BIOCHEMICAL AND STEREOLOGICAL ANALYSIS OF RAT LIVER MITOCHONDRIA IN DIFFERENT THYROID STATES

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ABSTRACT

The concentrations of the inner mitochondrial membrane markers cardiolipin and cytochrome a have been measured in liver homogenates and in purified mitochondria after thyroxine administration to thyroidectomized and normal rats. The biochemical results have been correlated with stereological electron micrographic analyses of hepatocytes in liver sections, and of isolated mitochondrial pellets. There were progressive and parallel increases in homogenate and mitochondrial cardiolipin concentration, and in mitochondrial cytochrome a concentration, after administration of 20 μg of thyroxine on alternate days to thyroidectomized rats, and of 300 μg on alternate days to normal rats. Electron microscope measurements showed marked differences in the shape of the mitochondria and in the number of cristae in different thyroid states. Hypothyroid mitochondria were shorter and wider than controls, and hyperthyroid mitochondria longer but of similar width. Mitochondrial volume per unit cell volume was virtually unchanged in hypo- and hyperthyroid animals. The most striking changes were a decrease in the area of the inner membrane plus cristae in thyroidectomized rats, and a substantial increase in membrane area after thyroxine administration. The biochemical and electron micrographic results indicate that, in rat liver, thyroid hormone administration leads to a selective increase in the relative amount of mitochondrial inner membranes, with little or no change in the mitochondrial volume per unit cell volume, or in total mitochondrial protein per unit total cell protein.

KEY WORDS   mitochondrial membranes · thyroid hormone · stereology · cardiolipin · cytochrome a

Administration of thyroid hormone to thyroid-depleted or normal animals results in considerable alterations in the synthesis (30, 38, 40, 41), turnover (13), and functional capacity (15, 18, 38, 40, 41, 47) of mitochondrial components. At very high, toxic doses, swelling and uncoupling of oxidative phosphorylation may occur (40). At physiological and moderately elevated levels, however, the hormone appears to participate in the control of normal mitochondrial development and in the regulation of the level of functional mitochondrial mass. Thyroxine administration to thyroidectomized rats leads to a rapid increment in muscle and hepatic mitochondrial respiratory enzyme activities (15, 18, 38, 41, 47) and to stimulation of amino acid incorporation (30),

RNA polymerase activity (9, 10), and RNA synthesis (2) in isolated mitochondria. Cytoplasmic protein synthesis (38, 40), nuclear RNA synthesis (39), and nuclear RNA polymerase activities (45) are also enhanced by thyroid hormone administration.

Alteration in the lipid composition of inner mitochondrial membrane has also been noted in hypothyroid animals (4. 5. 16). A low fatty acid unsaturation index is present in thyroidectomized rats which is rapidly corrected by thyroid hormone administration. The changes in oxidative phosphorylation observed in hypothyroidism have been ascribed to the altered inner membrane lipid environment.

Thyroid hormone function may be moderated by interactions with specific nuclear- (6, 23, 31, 35) and mitochondrial- (33, 34) binding proteins. Interaction with a nuclear receptor may lead to increased nuclear DNA transcription, resulting in enhanced mitochondrial membrane synthesis. On the other hand, a direct regulatory effect on oxidative phosphorylation has been postulated to result from the binding of the hormone to the inner mitochondrial membrane receptor (33, 34).

In a previous report from our laboratory, Gross investigated the effects of thyroxine on the turnover and accumulation of mitochondrial components in rat liver and heart (13). Using succinate cytochrome c reductase activity as an indicator of changes in the functional mass of inner mitochondrial membrane, he found that the 40% increment in total enzyme activity in liver could be accounted for by a similar increase in the enzyme activity per milligram protein of purified mitochondria. Thus, there appeared to be little or no increase in total mitochondrial protein per gram liver protein after thyroid hormone administration to thyroid-depleted or normal rats.

The findings of Gross did not resolve the question as to whether the increased respiratory enzyme specific activity in liver mitochondria reflected an activation of the respiratory enzymes, a change in the composition of inner membrane, or a quantitative increase in inner membrane mass relative to the matrix and outer membrane. Our purpose in this study was to try to differentiate between these possibilities by measuring the response to thyroid hormone administration of structural components localized exclusively in the inner mitochondrial membrane, namely, the acidic phospholipid cardiolipin (diphosphatidyl glycerol) (8, 11, 17, 25, 27) and cytochrome a

(32). Parallel changes in mitochondrial cardiolipin and cytochrome contents and respiratory enzyme activity suggest that an increase in the amount of inner membrane, relative to other mitochondrial proteins, accounts for the changes observed in the liver. The behavior of these biochemical markers of mitochondrial inner membrane in different thyroid hormonal states has been correlated with quantitative electron microscope analysis of mitochondrial size and shape, volume per cell, and inner membrane and crista area. Both biochemical and electron microscope analyses indicate that increased respiratory enzyme activity produced by thyroid hormone in the liver is, in large part, the result of enhanced development of the cristae infoldings of the inner mitochondrial membrane. Conversely, the depression of respiratory levels characteristic of thyroidectomized animals is correlated with a corresponding decrease in the area of the cristae. Although our studies on hepatocyte mitochondria in embryonic and newborn animals (17) indicated that cardiolipin-respiratory ratios can shift during development and maturation, we conclude that, in adult animals, the extent of folding of the inner membrane into cristae is a good correlate of mitochondrial cardiolipin content and respiratory activity.

MATERIALS AND METHODS

Experimental Design

Two similar experiments were performed. In the first, the hypothyroid state and its transition to the euthyroid or slightly hyperthyroid state were studied. In the second experiment, the differences between the normal and strongly hyperthyroid states were investigated. The thyroid state of each group was confirmed by two parameters: growth was measured by weekly weighing, and the heart rate was determined from an electrocardiographic tracing.

In the first experiment, Sprague-Dawley female rats surgically thyroidectomized (Hormone Assay Laboratories, Chicago, Ill.) at 10 wk of age were used. 10 days after thyroidectomy, each animal was given 50 μCi of ^{131}I intraperitoneally. The rats were maintained on a Purina rat diet (Ralston Purina Co., St. Louis, Mo.) ad lib., and on water supplemented with a small amount of $CaCl_2$, for 10 wk. Three groups of five animals each matched for weight were sacrificed 13 wk after thyroidectomy. Three other groups of five matched animals were given 20 μg each of L-thyroxine (Sigma Chemical Co., St. Louis, Mo.) on alternate days, by intraperitoneal injection, for 3 wk starting 10 wk after thyroidectomy. They were sacrificed at the end of this period.

In the second experiment, three groups of five normal

adult female rats weighing 250 g each were given 300 μg of L-thyroxine intraperitoneally on alternate days for 3 wk.

