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A CASE OF SEVERE HYPERMETABOLISM OF NONTHYROID ORIGIN WITH A DEFECT IN THE MAINTENANCE OF MITOCHONDRIAL RESPIRATORY CONTROL: A CORRELATED CLINICAL, BIOCHEMICAL, AND MORPHOLOGICAL STUDY

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Symptomatic hypermetabolism is often encountered in clinical medicine and is mostly due to thyroid diseases. In 1959, Ernster, Ikkos and Luft (1) presented preliminary biochemical studies on isolated skeletal muscle mitochondria from a patient with severe hypermetabolism of unknown origin. A defect of the biochemical function of these mitochondria was found, and it was suggested that this defect might be responsible for the hypermetabolic state of the patient.

It is now well established that mitochondria are the site of cellular respiration as well as of the process called oxidative phosphorylation by which energy liberated in respiration is conserved in the form of ATP [1] (2). Normally the respiration of isolated mitochondria is obligatorily coupled to the formation of ATP from ADP and inorganic phosphate, and thus cannot proceed at any appreciable rate if either of the latter components is absent from the incubating medium (3). This phenomenon, usually termed respiratory control, is generally considered to be a reflection of the capacity of the living cell to regulate its respiration according to its actual demand of energy. It was found that in the present patient this ability of the isolated skeletal muscle mitochondria was severely impaired (1). Thus, these mitochondria displayed a nearly maximal rate of respiration even in the absence of a continuous supply of ADP. On the other hand, they exhibited an almost normal extent of phosphorylation when ADP was supplied in excess. It appeared, therefore, that the coupling between respiration and phosphorylation in these mitochondria was not obligatory as in normal mitochondria but was separated in such a way that respiration proceeded at a high rate regardless of whether energy was conserved for metabolic purposes or wasted as heat. These results appeared to offer a logical background to the clinical syndrome, and therefore a further investigation of this mitochondrial defect was considered interesting.

At the time of the above studies, knowledge of the biochemical properties of skeletal muscle mitochondria in general was relatively poor and was entirely lacking as far as human muscle mitochondria are concerned. In order to evaluate properly the data obtained with the hypermetabolic patient, it was necessary to investigate the properties of normal human muscle mitochondria in some detail. Such a study has recently been completed (4). With this study as a basis, it has been possible to elucidate better the above biochemical defect. In addition, the morphology of the patient's as well as that of normal muscle mitochondria has been examined.

In the present paper a detailed report of both these lines of investigation is given together with the clinical findings. They have led to the conclusion that the present syndrome is related to a defect in the mitochondrial enzyme organization. The interest of the case lies both in the fact that it represents the first example of an endogenous

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[1] Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; DPN, diphosphopyridine nucleotide; PNA, ribonucleic acid; BMR, basal metabolic rate; PBI, protein-bound iodine.

disorder of the enzymic organization of the mitochondria and in the clinical syndrome per se which, to our knowledge, has not been described previously.

I. CLINICAL STUDIES

The Patient MBM, a 35-year-old single woman, has been intensively studied because of symptoms of increased perspiration, polydipsia without polyuria, polyphagia, decreased body weight, and asthenia, all of many years duration.

The first suggestion of her present illness was increased perspiration, chiefly at night, noted when she was still a child. This became more marked at the age of seven, when the perspiration was observed to be generalized, continuous, and sufficient to make necessary several changes of clothes daily. The perspiration was even further increased during physical and emotional stress. In addition she had increased thirst but normal urine volumes. She was underweight despite an increased appetite. She was abnormally tired. Bowel movements were frequent initially but later became normal. Her symptoms have persisted into adult life with little change except that the asthenia has become more pronounced, and she has been unable to do more than light housework.

Her general medical history appears unremarkable. She was the first child of healthy, non-consanguineous parents and born after a normal full-term pregnancy. Growth and development were normal. As a child she had rubeola, varicella, and pertussis without complications. At age 10, albuminuria was noted but no renal disease was found. At age 11, tonsillectomy was performed. Menses began at age 14 and have remained regular. At age 17, she was hospitalized for one month because of acute hepatitis; her recovery was apparently uneventful.

The patient was first hospitalized because of symptoms of her present illness in 1946 at the age of 19. The findings were: body height, 159 cm; weight, 42 kg; pronounced thinness with atrophy of subcutaneous tissue; marked generalized perspiration; increased skin temperature; normal secondary hair growth and breast development. The blood pressure was 135/75 mm Hg, and the heart rate 90 to 110 per minute. The thyroid gland was of normal size, and the eye signs and tremor of thyrotoxicosis were not present. Patellar reflexes could not be elicited, but neurological examination was otherwise negative. Urine analyses, examination of the cerebrospinal fluid, complete blood count, and electrocardiogram were within normal limits. BMR (basal metabolic rate) was + 172 per cent.

The patient was given methylthiouracil (Figure 1) and large doses of atropine. Her condition improved with this treatment, and in February, 1947, she reported a marked decrease in perspiration, asthenia, and polyphagia. Body weight had increased from 42 to 50 kg, and the BMR had decreased from + 170 per cent in October, 1946, to + 120 per cent. The thyroid gland was now moderately enlarged, and the tachycardia less pronounced (70 to 90 per minute). Thiouracil administration was stopped in March, 1947. In April BMR was again + 150 per cent, the increased perspiration and tachycardia had reappeared, and the body weight had decreased 2 to 4 kg.

When she was rehospitalized in August, 1947, her symptoms were as pronounced as in October, 1946. Body weight was 40 kg. The deep tendon reflexes could not be elicited. BMR was now + 170 per cent and plasma cholesterol 179 mg per 100 ml. Daily urine volumes were usually 0.5 to 1 L with a specific gravity of 1.014 to 1.034. Her daily food intake on free selection was calculated to be 2,500 to 3,000 calories daily. X-ray examinations of sella turcica, lungs, trachea, and heart were normal; air encephalography was also normal. Analyses of blood, urine, and cerebrospinal fluid were again normal.

The patient was treated initially with diiodotyrosine and thereafter with large doses of Lugol's solution and methylthiouracil (Figure 1). There was at first marked improvement with a concomitant decrease of the BMR to + 78 per cent (lowest value hitherto noted). This improvement was only transitory, however, and therefore a sub-total thyroidectomy was performed on December 4, 1947. The thyroid gland at operation was slightly enlarged but otherwise grossly normal. Histological examination showed the characteristic findings of colloid goiter without lymphocytic infiltration. The postoperative course was uneventful. The patient's condition improved somewhat but the symptoms soon recurred. Half a year after surgery the BMR was + 260 per cent.

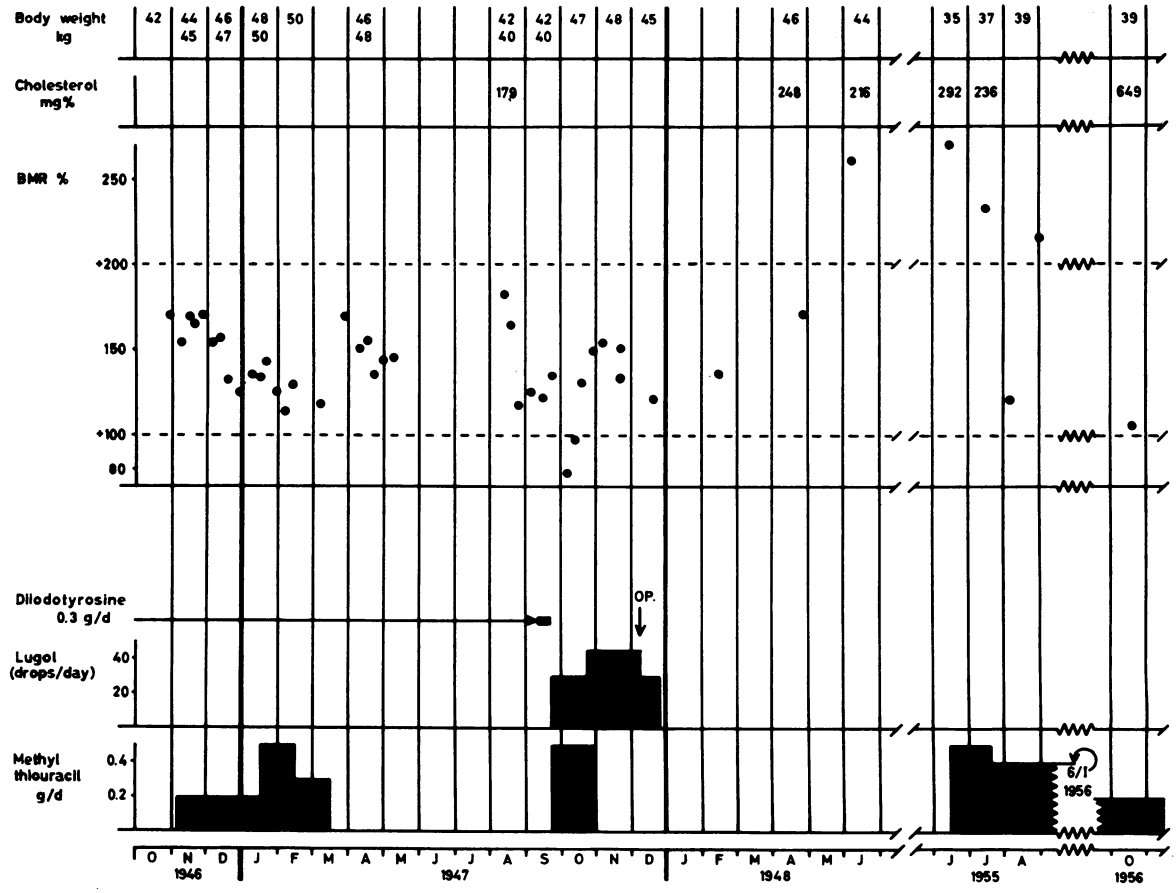

FIG. 1. BODY WEIGHT, SERUM CHOLESTEROL, AND BMR AS WELL AS TREATMENT OF THE PATIENT WITH HYPERMETABOLISM DURING THE PERIOD 1946–1956. Normal value for BMR, 0 per cent.

The patient was thereafter not seen by a physician until June, 1955. Her condition had remained practically unchanged. Body weight had decreased to 35 kg, the asthenia was severe, and she was complaining of exertional dyspnea. BMR was + 270 per cent and plasma cholesterol 292 mg per cent. She was given large doses of thiouracil from June, 1955, to November, 1956. In October, 1956 she appeared clinically hypothyroid with myxedematous facies, scanty hair growth on the head, appearance of lanugo hair on the trunk and extremities, dry skin, and amenorrhea for the last six months. Cholesterol was now 650 mg per cent. Despite this, the hypermetabolism persisted, as shown by BMR + 106 per cent and pulse rate 90 to 110 per minute. Her perspiration had decreased significantly.

The treatment was discontinued in November, 1956. The hypothyroidism disappeared, and the patient returned to her previous state.

The patient was referred to us in May, 1958. She had received no medication since 1956. Her complaints were, on the whole, unchanged. Her body weight was 37 kg; her height was 160 cm. She showed profuse perspiration and increased skin temperature at rest. Other signs of thyrotoxicosis (tremor, eye signs) were absent. The thyroid gland was not enlarged. Blood pressure was 125/65 mm Hg, and the pulse rate was around 100 per minute. Rectal temperature usually varied between 36.8° and 37.8° C but occasionally increased to as much as 38.4° C. A soft systolic murmur was audible over the whole precordium. Secondary hair growth and breast development were of female type. Her caloric intake on free diet was between 3,000 and 3,500

calories per day, while her body weight remained constant.

Neurological examination revealed poorly developed musculature with hypotony and hyperflexibility; muscular strength was decreased but pareses were not present. The patient while lying could not lift her head or sit up in bed without turning. Tendon reflexes were absent, abdominal reflexes were present, and Babinski's sign negative. There were no signs of myasthenia or myotonia. The electromyogram showed moderate voluntary activity generally consisting of narrow and polyphasic action potentials consistent with myopathy.

The results of the main laboratory examinations are summarized in Table I. The only abnormal findings were a BMR varying between + 140 and + 210 per cent and creatinuria of 240 to 400 mg per 24 hours. BMR was unchanged by administration of 100 μg triiodothyronine daily for six days. The following laboratory tests also gave results within normal limits: urinalysis, urinary amino acids, serum calcium, phosphorus, iron, sodium, potassium, chloride, CO_2, and total protein. Serum protein electrophoresis, liver function tests, peripheral blood picture, and bone marrow examinations were normal. X-rays of sella turcica and ophthalmoneurological and otoneurological examinations were negative.

