts.

The results showing a high lipid and phospholipid content in the light mitochondria become significant in view of the observation by Goldhor (1968) that during development of embryonic-chick liver, the mitochondrial protein/lipid ratio increases with a concomitant increase in mitochondrial density. Pollak & Woog (1971), however, observed a constant protein/lipid ratio during development of embryonic-chick liver.

Most of the cholesterol in mitochondria is associated with the outer membrane (Parsons *et al.*, 1967). The high cholesterol content of light mitochondria may thus be explained in terms of their relatively well developed outer membranes compared with their inner membranes, as seen by electron microscopy (Satav *et al.*, 1973; Subramanian *et al.*, 1973). It is noteworthy that Beattie (1969) has proposed that,

for biogenesis of mitochondria *in vivo*, prior synthesis of outer membrane is a prerequisite.

The low cardiolipin content of the light mitochondrial fraction can be correlated with the reported lack of oxidative phosphorylation and low rate of respiration in this fraction (Katyare *et al.*, 1970). In this respect, there is a striking resemblance between the light mitochondria and the promitochondria of anaerobic yeast, which also have less capacity for oxidation and a low cardiolipin content (Jakovcic *et al.*, 1968, 1971; Getz, 1970) Specific dependence of mitochondrial adenosine triphosphatase on linoleoyl cardiolipin is of relevance in this context (Lopez-Moratalla *et al.*, 1973). It is claimed that the content of cardiolipin in yeast (Jakovcic *et al.*, 1968, 1971) and rat liver (Jallow *et al.*, 1968) reflects the state of development of mitochondrial membranes.

[^{3}H]Glycerol incorporation studies revealed that both microsomal fractions and heavy mitochondria incorporate the label into their phospholipids at an identical rate (Figs. 1–3). Several workers have also observed that the rate of incorporation of labelled precursors into microsomal and mitochondrial lipids are somewhat similar (Gurr *et al.*, 1965; Taylor *et al.*, 1967; McMurray & Dawson, 1969; Stein & Stein, 1969). Thus Gurr *et al.* (1965), using ^{32}P as a precursor, found that 'specific activity of the phospholipids was of the same order in all sub-cellular fractions at times up to 19h after administration of the isotope'. Stein & Stein (1969) observed that with ^{32}P as a precursor, the activity of microsomal fractions was only slightly higher than that in mitochondria, with a ratio of 1.5, which did not change when [2-^{14}C]glycerol was used as a precursor. A direct precursor–product relationship between microsomal and mitochondrial phospholipid components thus could not be shown in such experiments, possibly because of a rapid transfer of lipids from the endoplasmic reticulum to mitochondria (Jungalwala & Dawson, 1970*a*). The low amount of incorporation into the cardiolipin fraction of heavy mitochondria observed in these studies has also been observed by others (Gurr *et al.*, 1965; McMurray & Dawson, 1969; Jungalwala & Dawson, 1970*a*).

The high incorporation of [^{3}H]glycerol into phospholipid components, particularly in the cardiolipin fraction of light mitochondria, is of significance in view of the fact that mitochondria are capable of synthesizing cardiolipin (Davidson & Stanacev, 1971; Hostetler *et al.*, 1971) and can be taken as indicative of its high synthetic activity and rapid turnover. This is consistent with similar observations on its protein-synthesizing activity (Satav *et al.*, 1973) and turnover (Katyare *et al.*, 1970). In agreement with our findings, Hallman & Kankare (1971) have reported a high ^{32}P incorporation into cardiolipin of their light mitochondrial 'M' fraction, which also has a low specific cardiolipin content. It should be pointed out here that phosphatidylcholine and phosphatidylethanolamine are synthesized in the endoplasmic reticulum and then transferred to mitochondria; these lipids are probably not synthesized by mitochondria themselves (Jungalwala & Dawson, 1970*a*,*b*).

The observations on the protein profiles obtained by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis show that light mitochondria have low amounts of high-molecular-weight proteins and an abundance of low-molecular-weight proteins. This is consistent with earlier observations by electron microscopy that inner membranes are relatively less developed than outer membranes (Satav *et al.*, 1973; Subramanian *et al.*, 1973). This supports the postulate that, during biogenesis of mitochondria *in vivo*, outer membrane is the first component to be synthesized (Beattie, 1969). The protein profile may be taken as truly representative of light mitochondria as, under these conditions, microsomal fractions show entirely different protein patterns (Plate 1 and Fig. 7) and distribution of proteins (Table 5). The same is true for lysosomes (A. S. Pappu, P. Fatterpaker & A. Sreenivasan, unpublished work).

The present findings, together with previous observations (Katyare *et al.*, 1970; Satav *et al.*, 1973), strongly support the earlier view (Katyare *et al.*, 1970) that the light mitochondria represent young mitochondria in the process of formation of stable heavy mitochondrial structures.

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