THE RELATIONSHIP BETWEEN THE STRUCTURE AND ACTIVITY OF RAT SKELETAL MUSCLE MITOCHONDRIA AFTER THYROIDECTOMY AND THYROID HORMONE TREATMENT

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ABSTRACT

The fine structure of rat gastrocnemius muscle fibers has been studied after changes were induced in the basal metabolic rate (BMR) by thyroidectomy and L-thyroxine administration under anabolic conditions. Biochemical analysis of skeletal muscle mitochondrial respiration and phosphorylation from the same tissue preparations has been summarized, details having been published earlier (3). As estimated from electron micrographs, the total amount of mitochondria from thyroidectomized animals was enlarged 1.5 times over that from normal controls. The total amount of mitochondria from thyroidectomized or normal animals made hypermetabolic with thyroxine was increased 2.5 to 3.5 times over that from their corresponding controls. In all cases, there was an increase in the mitochondrial population and the profile ratio of cristae to matrix was also considerably increased, thus indicating both relative and absolute enlargements of the entire surface of the cristae per unit fiber. The major structural changes persisted for at least 3 weeks after the cessation of thyroxine treatment, by which time the elevated mitochondrial respiratory and phosphorylative activity had declined to normal values. The hypertrophy and increase in mitochondrial population was more prominent in the perinuclear and subsacrolemmic regions near blood vessels than in the interstices of the fibrils. The very long interfibrillar mitochondria found in both the hypo- and hypermetabolic tissues are more likely to be derived from outgrowths of the original mitochondria rather than from a fusion of smaller ones. These findings are compatible with the ideas expressed elsewhere (see 1, 3, 10) that, under conditions close to the physiological, thyroid hormones control mitochondrial metabolic activity by a subtle alteration in mitochondrial composition with respect to their respiratory and phosphorylative constituents. The possible application of using thyroid hormones in the study of biogenesis of mitochondria and the synthesis of mitochondrial constituents are discussed.

The respiratory activity of skeletal muscle mitochondria would substantially account for the Basal Metabolic Rate (BMR) which is well known to be under the control of thyroid hormones (1). From biochemical studies on skeletal muscle and liver mitochondria, published 2 to 3 years ago, we concluded that the regulation of BMR by thyroid hormones *under anabolic conditions* could be best explained on the basis of a selective increase in the respiratory and phosphorylative units of the

mitochondria (2, 3). This conclusion was contrary to the frequently cited mechanisms of action involving direct effects on the efficiency of oxidative phosphorylation or mitochondrial stability, based on work *in vitro* or after the administration of catabolic doses of thyroid hormones (see 4–7). More recent work on amino acid incorporation into mitochondrial protein has also supported the idea that, under conditions close to the physiological, thyroid hormones exert a selective control upon mitochondrial composition without modifying the efficency of oxidative phosphorylation (8–10).

In our initial biochemical studies (2, 3) we had examined various parameters of respiration, phosphorylation, and stability of liver and skeletal muscle mitochondria isolated from normal and thyroidectomized rats after both chronic and acute administration of small doses of thyroid hormones. Pieces of tissue from which mitochondria were prepared were then fixed for electron microscopy. In this paper, we relate the fine structure of skeletal muscle mitochondria to an experimental modification of their biochemical activities as induced by thyroidectomy and by repeated administration of L-thyroxine. It will be shown that modification of respiratory activity is accompanied by very profound changes in the amount and structure of mitochondria *in situ*, but that the direction of these changes does not necessarily follow that of metabolic activity.

MATERIALS AND METHODS

Animals, Treatment, Preparation of Mitochondria, and Measurement of Respiration and Phosphorylation

Male Wistar rats, 3 to 6 months old and weighing 150 to 220 gm, were used in all experiments. Thyroidectomy was performed by ^{131}I administration 5 to 6 week before the experiments and hyperthyroidism was induced by the administration of 18 μg of L-thyroxine per 100 gm body weight to normal or thyroidectomized rats every 4th day for 3 weeks, as described by Tata *et al.* (3). The growth rate and basal metabolic rate (BMR) were periodically determined and a final measurement of the latter made within 2 hours before killing the animals. Skeletal muscle mitochondria were isolated from the hind leg and back muscle in the medium of Chappell and Perry (11) according to Azzone *et al.* (12). Mitochondrial respiration was measured both by the Warburg manometric technique and polarographically. Phosphorylation was measured by the esterification of ^{32}P phosphate also as described previously (3). The determination of respiratory quotient (Q_{O_2}), P:O ratio, respiratory control index, and all other biochemical and chemical determinations have also been described in the same communication (3).

Electron Microscopy

Small blocks of the gastrocnemius muscle from the same pooled material from which the mitochondria were isolated were rapidly immersed in an ice-chilled solution of 1 per cent osmium tetroxide according to Zetterqvist (13). The tissues were fixed for 2 hours and dehydrated in increasing concentrations of alcohol followed by propylene oxide before embedding in Epon 812 (14). In order to provide an acceptable basis for comparisons, all tissues were carefully orientated before sectioning. Silver- and light gold–colored sections were cut with an LKB Ultrotome ultramicrotome or a Servall Porter-Blum microtome. The sections were contrasted according to the method of Karnovsky (15), and examined in a Siemens Elmiskop I operating at 60 or 80 kv or a Hitachi HS-6 electron microscope. Micrographs were taken at initial magnifications of 2500 to 13000.

RESULTS

Effect of Thyroidectomy and Thyroxine Administration on the BMR Body Growth Rate, and Mitochondrial Oxidative Phosphorylation

In Table I is summarized the relationship between thyroid status and the metabolic rate and growth rate in the whole animal, on the one hand, and oxidative phosphorylation in skeletal-muscle mitochondria isolated from the same rats as those used for the electron microscopic studies, on the other.

It can be seen that thyroidectomy had lowered the BMR and considerably decreased the rate of growth. Repeated administration of thyroxine elevated the BMR by 70 and 100 per cent in normal and thyroidectomized animals, respectively, and at the same time caused the animals to gain weight. Thus, the stimulation of body oxygen

TABLE I

BMR, Growth Rate, and Muscle Mitochondrial Respiration and Phosphorylation in the Five Groups of Animals Used for Electron Microscope Studies, Described in Figs. 1 to 20

Skeletal-muscle mitochondria were isolated from groups of rats the same as those used for morphological studies of the skeletal-muscle mitochondria *in situ*. BMR measurements were made within 2 hours before killing the animals. All other details as described earlier (3).

Animals	BMR (ml O_2/g/hr)	Weight increase over last 6 weeks (expressed as % of original weight)	Mitochondrial activity: Substrate	Q_{O_2} (μg atoms O/hr/mg protein)	P:O	RC‡ Index
Normal	1.24	46.5	Glutamate	9.98	2.52	0.089
			Pyruvate + malate	12.61	2.61	0.161
			Succinate	10.45	0.86	—
Thyroidectomized	0.83	30.0	Glutamate	6.31	2.59	0.093
			Pyruvate + malate	6.90	2.51	0.101
			Succinate	8.12	0.93	—
Thyroidectomized + T_4*	1.80	59.4	Glutamate	15.60	2.45	0.108
			Pyruvate + malate	19.65	2.65	0.143
			Succinate	13.72	0.82	—
Normal + T_4	2.02	50.5	Glutamate	17.36	2.38	0.111
			Pyruvate + malate	21.40	2.72	0.135
			Succinate	15.35	0.95	—
Normal + T_4, 3 weeks after cessation of treatment	1.30	44.0	Glutamate	10.80	2.59	0.080
			Pyruvate + malate	11.38	2.70	0.121
			Succinate	9.10	0.81	—

* + T_4 refers to treatment with L-thyroxine for 3 weeks.

‡ Respiratory Control (RC) Index values averaged from manometric (ATP-glucose-hexokinase as phosphate acceptor) and polarographic (ADP as P acceptor) determinations and expressed as the ratio of respiration in the absence of P acceptor : respiration in the presence of P acceptor.

consumption was not produced under catabolic conditions although it must be added that a greater stimulation of growth rate was observed with lower doses of thyroxine (1, 3). In a fifth group of animals, the BMR had returned to the level found in normal animals, 20 days after treatment with thyroxine had been stopped. The respiratory quotient of muscle mitochondria isolated from normal, thyroidectomized, and thyroxine-treated rats paralleled the BMR values, with a variety of substrates. The actual percentage stimulation of mitochondrial Q_{O_2} observed after thyroxine administration, however, varied according to the substrate oxidized (from 35 per cent, with succinate, to 400 per cent, with glycerol-1-phosphate, of the values of mitochondria from thyroidectomized animals). Mitochondria from normal rats killed 20 days after thyroxine treatment had been stopped exhibited a return to normal Q_{O_2} values. Whatever the mitochondrial Q_{O_2} the P:O ratio and respiratory control index were normal and constant throughout. Hence, thyroidectomy or thyroxine treatment did not affect the degree of tightness of coupling of phosphorylation to respiration. Without going into further details, we have concluded from these and other biochemical studies that the increased respiration and phosphorylation of muscle and liver mitochondria brought about by thyroid hormones under anabolic conditions arise from a selective increase in the respiratory and phosphorylative units relative to other mitochondrial proteins (see 1–3, 10). A detailed study of individual mitochondrial dehydrogenases, pyridine nucleotides, and

cytochromes in different organs of hypo-, eu-, and hyperthyroid rats has recently been published by Kadenbach (16).

MORPHOLOGICAL OBSERVATIONS

I. *Skeletal Muscle from Normal Animal*

The observations on normal gastrocnemius muscle are essentially similar to those in earlier reports on striated muscles (17, 18). This muscle consists of white fibers (*cf.* reference 18 *a*). As mitochondria are the main object of this study, attention has to be focused on three regions of the muscle fibers in which mitochondria are located, namely the perinuclear region, the interfibrillar region, and the subsarcolemmic region in the vicinity of blood vessels. Synaptic regions have not been studied.

The perinuclear region of the fiber which is located close to the plasma membrane is the only major portion of the sarcoplasm free from myofibrils. In longitudinal[1] sections it usually contains a few scattered profiles of mitochondria (Fig. 1), in a wide range of size and shape. In a three-dimensional reconstruction these profiles would presumably represent fewer mitochondria. Elongated profiles do not show any preferential orientation to the axes of the fibers.

[1] The terms "longitudinal" and "transversal" are used in this paper with reference to the length axis of the muscle fibers.