Electrocardiograms showed sinus tachycardia and right deviation of electrical axis. Working capacity, measured according to Sjöstrand and Wahlund (5, 6), was markedly decreased to 200 kg per minute (normal value for body size, 550) with a normal reaction on ECG. Blood volume was increased to 4.31 L (normal value, 2.2) and total hemoglobin to 445 g (normal value, 305), while heart size in recumbency was normal, 540 ml. Heart catheterization demonstrated normal findings during rest, but during work the findings

TABLE I

*Laboratory findings in the patient with hypermetabolism during the period 1958–1960**

	1958	1959	1960
BMR, %	+165, +177, +139, +140 (narcosis)	+183, +170	+207, +191, +173
Respiratory quotient	0.74–0.79		
Cholesterol, *mg/100 ml*	284, 244	261	290, 278, 241
PBI, *μg/100 ml*	5, 3, 4.4, 2.5†	5.2	4.8, 3.2, 3.5, 4.5
Radioiodine test			
Uptake 4 hours, %	18		16
Uptake 24 hours, %	26, 11†	34	27
Urinary excretion 24 hours, %	77, 70†	53	46
PBI^{131} 48 hours, %/*L*		0.44	0.29
Urinary excretion per 24 hours			
17-Ketosteroids, *mg*	9, 9, 11, 11, 9, 20‡		9, 8
17-Ketogenic steroids, *mg*	10, 11, 14, 13, 11, 48‡		10, 11
Gonadotrophins, *mouse units*	13–53		
Norepinephrine, *μg*	10, 15, 14, 10		
Epinephrine, *μg*	3, 5, 4, 3		
5-Hydroxy-indolacetic acid, *mg*	4, 5		
Mandelic acid, *μg/mg* creatinine	7§		
Creatine, *mg*	329, 263, 240, 334, 397		397, 398
Creatinine, *mg*	400, 483, 558, 603		
Calcium, *mg*	114, 137, 196, 151		151, 134
Salicylate	neg.		
Blood analyses			
Hemoglobin, *g* %	13.1	12.7	11.8, 12.3
Red blood cells, *millions/cc*	4.2		
Body weight, *kg*	36–38	41–45	40–44

* Abbreviations: BMR = basal metabolic rate, PBI = protein-bound iodine.

† After triiodothyronine 100 μg daily for 6 days; BMR before +183 per cent and +172 per cent immediately after triiodothyronine.

‡ During i.v. ACTH (25 IU for 8 hours).

§ Normal values 2–8.

were of the type described as vasoregulatory asthenia (7), i.e., unchanged arteriovenous oxygen difference and increased minute volume.

X-ray examination of the lungs was negative. Spirometry demonstrated a pronounced increase in the ratio of functional residual capacity to total capacity and of residual volume to total capacity.

Fasting blood glucose, intravenous glucose tolerance test, insulin tolerance test (0.1 IU per kg body weight), and blood glucose after 36 hours of starvation were normal.

SUMMARY

A 35-year-old woman presented a clinical picture characterized by profuse perspiration, polydipsia without polyuria, thinness despite polyphagia, and progressing asthenia. This picture has clearly been present since the age of seven, and may have existed even earlier. The characteristic abnormal laboratory finding has been a markedly increased BMR. Other abnormal findings are those of myopathy with muscular wasting and weakness, absent deep tendon reflexes, pathological electromyogram, and creatinuria.

II. BIOCHEMICAL STUDIES

The biochemical studies were focused on the mitochondria, since this organelle is the site of cellular respiration. Mitochondria isolated from skeletal muscle were chosen for these investigations partly in view of the relatively large quantity of tissue required and partly because skeletal muscle accounts for the major part of body respiration.

The isolated mitochondria were investigated with regard to some basic properties relevant to their functional state, such as respiration, phosphorylation, respiratory control, and ATPase activity. In addition, a complete fractionation study of the muscle homogenate was performed, with the aim of obtaining a quantitative estimate of the total content of mitochondria in the tissue. Finally, determinations were made of certain chemical and enzymic constituents of the muscle as a whole.

A. TECHNIQUES

Biopsies. Specimens of skeletal muscle from the patient with hypermetabolism were taken from the sartorius muscles under local anesthesia with Xylocain without addition of epinephrine. Muscle specimens from subjects without metabolic diseases were obtained in connection with operations. The muscle specimens were placed, immediately after being excised, in cold 0.15 M KCl or 0.25 M sucrose and kept there for the transfer to the laboratory. The muscle was then blotted with filter paper, freed from fat and connective tissue, weighed, and very finely cut with scissors.

Studies with isolated mitochondria. For investigation of the isolated mitochondria, the homogenization was carried out with an all-glass Potter-Elvehjem homogenizer in the "Tris-KCl medium" devised by Chappell and Perry (8), containing 0.1 M KCl, 0.05 M Tris buffer, pH 7.4, 0.001 M sodium ATP, 0.005 M $MgSO_4$ and 0.001 M ethylenediaminetetraacetate. All operations were performed at 0 to 2° C. The homogenate was diluted with Tris-KCl medium to a volume of ten times the initial weight of the muscle and centrifuged in an International refrigerated centrifuge (rotor no. 840) at 650 G for 7 minutes. The supernatant fluid was decanted and centrifuged again at the same speed for another 10 minutes. After discarding the precipitate, the supernatant fluid was centrifuged for 10 minutes at 14,000 G either in a Model L Spinco ultracentrifuge (rotor no. 30) or in the multispeed attachment of the International refrigerated centrifuge (rotor no. 296). The mitochondrial pellet was then resuspended in Tris-KCl medium and recentrifuged as above. After discarding the washing medium, the surface of the tightly packed mitochondrial pellet was rinsed several times with 0.25 M sucrose in order to remove the medium. The pellet was finally resuspended in 0.25 M sucrose to contain mitochondria from 4 g of muscle per ml of suspension.

Respiration was measured by the conventional Warburg technique, mostly in vessels of a volume of 5 to 6 ml. The composition of the medium was as follows: 50 mM KCl, 25 mM Tris buffer, pH 7.5, 25 mM phosphate buffer, pH 7.5, 4 to 8 mM $MgCl_2$, 63 mM sucrose, 10 mM substrate, 0.0125 mM cytochrome c, and when indicated, 1 mM ATP, 30 mM glucose, and an excess of yeast hexokinase (Sigma, Type IV). The final volume was 1 ml per Warburg vessel. The amount of mitochondria added to each vessel varied between 0.7 and 1.4 g of muscle equivalent, according to the mitochondrial yield per gram of muscle. The temperature was 30° C. The vessels were thermo-equilibrated for 6 minutes, and the oxygen consumption was corrected by extrapolation for the time of thermo-equilibration. The reaction was stopped by the addition of 0.2 ml of 5 M sulphuric acid, and inorganic phosphate uptake was estimated according to the isotope distribution method described by Lindberg and Ernster (9).

The ATPase activity was tested with mitochondria from 200 mg (wet weight) of muscle in a medium of the following composition: 50 mM KCl, 25 mM Tris buffer, pH 7.5, 5 mM ATP, and 125 mM sucrose, in a final volume of 2 ml. The tubes were shaken gently at 30° C for 20 minutes. The reaction was stopped with 1 ml of 1 M

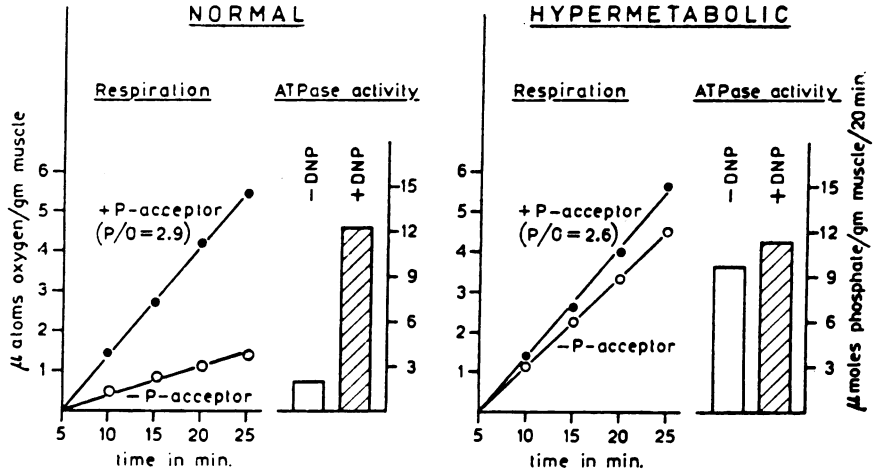

FIG. 2. RESPIRATORY CONTROL, PHOSPHORYLATIVE EFFICIENCY, AND ATPASE ACTIVITY OF ISOLATED SKELETAL MUSCLE MITOCHONDRIA OF A NORMAL SUBJECT (LEFT) AND OF THE HYPERMETABOLIC PATIENT (RIGHT). Substrate: pyruvate plus malate. ATP was added to all vessels. Hexokinase and glucose were added to samples marked "+ P—acceptor." DNP = 2,4-dinitrophenol. For experimental details, see reference (1).

perchloric acid, and inorganic phosphate was measured according to the modified Martin and Doty method (9).

Tissue fractionation studies. For complete fractionation of human skeletal muscle, the procedure recently described by Muscatello, Andersson-Cedergren, Azzone and von der Decken (10) for fractionation of frog skeletal muscle was adapted with minor modifications. The finely cut muscle was homogenized in 0.5 M cold sucrose solution (10 ml per g muscle) in an all-glass homogenizer. An aliquot of the homogenate was removed for assays (see below), and the remainder was centrifuged in the model L Spinco centrifuge, rotor no. 30, at 2,500 rpm (400 G) for 10 minutes. The supernatant fluid was decanted and recentrifuged in rotor no. 40 at 5,000 rpm (1,650 G) for 10 minutes. Six further centrifugations in rotor no. 40 were made, and each time the supernatant fluid obtained in the previous centrifugation was used: at 10,000 rpm (6,500 G) for 10 minutes; at 12,500 rpm (10,000 G) for 10 minutes; at 25,000 rpm (40,000 G) for 30 minutes; at 34,000 rpm (75,000 G) for 40 minutes; at 40,000 rpm (105,000 G) for 60 minutes; and at 40,000 rpm (105,000 G) for 180 minutes. Each of the eight pellets was suspended in 0.5 M sucrose, and aliquots of the suspensions as well as of the initial homogenate were assayed for protein and RNA content and for cytochrome oxidase activity. Protein was determined with the biuret method (11), RNA according to Schneider (12), and cytochrome oxidase activity by the procedure of Potter (13).

Miscellaneous analyses. The determinations of fat, water, sodium, potassium, and chloride in one of the muscle biopsies from the hypermetabolic patient was performed according to standard techniques (14–17). The specimen was freed from visible blood, fat, and connective tissue as far as possible. Total water content was measured by drying to constant weight at a temperature of 95° to 100° C. The fat content of the specimen was thereafter measured by extraction with ether for 16 hours in a Soxhlet apparatus. The dry, defatted specimen (usually 0.1 to 0.2 g) was consequently dissolved in 5 ml of 0.775 M HNO_3 and placed in a boiling water bath for one hour in stoppered tubes. Electrolytes in muscle were measured in the HNO_3 solution after appropriate dilution with redistilled water. Sodium and potassium were measured by flame photometry and chloride by electrometric titration. All muscle analyses were done in duplicate.

Creatine phosphokinase was assayed as follows. An aliquot of the final supernatant fraction obtained in the complete fractionations was incubated at 30°C in the presence of 10 mM creatine phosphate, 1 mM ATP, 8 mM $MgCl_2$, 20 mM glucose, 0.75 mg yeast hexokinase (Sigma, Type IV), and 50 mM Tris buffer, pH 7.5, in a final volume of 1 ml. Double sets of aliquot were removed at 1, 10, and 20 minutes of incubation and assayed for inorganic orthophosphate, both immediately after removal and after 30 minutes of exposure to 1 M sulfuric acid + 1 per cent ammonium molybdate at room temperature. The difference in orthophosphate content between the two samples was taken as an estimate of creatine phosphate (9). The disappearance of creatine phosphate from the incubation mixture, due to transfer of the phosphate to glucose-6-phosphate by way of the creatine phosphokinase and hexokinase reactions, was linear with time and with the amount of muscle extract used.

