FINE STRUCTURE OF LIPID-DEPLETED MITOCHONDRIA

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ABSTRACT

The fine structure of mitochondria and submitochondrial vesicles depleted of their lipid by extraction with aqueous acetone was studied. Thin sections of mitochondrial membranes depleted of more than 95% of their lipid retained the unit membrane structure. Densitometer tracings of the electron micrographs showed that the unit membrane of extracted mitochondria was, on the average, wider than that of unextracted controls and showed a greater variation in width. The outer membrane was lost in mitochondria from which 80–95% of the lipids was extracted. Inner membrane particles were present on submitochondrial vesicles depleted of up to 85% of their lipids. However, when more than 95% of the lipid was removed, few, if any, particles remained attached to the membranes but many particles were found unattached in the background. When lipid was restored to lipid-deficient preparations, the mitochondrial membranes were found to be devoid of inner membrane particles but were fully active with respect to succinate–cytochrome *c* reductase activity.

INTRODUCTION

It has been demonstrated that it is possible to remove 80–85% and more of the lipid from mitochondria without inducing gross changes in structure (1, 2). Preparations which retain 15–20% of the lipid show negligible enzymic activity; however, the electron transport chain can be fully reactivated when lipid is added to these preparations. The restoration of electron transport activity is associated with rebinding of lipid by the membrane protein (1, 2). The work described in this paper is an investigation of changes in the fine structure of mitochondrial membranes produced by the removal and restoration of lipid. Electron microscopy of unfixed and negatively stained membrane fragments and of thin sections of fixed and embedded material are the two techniques available which have revealed significant structural detail of mitochondrial membranes. Both have been used on all preparations described here. A preliminary report of this work has been presented (3).

METHODS

Preparation of Mitochondria and Submitochondrial Vesicles

Mitochondria were isolated from beef hearts and purified as described by Hatefi and Lester (4). Submitochondrial vesicles were prepared as follows: Suspensions of purified mitochondria were diluted in 0.25 M sucrose to a final concentration of 10 mg protein/ml and disrupted using a French pressure cell (American Instrument Co., Inc. Silver Spring, Md.) at about 4,000 lbs./inch^2. The preparations were passed through the cell twice, then centrifuged at 40,000 rpm for 30 min in a Spinco Model L. The resulting pellet contained two components, the major one was reddish and translucent, the minor one at

the bottom of the tube was dark brown. The red, translucent portion was taken up in 0.25 M sucrose and the centrifugation was repeated. The translucent portion was again resuspended in 0.25 M sucrose.

Extraction of Mitochondria and Submitochondrial Vesicles

Freshly prepared mitochondria were depleted of neutral lipids by extraction with 4% water in acetone as described previously (5). "Lipid-deficient" mitochondria, i.e. mitochondria depleted of neutral lipid and 80% of their phospholipids, were prepared according to Lester and Fleischer (6), by extraction with 10% water in acetone.

Mitochondria were rendered "lipid-free," i.e. depleted of more than 95% of their lipid, by the technique of Fleischer and Slautterback (2). This procedure is essentially the same as that used for obtaining lipid-deficient mitochondria, except that 12 μ liters concentrated ammonia (28% w/v) were added per 100 ml of the extraction medium.

For the lipid-extraction experiments, submitochondrial vesicles, at a concentration of 5–50 mg protein/ml in 0.25 M sucrose, were used and treated with 24 volumes of: dry acetone, or 10% water in acetone, or 10% water in acetone containing ammonium hydroxide as described for the extraction of whole mitochondria. The preparations were then sedimented once in 0.25 M sucrose and resuspended in the same solution. Centrifugation for 10 min at 18,000 rpm in a No. 30 rotor is usually adequate to sediment the extracted material from sucrose solutions. To recover vesicles extracted with acetone-water-ammonia may sometimes require longer centrifugation and higher speed when low concentrations of material are used.

When the lower concentrations of submitochondrial vesicles are treated with dry acetone, part of the phospholipids is extracted together with the neutral lipid. The degree of extraction can approach that of the "lipid-deficient" mitochondria. Three preparations having total phosphorus-to-protein ratios of 10.4, 7.2, and 5.9 μg/mg were thus obtained.

Enzyme Assays and Chemical Analysis

Whole mitochondria and submitochondrial vesicle preparations were assayed for succinate–cytochrome *c* reductase activity in the presence and absence of added lipids and coenzyme Q as described previously (1). The phospholipids and "total lipid" preparations were prepared from mitochondria and were "solubilized" as described (7). Lipid was restored to lipid-depleted fragments as described for whole mitochondria (1).

Protein was determined either by the Biuret method of Gornall, Bardawill, and David (8) or by the method of Lowry et al. (9). Phosphorus was determined according to Chen, Toribara, and Warner (10).

Electron Microscopy

A. SECTIONED MATERIAL: After centrifugation, small pellets were fixed in the centrifuge tube with 1% OsO_4 in 0.1 M veronal-acetate buffer, at pH 7.4, containing 2.4 mM $CaCl_2$ and 0.06 M NaCl. After being washed with the same buffer, the pellets were divided and transferred for 2 hr to a solution of 0.5% uranyl acetate in the same buffer at pH 6.0. The blocks were then dehydrated in a graded ethanol-water series and embedded in Epon. Sections were cut with diamond knives on an LKB Ultrotome and double-stained with uranyl magnesium acetate and lead citrate (11).

B. NEGATIVELY STAINED MATERIAL: 400-mesh grids were coated with a formvar film covered by a thin layer of evaporated carbon. The films were rendered hydrophilic by 1-min exposure to an AC glow discharge of 3 kv and 0.5 amp at a pressure of from 10^{-1} to 5×10^{-2} torr in an Edwards Model 12E6 vacuum evaporator. 2% phosphotungstic acid brought to pH 7.2 by addition of KOH was used as a negative stain. A drop of the suspended material was applied to the grid which was subsequently washed with several drops of water or phosphotungstate solution. The last drop of phosphotungstate was removed by touching the grid edge to a filter paper. After drying, a second thin layer of carbon was evaporated onto the specimen side of the grid.

Electron micrographs were taken on a Siemens Elmiskop I or a Hitachi HU 11B microscope at 40,000 or 80,000 magnification, using the double condenser.

Densitometer tracings of membranes were obtained from the original electron micrographs at 80,000 electron optical magnification. A Joyce Loebl Mark IIIC microdensitometer was used with a ratio of record/sample travel of 50:1, a slit width of 0.2 mm and the 3× objective lens.