Preparation of Tissue Fractions

Animals fasted overnight were sacrificed by decapitation, and the livers were excised immediately and rinsed with ice-cold 0.3 M sucrose, 0.01 M Tris (pH 7.4), 0.1 mM EDTA buffer. Livers from each group of animals were pooled and homogenized as described previously (14). The homogenate was centrifuged at 500 g for 10 min in the SS-34 rotor of an RC2-B Sorvall centrifuge (DuPont Instruments-Sorvall, DuPont Co., Wilmington, Del.). The pellet was discarded and the supernate centrifuged at 500 g for 10 min. The mitochondrial pellet was obtained by centrifugation at 12,000 g for 12 min and three subsequent washings at 9,000 g for 10 min.

Phospholipid Analysis

Phospholipids were extracted from each sample by the method of Letters (19). Lipid extracts containing ~25 μg of phosphorus were analyzed by two-dimensional thin-layer chromatography as described by Getz et al. (12). Phosphorus analysis was performed by the method of Bartlett (3).

Cytochrome Determination

Difference spectra (oxidized-reduced) of mitochondrial preparations solubilized with deoxycholate were obtained by the method of Williams (46), with a Cary model 14 recording spectrophotometer (Varian Associates, Instrument Div., Palo Alto, Calif.). Cytochrome a concentrations were calculated from these spectra according to William's method (46).

Protein Determination

Protein determinations were performed in duplicate by the procedure of Lowry et al. (22).

Microscopy and Stereology

Stereological studies were made on liver samples from the following 10 Sprague-Dawley female rats: one matched pair from exp I; another matched pair from exp II; and six additional animals, two of them thyroidectomized, two matched controls, and two intact and hormone-injected, from an earlier study (13). The results for normal animals and for thyroidectomized animals receiving 20 μg of thyroxine on alternate days were in agreement to within 10% and consequently were pooled under the designation "euthyroid." Studies on isolated mitochondria were made on pooled samples from thyroidectomized, normal, and hyperthyroid animals, as described by Gross (13). Minced liver samples or small pellets were fixed for 1 h in 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.4, at 2°C; in some cases, this was preceded by 2.5% glutaraldehyde in the same buffer. Samples were embedded in Epon.

For light microscopy, 1-μm Epon sections were cut on a Porter-Blum MT-1 microtome (DuPont Instruments-Sorvall, DuPont Co.) and stained in 0.5% azure B at pH 7 for 1 h at 60°C, rinsed in distilled water, air-dried, and mounted in Permount (Fisher Scientific Co., Pittsburgh, Pa.). Sections were also cut at 14 μm and stained before mounting with a modified Feulgen reaction, involving hydrolysis for 90 min at 5 N HCl at 20°C, and staining in Schiff's reagent for 6 h. This method worked well on some blocks, but produced pale staining in others. Micrographs of the azure B sections were photographed at × 200 on Pan X film with a Zeiss × 50 oil immersion objective and enlarged to × 1,000. Nuclei in the Feulgen-stained sections were measured directly by bright or phase-contrast microscopy at × 1,000 with a filar ocular micrometer. Polyploid frequencies were estimated visually on Feulgen-stained thick sections (28).

Electron micrographs were taken on thin silver sections stained with uranyl acetate and lead hydroxide. Low-power micrographs were made at × 1,750, and medium-power micrographs at × 16,700; these were enlarged to × 5,000 and × 50,000, respectively. All tissue samples were inspected first on 1-μm sections by light microscopy, so that their position within the lobule could be determined. Only midlobular areas were scored, since these comprise some 60% of the tissue volume and thus would be expected to match more closely the biochemical determination on the total liver. Furthermore, the peripheral 20% of the lobule has been found to match the midlobular region in structure. Only the central lobular region, comprising 20% of the tissue volume, appears to have significantly different mitochondrial dimensions (21).

The stereologic procedure used essentially followed those of Weibel and collaborators (42-44). Grids were drawn on transparent acetate sheets, and these were clipped to the 8 × 10 inch print for the scoring of point hits or intersections. Three different grids were used, one with 5 mm spacing, another with 20 mm, and a third with regularly spaced parallel lines, each 20 mm long, arranged in a staggered array 20 mm apart (43). For estimates of cell volume, intersection points were scored for hepatocyte hits on nuclei or cytoplasm, and for "other areas" including Kupffer cells, connective tissue, and tissue spaces using the 5-mm grid. A total of 18 points was scored, averaging 800 counts per print. Hepatocyte volumes were calculated by the formula:

$$V = (D - 2p + t)/N_A,$$

where D is the mean diameter, or average of the major and minor axes, for 100 nuclei; $2p$ is a correction factor for the visibility of nuclear segments within the section (21), taken here as $2p = 0.6$; t is the section thickness, taken as 1 μm; and N_A is the number of visible profiles of hepatocyte nuclei per unit area of hepatocyte (number of hits on hepatocytes times the area per grid square or 25 μm). Nuclear volumes were estimated on whole nuclei in the 14 μm-thick sections. Careful focusing

assured that only entire, uncut nuclei were included (36). Major and minor axes were measured to the nearest 0.2 μm and the nuclear volumes computed as prolate spheroids. Measurements were made on six different sections, with 50 nuclei per section.

Relative volumes of hepatocyte nuclei, mitochondria, microbodies, lipid droplets, ground cytoplasm, and other areas were scored on electron micrographs, which were enlarged to × 5,000 by use of the 20-mm grid. A total of 10 prints was scored per tissue sample, or 80 prints in all, at ~100 points per print.

The number of mitochondria per unit volume (N_v) was estimated on micrographs enlarged to × 50,000. The dimensions of mitochondrial profiles were determined by direct measurement with a transparent milli-meter rule, the longest length and width measurements being recorded on 10 prints for each tissue sample, for a total of 100 mitochondrial profiles per animal. The surface area and area of mitochondrial cristae were scored on the staggered grids as intersections and points hits, respectively (44). Sixteen prints were scored per sample, with ~200 measurements per print. As pointed out by Loud (20), estimates of cristae intersections are too low by a factor related to the section thickness, since membranes at a sufficient angle off perpendicular to the plane of section are no longer apparent (the Holmes effect). This error was corrected by scoring, on the same print, the number of clear and unclear transects of the mitochondrial-limiting membranes, according to the method of Yago et al. (48). Since transects through mitochondrial margins are evident, whether or not the membranes are distinct, it is easy to estimate the percent of transects of cristae that have not been counted due to their off-perpendicular orientation in the section. In this method, the assumption is made that mitochondrial-limiting membranes and cristae are equally distorted by the off-perpendicular orientation, which seems justified by their very similar morphology and dimensions. Correction factors computed in this way ranged from 16 to 23%, thus being considerably lower than that estimated by Loud (21). Such estimates can be expected to vary with section thickness and with the contrast of mem-branes relative to the surrounding matrix. The number of hits was recorded as being either cytoplasmic or within the mitochondria (Pi) and the number of transects across inner mitochondrial membranes (Ni) or cristae. The ratio $4Ni/Zpi$ was calculated, where Z is the length of the staggered line segment (4 cm or the equivalent of 0.8 μm). This gave the ratio Si/Vi, or surface area per unit volume. The surface area of the inner membrane per cell was calculated from the estimated mitochondrial volume per hepatocyte, and the area of the cristae was calculated from the ratio of transects of inner membrane and the corrected number of cristae intersections.