Thyroxine dehalogenase activity was measured in whole muscle homogenate according to the radiochromatographic method of Tata (18).

B. FINDINGS

Studies with isolated mitochondria. The main results obtained with mitochondria from the first muscle biopsy performed on the hypermetabolic patient and compared with the corresponding data in a normal case are summarized in Figure 2. Respiration was initiated with pyruvate and malate as added substrates (for other substrates, see Table II). The mitochondria were also supplemented with an excess of inorganic phosphate and a catalytic amount of ATP, and oxygen consumption was measured in the presence and absence of terminal phosphate acceptor. Glucose, in the presence of yeast hexokinase, was used as terminal phosphate acceptor, this system converting the ATP formed during oxidative phosphorylation into glucose-6-phosphate, whereby ADP is continuously regenerated.

As can be seen in Figure 2 there was no significant difference between the patient's and the normal mitochondria with regard to either the rate of respiration in the presence of phosphate acceptor or the efficiency of the accompanying phosphorylation. The latter, expressed in terms of P:O ratio (micromoles phosphate esterified per microatoms oxygen consumed), was 2.9 in the control and 2.6 in the patient's mitochondria, both these values being close to the generally accepted maximum of 3. However, the two specimens of mitochondria revealed a striking difference in their extent of "resting" respiration, i.e., when the respiration was measured in the absence of phosphate acceptor. Thus, when hexokinase and glucose were omitted from the incubating medium, the normal mitochondria exhibited, as expected, only a low rate of respiration (about 20 per cent of the maximum, "respiratory control ratio" = 0.20), whereas the mitochondria of the patient displayed a very weak respiratory control, the respiration without hexokinase and glucose amounting to about 80 per cent (respiratory control ratio 0.80) of the rate obtained in the presence of terminal phosphate acceptor.

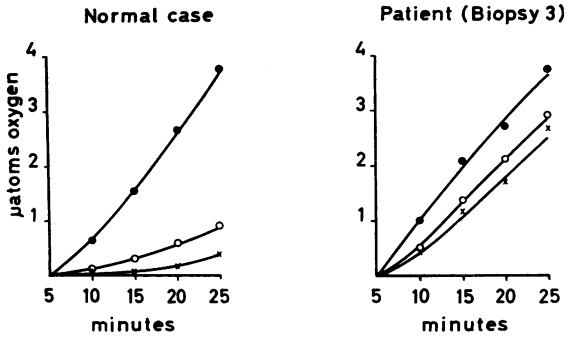

FIG. 3. RESPIRATORY CONTROL IN SKELETAL MUSCLE MITOCHONDRIA FROM A NORMAL SUBJECT AND THE HYPERMETABOLIC PATIENT (BIOPSY 3). ●——● ATP, hexokinase-glucose. ○——○ ATP, no hexokinase-glucose. ×——× no ATP, no hexokinase-glucose. For experimental details, see Techniques.

Another striking finding with the patient's mitochondria, also illustrated in Figure 2, was the presence of a relatively high ATPase activity, which could be stimulated only slightly by the addition of 10^{-4} M 2,4-dinitrophenol. Normally, under identical conditions, the endogenous ATPase activity of the isolated human skeletal muscle mitochondria was relatively low, and could be stimulated four- to fivefold, if this concentration of dinitrophenol (or other uncouplers of oxidative phosphorylation) was added.

The findings described above were consistent with the conclusion that the skeletal muscle mitochondria of the patient were in a so-called "loosely coupled" functional state. This state is characterized by a deficient capacity of the mitochondria to adjust their respiration according to the availability of phosphate, or phosphate acceptor, or both (deficient respiratory control), contrasted by an almost normal capacity for coupled phosphorylation when phosphate and phosphate acceptor are present.

This defect of the mitochondrial function, which has been known to occur experimentally under the influence of various agents of both endogenous and exogenous origin, may be considered as a transitory stage between the normal, "tightly-coupled" state and a state in which phosphorylation is "uncoupled," i.e., in which phosphorylation does not occur even if phosphate and phosphate acceptor are present (see Discussion). In the following studies carried out with mitochondria from three further biopsy specimens of the patient, an attempt was made to define this defect better in biochemical terms.

Previous studies with normal muscle mitochondria revealed the occurrence in these preparations of an ATPase, which is unrelated to the oxidative phosphorylation system (4), and probably origi-

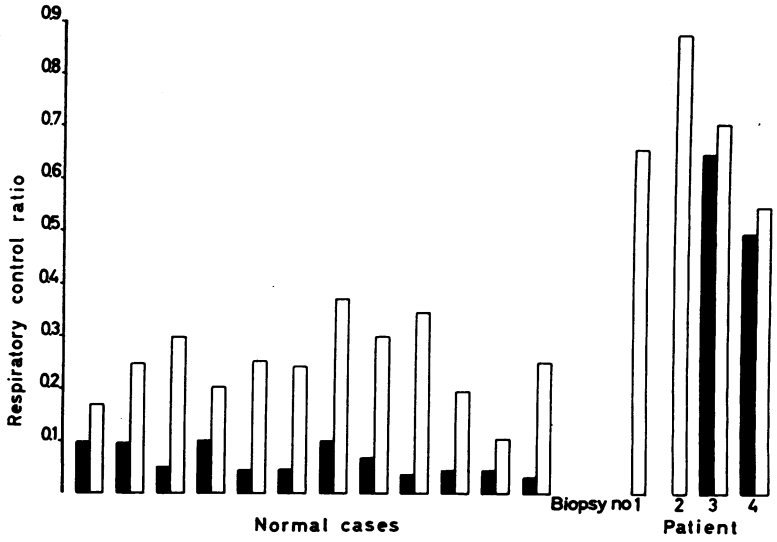

FIG. 4. RESPIRATORY CONTROL RATIOS IN SKELETAL MUSCLE MITOCHONDRIA FROM 12 NORMAL SUBJECTS AND FROM FOUR BIOPSY SPECIMENS OF THE HYPERMETABOLIC PATIENT. The respiratory control ratios were calculated by dividing the oxygen consumption obtained in the absence of hexokinase and glucose ("resting" respiration) by that obtained in the presence of hexokinase and glucose (maximal respiration). The resting respiration was determined under two conditions: without and with a catalytic amount of added ATP. Maximal respiration was always measured in the presence of a catalytic amount of ATP. The *black bars* indicate the respiratory control ratios calculated on the basis of the resting respiration as measured in the *absence* of added ATP, and the *white bars* the respiratory control ratios calculated on the basis of the resting respiration as measured in the *presence* of added ATP.

nates from contaminating sarcotubular fragments (10, 19). It was concluded (4) that the presence of this ATPase may obscure the true extent of respiratory control, if the resting respiration is measured, as above, in the presence of added ATP. It was therefore interesting to determine the extent of respiratory control in the patient's mitochondria under conditions where no ATP was added to the resting system. As reported in Figure 3, this measure resulted in a considerable decrease in the resting respiration of the normal mitochondria, whereas in the patient's mitochondria it influenced only insignificantly the rate of the resting respiration on a percentage basis.

Figure 4 summarizes the respiratory control ratios obtained with and without ATP added to the resting system in 12 normal cases and compares these with available data in the patient. In the case of the patient, the resting respiration without addition of ATP was measured only in two of the four biopsies investigated, since this effect of ATP was not recognized at the time of the first two biopsies. Mean values calculated on the basis of the data in Figure 4 reveal that the respiratory control ratio of the normal and hypermetabolic mitochondria was 0.25 and 0.69 respectively, when resting respiration was measured with added ATP, and 0.06 and 0.57 respectively, when measured without added ATP. The latter values may thus reflect the true extents of respiratory control.

Data reported in Figure 5 concern the effect of dinitrophenol on the ATPase activity of normal and "hypermetabolic" mitochondria in the presence of varying concentration of magnesium ions. In the normal mitochondria, the ATPase activity observed in the absence of dinitrophenol varied with the concentration of Mg^{++}, exhibiting maximal activity at a concentration of Mg^{++} of 0.5 to 1 mM. At all Mg^{++} concentrations, 10^{-4} M dinitrophenol exhibited a marked stimulation of the ATPase activity in accordance with earlier findings (4). In contrast, very little or no dinitro-

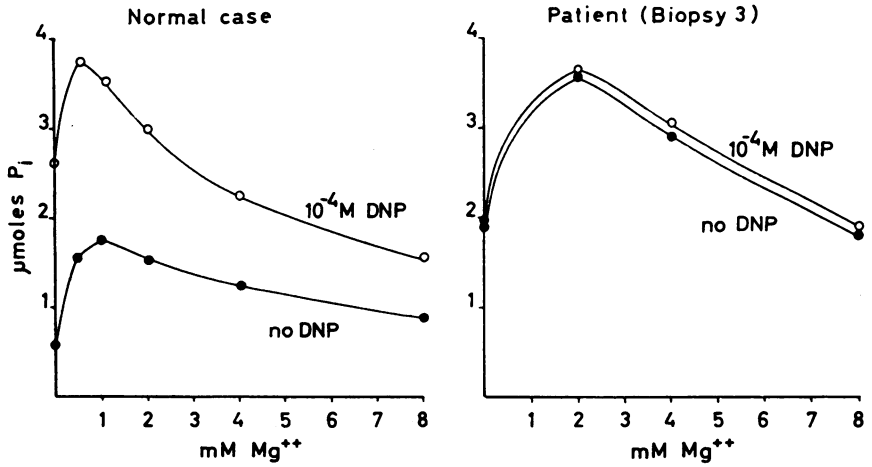

FIG. 5. Mg^{++}-STIMULATED ATPASE ACTIVITY WITH AND WITHOUT DINITROPHENOL (DNP) IN SKELETAL MUSCLE MITOCHONDRIA FROM A NORMAL SUBJECT AND FROM THE HYPERMETABOLIC PATIENT. For experimental details, see Techniques.

phenol stimulation of the ATPase activity was found in the patient's mitochondria, and the curve obtained without dinitrophenol in the presence of varying concentration of Mg^{++} was of the same height and shape as that found in the normal case with dinitrophenol. These findings are complementary to those reported in Figure 2 and show that the loosely coupled state of oxidative phosphorylation in the patient's mitochondria was accompanied by a nearly maximal manifestation of the truly mitochondrial ATPase activity. It should be mentioned that induction of loose-coupling in normal mitochondria *in vitro* has been found to be accompanied by an increase in ATPase activity only in the case of certain treatments, such as aging, but not with others, e.g., addition of dinitrophenol, where a fully uncoupling concentration is needed to elicit maximal ATPase activity (4).

Data concerning the respiration rate and P : O ratio of the patient's mitochondria in comparison with mitochondria from normal cases are reported in Table II. The respiratory data are primarily illustrative of the relative rates of oxidation with different substrates, the two α-ketoacids, pyruvate and α-ketoglutarate, as well as glutamate, giving relatively high respiratory rates, and succinate a relatively low one under the conditions used. In this respect, no deviation was found from the normal cases. Likewise, the amount of mitochondrial protein per gram of muscle, and thereby also the respiration per unit mitochondrial protein, fell within the normal range. However, as will be shown below (see Tissue fractionation studies), the total amount of mitochondria was much higher in the patient's than in the normal muscle. The failure to discover this difference with the present preparation procedure was probably due to the fact that the patient's mitochondria varied greatly in size (see Electron microscopy), and therefore the procedure yielded a lower recovery than normally found. Moreover, it will be seen that the respiratory capacity was also higher than normal, provided that only the cytochrome oxidase activity was measured, and not substrate oxidation which involves the entire respiratory chain.

The P : O ratios exhibited by the patient's mitochondria with various substrates were normal or somewhat lowered (Table II). These findings are concordant with the concept of the loosely coupled state of oxidative phosphorylation (see Discussion). A complete uncoupling of the oxidative phosphorylation could be induced in these mitochondria, precisely as in normal mitochondria, by the addition of 10^{-4}M dinitrophenol (see footnote to Table II).