RESULTS

Table I summarizes the results of acetone extraction on the phospholipid content and enzymic activity of beef heart mitochondria. As described earlier (5), extraction with 4% water in acetone removes neutral lipid from the mitochondria and results in a dependence on added coenzyme Q for succinate–cytochrome *c* reductase activity. Treatment with 10% water in acetone removes neutral lipid and about 80–85% of the phospholipid (6). Such mitochondria require addition of both coenzyme Q and phospholipid micelles for activity. A combination of water, acetone, and ammonia is an efficient solvent for removal of the residual

TABLE I

Phospholipid Content and Enzymic Activity of Beef Heart Mitochondria

Sample	Treatment	μg P/mg protein*	Succinate → cyt. *c* reductase‡ activity after addition of: None	CoQ	CoQ + MPL§
I. Mitochondria	None	16.8	0.59	0.52	0.53
II. "Neutral lipid-depleted" mitochondria‖	4% water in acetone	14.5	0.02	0.84	1.00
III. "Lipid-deficient" mitochondria¶	10% water in acetone	3.7	0.01	0.08	0.97
IV. III + MPL**		10.5	0.07	0.77	1.03
V. "Lipid-free" mitochondria‡‡	10% water in acetone + NH_3	2.2	0.01	0.01	0.39

* 1.7 μg P/mg protein is not extractable with chloroform-methanol (2/1) (v/v) and is probably not lipid phosphorus. 13 μg P/mg protein of mitochondria represents lipid phosphorus. "Lipid-free" mitochondria therefore contain less than 5% of the original lipid content.

‡ μmoles cyt. *c* reduced/min/mg protein at 30°C.

§ CoQ = coenzyme Q_{10}; MPL = mitochondrial phospholipid (7).

‖ An electron micrograph of this preparation is shown in Fig. 1.

¶ An electron micrograph of this preparation is shown in Fig. 2.

** Mitochondrial phospholipid was added to III and the unbound lipid was removed by centrifugation (1).

‡‡ An electron micrograph of this preparation is shown in Fig. 3.

acidic phospholipids (2). More than 95% of the phospholipid phosphorus can be extracted by this treatment. 86% of the total mitochondrial phosphorus (13 μg P/mg protein) is phospholipid phosphorus. 10% (1.7 μg P/mg protein) is not extractable with chloroform-methanol and is, therefore, not considered phospholipid phosphorus (1).

Complete reactivation of enzymic activity can be obtained with "lipid-deficient" preparations upon addition of lipid. However, as complete extraction of lipid is approached, the reactivation of enzymic activity becomes vanishingly small (2).

Treatment of submitochondrial vesicles with acetone-water mixtures resulted in preparations with phospholipid content and succinate-cytochrome *c* reductase activities similar to those obtained in preparations of extracted whole mitochondria (Table II). However, the phosphorus-to-protein ratios were usually found to be lower in submitochondrial vesicle preparations than in intact mitochondria extracted in the same way. This was, in part, due to the fact that lower concentrations of mitochondrial material were used in the extraction procedure. However, even in cases in which the same concentrations expressed in mg protein/ml were used, phosphorus-to-protein ratios tended to be lower for vesicles than for intact mitochondria. In vesicle preparations at concentrations of 10 mg protein/ml, it was possible to extract all of the phospholipid with the water-acetone-ammonia mixtures employed. Such preparations had lost all succinate–cytochrome *c* reductase activity and did not recover it when the assay medium was supplemented with lipid (see Table II).

Sectioned Material

In sections of isolated beef heart mitochondria prepared as described [under Methods], some rupture and swelling of mitochondria is usually observed. The matrix in most mitochondria appears light; in a few mitochondria which show a dense matrix, considerable swelling of the outer mitochondrial compartment is seen. However, many of the mitochondria which have a light matrix also show swelling, especially of the outer mitochondrial compartment. The cristae in the mitochondria with apparently intact envelopes show a considerable degree of disorder when compared to those of mitochondria fixed in the tissue immediately after removal from the animal. Many of the cristae appear to have been broken up into vesicular or tubular structures.[1] In places, where

[1] In one experiment, pieces of tissue were removed

TABLE II

Phospholipid Content and Enzymic Activity of Submitochondrial Vesicles

Preparation	Extraction	μg P/mg protein*	Succinate → cyt. *c* reductase* activity after addition of: None	CoQ	CoQ + MPL	§
I. Submitochondrial vesicles (S_v)‡	None	17.5	0.81	0.82	0.79	(3)
II. "Neutral lipid-depleted" (S_v)‖	(a) 4% water in acetone¶	10.4	0.03	0.86	1.25	
	(b) "	7.2				
	(c) "	5.9				
III. "Lipid-deficient" (S_v)	(a) 10% water in acetone¶	6.3	0.08	0.66	1.51	(1)
	(b) "	3.7	0.02	0.02	0.74	(3)
IV. III + TML**‡‡		16.5	0.80	1.51	1.63	
V. "Lipid-depleted" (S_v)§§	(a) 10% water in acetone¶ + NH_3	1.2–1.8	0.01	0.01	0.06	(4)
	(b) "	2.9	0.02	0.02	0.57	(1)
VI. V + TML**		25.3	0.45	0.60	0.72	

* *Cf.* footnotes * and ‡ in Table I.

‡ Electron micrographs of this preparation are shown in Figs. 5 and 8.

§ Numbers in parentheses refer to number of preparations studied.

‖ An electron micrograph of this preparation is shown in Fig. 9.

¶ By lowering the concentration of submitochondrial fragments in the extraction medium, "neutral lipid-depleted" fragments of varying phospholipid content can be prepared. The phospholipid content of some of these preparations can be as low as in "lipid-deficient" preparations. In the same manner, the phospholipid content of "lipid-deficient" and "lipid-depleted" fragments can be varied. Extraction time and amount of ammonia added are additional variables which affect the efficiency of lipid extraction.

** Total mitochondrial lipid, which contains some coenzyme Q (20), was added to the preparation; the unbound lipid was removed by centrifugation (1).

‡‡ An electron micrograph of this preparation is shown in Fig. 11.

§§ Electron micrographs of this preparation are shown in Figs. 6 and 10.

the membranes are sectioned at approximately 90°, the typical triple-layered "unit membrane" structure is clearly visible in both the outer and the inner membranes.

The neutral lipid-depleted preparations show a picture very similar to that of the unextracted control (Fig. 1). Moderately dense particles of ~90 A diameter are often seen on the surface of the cristae membranes. They may be poorly defined or entirely missing in some areas of cristae surface. In their shape, size, and location, they are similar to the inner membrane particles seen in negatively stained preparations. The unextracted controls show the same particles but less frequently and with poorer definition.

