The authors wish to express their thanks to Dr. T. H. Jukes and Dr. J. E. Casida for their support and interest in this work, to Dr. K. Sugeno for helpful suggestions and discussions, and to Mrs. Dorinne Tsuchiya for skillful technical assistance.

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Received July 1, 1968. P.S.E.B.M., 1968, Vol. 129.

Respiration Rate of Muscle Mitochondria from Genetically Dystrophic Chickens* (33371)

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Muscle mitochondria of genetically dystrophic animals have been observed to be abnormal morphologically (1, 2). Isolated mitochondria from chicken dystrophic muscle show greater efflux of protein during incubation in the absence of respiratory substrate than do similarly treated mitochondria from normal birds (3). There seem to have been few studies of the respiration of mitochondria from dystrophic muscle. Muscle mitochondria of dystrophic mice reportedly show normal oxygen uptake and normal coupling of oxidation and phosphorylation (4). There are descriptions of some myopathies in human subjects in which there is abnormal respiration involving loosely coupled oxidative phosphorylation in the muscle mitochondria (5, 6). In view of the lack of information on the subject the present investigation was conducted to determine if there is any abnormality in the respiration rate or in respiratory decline in mitochondria from dystrophic chicken muscle.

Materials and Methods. Mitochondria were isolated from the pectoral muscle of 11 normal and 11 genetically dystrophic New Hampshire cockerels. Birds of the two strains had been subjected to similar management and dietary practice since hatching and were one year old at the time of the experiment.

Approximately 15 g of muscle from each bird was immersed in ice-chilled 0.15 *M* KCl solution. The muscle was cut into small pieces and rinsed with the KCl solution. The tissue was then homogenized with 10 vol of ice-chilled Tris-KCl medium (7) using a Virtis homogenizer at a setting of 70 for 45 sec. Myofibrils, nuclei, and unbroken cells were removed by two successive centrifugations at 600*g* for 10 min at 0–4°. The final supernatant fluid was then centrifuged at 8500*g* for 10 min at 0–4°. The mitochondria were washed by resuspension in the isolation medium and recentrifuged at 8500*g*. The mitochondrial pellet was rinsed with 0.15 *M* KCl at a concentration of approximately 3 mg of mitochondrial protein per ml. Protein determination on the mitochondrial suspension was by the biuret reaction (8) after clearing with 0.5% sodium deoxycholate.

Oxygen consumption was measured manometrically. Each Warburg vessel contained 0.9 ml of mitochondrial suspension in a total volume of 3 ml. The medium contained, in addition to the suspension, 50 m*M* KCl, 25 m*M* Tris buffer, 0.02 m*M* cytochrome *c*, 10

* Supported by a grant from The Muscular Dystrophy Association of Canada.

TABLE I. Respiration Rates of Mitochondria from Normal and Dystrophic Muscle.[a]

Incubation period (min)	Incubation at 30° (μl O_2)		Incubation at 42° (μl O_2)	
	Normal	Dystrophic	Normal	Dystrophic
0– 30	8.08 ± 2.24	9.77 ± 3.01	10.76 ± 4.69	14.92 ± 7.50
30– 60	5.60 ± 2.24	6.89 ± 3.01	4.43 ± 2.83	7.31 ± 2.76
60– 90	3.79 ± 2.63	6.12 ± 4.32	2.57 ± 2.34	2.93 ± 4.50
90–120	3.28 ± 2.39	5.54 ± 3.21	1.27 ± 2.68	1.55 ± 2.10

[a] Expressed as μl O_2 uptake per mg mitochondrial protein (± sample standard deviation) during successive 30-min periods.

m*M* α-ketoglutarate, 1 m*M* ATP (diNa salt), 6 m*M* $MgCl_2$, 30 m*M* glucose, and 100 units Sigma hexokinase Type V per ml. The pH of the medium was 7.4

The respiration of each mitochondrial preparation was measured simultaneously at 30 and 42° with duplicate determinations at each temperature. Respiration rate was followed for two hr.

Results. The amount of mitochondrial protein isolated per gram of muscle was similar for the normal and the dystrophic tissue. The respective average amounts were 0.87 and 0.85 mg per g of muscle.