Included in Table II are also data showing that the respiration of the patient's mitochondria with pyruvate plus malate as substrate could not be stimulated by added DPN; this finding, together with the high respiration observed without added DPN, suggested that the mitochondria were not deficient in DPN. This result may be of some

significance, since it has been found that mitochondria which have been exposed to aging (20) or other treatment *in vitro,* leading to a loose-coupling of phosphorylation from respiration (see 21), often lose their bound DPN and require added DPN to exhibit maximal respiration. It may also be of some interest in this connection that the patient's mitochondria did not become DPN-deficient even after three hours of storage in 0.25 M sucrose at 0° C, nor did addition to the fresh mitochondria of serum albumin, a known protective agent of mitochondrial structure (22, 23), improve their capacity for respiratory control. Thus, the lack of respiratory control in these mitochondria did not seem to be correlated with a general labilization of the structural organization but rather to be of a more specific nature.

It further emerges from the data in Table II that the respiration of the patient's mitochondria, as measured with glutamate as substrate, was strongly inhibited by Amytal. This finding is in agreement with findings with normal mitochondria and eliminates the possibility that the lack of respiratory control in these mitochondria might be due to the activation of a non-phosphorylating pathway of electron transport, which is inducible in normal mitochondria by structural damage and which is characterized by an insensitivity to Amytal (24, 25).

The lack of respiratory control in the patient's mitochondria could further be studied by investigating the effect of oligomycin. Lardy, Johnson and McMurray (26) found in 1958 that oligomycin inhibits mitochondrial respiration under phosphorylating conditions but not when phosphorylation is uncoupled by dinitrophenol. From these and subsequent studies (27–30) oligomycin proved to be a potent and selective inhibitor of tightly coupled mitochondrial respiration, leaving loose or uncoupled respiration totally unaffected, its mode of action probably consisting of a specific inhibition of the reaction of inorganic phosphate with the primary high-energy intermediates of the respiratory chain (see Discussion). In view of this property, oligomycin appeared to be a tool of choice in our studies.

In Figure 6, the effect of increasing concentrations of oligomycin on the respiration of the patient's mitochondria (as measured with pyruvate plus malate as substrate) is compared with corresponding data from two normal cases. It can be seen that in the normal mitochondria, under the experimental conditions used, 0.1 to 0.2 μg per ml oligomycin inhibited respiration to an extent of

TABLE II

Respiration and phosphorylation with various substrates in skeletal muscle mitochondria from four normal subjects and from patient with hypermetabolism *

Case	Mg protein per mitochondria from 1 g of muscle	Substrate	μAtoms of oxygen per minute per mitochondria from 1 g of muscle	P:O ratio
Normal 1	2.5	Pyruvate-malate	0.20	3.0
		α-Ketoglutarate	0.16	2.7
		Glutamate	0.09	2.7
		Succinate	0.05	2.3
Normal 2	1.83	Pyruvate-malate	0.26	2.8
Normal 3	1.74	Pyruvate-malate	0.57	2.5
Normal 4	1.51	Pyruvate-malate	0.40	2.9
Hypermetabolic patient				
Biopsy 1	2.7	Pyruvate-malate	0.22†	2.5
		α-Ketoglutarate	0.28	2.1‡
		Glutamate	0.11§	1.8
		Succinate	0.04	
Biopsy 2		Pyruvate-malate		2.2
Biopsy 3	3.4	Pyruvate-malate	0.27	1.5
Biopsy 4		Pyruvate-malate	0.38	2.0

* For experimental details, see Techniques and reference (1).

† When 1.5 mM DPN was added the oxygen uptake was unchanged, 0.22 μatoms.

‡ When 10^{-4} M dinitrophenol was added the P:O ratio was lowered to 0.2; the respiration was virtually unchanged, 0.26 μatoms.

§ When 2.0 mM Amytal was added the oxygen uptake decreased to 0.008 μatoms.

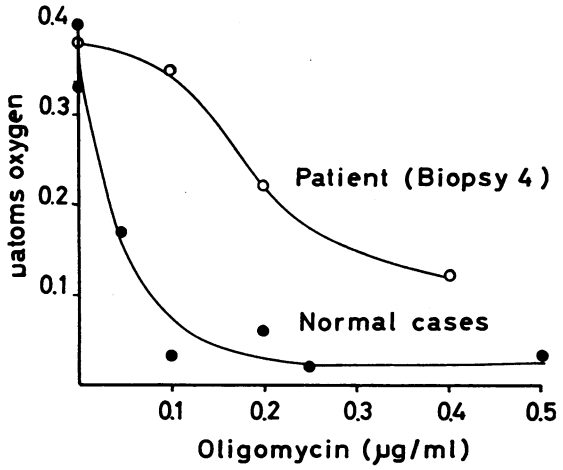

FIG. 6. EFFECT OF OLIGOMYCIN IN THE RESPIRATION OF SKELETAL MUSCLE MITOCHONDRIA FROM TWO NORMAL SUBJECTS AND FROM PATIENT WITH HYPERMETABOLISM. Substrate: pyruvate plus malate. ATP and hexokinase-glucose added to all vessels. For experimental details, see Techniques.

80 to 90 per cent. The maximal inhibition by oligomycin was about 95 per cent which is in accordance with the average respiratory control ratio of 0.06 found in these mitochondria (see Figure 4). In the patient's mitochondria, under the same experimental conditions, no significant inhibition of the respiration occurred with 0.1 μg per ml, and an inhibition of about 50 per cent with 0.2 μg per ml oligomycin. Above this concentration the inhibition tended to level off. In the same biopsy (no. 4) the respiratory control ratio was about 0.5 (see Figure 4), which conforms to the above concept that oligomycin selectively inhibits tightly coupled respiration.

We have briefly reported that skeletal muscle mitochondria from two patients with thyrotoxicosis exhibited biochemical alterations similar to those observed in the present patient (1). In extending these studies to three further cases, however, it was found that the occurrence of these changes in the muscle mitochondria of thyrotoxic patients is not a consistent phenomenon. Thus, only two out of five cases clearly revealed a loose coupling of the oxidative phosphorylation, one exhibited a slightly subnormal respiratory control, and the two remaining cases showed a fully normal picture. No correlation was found between the severeness of the thyrotoxicosis and the state of respiratory control. Possible reasons for this inconsistency will be discussed later (see Discussion).

Tissue fractionation studies. Electron microscopical examination of sections of the muscle specimens from the patient revealed a large increase in the number of mitochondria as well as great variations in the individual mitochondrial size (see Morphological studies). Prompted by these observations, complete tissue fractionation of one biopsy specimen (no. 4) from the patient and nine specimens of normal muscle were performed. The procedure recently worked out by Muscatello, Andersson-Cedergren, Azzone and von der Decken (10) for fractionation of frog skeletal muscle was adapted (see Techniques). Each pellet was analyzed for protein content and cytochrome oxidase activity. The results, expressed in milligrams of protein per gram of muscle and in microliters of oxygen per milligram of protein, respectively, are plotted against the logarithms of the total applied centrifugal force (gravity × minutes) in Figure 7.

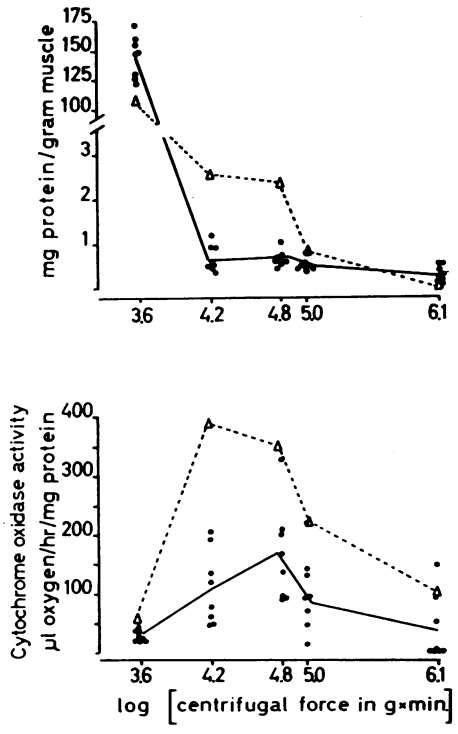

FIG. 7. PROTEIN CONTENT AND CYTOCHROME OXIDASE ACTIVITY OF VARIOUS FRACTIONS OF SKELETAL MUSCLE HOMOGENATE. ●——● normal subjects. △----△ hypermetabolic patient. For experimental details, see Techniques.

TABLE III

Miscellaneous biochemical findings in skeletal muscle from normal subjects and from patient with hypermetabolism *

	Normal subjects		Hypermetabolic patient Biopsy no. 4
	No. of cases	Mean value and range	
Protein, *mg/g* muscle	9	176 151–202	170
RNA, *mg/g* muscle	8	52.9 20.5–101.9	108.9
Water, *ml/kg* fat-free muscle	20	787 770–806	787
Fat, *g/kg* muscle (wet weight)	20	30.5 10.0–74.0	66.5
Sodium, *mEq/kg* fat-free muscle (dry weight)	20	143 108–185	160
Potassium, *mEq/kg* fat-free muscle (dry weight)	20	370 335–433	365
Chloride, *mEq/kg* fat-free muscle (dry weight)	20	105 71–143	104
Creatine phosphokinase, *μmoles* creatine phosphate/*min/mg* protein	2	250 218–282	240
Thyroxine dehalogenase, *mμmoles* thyroxine dehalogenated/10 *min/g* muscle	2	5485 4850–6120	3980

* For experimental details, see Techniques.

As can be seen, both set of values were considerably higher in the patient's muscle than in the controls. Moreover, the increase in protein content per gram of muscle was confined to those fractions which contained the bulk of the cytochrome oxidase. Thus the heaviest fraction, which accounted for the major part of the total protein but was relatively poor in cytochrome oxidase, had an unchanged or even slightly lower protein content per gram of tissue in the case of the patient's muscle as compared with the control specimens. Likewise, the lightest fractions, as well as the final supernatant fluid, which were completely devoid of cytochrome oxidase and are not plotted in the figure, had roughly equal protein contents in the patient's muscle and the controls. These results, therefore, are in agreement with the conclusion that the patient's muscle contained an increased amount of mitochondrial protein which, in turn, is consistent with the electron microscopical findings. From the above data it appears that the increase in mitochondrial protein is about three- to fourfold as compared with the controls.

From Figure 7 it is evident that the cytochrome oxidase activity per milligram of protein was also increased. This was especially conspicuous in the fraction obtained at $10^{4.2}$ G × minutes, which probably contained the largest mitochondria. As shown in the electron microscopic section (see Morphological studies), the large mitochondria in the patient's muscle were especially abundant in cristae.

Miscellaneous analyses. Table III summarizes data on protein, RNA, water, fat, sodium, potassium, and chloride contents, as well as creatine phosphokinase and thyroxine dehalogenase activities of the patient's muscle as compared with those of a number of normal muscle specimens. With the possible exception of the RNA and fat content, which were both at the upper margin of the normal range, all values were normal. Determination of the creatine phosphokinase activity was of interest in view of the marked creatinuria. The finding of a normal activity of this enzyme appears to exclude the possibility that the mechanism of creatine phosphate formation as such was impaired. It appears therefore more likely that the high creatinuria is a secondary consequence of an impaired ability to maintain a normal level of creatine phosphate in the muscle.

The normal level of thyroxine dehalogenase activity is in accordance with the nonthyroid origin of the hypermetabolic state of the patient. Tata has shown that this enzyme is elevated only in hypermetabolism induced by thyroid hormone (31).

Preliminary measurements have also been made of the activity of a third enzyme, the so-called "sarcotubular ATPase" (10), which has recently been implicated in the process of muscle relaxation (19). These measurements were carried out in the postmitochondrial tissue fractions, according to the procedure described by Muscatello, Andersson-Cedergren, Azzone and von der Decken (10). Markedly low activities were found in the patient's muscle as compared with a number of normal muscle specimens. However, a more detailed investigation of this finding, involving examination of the individual tissue fractions by electron microscopy in order to ascertain the degree of contamination of the myofibrillar fraction with sarcotubular fragments (10), is needed before its significance can be settled.

C. SUMMARY

Studies with isolated skeletal muscle mitochondria reveal a loosely coupled state of the oxidative phosphorylation in the patient's mitochondria, characterized by a nearly maximal rate of respiration in the absence of phosphate acceptor, parallel to an essentially normal P:O ratio when phosphate acceptor is present. The sensitivity of the respiration to oligomycin is only partial. The mitochondria exhibit a high Mg^{++}-dependent ATPase activity, which is only slightly stimulated by dinitrophenol.