The lipid-deficient (Fig. 2) and lipid-depleted preparations (Fig. 3) exhibit no significant morphological differences and will be described together. The gross morphology of the mitochondria is generally preserved; however, the outer membrane of the envelope is completely missing. The arrangement of the cristae appears, on the average, actually less distorted than in the unextracted controls. The unit membrane structure is clearly visible in many places, but the contrast between the light and dense bands is low. Closer inspection reveals many breaks across the membranes which are virtually absent from the unextracted controls and very rare in the neutral lipid-depleted preparations. On the surface of the cristae membrane and between the cristae a moderately dense and particulate material is visible, which may correspond to the ~90 A particles of the neutral lipid-depleted preparations. It appears slightly denser, more irregular in shape, and more abundant than in those preparations.

47 densitometer tracings across the membranes

from beef hearts in the laboratory, immediately before isolation of the mitochondria. Thin sections of this material showed essentially the same morphological changes of the mitochondrial structure in situ that were observed in the isolated mitochondria.

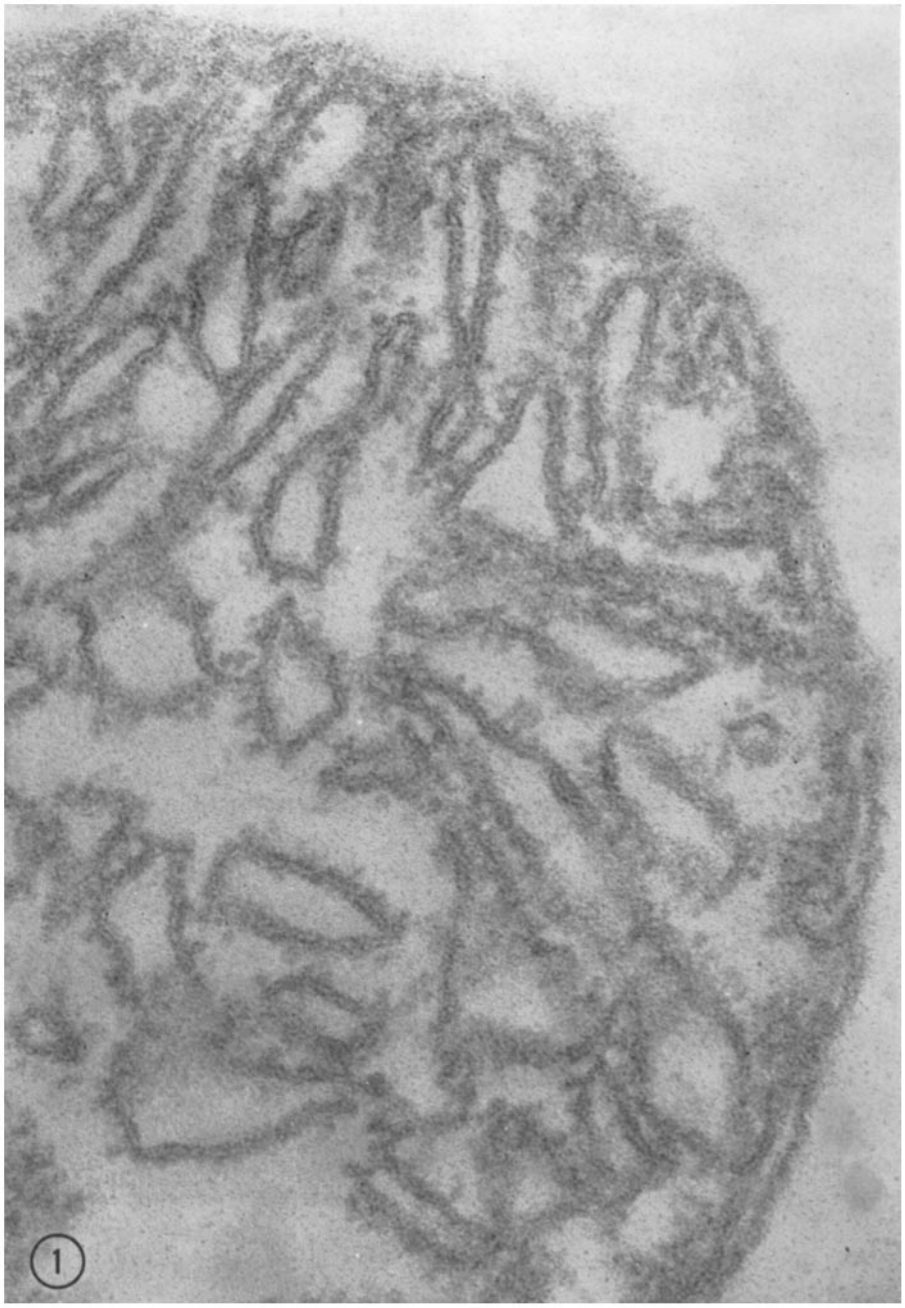

FIGURE 1 Electron micrograph of a portion of a "neutral lipid-depleted" mitochondrion (see Table I). The "unit membrane" structure of the cristae membranes is clearly visible. Associated with the cristae are small particles which may be identical with the inner membrane particles observed in "negative staining." The outer membrane is retained. × 192,000.

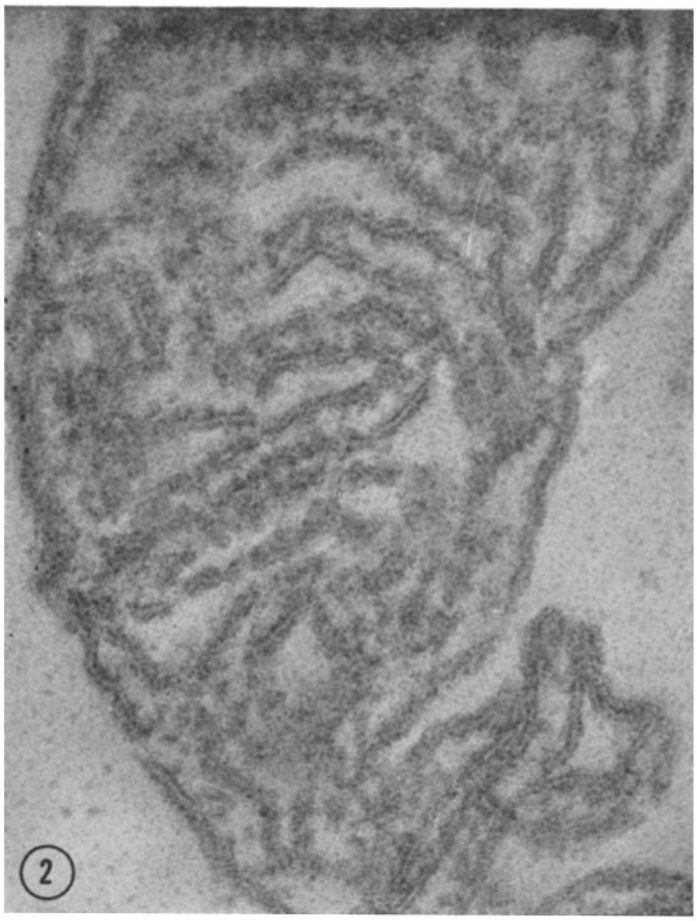

FIGURE 2 Electron micrograph of a portion of a "lipid-deficient" mitochondrion (see Table I). The "unit membrane" structure of the cristae membranes is clearly visible. Frequent breaks across the membrane are apparent. The small particles on the surface of the membranes are not so clearly discernible as in Fig. 1. The outer membrane has been lost. × 192,000.