Mitochondrial number was estimated by the method of DeHoff and Rhines (7), in which the mitochondria are considered to have the shape of cylinders or of prolate spheroids. These methods are very inexact when they are applied to biological systems, since the mito-chondrial dimensions are not gaussian in their distribu-tion (1); marked differences in shape are clearly shown for different treatments, as is evident in Fig. 2–9. More accurate estimates would require a careful reconstruction of the mitochondrial population from serial sections. For this reason, it seems appropriate also to present the raw data of mitochondrial profiles in graphic form (Fig. 12).

RESULTS

Cardiolipin Content

The acid phospholipid cardiolipin is localized predominantly, if not exclusively, in the inner mitochondrial membrane in rat liver (25, 27). The influence of the thyroid hormone status on cardi-olipin concentrations in liver homogenates and purified mitochondria is shown in Fig. 1. The cardiolipin concentration in the homogenate in-creased by ~36% when small doses of thyroxine (20 μg on alternate days) were given to thyroid-ectomized animals for 3 wk. Similarly, a 23% increase in homogenate cardiolipin concentration was obtained after large doses of thyroxine (300 μg on alternate days) to normal animals (Fig. 1). Thus, thyroid hormone administration leads to substantial increases in homogenate cardiolipin content, whether the hormone is given in small doses to thyroidectomized rats or in large doses to normal animals.

The identical pattern of increase in cardiolipin content per milligram of purified mitochondrial protein was observed under the influence of thy-roid hormone (Fig. 1). Cardiolipin concentration in isolated purified mitochondria and in homoge-nates increased in parallel and approximately to the same extent, as illustrated by the almost constant ratio of mitochondria to homogenate cardiolipin concentration (Fig. 1). It may there-fore be concluded that there was little change in the mitochondrial fraction of total liver protein. Thyroid hormone treatment instead led to an increase in the mitochondrial concentration of the phospholipid cardiolipin in the inner membrane.

Cytochrome Content

The effect of thyroid hormonal status on mito-chondrial inner membrane development was fur-ther evaluated by spectral measurements of cyto-chrome a concentration in purified mitochondria (Table I). Cytochrome a content per milligram of mitochondrial protein increased 42 and 22% after administration of small doses of hormone to thy-
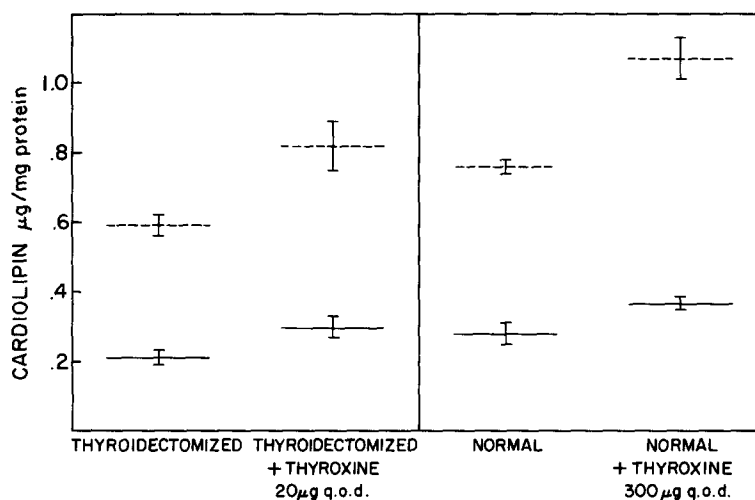
FIGURE 1 Changes in cardiolipin content of rat liver homogenates and of purified mitochondria after thyroxine administration to thyroidectomized (exp 1) and normal rats (exp 2). Cardiolipin was measured in liver homogenates (−) and in isolated purified mitochondria (- - -) as described in Materials and Methods. The values (I) represent the mean ± SD of four separate duplicate determinations. Differences from controls were statistically significant ($P < 0.01$). The ratios of mitochondria to homogenate cardiolipin concentration in exp 1 are 2.7 and 2.7, and in Exp 2 are 2.8 and 3.1.

TABLE I

Cytochrome a Content of Purified Mitochondria after Thyroxine Administration to Thyroidectomized and Normal Rats

	Cytochrome a	Change from controls
	(nmol/mg protein)	*%*
Exp 1		
Thyroidectomized	0.19 ± 0.009*	42
+20 µg Thyroxine q.o.d.	0.27 ± 0.020*	
Exp 2		
Normal	0.27 ± 0.021*	22
+300 µg Thyroxine q.o.d.	0.33 ± 0.01*	

Values represent mean ± SD of four separate determinations.
* $P < 0.01$.

roidectomized animals and larger doses to normal animals, respectively. The changes were similar in magnitude to those observed for mitochondrial cardiolipin concentration (described above) and for succinate cytochrome c reductase activity (13). Cytochrome a values were somewhat lower than reported by Kadenbach (18) but similar to those of Williams (46). The results further support the hypothesis that thyroid hormone leads predomi-

nantly to an increase in the relative amount of inner mitochondrial membrane in rat liver.

Microscopy and Stereology

Typical sections of hypothyroid and hyperthyroid tissues are shown in Figs. 2 and 3, stained in azure B, and printed at × 1,000. Stereological measurements are shown in Table II. Hepatocyte volume was altered only slightly, being increased by 6% in the hypothyroid tissues, and by 4% in hyperthyroid animals. Nuclear volumes in the hypothyroid animals were ~30% less than in the controls, in part associated with a reduced polyploid frequency. Feulgen preparations, scored by eye for diploid, tetraploid, and octoploid hepatocytes (28), gave the following values based on 200 nuclei per sample: controls, 2N 32%, 4N 62%, and 8N 6%; hypothyroid, 2N 28%, 4N 72%, 8N none; hyperthyroid, 2N 20%, 4N 64%, and 8N 16%.