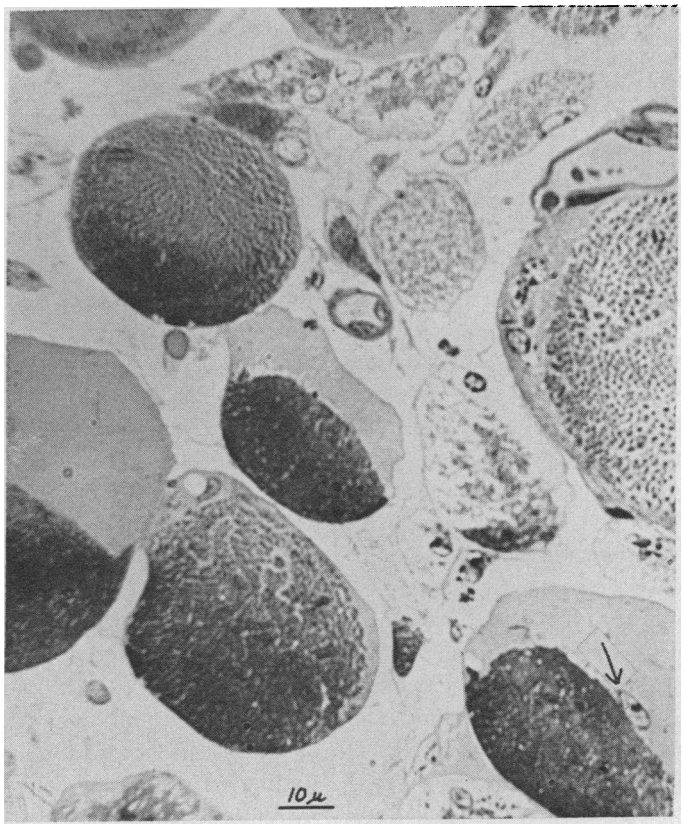

FIG. 8. CROSS-SECTION THROUGH SKELETAL MUSCLE FIBERS FROM THE HYPERMETABOLIC PATIENT, STAINED WITH BASIC FUCHSIN AND CRYSTAL VIOLET AND PHOTOGRAPHED IN A LIGHT MICROSCOPE. All fibers have a rounded cross-section and many of them contain a crescent-shaped zone of a diffuse substance that is only faintly stained. Magnification: 700×.

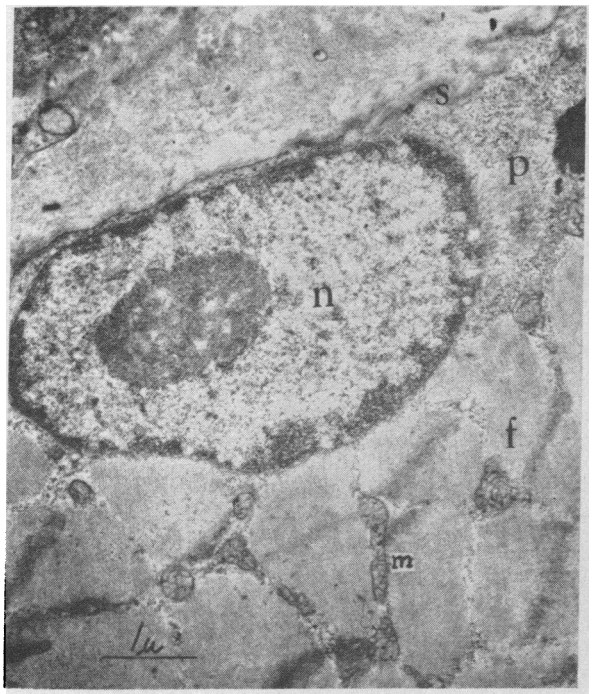

FIG. 9. ELECTRON MICROGRAPH OF A THIN SECTION THROUGH THE PERIPHERAL PORTION OF A MUSCLE FIBER FROM A HEALTHY SUBJECT. The myofibrils (f) are tightly packed and there are only a few mitochondria (m) between the myofibrils or between the sarcolemma (s) and the myofibrils. The nucleus (n) with its dense nucleolus is seen close to the sarcolemma. Numerous dense particles (p) surround the nucleus. Magnification: 12,000×.

Complete centrifugal fractionation of the muscle homogenate reveals the presence of a three- to fourfold increase in total mitochondrial protein, together with an increased cytochrome oxidase activity per unit mitochondrial protein.

III. MORPHOLOGICAL STUDIES

A. LIGHT MICROSCOPY [2]

Findings

The cross-sectioned muscles from the hypermetabolic patient showed individual fascicles of normal appearance (Figure 8). A few of the muscle fibers were lacking and replaced by collageneous connective tissue.

The diameter of the individual fibers varied; they were of normal size or somewhat smaller than normal but in no instance hypertrophic. The cross-sectional outline of a muscle fiber was usually rounded instead of the normal polygonal shape. The sarcolemmal nuclei as a rule took a normal hypolemmal position. In some of the fibers, however, there were centrally located nuclei; these were of normal size or somewhat smaller and denser. In the thin fibers there was a relative increase in the number of nuclei.

A certain proportion of the muscle fibers (approximately 10 to 50 per cent in different fascicles) showed signs of degeneration. The fibrils of many fibers were absent within a peripheral zone; this zone was crescent or half-moon shaped or of irregular shape. The size of the zone was quite variable.

In sections stained with basic fuchsin and crystal violet (32) this zone was made up of a faintly stained diffuse material. The nuclei were not infrequently found in the interior of the fibers at the border between this diffuse zone and the regular portion of the fiber (arrow in Figure 8). In sections stained with hematoxylin and eosin this diffuse zone either did not take stain or contained some eosinophilic granules or filaments. In sections stained with the PAS (periodic acid-Schiff) technique some of the diffuse zones contained PAS-positive granules; the granules in general were sparsely distributed, but in a few fibers they had fused together to form a rather large homogeneous mass. The myofibrils in the degenerated fibers with diffuse zones either were of a normal appearance or were fused together.

The endomyseal connective tissue contained

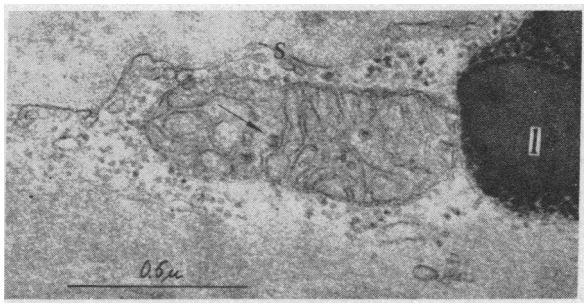

FIG. 10. A PORTION OF THE SAME MUSCLE AS SHOWN IN FIGURE 2, REPRODUCED AT HIGHER MAGNIFICATION SO AS TO SHOW THE DETAILS OF THE MORPHOLOGY OF THE MITOCHONDRION. The outer membrane and the many inner membranes (cristae) can be seen. There are some electron dense particles in the mitochondria (arrow). To the right is a so-called lipofuscin body (1). Sarcolemma at s. Magnification: 48,000×.

[2] Performed by Dr. K. E. Åström.

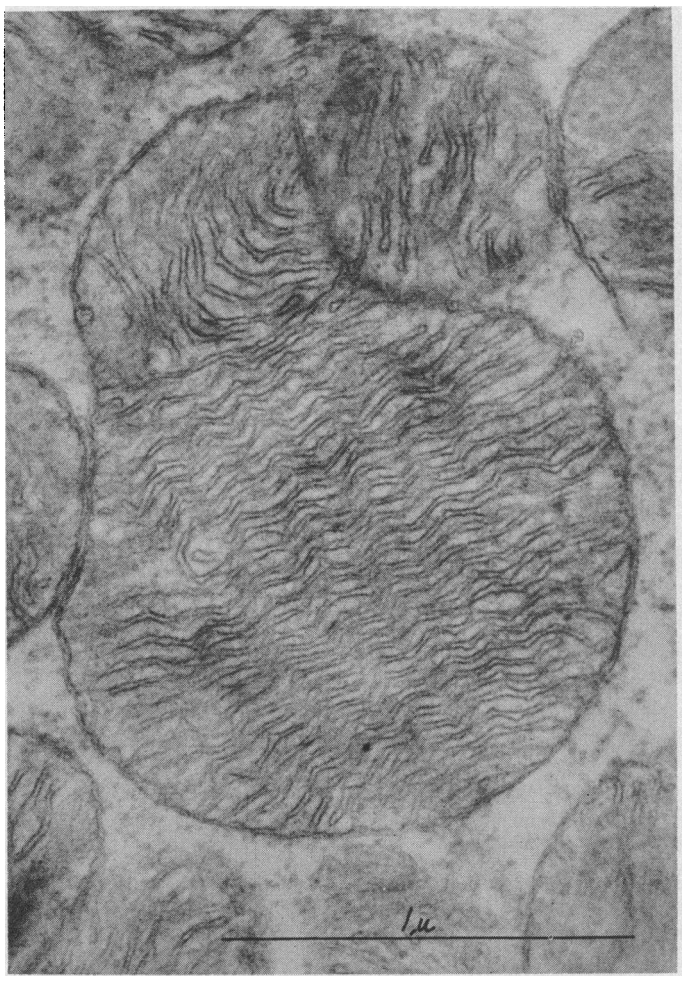

FIG. 11. MITOCHONDRIA FROM THE MITOCHONDRIAL ZONES (SEE TEXT) OF THE HYPERMETABOLIC PATIENT. The zigzag-like arrangement of the densely packed cristae is evident. It can also be noted that the cristae may take a semicircular course due to the membranes taking several turns in the same direction (as in the upper right of the large mitochondrion). There are no electron-dense particles inside the mitochondrion. Magnification: 54,000×.

some round cells. The blood vessels were of a normal appearance.

Comments

The light-microscopical examinations demonstrated degenerative changes of a moderate degree in some muscle fibers, indicating primary muscle damage. These changes do not indicate muscle dystrophy or myositis. They were rather similar to those seen in certain metabolic disturbances, e.g., thyrotoxicosis (33). The nature of the lesions could not be interpreted on the basis of the techniques employed; this especially holds true for the half-moon-shaped hypolemmal formations. There was a discrepancy between the pronounced clinical and the only moderate histological findings. No or hardly any loss of muscle fibers could be seen, which is remarkable in view of the prolonged duration of the disease. Thus, the degenerative changes were reversible.

B. ELECTRON MICROSCOPY

Muscle studies

Relatively little is known about the ultrastructure of normal human skeletal muscle (34, 35).

It was therefore necessary to study specimens from the sartorius muscle of healthy subjects of the same sex and age as our patient.

Techniques

Small (2 mm) cubes were cut from the biopsies and rapidly immersed in a fixative consisting of 1 per cent OsO_4 in a buffered and balanced saline medium essentially according to Sjöstrand (36). After dehydration in a graded alcohol series the samples were embedded in methacrylate (37), Araldite (38), or Epon (39). The tissue blocks were sectioned with a Sjöstrand microtome (40) or with an LKB-Ultrotome and examined in a Siemens Elmiskop I microscope.

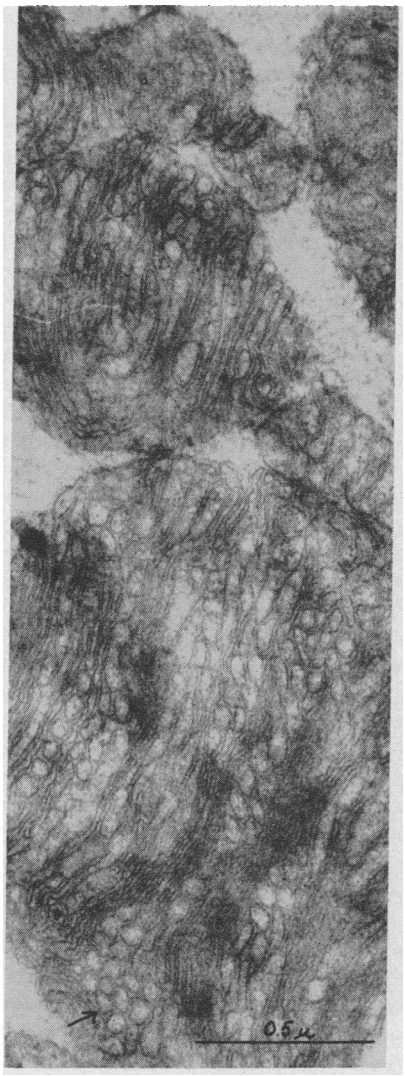

FIG. 12. MITOCHONDRIA OF "TUBULAR" TYPE IN MUSCLE FROM THE HYPERMETABOLIC PATIENT. Instead of cristae of a parallel laminar type there are tubes, which in cross-section give rise to the circular profiles that are visible at the arrow. Magnification: 48,000×.