of an unextracted preparation (Fig. 4 *a*) show a mean width, measured between the density peaks of the unit membrane, of W_d = 48.8 *A* (s = ±6.5 *A*). The width of the light band, measured as the horizonal distance between points on the inner slope of the tracing at one-half the maximum density of the peaks, is: W_1 = 24.5 *A* (s = ±4.8 *A*). The mean widths found for 77 measurements on the lipid-depleted mitochondria (Fig. 4 *b*) are: W_d = 54.3 *A* (s = ±8.3 *A*) and W_1 = 28.8 *A* (s = 5.3 *A*). The differences in the means for both W_d and W_1 are statistically significant ($P < 0.001$). The total width of the membrane could not be measured satisfactorily because of the irregularly distributed particulate material attached to the outer surface of the cristae membrane. Especially in the extracted preparations it is abundant and the contrast between this material and the dense bands of the unit membranes is low. The density profile of the lipid-depleted membranes is consistently shallower than that of the control, but absolute values for the density of the maxima and minima of the curve are not considered significant because differences in section thickness cannot be controlled accurately enough and differences in the staining properties before and after lipid extraction must be expected to occur.

After passage of the isolated mitochondria through a French pressure cell followed by centrifugation and resuspension, sections show few, if

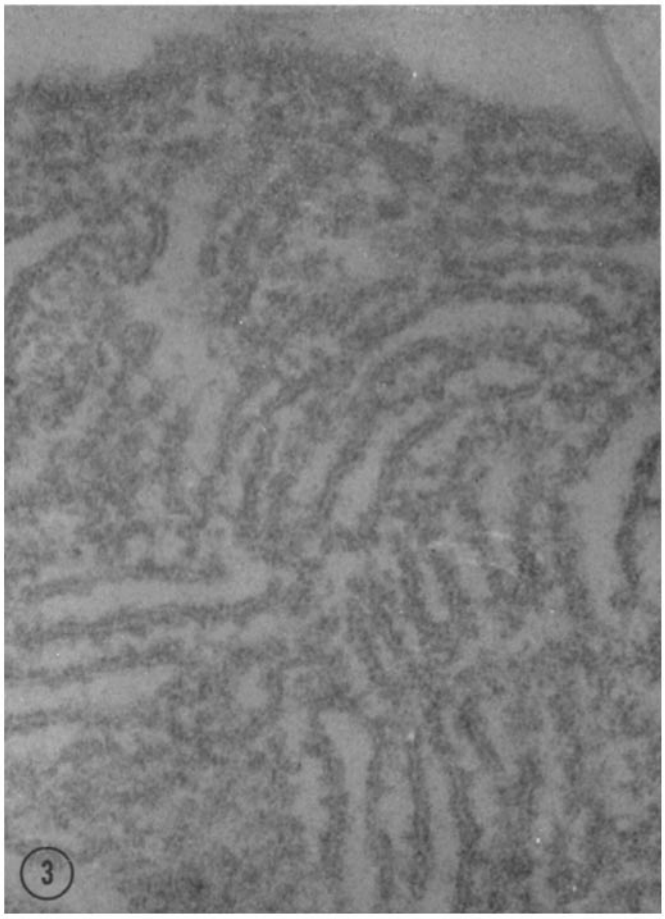

FIGURE 3 Electron micrograph of a portion of a "lipid-free" mitochondrion (see Table I). Its appearance is essentially the same as that of a "lipid-deficient" mitochondrion. × 192,000.

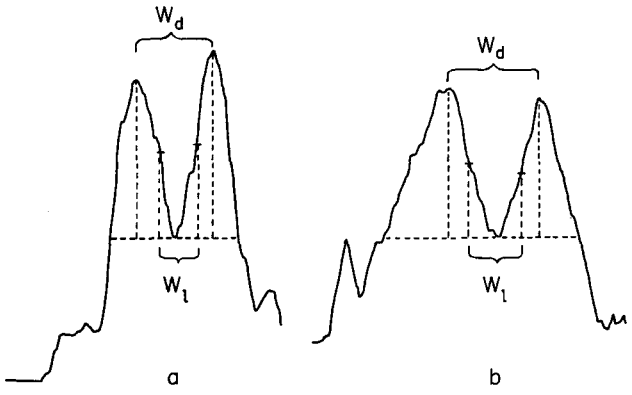

FIGURE 4 Typical microdensitometer tracings of mitochondrial membranes; (*a*) of untreated control mitochondria (*b*) of "lipid-free" mitochondria.