Electron micrographs showed marked differences in the shape of the mitochondria (Figs. 4 and 5) and in the number of cristae (Figs. 6-11). Mitochondria of hypothyroid tissues were round or short oval. A number of "partitioned" mitochondria were evident; presumably, these were in the processing of dividing (37). The hypothyroid tissue also had less glycogen than either the controls or hyperthyroid animals, and more extensive
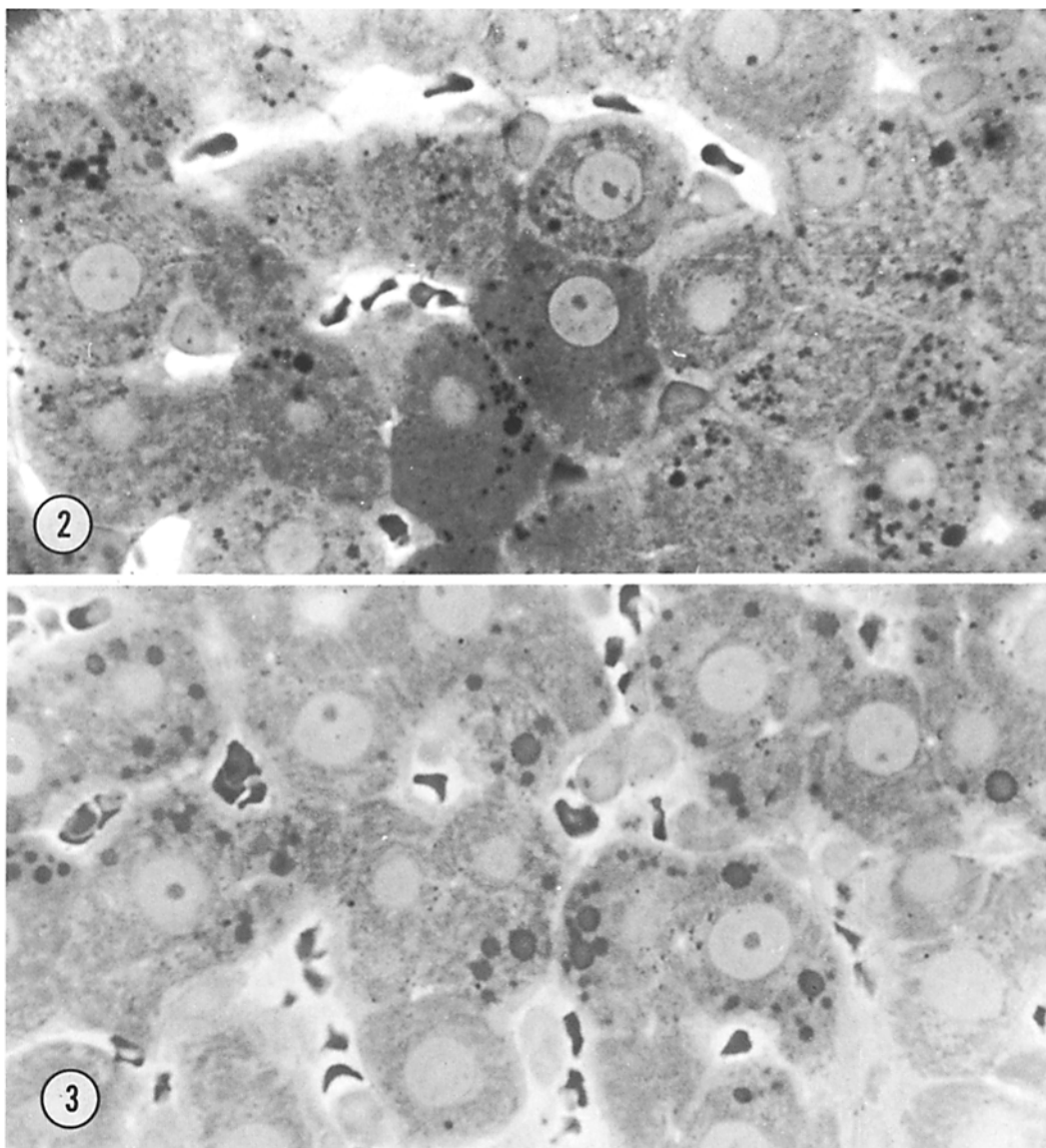
FIGURES 2 and 3   Light micrographs of 1-μm thick sections of liver, stained with azure B. Micrograph in Fig. 2 is from a hypothyroid animal. Fig. 3 is from a hyperthyroid animal. Cell sizes in the two samples are similar, but nuclear dimensions are slightly smaller in the hypothyroid tissue. Large lipid droplets are present in hyperthyroid hepatocytes. × 1,000.

regions of smooth endoplasmic reticulum. Hyperthyroid animals were clearly distinguishable by differences in mitochondrial shape and the presence of a larger number of cristae. They also showed an increase in lipid droplets (Fig. 3).

Mitochondrial volume per unit cell volume was virtually unchanged (Table II), showing nonsignificant increases of ~7% in both hypo- and hyperthyroid animals. Mitochondrial dimensions and

mitochondrial numbers can be estimated only if several simplifying assumptions are made as to their shape distribution. For this purpose, mitochondria have been treated as spheres (42) and cylinders (21), neither of which provide more than rough approximations. They can also be treated as prolate spheroids and their number calculated by the method of DeHoff and Rhines (7). We have graphed the length and width measurements

for 300 mitochondrial profiles in Fig. 12; these indicate clear differences in mitochondrial shape after treatment. Hypothyroid mitochondria were shorter, but also wider than the controls, and the mitochondria in hyperthyroid animals were little changed in width, but noticeably longer. Means of these values are given in Table III, together with estimates of mitochondrial volume and the number per nucleus.

DISCUSSION

The biochemical and stereological electron microscope observations presented in this paper indicate that thyroid hormone regulates the development of inner mitochondrial membrane in rat liver. There was excellent correlation between thyroid hormonal status and the density of packing of mitochondrial cristae, as indicated directly from quantitative electron micrographic analysis of hepatocytes, or derived from the mitochondrial concentrations of inner membrane markers, cardiolipin, and cytochrome a (Fig. 13 and Table I). Increases of inner membrane and cristae area have also been observed after triiodothyronine administration by Reith et al. (29). However, those authors report a dissociation of cytochrome content and inner membrane area in normal and riboflavin-deficient animals given riboflavin and triiodothyronine. Thyroid hormone appears to affect the size and shape of liver mitochondria, since they were wider and shorter in thyroidectomized animals. The number of mitochondria per square micrometer of cell was essentially unchanged, but, since hepatocyte volume was slightly increased in hypothyroid livers, there were 7% more mitochondria per cell. It should be

noted that there was no significant change in the ratios of mitochondria to cytoplasmic volume measured from electron micrographs, or in the fraction of total liver protein composed of mitochondrial protein as estimated from homogenate and mitochondrial cardiolipin concentration. Similarly, Kadenbach (18) and Gross (13) reported no changes in total mitochondrial protein content after thyroid hormone administration, based on respiratory enzyme activity. Therefore, the increased respiratory enzyme activities per gram of liver protein previously noted after thyroid hormone administration (13, 15, 18, 40, 41) seem to result from the appearance of a population of mitochondria having a greater fraction of its structure composed of inner mitochondrial membrane respiratory units, rather than resulting from increased mitochondrial number or size per unit volume of liver cell.