The average respiration rates during four successive 30-min periods of the mitochondria from the birds for each strain are shown in Table I. The oxygen uptake of the mitochondria from the dystrophic muscle was consistently higher than normal during the 2-hr period over which the measurements were made. The differences were apparent at both incubation temperatures. The difference in oxygen uptake over the 2-hr period of mitochondria from the normal and dystrophic muscle was significant for the mitochondria maintained at 30°. At 42° the difference between the strains was significant only for the first 30-min period. Although the average rates of oxygen uptake over the 2-hr period were similar at the two temperatures, there was a faster rate of decline in oxygen uptake in the mitochondria incubated at the higher temperature. The relative response in respiration of the mitochondria from the normal and the dystrophic muscle to the two temperatures was similar.

Discussion. The increased rate of oxygen uptake of mitochondria from dystrophic muscle observed in this experiment when the mitochondria were incubated in the presence of Mg^{2+} and ATP is significant in relation to a number of other reports. It has recently been reported that Mg-ATPase activity is elevated in fragmented sarcoplastic reticulum preparations of muscle from human subjects with progressive muscular dystrophy (9) and from dystrophic mice (10). Corroborative evidence of involvement of enhanced ATPase activity in dystrophic muscle is provided by the observation that ATP level in mouse dystrophic muscle is low (11) and that the ATP:ADP ratio is low in human dystrophic muscle (12). Lehninger (13) has hypothesized that when the ratio of ATP:ADP in the mitochondria is low, the conformation of the respiratory assembly is swollen and that when the ratio is high it is contracted. The high rate of respiration in dystrophic muscle mitochondria in the presence of Mg^{2+} and ATP observed in the present study, together with the evidence noted from the literature, suggest that a low level of mitochondrial ATP, as a result of elevated ATPase activity, may be responsible for the microscopic appearance (1,2) and altered permeability (3) of mitochondria from dystrophic muscle.

Summary. Pectoral muscle mitochondria were isolated from normal and genetically dystrophic year-old New Hampshire cockerels. The mitochondria from the dystrophic muscle showed an elevated rate of oxygen uptake when incubated in a medium containing Mg^{2+} and ATP over a 2-hr respiration period. It is suggested that accelerated loss of ATP from mitochondria in dystrophic muscle

is responsible for the abnormal permeability of the mitochondrial membrane and the altered microscopic appearance.

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Received May 13, 1968. P.S.E.B.M., 1968, Vol. 129.

Gold Thiomalate–Induced Weight Gain in Guinea Pigs* (33372)

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Obesity can be induced in mice with a single dose of gold thioglucose (1). After injection, gold localizes in the neurones of the ventromedial area of the hypothalamus, presumably due to the special affinity of these cells for the glucose moiety (2–4). The animal becomes hyperphagic and obese when this hypothalamic area concerned with the regulation of food intake is damaged by the heavy metal. Gold compounds other than gold thioglucose are not effective (5, 6). Thus far, gold-induced obesity has been observed only in mice.

In a previous study of the effects of gold sodium thiomalate on the immune response of guinea pigs, treated animals were observed to gain weight at a faster rate than controls (7). This observation was investigated to determine whether the weight increase observed in gold thiomalate–treated guinea pigs was due to a mechanism comparable to that of gold thioglucose–induced obesity in mice.

Materials and Methods. Male Hartley strain guinea pigs were singly caged and given Purina chow and tap water *ad libitum*. Animals were randomly divided into two groups. Gold sodium thiomalate (Myochrysine, Merck) was administered intramuscularly to nine guinea pigs at a weekly dose of 2.1 mg. Ten control animals received a comparable dilution of sodium thiomalate in the same vehicle, but without gold. All animals received a weekly intramuscular injection and were handled identically throughout the experiment. The animals were weighed weekly and observed closely for any abnormalities. No toxic manifestations were observed in either group.

After nine weeks of treatment (ten injections), the animals were sacrificed and examined for retroperitoneal fat deposits. The omentum was carefully dissected, weighed, and repeatedly homogenized in a 2:1 chloroform to methanol mixture (8). The extracta-

* Supported by NIH Research Grant AM 09209 and PHS Training Grant AM 05217.

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