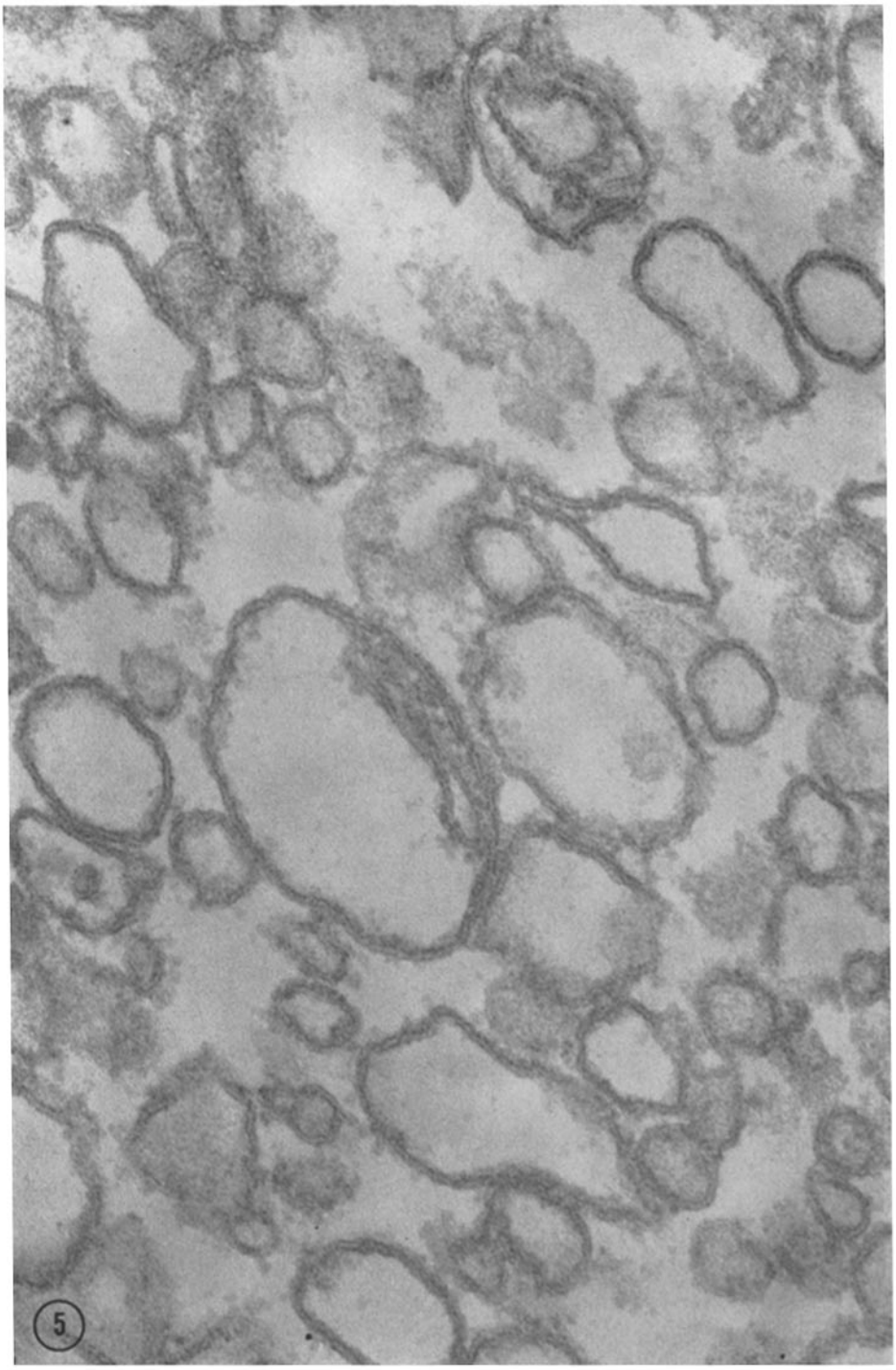

FIGURE 5 Electron micrograph of submitochondrial vesicles prepared by disrupting mitochondria in a French pressure cell. The "unit membrane" structure of the membrane is clearly visible. × 192,000.

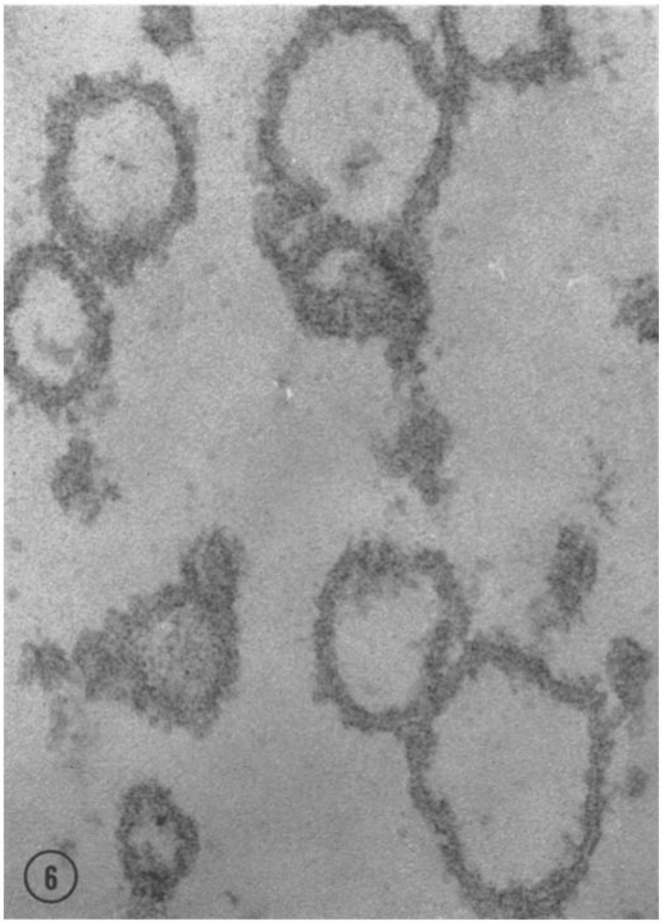

FIGURE 6 Electron micrograph of "lipid-depleted" submitochondrial vesicles (see Table II). The "unit membrane" structure of the membrane is clearly visible. × 192,000.

any, intact mitochondria. The bulk of the preparation consists of vesicles of varying size bounded by a unit membrane. The smallest vesicles always have roughly circular cross-sections and, when they clearly show the unit membrane and, therefore, are presumably sectioned equatorially, they have a diameter of ~300 A. The bigger vesicles are usually more irregular in shape and have diameters up to 2000–3000 A. Very few, if any, of the low contrast 90 A particles present in sections of intact mitochondria can be seen on the outer surface of the vesicles (Fig. 5).

After lipid-extraction, submitochondrial vesicles show qualitatively the same changes in "unit membrane" appearance that have been observed in intact mitochondria, but no measurements or densitometer tracings have been carried out. The proportion of small to large vesicles appears higher in the extracted preparations than in the unextracted controls, and they seem to contain less of the low density particulate material on the outer membrane surface than do the intact mitochondria (Fig. 6).

Negatively Stained Material

Isolated mitochondria after negative staining show the familiar picture of membrane fragments studded with inner membrane particles. After lipid extraction, the material is usually aggregated in big irregular clumps, which do not allow observation of details. Even the few smaller fragments found are not flattened out and appear mostly electron opaque. The neutral lipid-depleted mitochondria show these changes to a much