The concept that thyroid hormone selectively influences the synthesis of inner membrane respiratory units was first suggested by Roodyn et al. (30), who noted increased amino acid incorporation into inner mitochondrial membranes of liver mitochondria isolated from rats given thyroxine. Gross (13) arrived at a similar conclusion. based on parallel increases in liver homogenate and mitochondrial succinate cytochrome reductase activities after thyroxine administration.

In heart, McCallister and Page (24, 26) have also observed that thyroid hormone increases inner mitochondrial and cristae membrane areas. A significant increase in the fractional cell volume occupied by mitochondria was noted as well. Inner mitochondrial crista membranes in heart are considerably more densely packed than in liver, as

TABLE II

Stereological Measurements of Hepatocytes

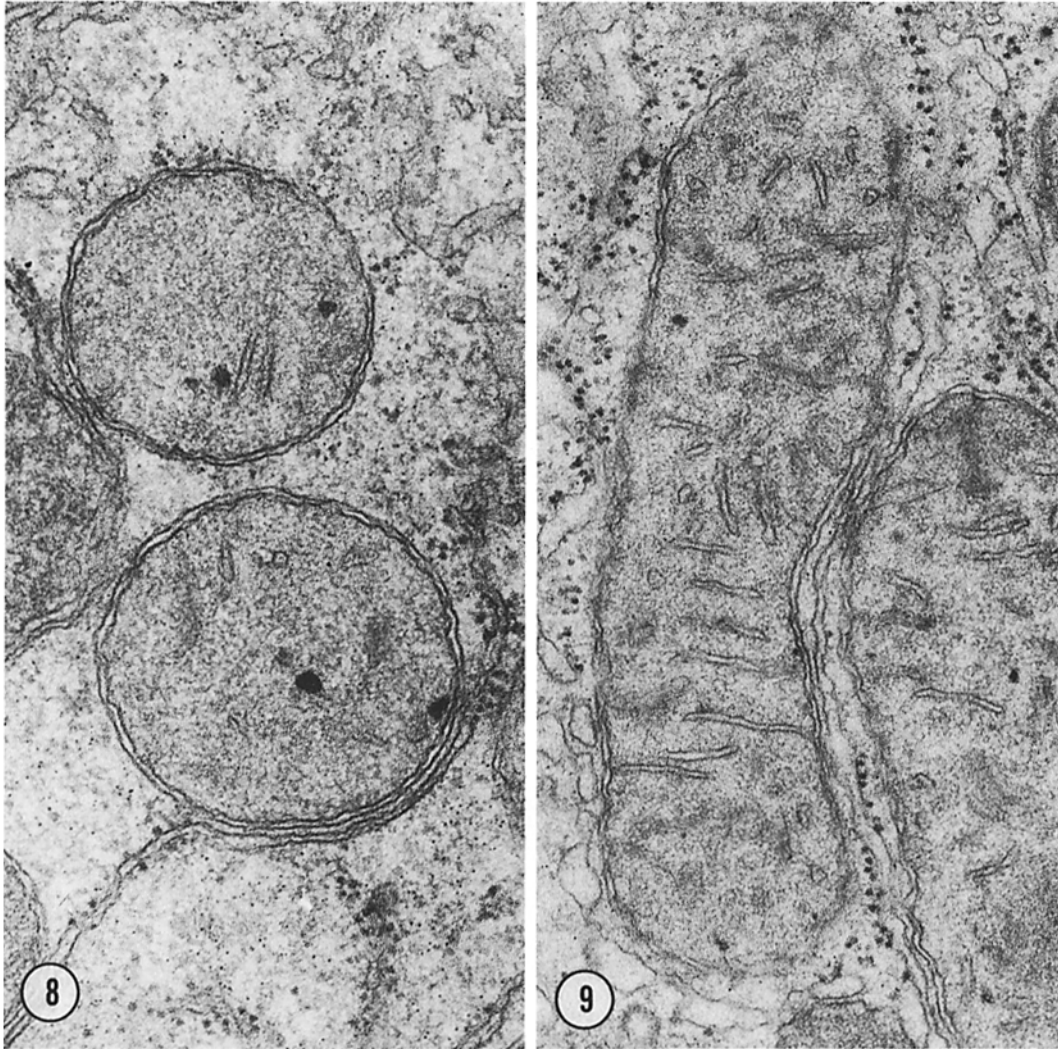|	Hypothyroid	Euthyroid	Hyperthyroid
Cell volume (μm^3)	5,550 ± 233	5,260 ± 280	5,450 ± 152
Nuclear volume (μm^3)	244 ± 16	390 ± 28	339 ± 27
Cytoplasmic volume (μm^3)	5,310 ± 238	4,870 ± 287	5,110 ± 169
Nuclear/cytoplasmic ratio	0.046	0.080	0.066
Mitochondrial/cytoplasmic ratio	0.265 ± 0.029	0.268 ± 0.037	0.275 ± 0.019
Mitochondrial volume per cell (μm^3)	1,400	1,310	1,410
Envelope membrane area per μm^3	5.66	6.43	5.87
Envelope membrane area per cell (μm^2)	7,950	8,390	7,840
Area of cristae per cell (μm^2)	7,140	18,600	30,400
Inner membrane + cristae (μm^2)	15,100	26,900	38,200
Isolated mitochondria			
Inner membrane + cristae (μm^2)	22,200	29,800	37,600