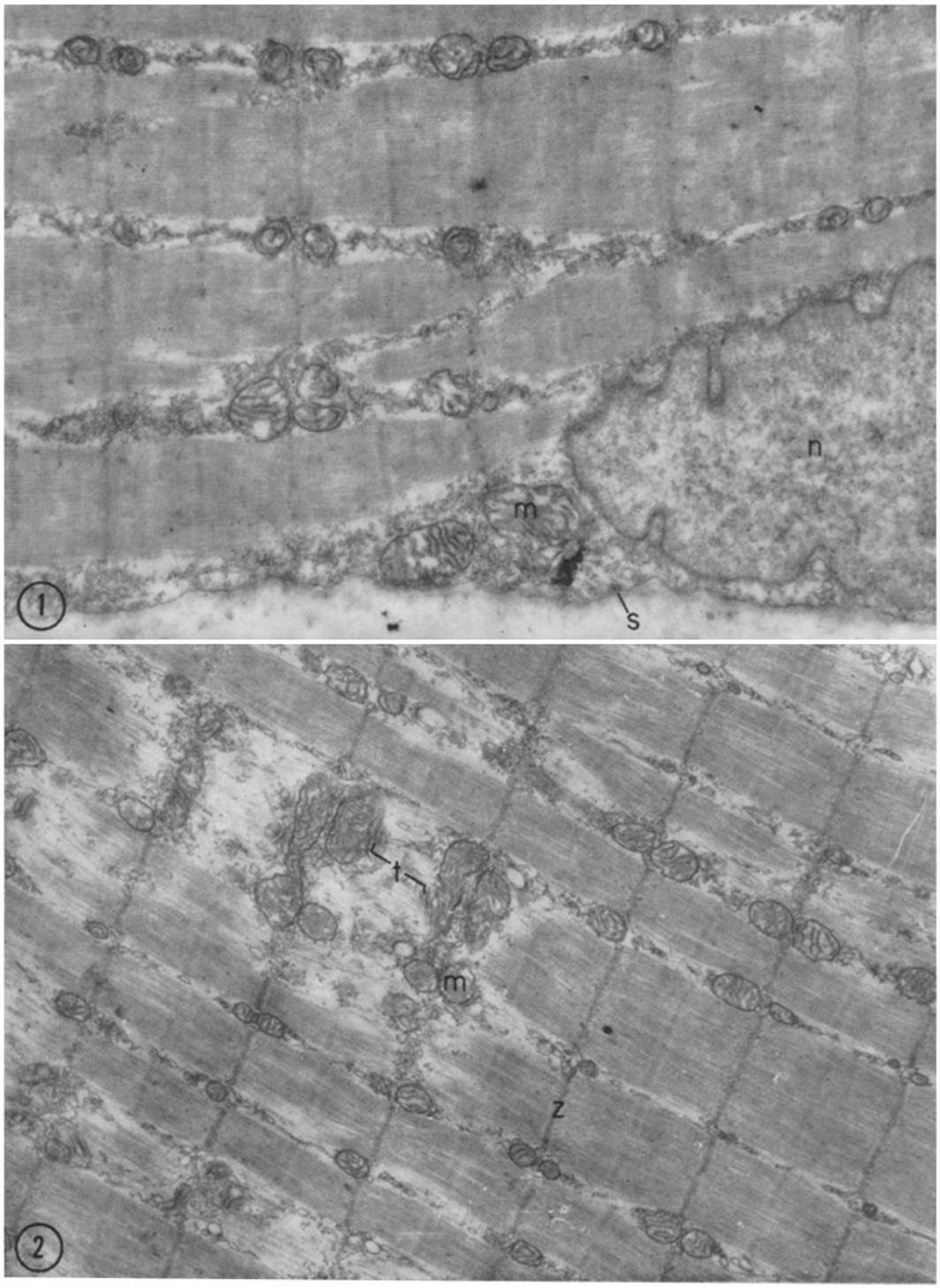
As is evident in Fig. 2, a longitudinal section through the central portion of a fiber depicts the familiar pattern of interfibrillar mitochondria. Small bodies of spherical or elongated shape are distributed in the sarcoplasm at levels immediately above and below the Z lines of the contracted myofibrils. Most individual profiles around the Z line levels do not usually extend longitudinally beyond a level in the sarcomeres which is opposite the sarcotubular T system (see *t* in Fig. 2). Longitudinal connections between mitochondria are exceptional. However, the symmetrical arrangement of the mitochondria in pair formations (or apparent pair formations) around the Z lines of the fibers are not always as regularly encountered. When a large expansion of interfibrillar sarcoplasm is included in the plane of section, it usually contains one or two elongated mitochondria orientated perpendicularly to the fibrils. This well known observation (17) only demonstrates the fact that most profiles of mitochondrial doublets represent cross-cut portions of oblong mitochondria running transversely or circumferentially to the fibrils.

Subsarcolemmic regions in the vicinity of blood vessels only occasionally contain a few profiles of mitochondria beneath the plasma membrane (Fig. 3).

The mitochondria of the fibers examined are frequently characterized by a heterogeneous matrix. They contain cristae of varying configura-

FIGURE 1 Longitudinal section through the peripheral portion of leg muscle fiber (M. gastrocnemius) from a normal rat showing mitochondria in the perinuclear region. The plasma membrane of the fiber (*s*, sarcolemma) is seen near the bottom of the figure as a long sinuous line. The narrow zone above is part of the perinuclear region with the nucleus (*n*) visible to the right. This region borders the myofibrils, which occupy the main portion of the micrograph. Note the size of the mitochondrial profiles (*m*) in the perinuclear sarcoplasm. The limited number and the lengths of the cristae of the mitochondria are also to be noted. The mitochondrial matrix appears heterogeneous in its density. × 18,000.

FIGURE 2 Longitudinal section through the central portion of a muscle cell (contracted) from same material as in Fig. 1. This figure shows the familiar pattern of distribution of interfibrillar mitochondria. The fibrils run parallel to the diagonal from the upper left to the lower right corner. The small, mainly round or oval profiles of mitochondria in the interstices of the myofibrils are mostly seen as pairs located directly above and below the Z line levels of the fibrils (*z*). In the upper left quadrant, an expansion of interfibrillar sarcoplasm is included in the section (the major translucent areas). The extensions of the mitochondria circumferentially to the myofibrils are well demonstrated by the transversally elongated profiles in this area. Note the location of the sarcotubular T system (*t*) relative to the mitochondria and the Z lines in the same area. The number of cristae and the heterogeneous density of the mitochondrial matrix are also to be noted. × 18,000.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tions and with less regular dispositions than is the case in other, more cristae-rich muscle mitochondria (19). Thus, there is a marked variation of their length, orientation, and individual course (Fig. 2). A detailed analysis of the membrane components constituting the cristae shows in some places the same characteristic angulation of the sheets as has been described earlier for various other tissues (Fig. 4, *cf.* 20, 21). Within the matrix of the mitochondria there are small annular profiles averaging 250 A in diameter (Fig. 4) which are believed to represent tubular structures, although these have not been observed in association with other inner membrane structures.

II. *Skeletal Muscle from Thyroidectomized Animals*

The arrangement of the mitochondria in longitudinal sections of muscle fibers from thyroidectomized rats is essentially similar to that in the normal animals. In the interfibrillar sarcoplasm, mitochondria predominate at levels immediately above and below the Z lines of the fibrils. In a few fibers, however, the profiles of the mitochondria exhibited a wider range of sizes than found in normal animals as well as an additional number of them. This latter feature is most often encountered in the peripheral portions of the fibers, particularly in the perinculear regions (Fig. 5) and beneath the plasma membrane in the vicinity of blood vessels (Fig. 7). Thus, it is not uncommon to find in these regions a large number of tightly packed mitochondria. Interfibrillar mitochondria in adjacent regions frequently show longitudinal extensions and occasionally form end-to-end columns which may extend over a few sarcomere lengths, as depicted in Fig. 6.

The internal structure of mitochondria is not much different from that in the normal controls. In fibers, however, in which the mitochondria are found to be locally enlarged, they show an increased profile ratio of cristae to matrix which is most prominent in the interfibrillar region (Fig. 6). This increase in the number of cristae is usually accompanied by more strict arrangement of the cristae in parallel arrays. Another, although less frequent, anomaly is the honeycomb-patterned cristae (Fig. 8, *cf.* 20). These formations may be derived from two or more parallel zigzag cristae, which get displaced so that a few sharp angles of one cristae oppose those of the other. These potential points of bridging when approaching each other tend to anastomose, thus resulting in a division of the available matrix space into several partially or entirely separate compartments. The annular profiles within the mitochondrial matrix appeared with higher frequency in this group of animals than in the normal controls (Fig. 8). When the enlargement of the mitochondrial apparatus in muscle fibers of thyroidectomized rats was estimated quantitatively by Loud's method (22) for the determination of surface-linear relationships in morphological components, an average increase (different fibers included) was found over the normal controls by a factor of 1.5.

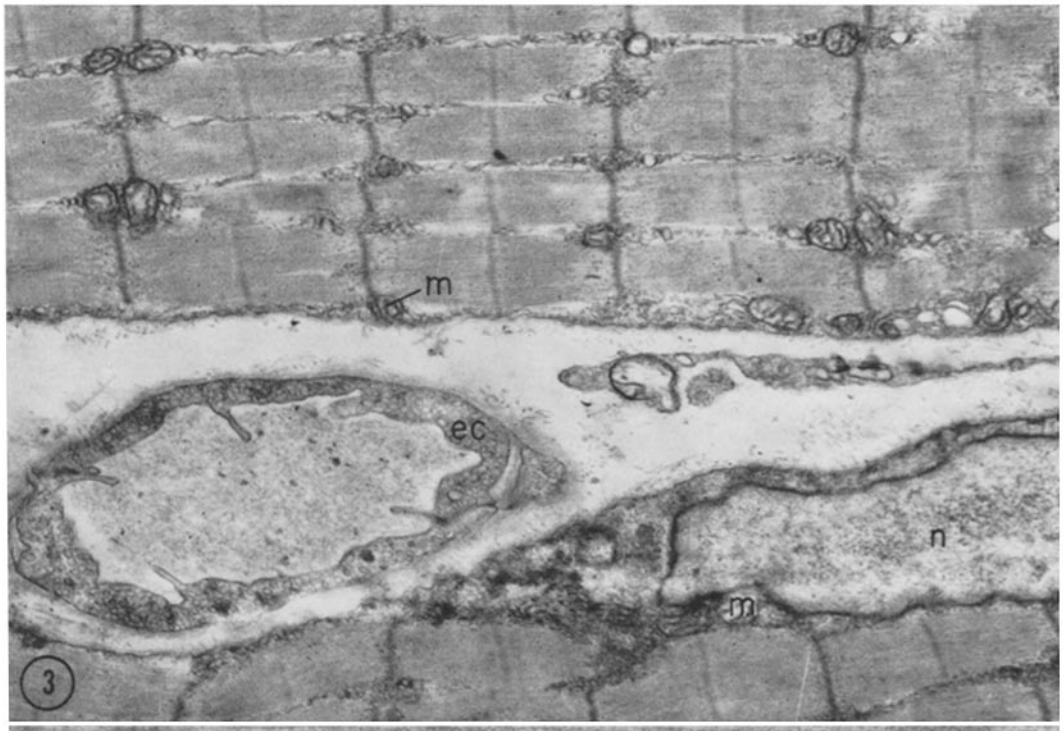
III. *Thyroidectomized and Normal Animals Treated with L-Thyroxine*

Mitochondria of fibers from thyroidectomized or normal rats treated with thyroxine show drastic alterations as compared to those from untreated animals, bearing in mind that thyroidectomy itself produces structural changes.