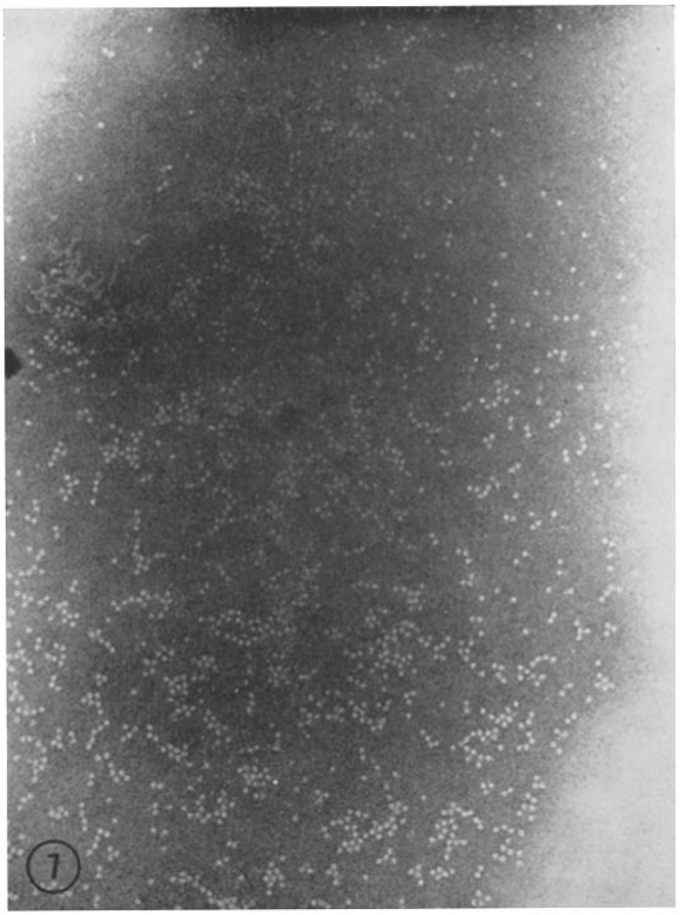

FIGURE 7 Free 85 A particles seen in electron micrographs of whole "lipid-depleted" mitochondria after negative staining. × 112,000.

lesser degree than the more thoroughly extracted preparations. Their appearance is very similar to that of the unextracted controls. We assumed that the increased rigidity of the lipid-extracted material was at least partly responsible for the unsatisfactory results, and this led to the use of the French pressure cell in an attempt to break the membranes before extraction into particles small enough to be observed with negative staining. In the preparations from whole mitochondria, wherever fragments small enough for the observation of detail were found, the fine structure seen was consistent with the results obtained from material passed through the French pressure cell before extraction, which will be described below. One interesting observation was made on negatively stained preparations of lipid-depleted intact mitochondria. Where the phosphotungstate was thinly spread, areas were found that contained large numbers of uniform particles roughly spherical with a diameter of ~85 A (Fig. 7), presumably detached inner membrane particles (see Discussion).

Negatively stained preparations of mitochondria disrupted with a French pressure cell showed membrane fragments, apparently flattened vesicles of varying size and shape, most of them bearing large numbers of inner membrane particles on their surface (Fig. 8). Few detached particles were seen in the background.

Neutral lipid-depleted submitochondrial vesicles, which had also lost part of their phospholipid (see Methods), showed considerable aggregation. However, enough regions could be found in which the finer details of the membranes were clearly

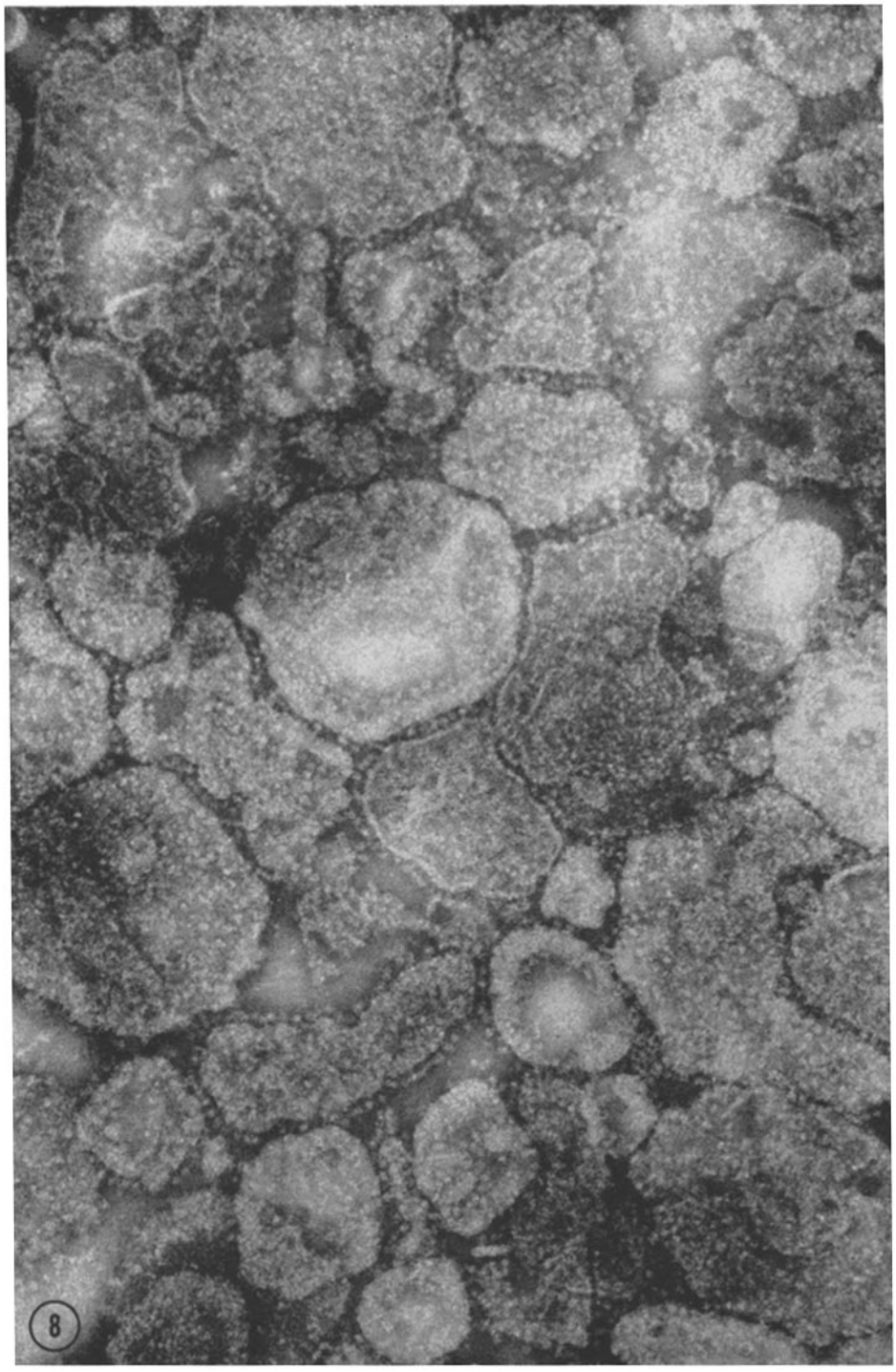

FIGURE 8 Electron micrograph of submitochondrial vesicles after negative staining with phosphotungstate. The inner membrane particles are clearly visible, attached to the membranes. × 135,000.