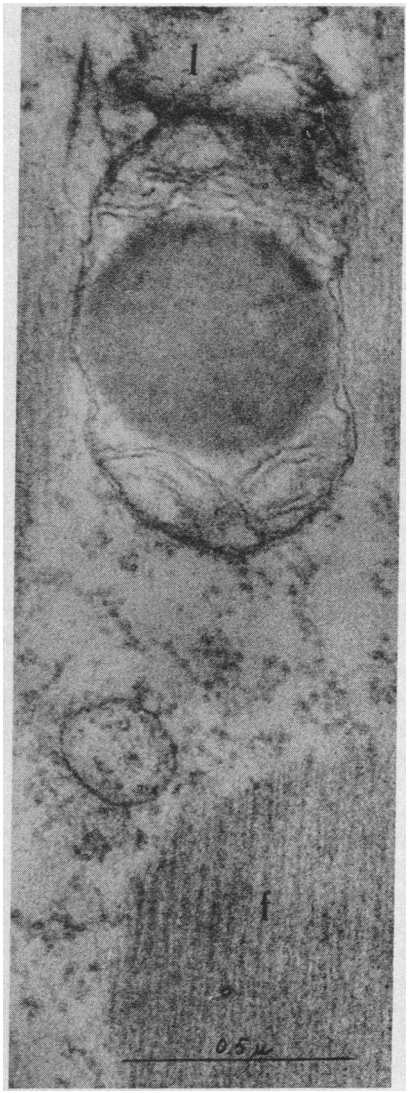

FIG. 13. THIS PORTION OF THE MUSCLE OF THE HYPERMETABOLIC PATIENT SHOWS A MITOCHONDRION BETWEEN TWO MYOFIBRILS (f). In the mitochondrion there is a large dense globule. The mitochondrion is in contact with a particle that presumably represents a lipid droplet (l) at the top of the figure. In the lower part of the figure there is a circular profile with a granular content. This profile is interpreted as being a section through the sarcotubular system. Magnification: 60,000×.

Findings

Normal muscle. A short description follows of the findings in a biopsy from the sartorius muscle of a healthy woman of the same age as the patient.

As seen in Figure 9, the myofibrils were closely packed, and small mitochondria and granules as well as membrane-bounded narrow tubules—sarcotubuli of Andersson-Cedergren (41) or sarcoplasmic reticulum of Porter and Palade (42)—occupied the space between these fibrils. The nuclei took an excentric position near the sarcolemma (or plasma membrane). In suitable sections the nucleus could be seen to contain a prominent nucleolus. Two other types of inclusion were seen in most cells, fat droplets and lipofuscin bodies (Figure 10). The sarcolemma was surrounded by a homogeneous basement membrane.

The mitochondria were of moderate number and uniformly dispersed throughout the muscle fiber (Figure 9). They appeared isolated or in small groups; up to 20 closely packed mitochondria have been found in one section. They had an

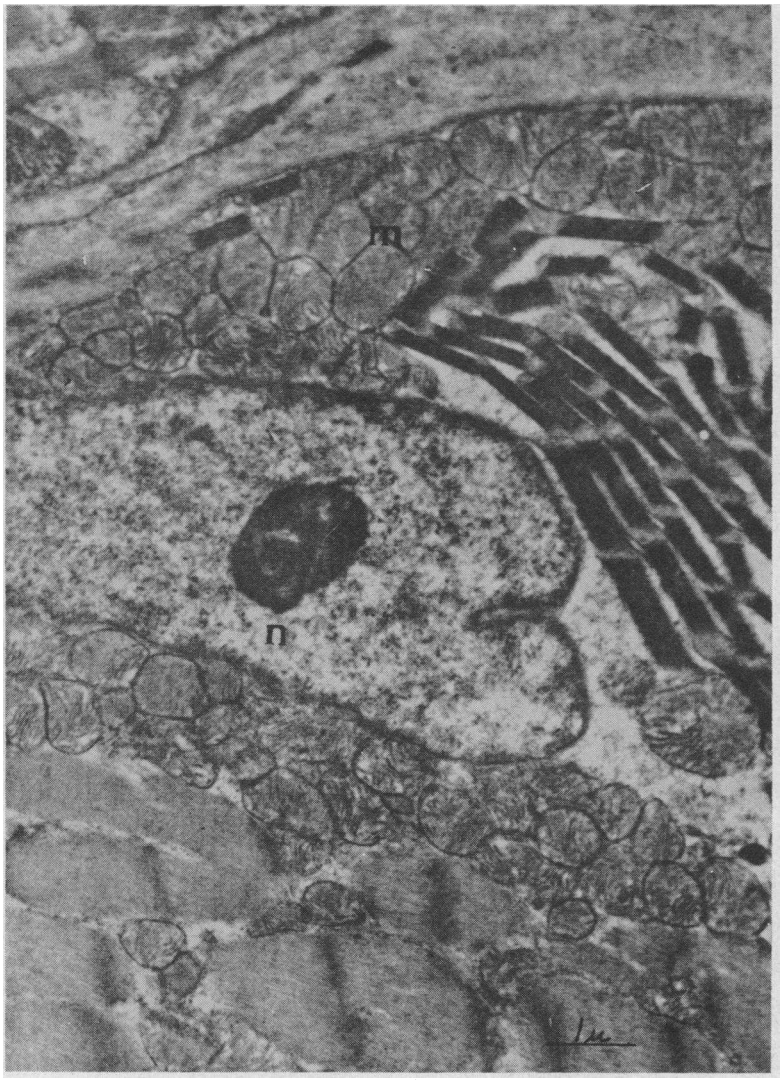

FIG. 14. LOW MAGNIFICATION ELECTRON MICROGRAPH FROM A MUSCLE FIBER OF THE HYPERMETABOLIC PATIENT. The figure shows the cell nucleus (n) in the center and a multitude of mitochondria (m) surrounding it. Below this mitochondrial zone there are the myofibrils with some mitochondria intermingled. In the right part of the figure there is a bundle of dense cell inclusions of unknown character; this type of inclusion is shown at higher magnification in Figures 15 and 16. Magnification: 10,000×.

outer limiting double membrane averaging 170 Å (Figure 9) and a system of inner double lamellae —or cristae mitochondriales; see Palade (43)— averaging 210 Å. The cristae were loosely packed and embedded in a diffuse mitochondrial matrix. The latter also contained opaque bodies with a size of 130 to 260 Å. The size of the cross-sectioned mitochondria rarely exceeded 0.5 × 1.0 μ.

The mitochondrial structure described here agrees well with that of muscle mitochondria from other mammals. The dimensions of the membranes as measured here are the same as those found by Sjöstrand (44).

Muscle from the hypermetabolic patient. Material from four biopsies of the hypermetabolic patient was examined. Twelve of the resulting embeddings were sectioned, and more than 500 electron micrographs taken. There were certain differences in the appearance of the tissue when one embedding was compared with the other. This variation was not due to systematic differences between blocks from the four biopsies but seemed to depend either upon marked local variation in the muscle or on changes brought about in the individual embeddings by factors beyond the control of the investigators. This variability contrasted with the much greater conformity found in the examined embeddings from biopsies of healthy subjects or from those of other patients with metabolic disorders.

The significant features (Figures 11–20) were confined to the appearance of the mitochondria and their derivatives and to the proportion of myofibrils to interfibrillar regions. The appearance of the myofibrils, basement membrane, sarcolemma, sarcotubular system, small granules, fat droplets and lipofuscin bodies, Golgi equivalents, and nuclei was found to be the same as in the normal muscle.

The ratio between the myofibrils and the interfibrillar space was relatively low in muscle fibers from the patient (Figures 13 and 14). This was partly due to the rounded cross-sectional appearance of the myofibrils which gave the interfibrillar substance more space than in normal muscle, the latter having contiguous myofibrils with a polygonal cross-section. Also, there was in a great many muscle fibers a peripheral segment free of myofibrils, usually located around the nucleus, where an abundance of tightly packed mitochondria occupied the space. This region will here be called the "mitochondrial zone" (Figure 14). Such zones of mitochondria with particular appearance were not observed in specimens from normal muscle.

The mitochondria from the four muscle biopsies examined showed a much greater variation in size than the mitochondria from normal muscle (see Figures 10 and 11). This was especially so with the mitochondria from the mitochondrial zones. Large mitochondria were found with cross-sectional dimensions up to 2.6 × 1.9 μ, and small mitochondria with diameters down to about 0.07 μ. It is likely that all or most of the very small mitochondria seen in the sections in reality represented cross-cut, beak-like extensions from large mitochondria. It is notable that the maximal mitochondrial cross-sections were nearly ten times larger than mitochondria from normal human muscle.

A distinction must be made between the mitochondria in the interfibrillar space and those in the peripheral mitochondrial zones. Only those in the mitochondrial zones differed markedly from the mitochondria in the normal muscle specimens. The distinguishing features of these mitochondria resided in an increased number of mitochondrial cristae, in the zigzag-like course taken by the cristae, and in a complete lack of the opaque bodies commonly seen in the mitochondrial matrix (Figure 11). The cristae were tightly packed, and their ratio to matrix was greatly increased.

The zigzag-like pattern of the cristae was likewise a very characteristic feature (Figure 11). The separate segments in the zigzag-bent cristae had fairly regular length dimensions—around 700 Å. Occasionally the cristae showed swelling about 700 Å from the closest knee, without making a new turn, however. At other places in some mitochondria, the cristae did not take a zigzag-shaped arrangement but produced circles or spirals by having several consecutive segments deviating in the same direction. These observations indicate that the mitochondrial cristae contained segments of a definite length.

In thickness the mitochondrial membrane did not differ significantly from that in mitochondria from the healthy subject.

The matrix of the patient's mitochondria was

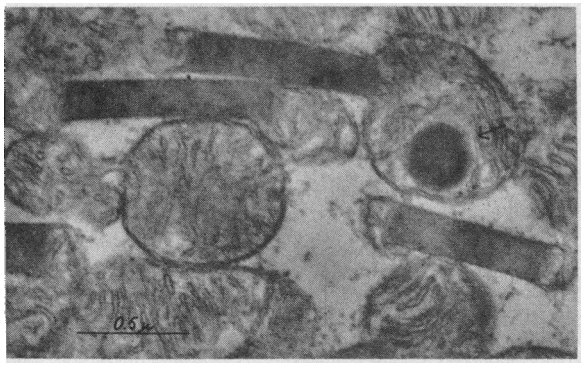

FIG. 15. A UNIQUE TYPE OF CELL INCLUSIONS FOUND IN THE MUSCLE CELL OF THE HYPERMETABOLIC PATIENT. This type of particle has some connection with the mitochondria, as it is located in the mitochondrial zones and has ends of a fairly traditional mitochondrial morphology. The particle in the upper part of the figure (at the arrow) has a mitochondrial body containing a round interior globule and, at the left side, a dense rodlike structure which again ends by a mitochondrion-like knob. Magnification: 29,000×.

quite electron-translucent. As already mentioned, no small opaque bodies were noted in the matrix (Figure 11), in contrast with the situation in the normal muscle (Figure 10) and in most mitochondria studied so far (45). On the other hand, another type of inclusion in the mitochondrial matrix was found: a spherical and moderately electron-dense body (Figure 13). The diameter of this inclusion varied between 0.2 and 0.85 μ. It was thus of a size suitable for light-microscopical investigations, although attempts to see them by light microscopy were unsuccessful. These spherical inclusions were rather rare, although they were found in most of the embeddings, and both in mitochondria from the mitochondrial zones and in those of the interfibrillar regions.

Three rather unique cytoplasmic structures were seen in the biopsies from the hypermetabolic patient, all of which are believed to represent modified mitochondria.