It is evident from the longitudinal sections that the number of mitochondrial profiles as well as the range of their sizes is markedly increased over

FIGURE 3 Longitudinal section of peripheral portions of two adjacent muscle fibers and cross-cut blood vessel endothelial cells (*ec*) from the same specimen as in Fig. 1. Note the sparsely occurring mitochondrial profiles (*m*) in the subsarcolemmic regions of the two neighboring fibers. A nucleus (*n*) is seen at lower right. × 18,000.

FIGURE 4 Higher power micrograph of longitudinal section of myofibrils from the same sample as in Fig. 1. In the two zones of interfibrillar sarcoplasm there are profiles of mitochondria (*m*) and elements of the sarcoplasmic T system (*t*). Note the angular configurations of the cristae demonstrated at lower left (arrow). Small annular profiles are occasionally visible in the mitochondrial matrix, and they lack any apparent association with the cristae (see upper right arrow). z, Z line. × 44,000.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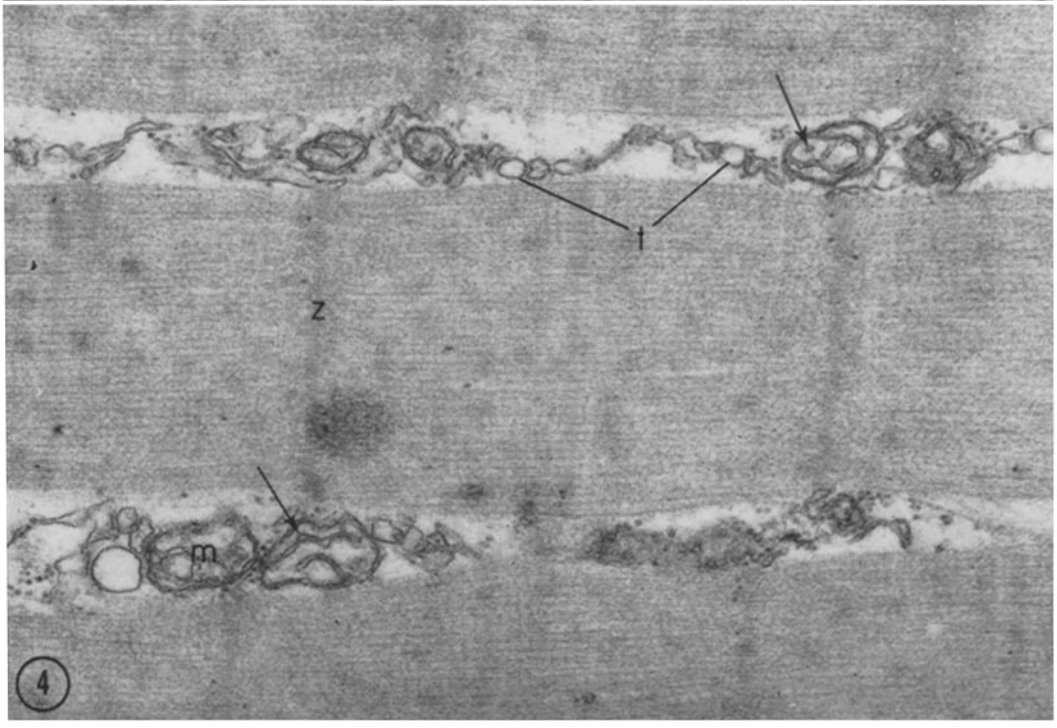
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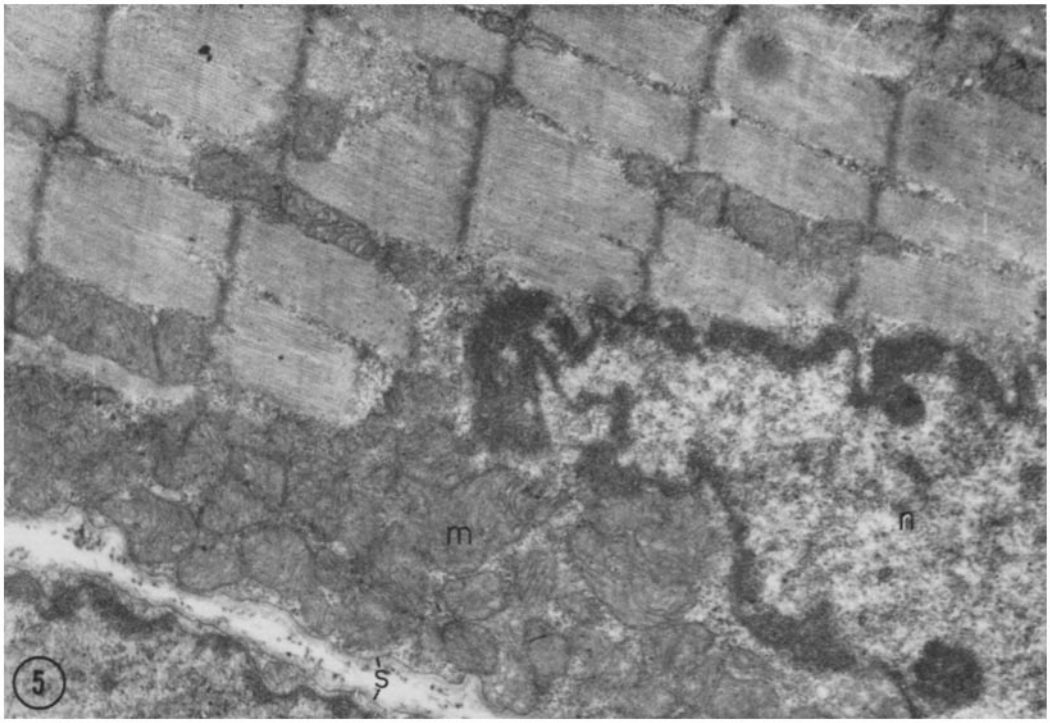
that found in the non-thyroxine–treated counterparts. An additional mitochondrial component is observed in the micrographs opposite the middle level of the sarcomeres, which results in the formation of rows of continuous profiles, often of several sarcomere lengths (Figs. 10 and 14). At places, only one of the mitochondria of the pair shows longitudinal extensions, also resulting in long end-to-end columns. It is frequently observed that indivudual profiles abut upon each other opposite certain definite levels of the fibrils, namely at and within 0.4 μ of the Z line level (Fig. 10). It is interesting to note that the latter levels closely coincide with the location of the T system (transverse elements) of the sarcoplasmic reticulum (Fig. 10, arrows). Occasionally, individual mitochondrial profiles of 2 to 3 sarcomere lengths are encountered (Fig. 14). In these elongated interfibrillar mitochondria, indentations are also often observed at the levels of the sarcotubular T system. A longitudinal-tangential section through an individual indented mitochondrion may create the impression of a row of profiles with diffuse borders at the T system levels. This seems to be the explanation of the row of apparently separate mitochondria seen in a preparation from a thyroxine-treated normal animal shown on the left in Fig. 14. In the majority of cases, however, the transverse borders between the mitochondrial profiles appear to be more closely cross-cut and there is usually very little interspace between the individual profiles. In all likelihood, therefore, these borders represent boundaries between separate mitochondria. The maintenance of individual integrity of abutting mitochondria is even better demonstrated on the right side of Fig. 14 by the relation of the L-shaped mitochondrial profile to its adjacent longitudinal partner. It is, therefore, suggested that most of the very long mitochondria are derived from outgrowths of the original mitochondria along certain preferential passages rather than representing fusions of several smaller ones. Yet, fusion of mitochondria cannot definitely be excluded.

The induction of a hypermetabolic state by thyroxine treatment caused a marked increase in the number of mitochondrial profiles in both normal and thyroidectomized animals. This was particularly marked in the perinuclear regions (Figs. 9 and 13) and in the sarcoplasmic expansions beneath the sarcolemma in the vicinity of blood vessels (Figs. 11 and 15). The individual profiles generally resembled those found in the untreated thyroidectomized controls described above. Small vesicular inclusions were occasionally encountered in the sarcoplasm immediately adjacent to the nucleus. Some of these inclusions are limited by a single sharp membrane, while others may be clearly enclosed by two membranes. These elements are, in some instances, closely associated with extensions of the outer membrane of the nuclear envelope, as shown in Fig. 12 for a preparation from normal rats treated with thyroxine. The vesicular inclusions often contain a substance of heterogeneous opacity and membranous profiles, which are reminiscent of cristae (see inset, Fig. 12).

The internal structure of mitochondria is quite similar whether thyroxine has been administered to normal or thyroidectomized rats, but it differs from that in untreated normal animals in the increase in the number and length of cristae. Be-

FIGURE 5 Micrograph showing portions of two adjacent leg muscle fibers from a thyroidectomized animal. The appearance of the perinuclear region can be compared to that in Fig. 1. The sarcoplasm adjacent to the nucleus (n) is almost entirely filled up by mitochondria (m). The mitochondrial matrix is denser than it is normally and the cristae more closely packed. The border of the fiber is marked at s. × 18,000.