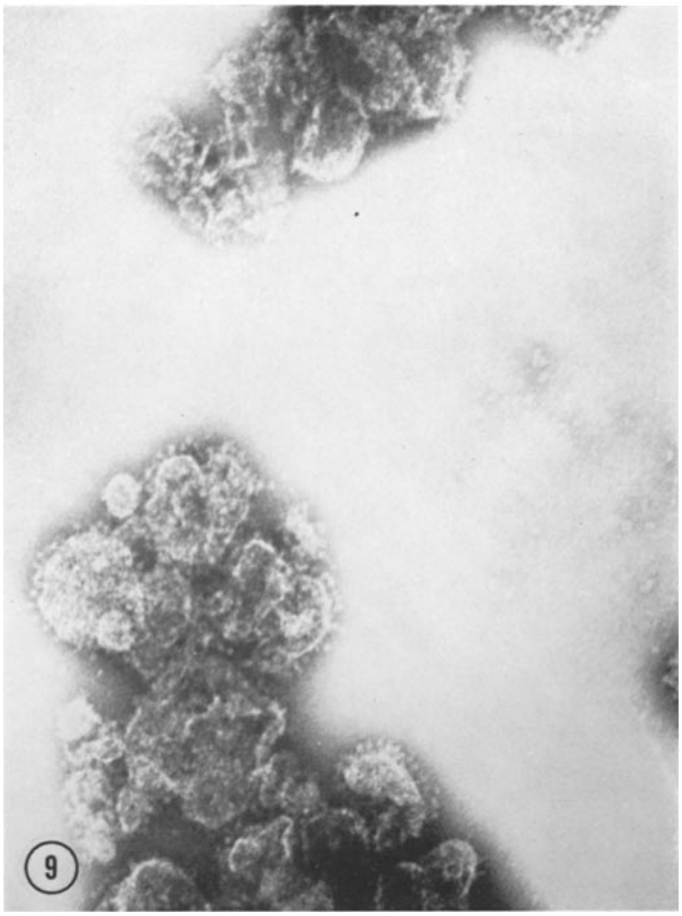

FIGURE 9 Electron micrograph of submitochondrial vesicles which have been extracted with 4% water in acetone. The initial concentration of mitochondrial material was low. This resulted in the removal of neutral lipid and approximately 70% of the phospholipids. Most of the inner membrane particles are still attached to the membranes. A few unattached particles can also be seen in the background. × 135,000.

visible. They showed no significant differences from the unextracted controls, i.e. inner membrane particles were present in approximately the same number and unchanged in morphology (Fig. 9).

The lipid-deficient preparations were still more aggregated. At the edges of the aggregates or in the few areas containing well dispersed material, the membranes of the vesicles were often not so clearly outlined as in the unextracted controls. They seemed to carry an ill-defined "fuzz" consisting of very short filaments or rods on their surface. The inner membrane particles were still present, though usually in reduced numbers and often without a clearly visible stalk. The morphology of these preparations varied considerably: sometimes their appearance was very similar to that of the neutral lipid-depleted material, sometimes it resembled more the lipid-depleted preparations.

The most thoroughly extracted preparations showed the "fuzzy" appearance of the membrane over most of their surface. Occasionally, inner membrane particles were found in small groups or as single particles still attached to the membrane. Often, though not consistently, many more particles were seen free in the background (Fig. 10). In all extracted preparations, only small vesicles were observed which appeared not so flat as the

vesicles in unextracted controls. Whether this is caused by absence of the bigger vesicles or whether those are not observed because they are hidden in the big opaque clusters cannot be determined from the negatively stained preparations. However, their absence or smaller number in sectioned material suggests that further comminution of the membrane fragments occurs during lipid extraction.

Negative staining of material after lipid extraction and rebinding of lipid did not give clear-cut results. The membranes were changed little by the addition of lipid, except that they appeared somewhat less rigid and better dispersed. Evaluation of this feature, however, was complicated by the presence of some myelin figures and vesicle-like structures probably consisting of free lipid (Fig. 11). The presence of free lipid is not necessarily due to incomplete washing. The "reconstituted" membranes may be labile and some breakdown may occur during negative staining. It should be noted that in lipid-deficient and lipid-depleted preparations no inner membrane particles, either free or attached, could be found after addition of lipid sedimentation and resuspension as described (1).

DISCUSSION

The most widely accepted model for the structure of cellular membranes is that of Danielli which describes the molecular arrangement of lipid and proteins as a bimolecular lipid layer covered on both surfaces with protein. The general arguments for this model and its applicability to mitochondrial membranes have recently been discussed (12). The persistence of the "unit membrane" structure in the inner mitochondrial membranes after virtually complete extraction of the lipids is difficult to reconcile with the model. One would expect that removal of the lipid would lead to a collapse or a complete separation of the two dense bands of the unit membrane which in the Danielli model are assumed to represent the two surface layers of protein with some contribution by the hydrophilic groups of the lipids. If stable cross-links between the two protein layers of the mem-

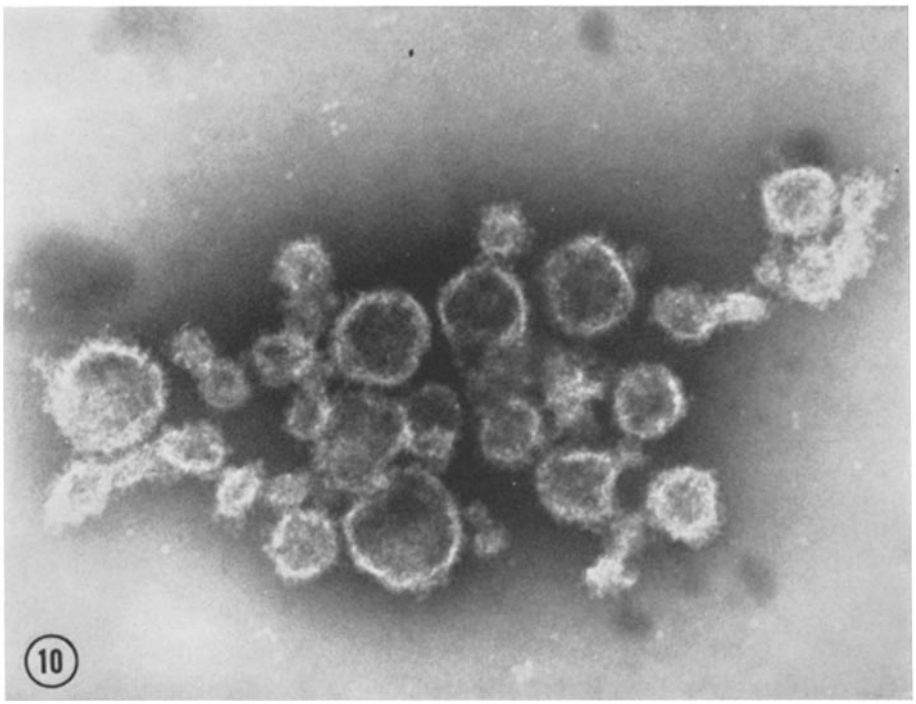

FIGURE 10 Electron micrograph of "lipid-depleted" submitochondrial vesicles after negative staining with phosphotungstate. The membrane surface appears frayed with no particles attached. Free particles are present in the background. × 135,000.