The first category (Figure 12) was a mitochondrion of a "tubular" type. It was characterized by many tubules, 450 to 600 Å wide, which to a large extent replaced the normal cristae. When densely packed the tubules occupied a great volume of the mitochondrion, giving the cross-sectioned area a honeycomb-like appearance. It was common to find a row of cross-sectioned tubules, separated from another row of tubules by two or several membranous layers. At other places in these mitochondria there were closely packed membranes.

The second category of mitochondria or mito-

FIG. 16. ANOTHER FIELD CONTAINING THE DENSE RODLIKE PARTICLES. It is believed that the periodic particle at the bottom of the figure and the elongated particles at the top represent projections of the same type of particles sectioned in different planes (see text). Magnification: 52,000×.

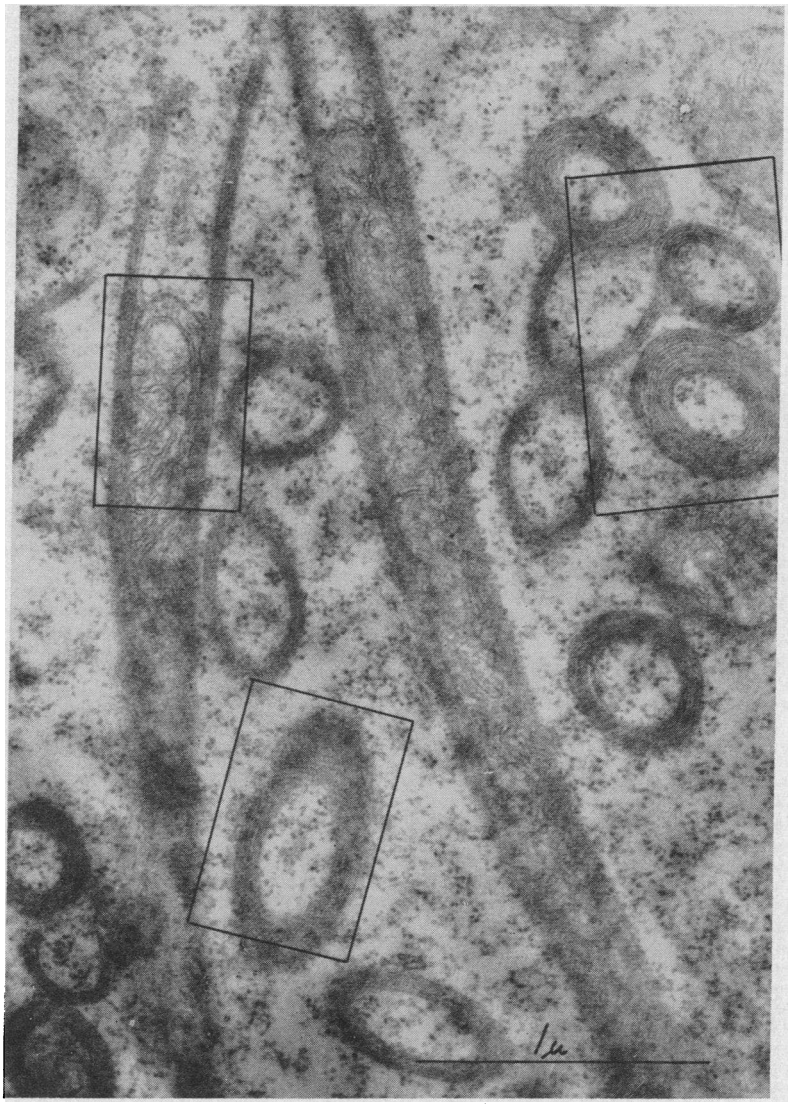

FIG. 17. ELECTRON MICROGRAPH SHOWING SOME CYLINDER-SHAPED INCLUSIONS IN THE MUSCLES OF THE HYPERMETABOLIC PATIENT. One length-sectioned cylinder is shown to the left, and another occupies the central part of figure (both oriented vertically in the figure). It is seen that a mitochondrion-like structure occupies a large portion of the central area of the cylinders. Some ten cross-sectioned cylinders can also be seen. Enlargements of the three marked areas appear in Figures 18, 19, and 20. Magnification: 39,000×.

chondrial derivates were the rodlike inclusions shown in Figures 14, 15, and 16. They consisted of two parts, a dense portion having a paracrystalline nature and a less dense portion with the appearance of a typical mitochondrion. In most cases the less dense portion was quite inconspicuous and formed small mitochondrial knobs at both ends of the rod. By analysing the different shapes that these inclusions could take when sectioned at different angles, it was found that one particular three-dimensional arrangement could account for all the different appearances found. This structure can best be compared with a pile of boards, some 50 boards piled on top of each other (Figure 16). The piles are seen cross-sectioned in Figure 16 and longitudinally cut in Figures 14

and 15. In conclusion, this body can be described as a modified mitochondrial rod, the inner matrix of which has been replaced by an electron-dense regularly layered core.

The third category of structure had a less clear connection to the mitochondria. It had a cylindrical shape, and a wall consisting of three to fifteen concentric layers (Figures 17–20). The length of a cylinder could approach 5 μ, the diameters ranging from 0.3 to 0.5 μ. The cylinders were open, as the layers ended freely at both sides. In the center of the cylinder there were membranes with the appearance and size of typical mitochondrial cristae. The cristae sometimes had a zigzag course. The most interesting aspect of the cylinders was the periodic regularity of the outer layers. Regular periodicities of 100 Å, 130 Å, 170 Å, and 190 Å were found. Often two systems of

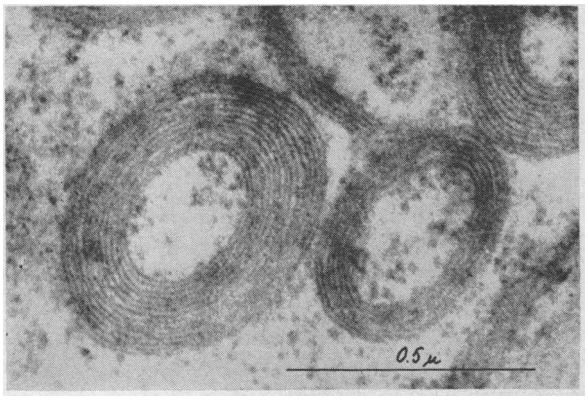

FIG. 18.

FIG. 19.

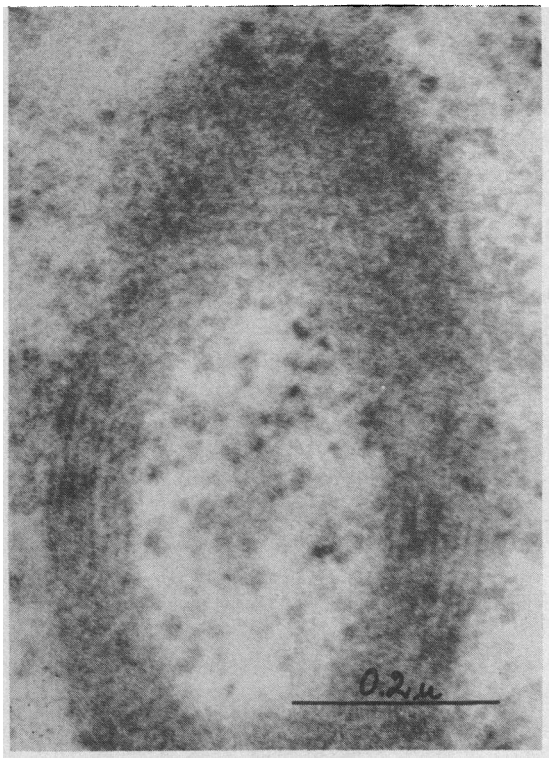

FIG. 20.

FIGS. 18–20. ENLARGEMENTS FROM FIGURE 17. The cylinder-shaped structures are shown in cross-section to contain concentric layers of great regularity (Figure 18). When sectioned lengthwise, the dense layers consist of small granules in periodic arrays (Figure 19, arrow). When sectioned obliquely the cylindrical layers show a checkered pattern due to overlapping of the regularly spaced granules in individual layers (Figure 20). Magnifications: Figure 18, 65,000×; Figure 19, 137,000×; Figure 20, 135,000×.

banding crossed each other at an angle to form a checkered pattern (Figure 20). Such an effect cannot be produced by uniform membranes but has to be explained by regularly spaced densities in the constituting membranes. In contrast to the mitochondrial or the other cytoplasmic membranes, the concentric layers of the cylinders were granular and in thin cross-sections could be depicted as lines of 80-Å thick granules (Figure 19). The cylinders can be regarded as abnormal mitochondria surrounded by concentric sheaths of globular macromolecules of uniform diameter and spaced with crystal-like regularity.

Comments

The following analogies seem relevant. A very high number of cristae in the mitochondria has been described in diaphragm and heart muscle from rats (43, 46) and flight muscle from insects (47), all muscles with high activity. Palade (43, 46) concludes that this finding may be consistent with an increased amount of oxidative enzymes in the mitochondria. A regular zigzag course of the cristae has not been described earlier, but obviously such an arrangement also increases the surface area of these cristae within a given mitochondrial volume.

The absence of opaque granules in the mitochondrial matrix is a most striking phenomenon. As it has only rarely been possible to influence the number of such granules by experimental means (48–50), an evaluation of this lack cannot be made.

Tubules instead of cristae are considered typical of the mitochondria of certain cell types, for instance, most protozoans (51–53). Occurrence of intramitochondrial tubules has also been described in adrenal cortex and other steroid-secreting tissues (54–56) as well as in liver (57). The view has been advanced that tubules may represent the phylogenetically basic type of intramitochondrial structure (58).

The presence of large round globules inside the mitochondria has been noted on rare occasions (59–62). Paracrystalline inclusions have been described from some types of mitochondria, such as those of the retina (63), the brown fat body (64), human liver cells in jaundice (65–67), and frog embryos (68). There are also paracrystalline inclusions in the cytoplasm of the thyroid cells (69) which show a very similar ultrastructure. The cylindrical type of mitochondria characterized by macromolecules in regular periodicity is reminiscent of similar structures in snail spermatids (70).

A combination of all the above modifications of the mitochondria in the skeletal muscle mitochondria of this hypermetabolic patient is most remarkable, and has, to our knowledge, no counterpart in the literature.

Other studies

Skin from the upper arm of the patient was studied in the electron microscope by Dr. I. Brody. The mitochondria did not differ from those in epidermis from healthy subjects as to size, structure, frequency, and electron density (71).

Furthermore, a specimen of the uterine mucosa was removed during day 18 of the patient's menstrual cycle and examined with the electron microscope by Dr. O. Nilsson. The appearance of the glandular cells corresponded to that of the epithelial cells in normal uterine gland from a similar menstrual phase (72).

C. SUMMARY

In the skeletal muscle from the hypermetabolic patient there is an increased number of mitochondria, most of which are collected in mitochondrial zones. The mitochondria are of extremely variable size and contain an increased amount of densely packed cristae, which produce zigzag formations. Furthermore, the mitochondria lack the normally occurring small opaque bodies, but sometimes show rather large electron-dense globules. Finally, there appear markedly altered mitochondria of three different types: some mitochondria with a tubular inner structure, others with an electron-dense regularly layered core, and cylindrical mitochondria surrounded by small granules forming concentric sheaths.

IV. DISCUSSION

The syndrome described in this paper is of great interest both from clinical and general biological viewpoints. Clinically, it represents a new entity,

consisting of endogenous hypermetabolism of non-thyroid origin and of a degree which has not been reported before in the literature. From the point of view of biological interest, the present case would appear to be the first instance of a spontaneous functional defect of the mitochondrial enzyme organization and perhaps of an organized enzyme system in general. In addition, the biochemical and morphological findings constitute a logical background to the clinical picture.

This patient manifested as her most characteristic finding a hypermetabolism, which was exceptional in its severity, duration, and origin. For many years the BMR was well over + 100 per cent, and occasionally values around + 250 per cent were observed. Values of such a magnitude have, to our knowledge, never been reported in humans. According to Means (73), in extreme thyrotoxicosis BMR may reach + 100 per cent, but is usually lower.