Cell volume determination was made on six tissue samples from three different animals per treatment. Nuclear volumes were made on 100 nuclei measured on entire nuclei in 14-μm sections, per treatment.
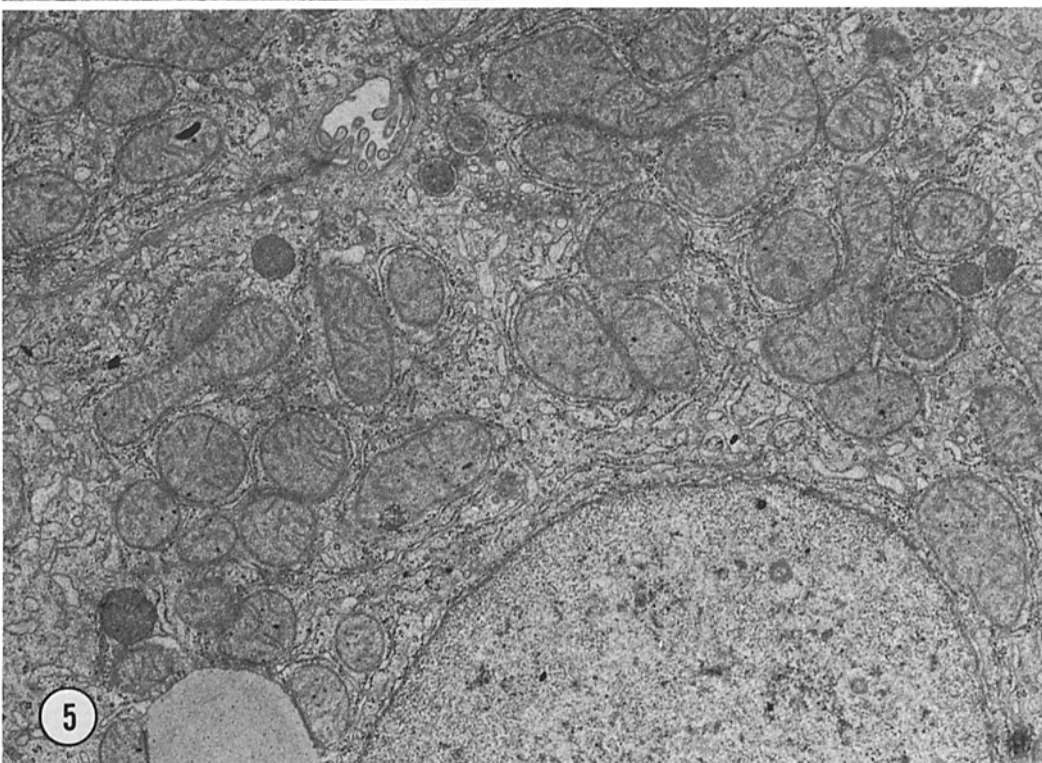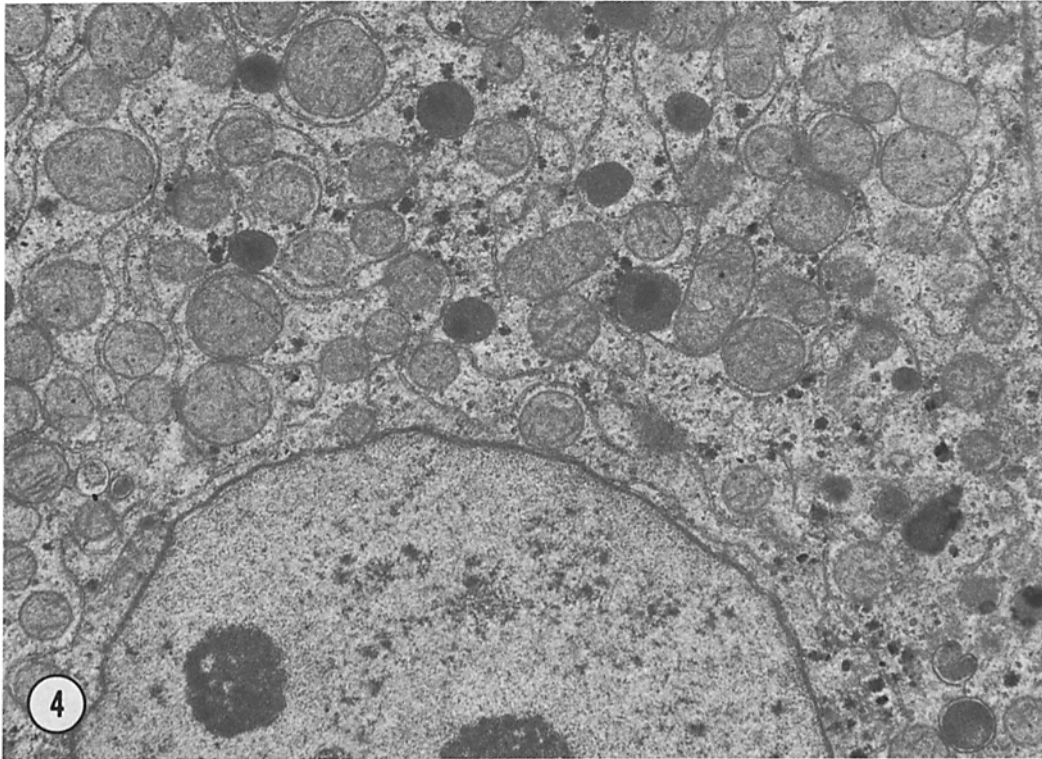
FIGURES 4 and 5 Representative electron micrographs of thin sections of hepatocytes. In Fig. 4, mitochondria from a hypothyroid rat show regular, round, or slightly oval profiles; cristae are rare. The ground cytoplasm contains numerous peroxisomes and glycogen granules. In Fig. 5, a portion of cytoplasm from a hyperthyroid animal contains many long and irregularly shaped mitochondrial profiles. Cristae are abundant and platelike. Rough endoplasmic reticulum is well developed; glycogen is not observed. × 15,000.
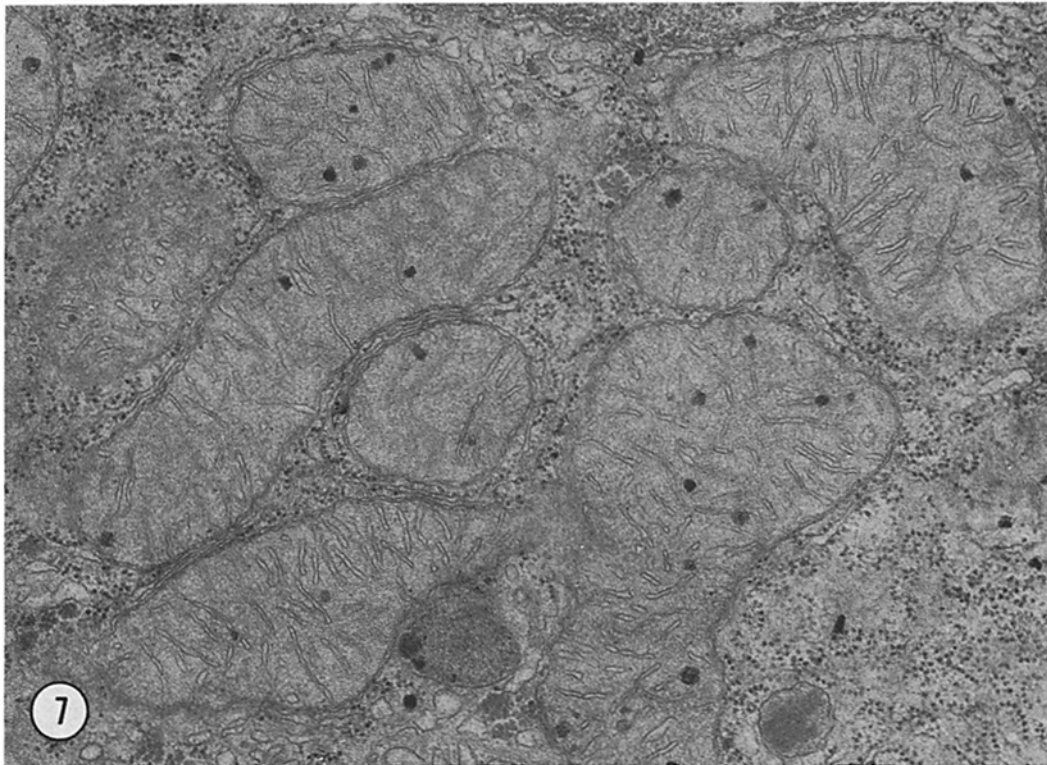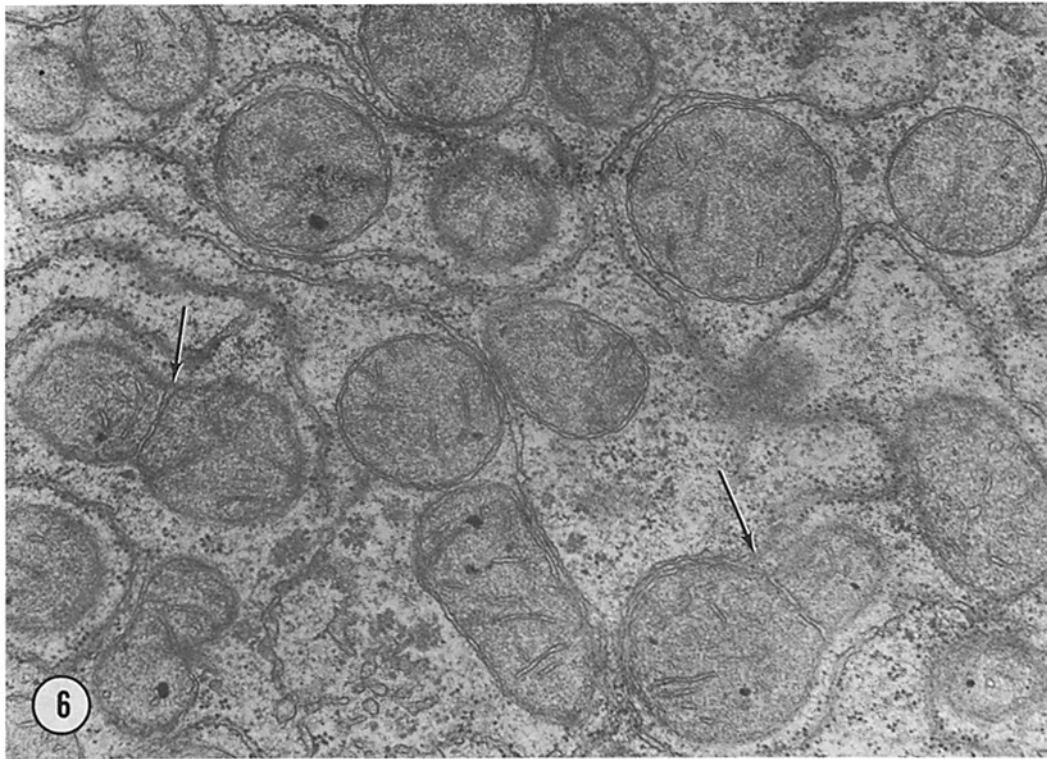
Figures 6 and 7 Comparison of the morphology of hepatocyte mitochondria. The cell in Fig. 6 from a hypothyroid animal contains mitochondria with round or slightly elongated shapes and few cristae. Membranous "partitions" in two profiles (arrows) are suggestive of mitochondrial division. In Fig. 7, the mitochondria of a hepatocyte from a hyperthyroid animal have clearly elongated forms and they contain numerous cristae. × 45,000.