FIGURE 6 Longitudinal section through the central portion of muscle fiber from thyroidectomized animal. The interfibrillar region shown can be compared to that in Fig. 2. In the lower left part of the figure, the section includes a few profiles of longitudinally extended mitochondria averaging one sarcomere length. Some mitochondria line up to form a continuous row parallel to the fiber. A portion of interfibrillar sarcoplasm at lower right also shows some transversally orientated mitochondrial profiles. Note the abundance of long cristae. × 18,000.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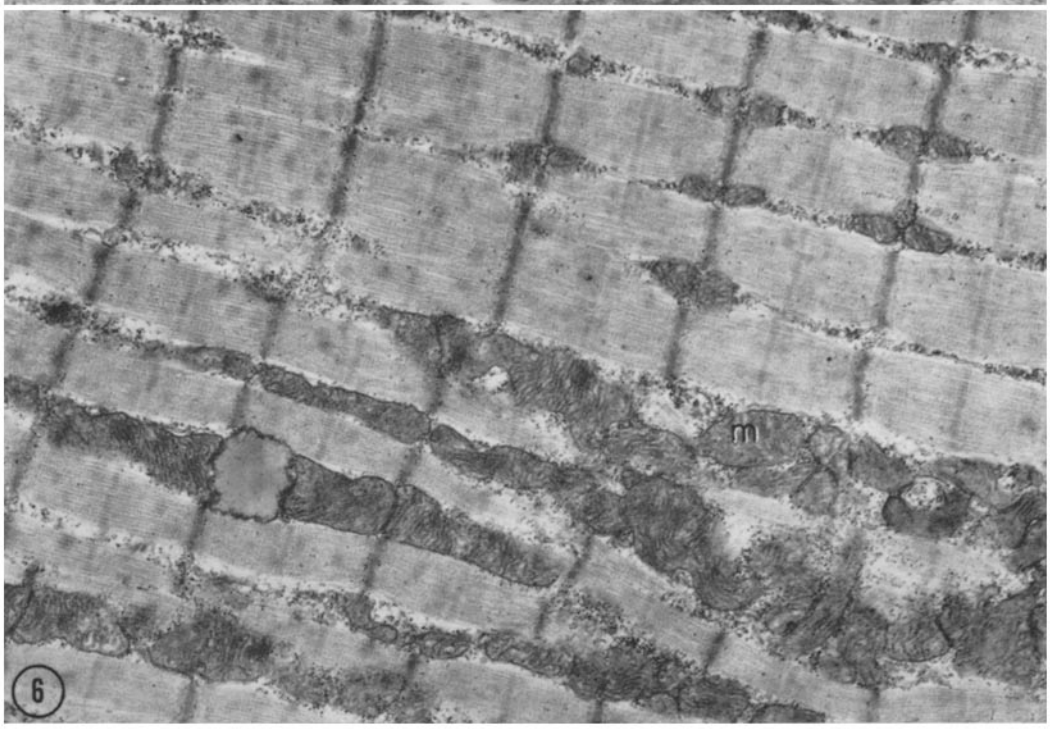
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sides tighter packing of cristae, mitochondria from hypermetabolic animals also showed a higher frequency of angular or zigzag configuration of the cristae across the mitochondria (Fig. 10). Unlike the preparations from untreated thyroidectomized animals, these mitochondria did not show cristae of the honeycomb type and there were markedly fewer annular structures within the mitochondrial matrix. Another feature of structural changes was the high frequency of small dense granules in the matrix, as shown in Fig. 16 for mitochondria from normal rats treated with thyroxine.

Estimation of the total mitochondrial cross-section area by Loud's method showed that thyroid hormone treatment of thyroidectomized animals led to a 3-fold increase over the values in normal controls and a 2-fold increase compared to the untreated thyroidectomized animals. In normal rats that were made hypermetabolic, a 2.5-fold increase was observed over their corresponding untreated controls.

IV. *Persistence of Structural Changes after Cessation of Thyroxine Administration to Normal Rats*

Three weeks after thyroxine treatment was stopped, the mitochondria had retained the structural changes found in the hypermetabolic rats. This is true for the increase in their number and size as well as for their distribution and shape (Figs. 17-19). The retention of mitochondrial hypertrophy is interesting in view of the fact that the previously elevated mitochondrial and respiratory activity had returned to normal levels 3 weeks after the cessation of hormonal treatment (see Table I). In addition to the abundance of cristae in mitochondria from this group of animals, there are numerous small annular profiles similar to those less frequently found in mitochondria from normal animals (Fig. 20). The small dense granules in the matrix are, however, less frequently found in these mitochondria than in those from hypermetabolic animals. The total mitochondrial mass still is three times larger than that from untreated normal controls.

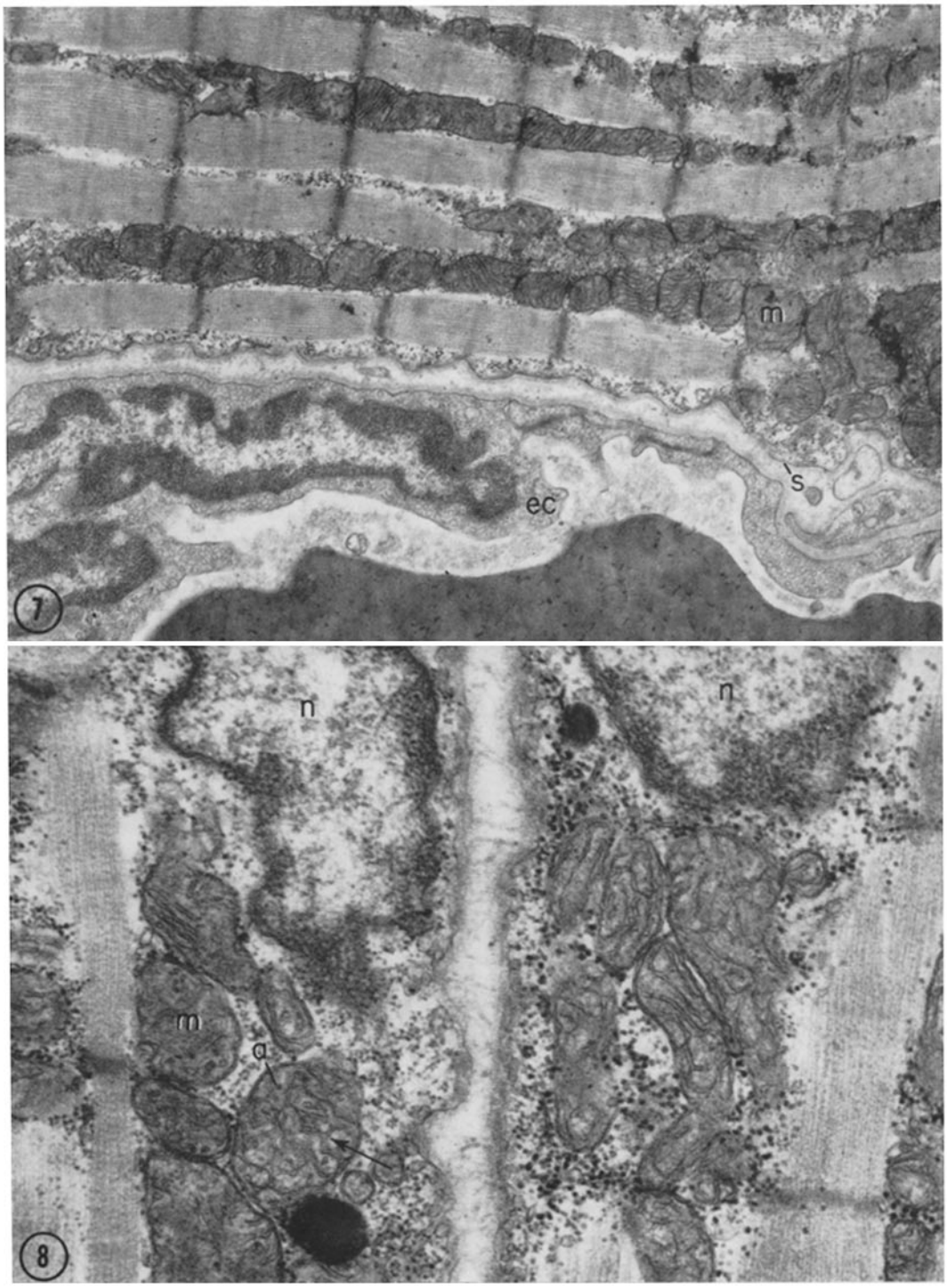
DISCUSSION

A change in thyroid status of the animal, either after thyroidectomy or thyroid hormone administration, results in an impressive alteration of the structure of skeletal muscle mitochondria. There is, however, no simple correlation between the changes induced in metabolic activity as measured in isolated mitochondria and their structure in the intact tissue observed under the electron microscope. Fig. 21 schematically summarizes some of the structural changes under the various experimental conditions, which are as follows:

Thyroidectomy caused a marked reduction in the growth rate and BMR of the whole animal, as well as lowered mitochondrial respiration and phosphorylation per unit protein (see Table I). This was accompanied by a marked hypertrophy and increase in the number of mitochondria (compare Fig. 21, *a* and *b*), thus suggesting that at 6 weeks after thyroidectomy, the over-all composition of muscle mitochondria has been altered in the sense that there are fewer active respiratory and phosphorylative units relative to total mitochondrial protein or mass. Administration of moderate amounts of thyroxine over a period of 3 weeks nearly doubled the growth rate of thyroidectomized rats and also considerably increased the BMR and the oxidative phosphorylation in isolated mitochondria in both normal and thy-

FIGURE 7 Peripheral portion of muscle fiber near blood vessel from the same specimen as in Fig. 5. This micrograph is to be compared to that in Fig. 3. Note the cluster of mitochondrial profiles at *m*. This subsarcolemmic region faces a blood vessel (*ec*) at bottom. *s*, sarcolemma. × 18,000.