The common cause of long-standing hypermetabolism, i.e., thyroid hyperfunction, could be excluded: eye signs of thyrotoxicosis were constantly absent; the histology of the thyroid gland removed at operation in 1947 only showed changes of colloid goiter; the BMR remained above + 100 per cent after subtotal thyroidectomy; intense treatment with thiouracil during 1955 to 1956 induced typical clinical myxedema, but in spite of this the hypermetabolism persisted; and finally, measurements of PBI (protein-bound iodine) studies with radioiodine, including chromatographic analyses of serum organic iodine, gave normal results before and during administration of triiodothyronine. One might speculate whether the hypermetabolic state could be due to an abnormal peripheral sensitivity to thyroid hormone. This possibility can be excluded, however, from the fact that serum cholesterol was normal despite the hypermetabolism, and that the hypermetabolism persisted during the myxedematous state induced with thiouracil when cholesterol rose to 649 mg per 100 ml. Furthermore, the capacity of the muscle to dehalogenate thyroxine, which in experimental animals is closely related to thyroid hormone-stimulated BMR (31), was within normal limits.

None of the known extrathyroidal causes of hypermetabolism could be demonstrated in the patient: acromegaly, anemia, leukemia, and other blood diseases, pulmonary and cardiovascular diseases; pheochromocytoma, and carcinoid tumors could be excluded on the basis of clinical and laboratory findings. A psychogenic basis could be excluded because of the magnitude and duration of the hypermetabolism as well as from the results of BMR measurements during general anesthesia. Chronic intoxication with agents known to induce hypermetabolism (such as dinitrophenol, thyroid hormones, epinephrine, caffeine, or benzedrine) seemed highly improbable, since the disease appeared to have started during early childhood. Chronic salicylate intoxication could be excluded by urinalysis, and thyrotoxicosis factitia from PBI values and tests with radioiodine. Thus, none of the known causes of hypermetabolism could be established in our patient.

The other group of outstanding findings were those referable to myopathy: pronounced muscular weakness, pathological histological findings, muscular atrophy, absence of some tendon reflexes, abnormal electromyogram, and creatinuria. The myopathy was similar to the one in thyrotoxicosis but it could not be classified as one of the other known types of muscular diseases. It is conceivable that the myopathy might have been due to the longstanding hypermetabolism, although it cannot a priori be excluded that myopathy and hypermetabolism were parallel manifestations of one common cause.

The other manifestations of the disease—slight increase in body temperature, increased perspiration, increased thirst, polyphagia, and thinness—could adequately be attributed to the hypermetabolism per se. The persistence of normal thyroid, gonadal, and adrenal function in the presence of the hypermetabolism is remarkable.

Studies with isolated skeletal muscle mitochondria from the patient have added valuable information to the nature of the present disease. The biochemical findings reported in this paper clearly reveal a functional defect of these mitochondria. This defect is characterized by an insufficient, in fact almost absent, ability of the mitochondria to adjust the respiratory rate according to their access to phosphate acceptor. Since this ability of the mitochondria may on good grounds be considered as a basis for the ability of the organism as a whole to adjust its respiration according to the actual demand of energy, this biochemical

finding is in excellent accordance with the clinical picture. The fact that the isolated mitochondria, in spite of their defective capacity for respiratory control, still can produce ATP when phosphate acceptor is present, i.e., that they are "loosely coupled" but not "uncoupled," also fits with the clinical observation that the patient is capable, even though to a limited extent, of performing work. Very probably, a complete uncoupling of phosphorylation from respiration would be incompatible with life.

According to a widely held opinion, a tight coupling between respiration and phosphorylation requires, in addition to an assembly of active enzymes, a proper arrangement of these enzymes within the mitochondrial structure (74). It appears highly probable today that it is these accessory structural factors, rather than the very enzymes, that are in a state of malfunction when mitochondrial respiratory control is impaired. Perhaps the strongest argument for the validity of this concept actually comes from the phenomenon of loose-coupling, since in this state, as also illustrated by the present case, the mitochondria are still capable of a nearly normal extent of oxidative phosphorylation even though their capacity for respiratory control is almost lacking. It may therefore seem safe to conclude that the functional anomaly found in the patient's mitochondria is not due to an enzymic defect in the true sense, at least not so far as the enzymes involved in respiration and phosphorylation are concerned, but rather is a reflection of a malfunction of those accessory structural factors which are responsible for the functional coordination of these enzymes.

The precise chemical mechanism by which these coordinative structural devices operate is not yet understood, and hence, a closer definition of the site and nature of the above defect is not possible at the present time. According to current hypotheses, most of which are based on studies with various uncoupling agents *in vitro,* loss of mitochondrial respiratory control involves a hydrolytic cleavage (75–77), or alternatively, the lack of formation (78, 79) of some intermediates in the process of oxidative phosphorylation which may manifest itself as a loose-coupling or uncoupling, according to the severeness of the defect. A detailed reaction mechanism accounting for the phenomena of loose-coupling and uncoupling, based on studies of the effect of dinitrophenol on normal human skeletal muscle mitochondria, has previously been proposed from this laboratory (4). A further elaboration of this mechanism has been published recently (30) and will not be dealt with in the present context. The following discussion will instead be focused on a consideration of possible endogenous agents which may be responsible for the type of mitochondrial defect found in the present case.

For years, interest has been centered upon thyroid hormone as the regulator of mitochondrial respiratory control in the warm-blooded animal organism. Uncoupling effects observed on mitochondria either treated with thyroxine *in vitro* or isolated from experimentally thyrotoxic animals appeared to support this concept (see 21, 80, 81), and it was in fact with liver mitochondria from thyrotoxic rats that the phenomenon of loose-coupling was first described by Lipmann and coworkers (82, 83). All these findings, however, failed in reproducibility from one experiment to another (84), just as did the observations with the clinical cases of thyrotoxicosis mentioned earlier in this paper (see Biochemical studies). Moreover, recent experiments conducted in our laboratory with liver and skeletal muscle mitochondria from hyperthyroid rats under carefully controlled conditions have given consistently negative results (85). Thus, even if hyperthyroidism were connected with a loosened respiratory control *in vivo,* this state appears not to be consistently preserved at the level of the isolated mitochondrion.

Reports have appeared during recent years from several laboratories describing the occurrence of endogenous uncouplers in both mitochondria (75, 77, 86–88) and the extramitochondrial space of the cell (89) . These agents, characterized chemically as fatty acids, lipids, or proteins, have been considered as inactive in exerting their uncoupling effect under normal conditions but able to become activated under certain conditions. The idea has been proposed (21, 89) that such endogenous uncouplers, in fact, may be the instruments for regulating the metabolic rate of the organism. It is not inconceivable, therefore, that in the present case we have to deal with an undue activation of such an endogenous uncoupler. However, better knowledge about the nature and mode of action of

these agents is needed before this question can be settled.

Attempts to reconstruct the organized enzyme system of electron transport and oxidative phosphorylation by recombination of single enzymic units isolated from mitochondria have met with considerable success during recent years (90, 91). It is increasingly obvious from these studies that besides the enzymes additional components, mostly lipids or lipoproteins, are needed for such a reconstruction. The role of these may be to ensure the proper arrangement of the catalysts. There are also indications that these structural components may be endowed with a high degree of chemical specificity (90). In the light of this reasoning it might not be inconceivable that a defect of the coupling between oxidation and phosphorylation of the type observed in the present case might be connected with a chemical aberration—possibly genetic in nature—of some structural component of the mitochondria.

In this connection it may be relevant to discuss the structural changes observed in the mitochondria of the patient. The striking features of these changes were an enormous enlargement of the mitochondria in certain regions of the skeletal muscle, as well as a pronounced abundance of cristae in these mitochondria. It should be pointed out that although characteristic structural changes can be induced in liver mitochondria by various agents both *in vitro* (92–94) and *in vivo* (95–97), these changes are very different from those found here; they consist of a swelling of the mitochondrial body, connected with an uptake of water and characterized by a decrease rather than an increase in the tightness of the cristae. The morphologically abnormal mitochondria of the patient rather resemble mitochondria from tissues with very high metabolic activity. The question, therefore, must be left open, whether the observed morphological alterations are connected with a biochemical defect in a primary fashion or whether they are secondary consequences of this defect. It is conceivable that the increase in size and number of cristae as well as in the total number of mitochondria may be the result of a compensatory mechanism by which the muscle cell attempts to overcome the handicap imposed by the decreased biochemical efficiency of the mitochondria.

Finally, the question must be considered whether the present defect in mitochondrial function is confined to skeletal muscle only, i.e., if the patient's disease belongs to the group of muscle disorders. To judge from the electron microscopical pictures taken on specimens of skin and uterine mucosa, both of which revealed normal structure of the mitochondria, this would seem to be the case. It is questionable, however, whether these additional findings are sufficient to give a definite answer to the problem. Furthermore, no biochemical investigation could be performed with tissues other than skeletal muscle from the patient.

SUMMARY

The case of a 35-year-old woman with severe hypermetabolism (BMR usually between + 150 and + 200 per cent) since the age of seven, or probably even longer, is described. The clinical picture is characterized by profuse perspiration, polydipsia without polyuria, thinness despite polyphagia, and progressing asthenia. In addition, there is muscular wasting and weakness, a pathological electromyogram, and creatinuria.

Thyroid function is normal, and measures that normally depress thyroid hormone production (subtotal thyroidectomy and administration of iodine and thiouracil) have no or only moderate effect on the BMR. None of the known extrathyroidal causes of hypermetabolism can be demonstrated. The findings are not consistent with any of the known muscular diseases.

Biochemical studies with isolated skeletal muscle mitochondria reveal a loosely coupled state of the oxidative phosphorylation in the patient's mitochondria, characterized by a nearly maximal rate of respiration in the absence of phosphate acceptor, parallel to an essentially normal extent of phosphorylation when phosphate acceptor is present. The sensitivity of the respiration to oligomycin is only partial. The mitochondria exhibit a high Mg^{++}-dependent ATPas activity that is only slightly stimulated by dinitrophenol.

Complete centrifugal fractionation of the muscle homogenate reveals the presence of a three- to fourfold increase in total mitochondrial protein, together with an increased cytochrome oxidase activity per unit of mitochondrial protein.

Electron microscopic examination of the patient's skeletal muscle reveals an increased amount

of mitochondria in the perinuclear areas of the fibers. The mitochondria are of extremely variable size and contain a vast amount of densely packed cristae. Also other structural changes of the mitochondria are observed, such as tubular inner structures instead of cristae, lack of opaque bodies, presence of dense, regularly layered cores, and occurrence of cylindrical mitochondria surrounded by concentric sheaths.

It is concluded that the hypermetabolic state of the patient is caused by a defect in the mitochondrial enzyme organization, resulting in a severely lowered capacity for respiratory control. The present case appears to represent the first clinical instance of an endogenous defect at this level of biological organization.

ADDENDUM

Since the submission of this paper, a fifth muscle biopsy has been performed on the patient. The main results obtained with this specimen may be briefly summarized as follows.

1. The isolated mitochondria revealed an even more deficient respiratory control than on earlier occasions, the respiratory control ratio (respiration without/with ATP, glucose, and hexokinase) being 0.9. The P : O ratio with pyruvate-malate as substrate was 1.6. As could be anticipated, oligomycin inhibited the respiration only to a maximal extent of 10 per cent, whereas it abolished phosphate uptake completely.

2. No improvement of the respiratory control was observed when inorganic phosphate was omitted from the incubating medium or when endogenous phosphate from the mitochondria was exhausted by means of ATP, glucose, and hexokinase. This finding indicates that the site of the defect is above the level of entrance of phosphate—probably at the level of the primary high energy intermediates—which is in accordance with current views about the site of action of oligomycin (26–30).

3. Determinations were made of the content of coenzyme Q_{10} [by the procedure of Beyer, Noble and Hirschfeld (98)] and of the antimycin A (99) and Rotenone (100) titers of the mitochondria. No significant difference was found in comparison with corresponding values obtained with normal human muscle mitochondria.

4. An extra fraction of "heavy" mitochondria was isolated and compared with the mitochondrial fraction obtained in the usual range of centrifugal force with regards to respiratory control and P : O ratio. No difference was found between the two mitochondrial fractions.

5. The effect of the supernatant fraction from the patient's muscle homogenate on the respiratory control of normal mitochondria was investigated by using the oligomycin sensitivity of the respiration of these mitochondria as a test. No effect was observed, which indicated that the supernatant fraction of the patient's muscle contained no endogenous uncoupling factor.

The details of these findings will be reported separately.

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