FIGURES 8 and 9   Higher magnification micrographs of typical hepatocyte mitochondria. In Fig. 8, the liver mitochondria of a hypothyroid animal are round and they contain few cristae. In Fig. 9, two elongated mitochondria from a hyperthyroid animal display numerous, platelike cristae. × 80,000.
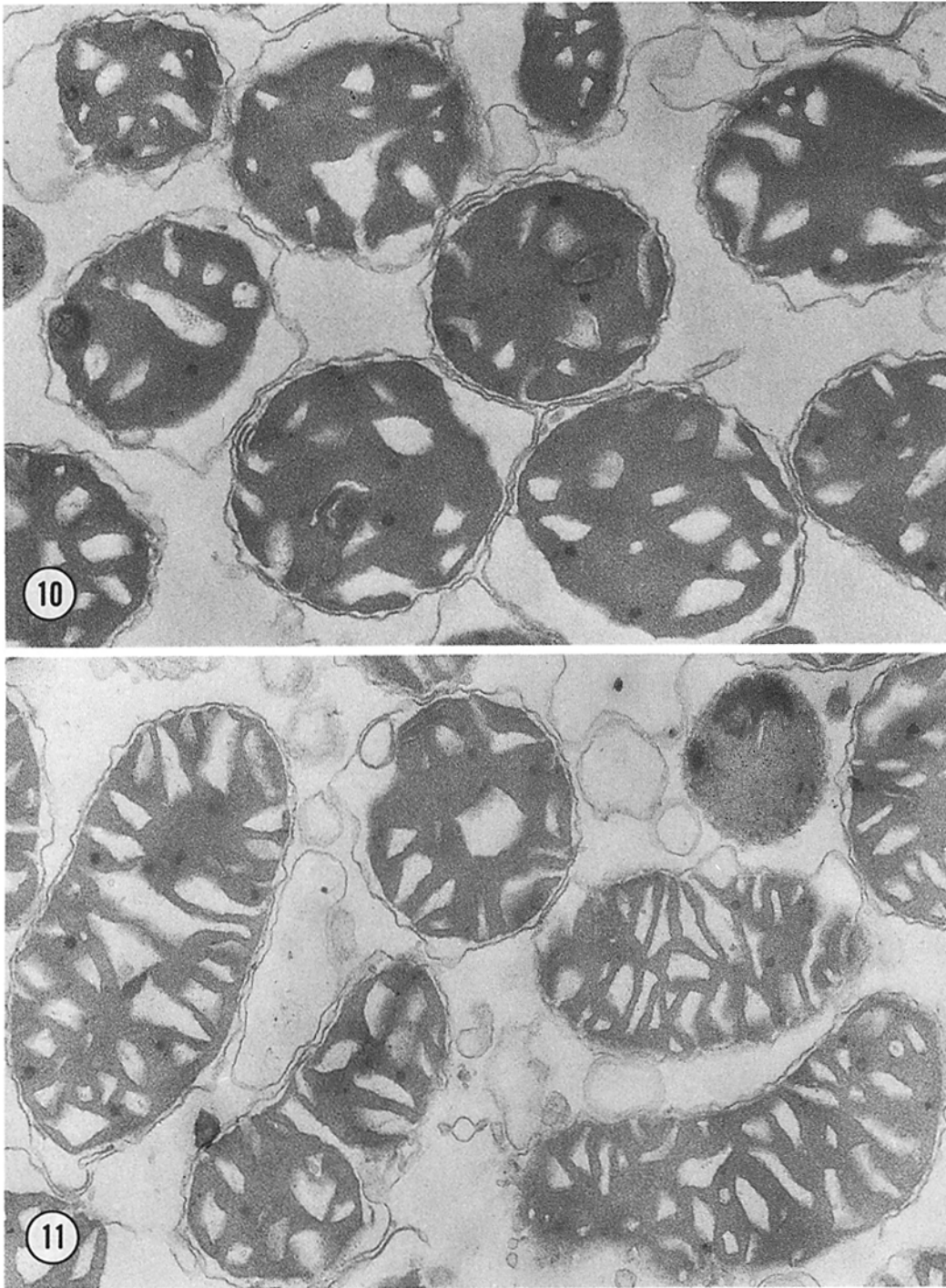
FIGURES 10 and 11   Examples of thin-sectioned pellets of isolated liver mitochondria. Fig. 10 shows mitochondria isolated from hypothyroid animals. The profiles are mainly round, with few, short cristae. The mitochondria in Fig. 11 are isolated from hyperthyroid animals. Their profiles are mainly elongated, and they contain numerous and longer cristae. × 60,000.
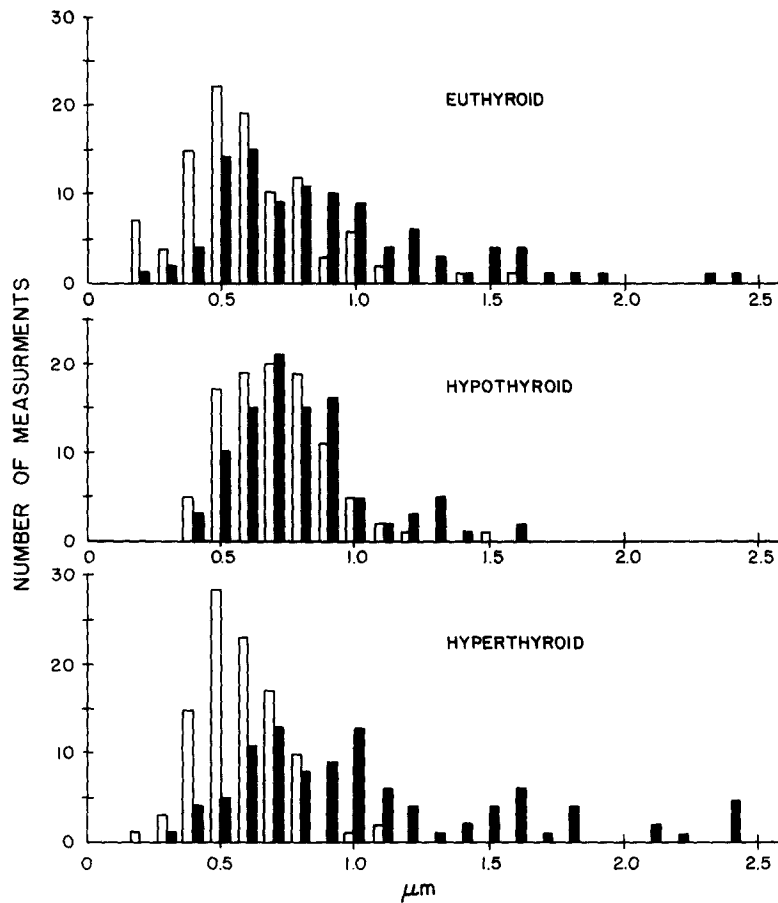
FIGURE 12 Histogram of the dimensions of individual mitochondrial profiles from euthyroid, hypothyroid, and hyperthyroid animals. White bars = width; black bars = length.