FIGURE 8 Higher power micrograph of two perinuclear regions in adjacent muscle cells from the same material as in Fig. 5. This figure demonstrates an unusual cristae configuration encountered in thyroidectomized animals. Instead of running more or less parallel to each other, the cristae tend to branch and anastomose (arrow), resulting in a pattern similar to a honeycomb structure. Small annular structures (*a*) are seen in the matrix. × 45,000.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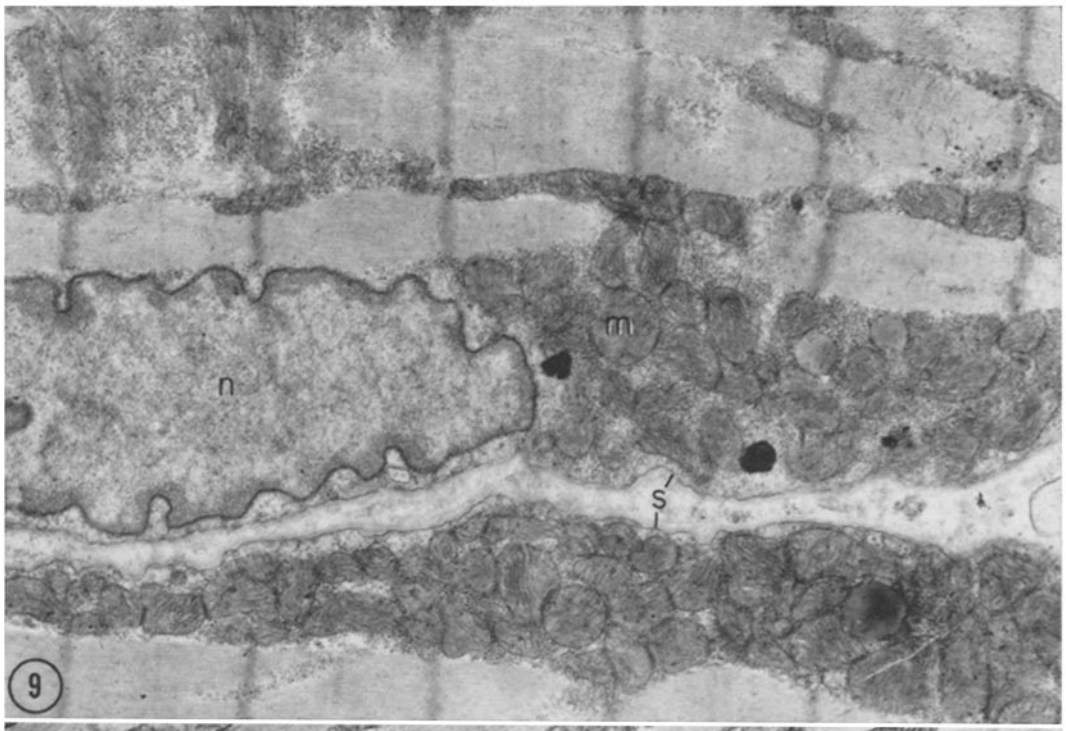
roidectomized rats. Under these conditions, there was again a marked mitochondrial hypertrophy and increase in population (Fig. 21, *c* and *d*). Although both thyroidectomy and hyperthyroidism seemed to produce similar structural changes, there were some significant differences, such as the frequency of the appearances of honeycomb type of cristae, annular structures, and small dense granules, within the mitochondrial matrix. There is also an important difference between the mitochondria in hypo- and hypermetabolic states. The change in over-all composition of mitochondria of hypermetabolic rat muscle is in the direction opposite to that seen after thyroidectomy; *i.e.*, an increase in the respiratory and phosphorylative units relative to the rest of the mitochondrial protein. It has already been shown that liver mitochondria from hyperthyroid rats have a higher content of cytochrome *c* and glycerol-1-phosphate dehydrogenase than those from normal or thyroidectomized animals (3, 10, 23, 24).

Our interpretation of the above findings is that there are other factors besides thyroid hormones that control the activity, growth, and multiplication of skeletal muscle mitochondria (see Lehninger, 25). Depriving the animal of thyroid hormones slows down metabolic activity, and the resultant hypertrophy and increase in number of mitochondria may be due to the "compensatory" stimulation of mitochondrial growth by some of these other regulatory factors which do not have the same selective action that thyroid hormones have in regulating the content of respiratory and phosphorylative components. Mitochondria from such animals would still respond to thyroxine in the same way as those from normal animals, resulting in a further increase in size and population. Thus the thyroid hormone would cause an even greater increase in mitochondrial respiratory units per gram skeletal muscle than per gram mitochondrial protein. It is, of course, impossible to say, from the present observations on long-term effects of hormone administration, whether the fine structural changes are a cause or consequence of the control of metabolic activity of mitochondria by thyroid hormones.

The type of structural changes that we have reported here are quite distinct from the well known phenomenon of mitochondrial swelling produced *in vitro* by incubation with high concentrations of thyroxine or the administration of toxic or catabolic amounts of the hormone and its analogue (see 25, 26, 6, 27). Swelling represents an increase in mitochondrial volume due to water imbibition whereas under our anabolic conditions of thyroxine treatment the increase in mitochondrial volume, and in number and packing of cristae, clearly is a phenomenon of growth and formation of new units. Furthermore, unlike liver and kidney mitochondria, skeletal muscle mitochondria appear to be insensitive to the swelling action *in vitro* or *in vivo* of thyroid hormones (28); at the dose of thyroxine used by us, liver mitochondria also were neither swollen nor were they

FIGURE 9 Longitudinal section of peripheral portions of two muscle fibers from a thyroidectomized animal treated with L-thyroxine for 3 weeks. The middle left part of the micrograph depicts an indented nucleus (*n*) as it is frequently observed. A portion of the perinuclear sarcoplasm to the right contains a cluster of tightly packed mitochondria (*m*), with a high number of long and virtually parallel cristae. A similar region is evident beneath the sarcolemma (*s*) in the fiber at the bottom of the figure. This micrograph is to be compared to that in Fig. 5. × 18,000.

FIGURE 10 Longitudinal section through central portion of fiber from same material as shown in Fig. 9. The section is to be compared with that shown in Fig. 6 from untreated thyroidectomized rats. The profiles of the mitochondria in the interstices of the myofibrils border on each other, forming end-to-end columns of several sarcomere lengths (see row at *m*). Note that most individual profiles abut upon each other at certain definitive levels of the fibrils: at the Z line level (*z*) and 0.4 μ above or below it (see arrows). These levels coincide approximately with the location of the T system (see longitudinal profiles at *t*). Note also at lower right of the section that the positions of the indentations of the elongated mitochondria are at the levels of the T system. Most mitochondria have long and angulated cristae in a predominantly parallel arrangement. × 18,000.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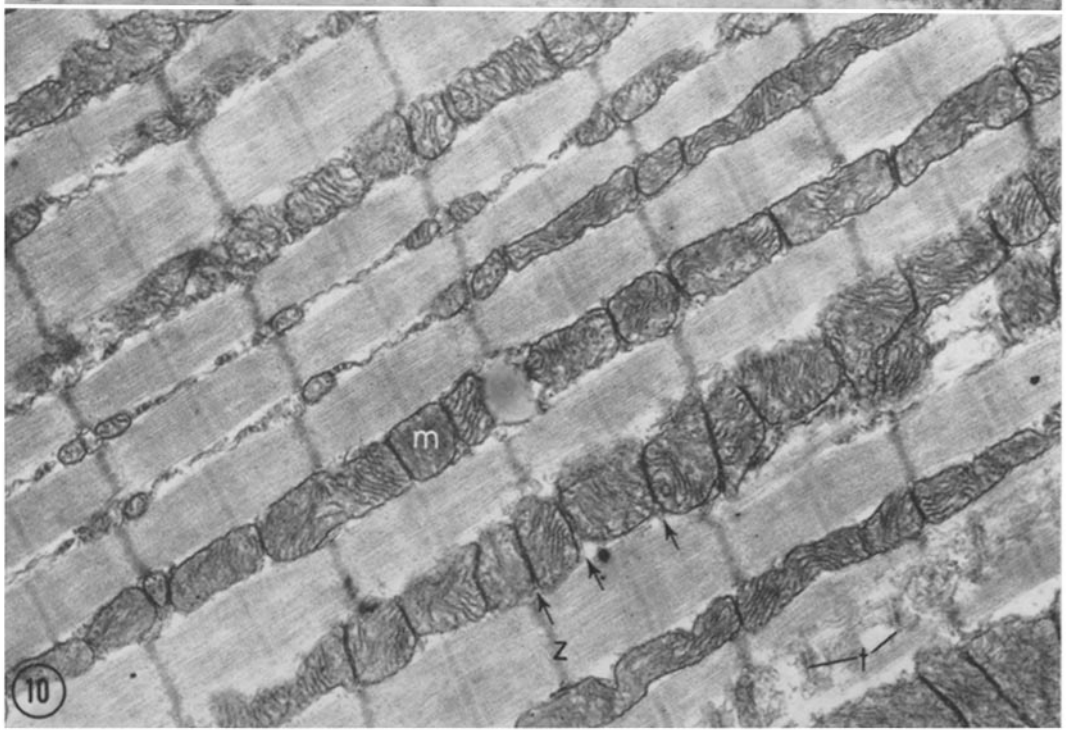
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more susceptible to swelling agents *in vitro* than normally (3). The fact that the structural changes we have observed represent a growth effect is compatible with the idea expressed elsewhere (see 1, 3, 7) that the regulation of BMR by thyroid hormones under physiological conditions is only one facet of the anabolic or growth-promoting and developmental actions of thyroid hormones. This is further supported by the increase in the amount of mitochondrial dehydrogenases and cytochromes (3, 10, 23, 24), stimulation of protein synthetic capacity of mitochondria (8–10) and of nuclear and cytoplasmic RNA synthesis brought about by thyroid hormone administration (29, 30).

A clear dissociation of changes in mitochondrial activity from those in structure was observed after the cessation of thyroxine administration. In these animals, the structures found in hypermetabolic tissues were still retained although the respiratory activity had declined to the original levels (compare Fig. 21, *d* and *e*). It may be that after cessation of hormone treatment, the lowering of the metabolic activity may result from a relatively more rapid "disintegration" of respiratory units in the cristae (see 25, 31, 32) compared to the other constituents. In other words, the respiratory components may disappear at a more rapid rate than other structural units. In this connection, it is of interest to quote the earlier findings of Fletcher and Sanadi (33) that, in rat liver mitochondria, lipids, soluble and insoluble proteins, and cytochrome *c* were all replaced at nearly equal rates with a half-life of 10.5 days. If such a synchrony of the turnover of mitochondrial constituents also exists in muscle, then the intriguing possibility arises that thyroid hormones, *i.e.*, the euthyroid state, might constitute a factor responsible for this synchrony. Striking examples of metabolite-induced shifts in mitochondrial composition are found in recent work of Linnane (34) with yeast and of Luck (35) with *Neurospora*.