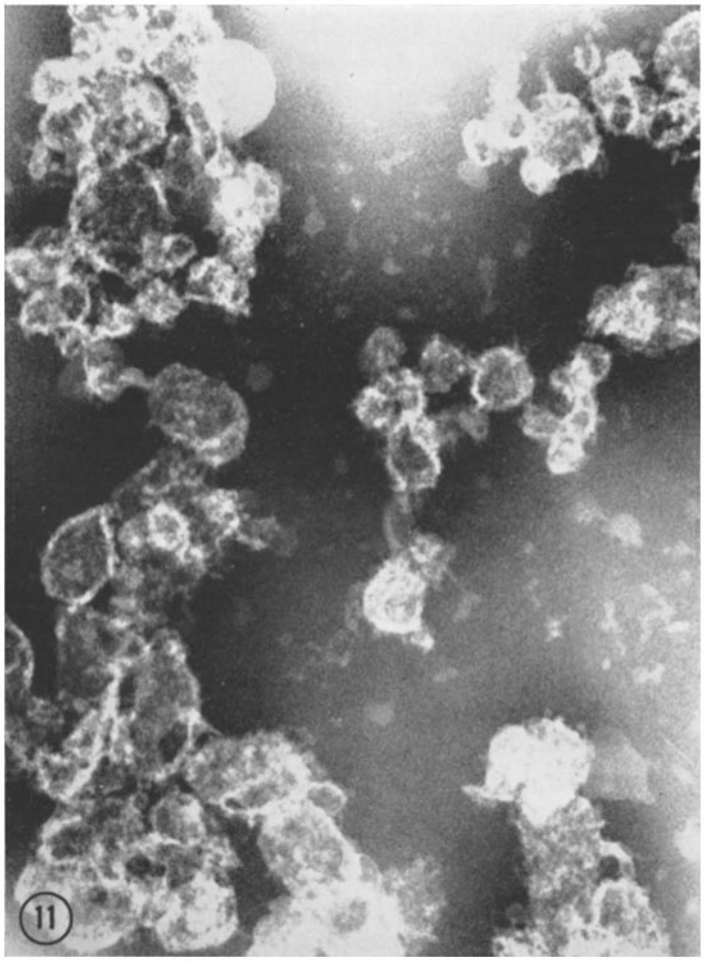

FIGURE 11 Electron micrograph of mitochondrial vesicles extracted with 10% water in acetone, reactivated by addition of lipid, and negatively stained with phosphotungstic acid. The inner membrane particles are lacking. This preparation has full succinate-cytochrome *c* reductase activity (see Table II). × 135,000.

brane could be demonstrated, the difficulty would be resolved. These could be formed by a small remaining fraction of unidentified lipid or by protein. The observed wider variation in the dimensions of the membranes and their greater average width after lipid extraction is compatible with the assumption of cross-links that would allow some swelling or collapse of the remaining protein layer, depending on the degree of penetration by other material, e.g. the embedding medium, into the space originally occupied by the lipid. Removal of the staining groups of the lipid molecules and possible changes in the stainability of the protein could also affect the apparent width of the membrane. The persistence of organized protein layers after lipid extraction of the membranes makes it easier to understand the reactivation of electron transport activity through the addition of lipid to these preparations.

Sjöstrand has described a structure in mitochondrial membranes that could be interpreted as evidence for numerous equally spaced cross-links (13). There are, however, other possible interpretations of his observations (12), and one would expect the cross-links to be clearly visible after lipid extraction. This is not the case. Moreover, the

outer membrane, which disappears after lipid extraction, shows, according to Sjöstrand, the same structure as the inner membranes. The problem, therefore, remains at present unresolved. We do not think that our observations disprove the Danielli model or strongly favor another model. Most other proposed models also would be expected to disintegrate after removal of the lipid. However, at least some modification of the Danielli model for the inner mitochondrial membranes will be required.

Preliminary experiments on isolated membranes of rat liver endoplasmic reticulum, erythrocyte ghosts, and isolated chloroplasts show that these membranes collapse when lipids are extracted by the technique used here for mitochondria. Finean and Martonosi (15) have recently reported persistence of membrane structure in preparations of endoplasmic reticulum treated with phospholipase *C*.

Our observations on negatively stained preparations show that the inner membrane particles persist through lipid extraction, but may become detached when most of the lipid is removed. At least some of the particles may only be detached when the material is prepared for electron microscopy. Otherwise, it would be difficult to explain their presence in these preparations, because the centrifugation of submitochondrial vesicles which follows the lipid extraction should not sediment these small particles. In lipid-depleted whole mitochondria, the high concentrations of particles found in some areas may be caused by release of particles from mitochondria which break during the drying process on the electron microscope grid.

The results reported here seem to rule out the possibility that the inner membrane particles are lipid micelles resulting from a breakdown of the mitochondrial membranes, as has been suggested (16, 17). No correlation has been observed between the presence of the particles, attached or detached, and the electron transport activities of the preparations. This makes their participation as necessary components of the electron transport chain from succinate to cytochrome oxidase highly unlikely. They may, however, be identical with the soluble ATPase, as indicated by the work of Racker and his collaborators (18).

The swelling observed in sectioned whole mitochondria cannot be evaluated because it is not known how much of it is present in the fresh material and how much is introduced by fixation and preparation for electron microscopy. The 90 A particles of low contrast seen on the cristae in sections may be identical to the inner membrane particles. Their location, morphology, and their persistence through lipid extraction is consistent with this assumption, but no direct proof for it exists. It should be pointed out that their low contrast would make it difficult to detect them in mitochondria with even a moderately dense matrix, and this could explain why they are usually not seen in sectioned material (see, however, references 14 and 19). It is also possible that pretreatment with acetone stabilizes the particles against the detrimental action of the subsequent fixation and embedding process. The loss of the outer membrane in lipid-extracted mitochondria shows that its composition and/or structure must be different from that of the inner membranes.

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