TABLE III

Mitochondrial Dimensions

	Euthyroid	Hypothyroid	Hyperthyroid
Mitochondrial profiles (mean width × mean length in μm)	0.65 × 0.91	0.73 × 0.82	0.61 × 1.12
Estimated actual dimensions (μm)	0.8 × 1.5	0.9 × 1.3	0.8 × 1.6
Estimated volume (μm³) (ellipsoid)	0.502	0.551	0.536
Estimated volume (μm³) (cylinder)	0.704	0.763	0.804
Estimated number (per nucleus)	1,860–2,610	1,830–2,540	1,750–2,630

Means of the values plotted in Fig. 12 are shown. Standard errors are not given, since the distribution is not gaussian. From these, estimates of dimensions have been made and the volumes calculated as prolate ellipsoids and as cylinders. True values fall somewhere between these limits. The number of mitochondria per hepatocyte nucleus, based on these two volumes, is also given.

indicated by stereological electron micrographic measurements (26), and by cytochrome (46) and respiratory enzyme concentration. Thus, inner mitochondrial membrane packing normally may be nearer maximal levels in the heart, so that further synthesis results in enlargement or increased number of mitochondria. as well as in increased development of cristae. The effect of thyroid hormone on skeletal muscle mitochondria (15) also appears to involve increases both in
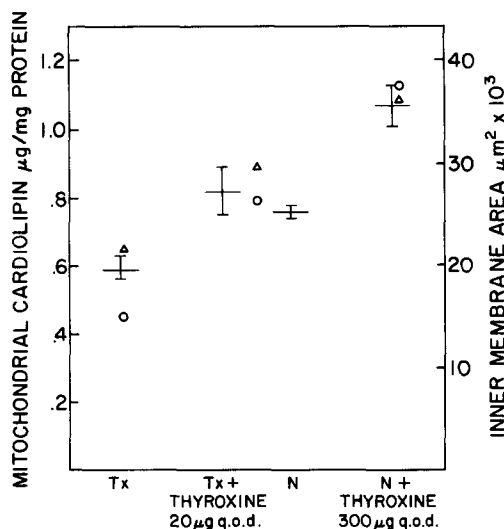
FIGURE 13 Correlation between mitochondrial cardiolipin content and inner mitochondrial membrane area in different thyroid states. Thyroidectomized rats (Tx) were given 20 μg thyroxine every other day (q.o.d.). Cardiolipin data (I) are derived from Fig. 1. Mean values for the area of mitochondrial inner membrane and cristae determined by stereological measurements are given separately for two sets of experiments. (○) Thyroidectomized animals given supplemental ^{131}I; (△) earlier experiments in which no ^{131}I was administered to the thyroidectomized rats. For stereological analysis, normal rats and thyroidectomized animals receiving 20 μg of thyroxine q.o.d. were pooled. These data are therefore plotted midway between the cardiolipin data for the two groups.

inner membrane packing and in relative mitochondrial volume.

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