The foregoing consideration points to another implication of our work, namely, the possibility of using thyroid hormones as convenient tools in studying mitochondrial biogenesis in higher organisms. Earlier work of Luck (36) on *Neurospora* has already opened up one approach of isotopic labeling of mitochondria under different growth conditions. We have shown in this paper that thyroxine is an excellent agent for experimentally changing the composition of total mitochondrial population, for stimulating the growth rate of already formed mitochondria, and for the formation of new mitochondria. The value of experimentally causing the formation of new mitochondria or their constituents is obvious in connection with the problem of origin of mitochondria and the role of the cell nucleus in this process. The administration of small doses of thyroid hormones has also been shown to affect the rate of incorporation of amino acids into mitochondrial protein immediately preceding alterations in the metabolic activity of the particles (10). In view of the role of the membranes in mitochondrial activity, it would be interesting to analyze the structural changes produced as a function of time after the administration of thyroid hormones, with a view to seeing whether the increase in protein synthetic capacity and metabolic activity of mitochondria precedes or follows formation of new membranous elements. A reverse time-course analysis after the cessation

FIGURE 11 Survey micrograph of subsarcolemmic portions of two fibers around a blood vessel from the same material as in Fig. 9. A blood vessel (*ec*) is seen in the center of the figure with portions of two longitudinally sectioned fibers in apposition to it. In the sarcoplasmic expansions partially surrounding the vessel, tightly packed clusters of mitochondrial profiles (*m*) are evident. A nucleus is seen at *n*. This micrograph is to be compared with that in Fig. 7. × 18,000.

FIGURE 12 Micrograph of nuclear region of muscle fiber from a normal animal rendered hypermetabolic by treatment with L-thyroxine for 3 weeks. This figure is aimed at showing a small vesicular structure located in an indentation of the nuclear envelope (see encircled area). In the inset, this structure can be seen to be bounded by a double membrane, which is in association with an outpocketing of the outer nuclear membrane. It contains membranous structures reminiscent of cristae. *n*, nucleus. × 28,000. Inset, × 60,000.


ec
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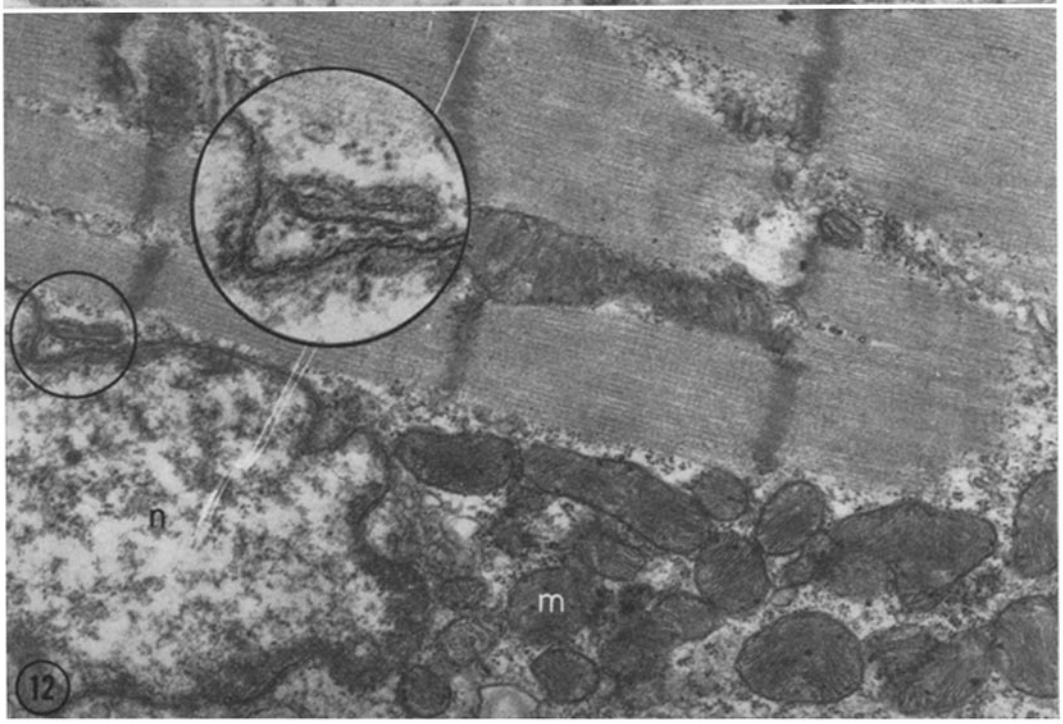
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of hormone administration would also be valuable in deciding to what extent the respiratory or other functional units of mitochondria are turned over at rates different from those of structural elements observed in the electron microscope.

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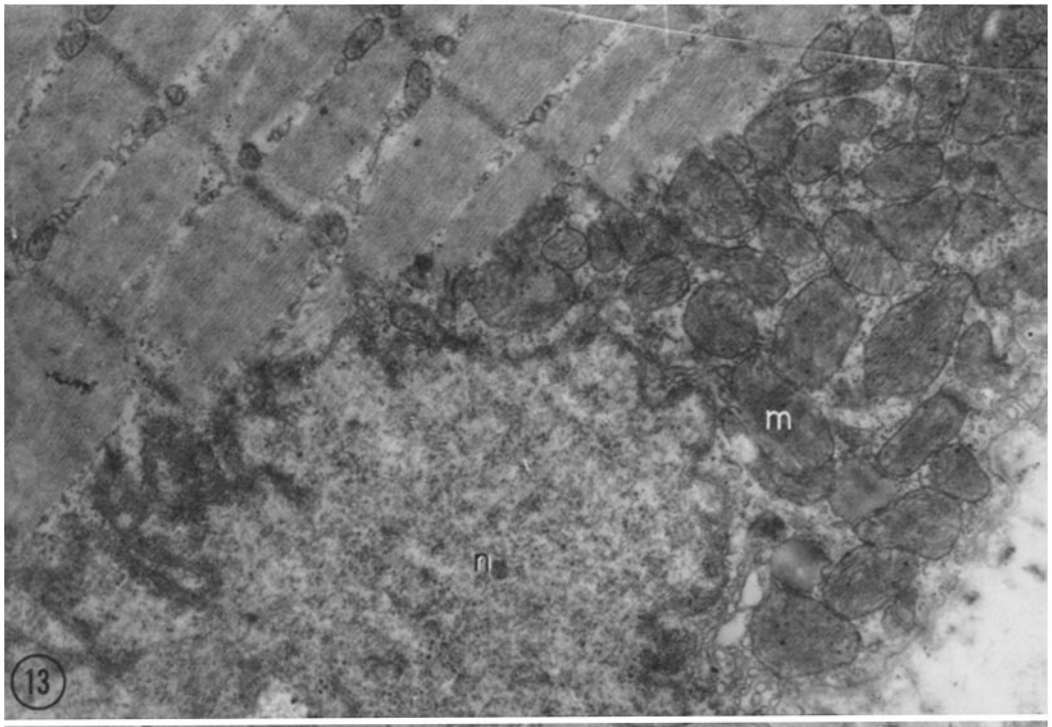
FIGURE 13 Longitudinal section showing a perinuclear region of muscle fiber from the same material as shown in Fig. 12. In the large sarcoplasmic zone surrounding the nucleus (*n*), the great number of mitochondrial profiles are prominent as is the abundance of long cristae. Note the small dense granules in the matrix between the cristae (seen to better advantage in Fig. 16). This micrograph is to be compared with that of Fig. 1. × 18,000.

FIGURE 14 Longitudinal section of a central portion of fiber from the same specimen as in Fig. 12. It is evident that the profiles of the interfibrillar mitochondria (*m*) show a pronounced variation in size, mainly due to their longitudinal extensions. Thus, the long mitochondrial profile in the center of the figure measures almost three sarcomere lengths. Note the characteristically repeated positions of its indentations (arrows), as well as those of the transverse borders between the mitochondrial profiles. In the mitochondrial column to the upper right, an L-shaped profile is evident. It is believed that this mitochondrion represents an outgrowth along certain favored passages, rather than a fusion of several smaller mitochondria. The same interpretation is proposed to explain the elongated mitochondria. Most mitochondria have tightly packed angulated cristae. This micrograph is to be compared to that of Fig. 2. × 18,000.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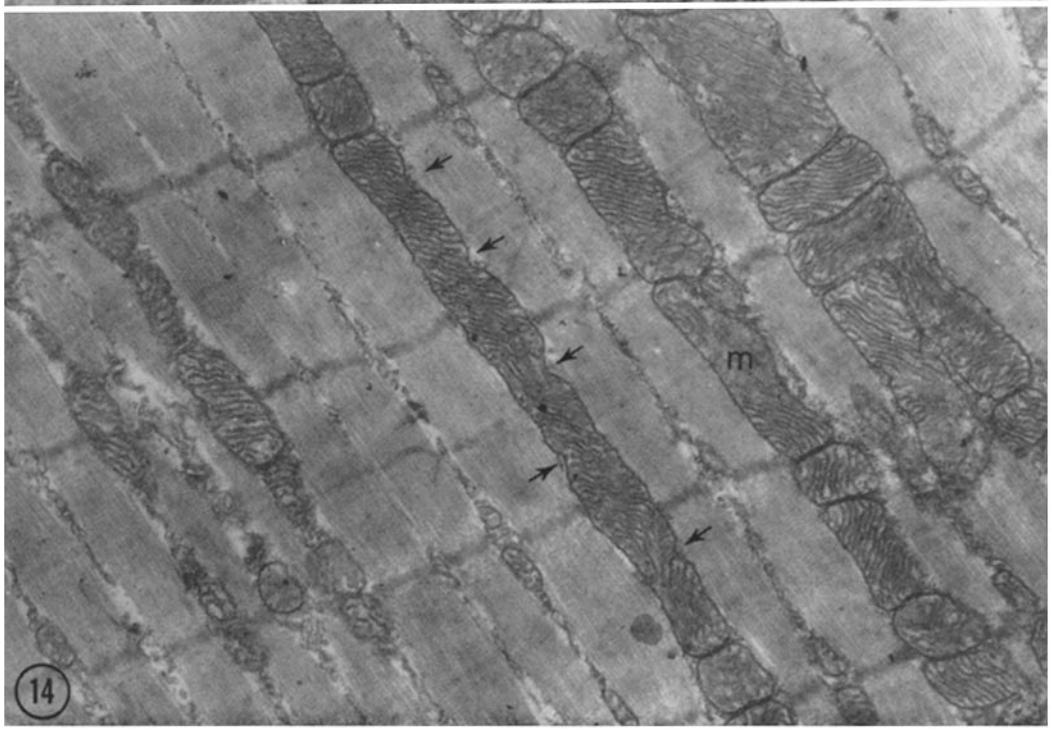
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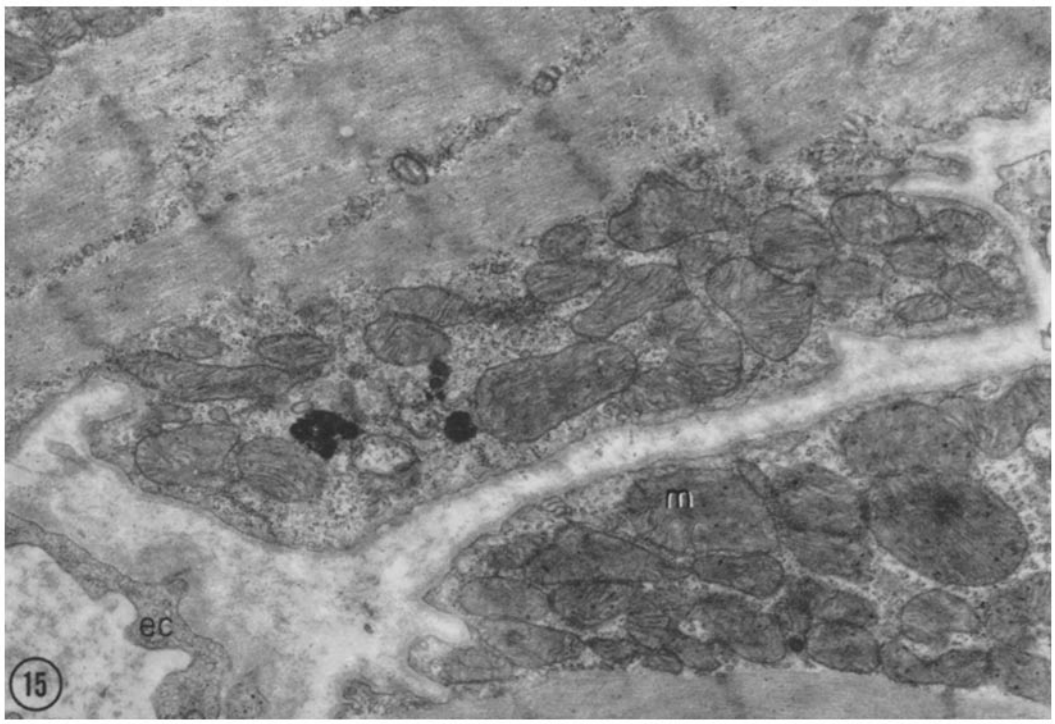
FIGURE 15 Longitudinal section through peripheral portions of two fibers and interjacent blood vessel of the same specimen as in Fig. 12. The blood vessel (*ec*) is seen at lower left. In both fibers there is an expansion of sarcoplasm in the vicinity of the vessel. It is evident that these regions contain large clusters of mitochondrial profiles (*m*) which are rich in cristae. This figure is to be compared to Fig. 3. × 18,000.

FIGURE 16 Higher power micrograph of perinuclear region of muscle fiber from a hyperthyroid animal similar to that in Fig. 12. The figure is intended to show, at a greater enlargement, the frequency of the small dense granules in the mitochondrial matrix of this material (arrow). *m*, mitochondrion. × 45,000.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ec
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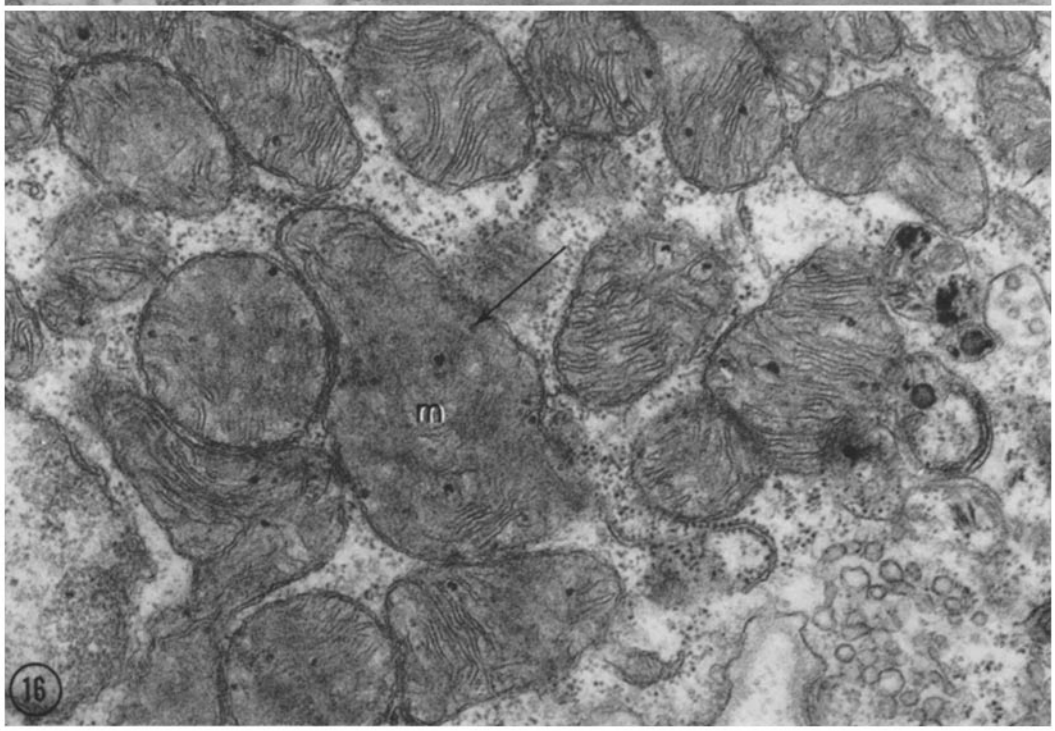
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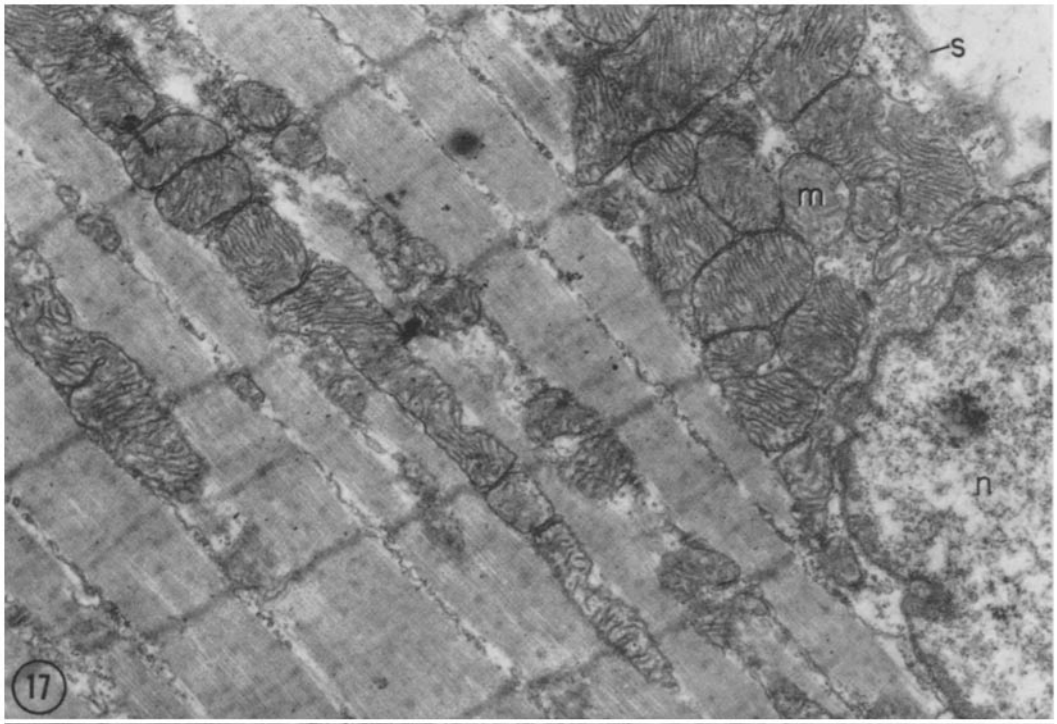
FIGURE 17 Longitudinal section through peripheral portion of muscle fiber from a normal animal 3 weeks after the cessation of a 3-weeks' treatment with L-thyroxine. By this time, the metabolic activity of mitochondria had declined to the original normal values. The figure shows the appearance of mitochondria (*m*) in a perinuclear region which can be compared to those in Figs. 1 and 13. A nucleus (*n*) is visible to the right and the adjacent sarcoplasmic region contains a large cluster of tightly packed profiles of mitochondria. Elongated cristae almost completely fill up the mitochondria. *s*, sarcolemma. × 18,000.

FIGURE 18 Longitudinal section of central portion of a fiber from the same material as that in Fig. 17. This micrograph is to be compared with that in Fig. 2. In the lower half there are two rows of mitochondrial profiles (*m*), many of which are quite extended longitudinally, and abutting onto each other. Note the fixed levels at which the mitochondrial profiles border on each other or are characteristically indented (*cf.* Figs. 10 and 14). × 18,000.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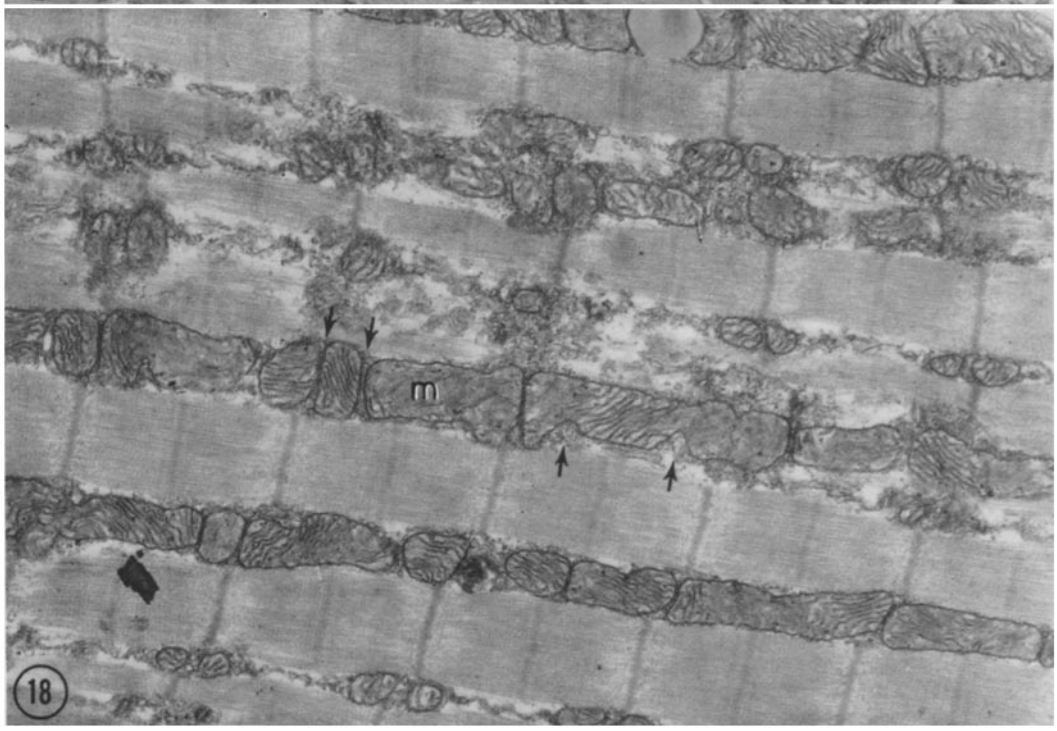
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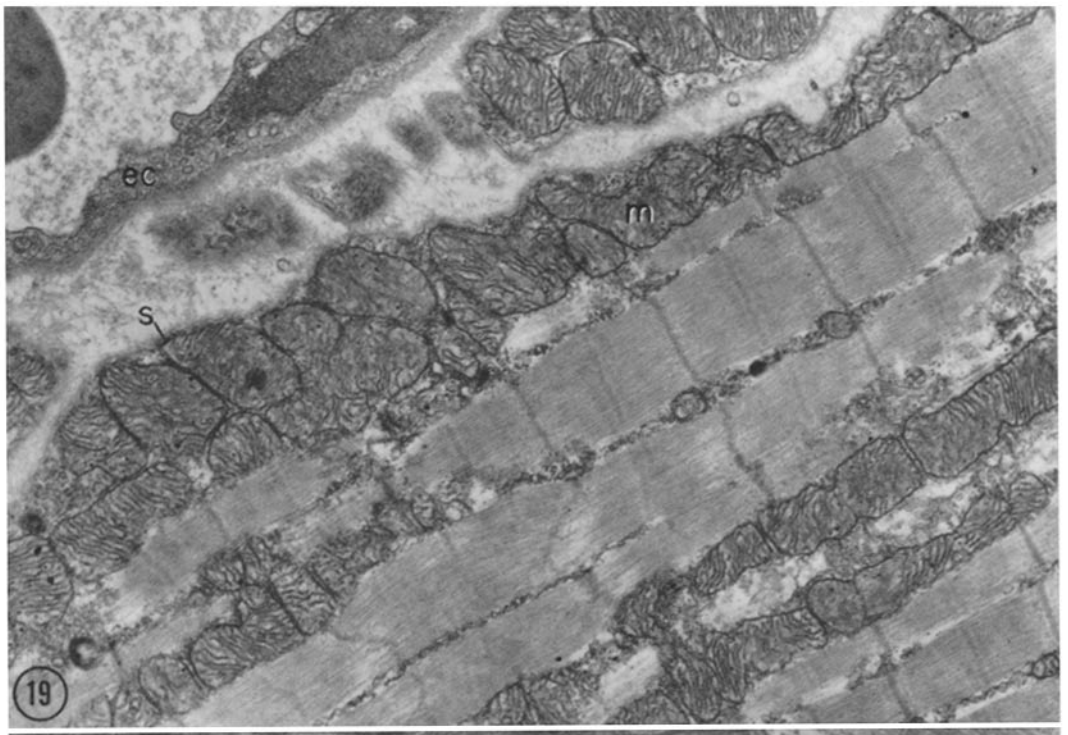
FIGURE 19 Longitudinal section of marginal portion of a muscle fiber and adjacent blood vessel (*ec*) from same animal as in Fig. 17. The sarcoplasm under the sarcolemma (*s*) contains an abundance of large mitochondrial profiles (*m*). When favorably orientated, the mitochondria show numerous cristae. This figure demonstrates a region comparable to that in Fig. 3. × 18,000.

FIGURE 20. Higher power micrograph of a longitudinal section showing a few interfibrillar mitochondria from the same muscle fiber as in Fig. 17. The large mitochondrial profile at upper left contains a great number of long cristae, which roughly parallel each other and run diagonally to the orientation of the fiber. There is a marked tendency to angulation or zigzag configuration of the cristae (see left arrow). Small structures of annular appearance are frequent within the matrix in this material (see right arrow). This figure is to be compared to Fig. 4. *m*, mitochondrion. × 48,000.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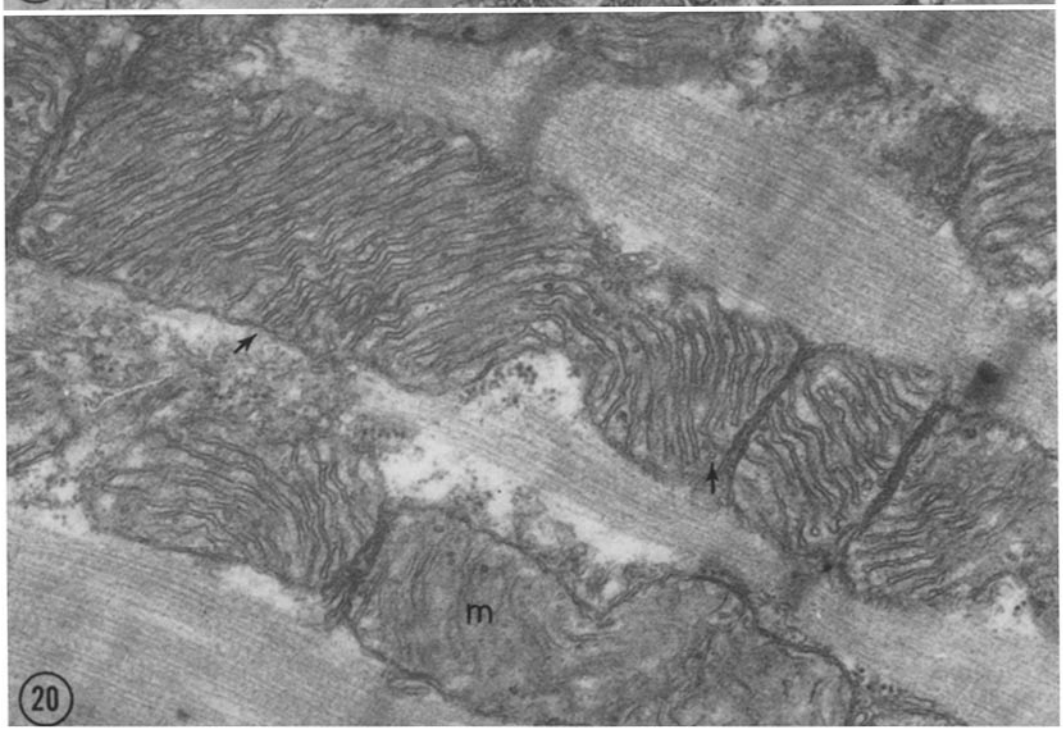
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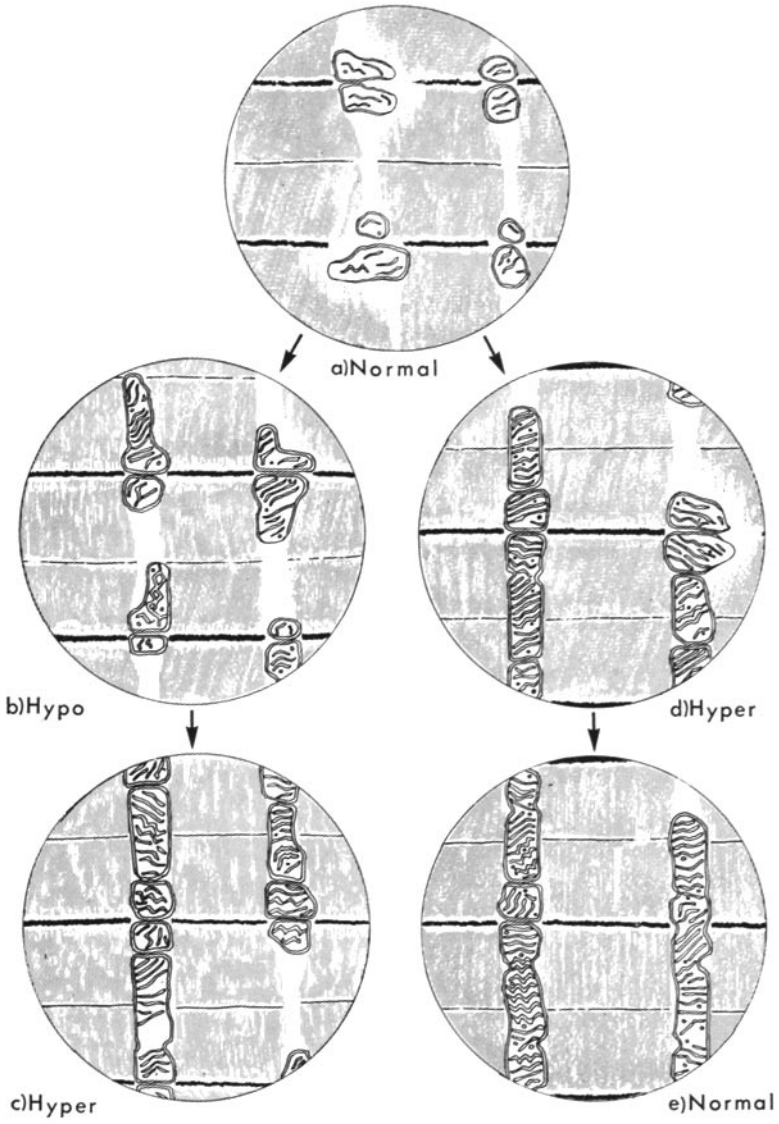

FIGURE 21 Schematic summary of the structural changes in skeletal muscle mitochondria accompanying alterations in their metabolic activity (Table I) induced by thyroidectomy and treatment with L-thyroxine. The terms "normal," "hypo," and "hyper" refer to the thyroid state. For explanation, see text under "Discussion."