# Thyroxine-Binding by Muscle Mitochondria from Normal and Genetically Dystrophic Chickens[1] (34211)

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There are disparate observations in the literature which suggest that respiration is abnormal in genetically dystrophic muscle and that the course of the disease may be temporarily retarded by treatments which increase the availability of oxygen to the tissue or facilitate the biosynthesis of ATP by the oxidative–phosphorylation system. Mitochondria from dystrophic muscle are abnormal morphologically (1, 2). Mitochondria from dystrophic chicken muscle show an elevated rate of oxygen uptake when incubated in the presence of $Mg^{2+}$ and ATP (3). A low ATP level in dystrophic muscle (4, 5) and the possibility that a low ATP:ADP ratio results in a swelling of the respiratory assembly in mitochondria (6) led to the suggestion (3) that accelerated loss of ATP from mitochondria in dystrophic muscle is responsible for the abnormal permeability of the mitochondrial membrane (7) and the altered microscopic appearance. In studies with canary and rat liver mitochondria thyroxine was found to stimulate ATPase activity (8-10), concomitantly with the induction of mitochondrial swelling. In view of the above it is interesting that administration of large doses of thyroxine to dystrophic mice improves movement (11), and that a high-oxygen environment retards the progress of muscular dystrophy in chickens (12, 13) although only temporarily (14). Treatment of dystrophic mice with coenzyme Q has also been reported to improve their condition (15, 16) and it may be significant that experimental thyrotoxicosis markedly increases the level of coenzyme Q in rat liver mitochondria (17). Evidence of mitochondrial abnormality in muscular dystrophy and the effect, albeit temporary, on the progress of the disease of treatments which appear to relate to augmentation of energy available from mitochondrial function, suggested the following experiments to determine if thyroxine-binding is normal in the mitochondria of dystrophic muscle.

*Materials and Methods.* Mitochondria were isolated as previously described (3) from the pectoral muscle of normal and genetically dystrophic New Hampshire cockerels. The birds of the two strains were approximately 22 months old and had been subjected to similar management and dietary practice since hatching. The final mitochondrial pellet was suspended in Chappell–Perry medium (18) at a concentration of approximately 15 mg of mitochondrial protein/ml. Protein concentration was determined on the mitochondrial suspension by the biuret reaction (19) after clearing with 0.5% sodium deoxycholate.

Thyroxine-binding was determined in four separate tests using mitochondria from two normal and two dystrophic birds each time. In Expt. 1 L-($^{131}$I)thyroxine[2] was bound to chicken serum protein (1 ng of thyroxine/ml of serum) before addition to the mitochondrial suspension. This was accomplished by incubating the thyroxine with chicken serum for 15 min at room temperature. Incubation of the mitochondria with thyroxine was carried out in centrifuge tubes which could be placed directly in the deep-well scintillation detector. The incubation mixture contained 2.4 ml of serum containing the thyroxine, 2.4 ml of Chappell–Perry medium, and 0.2 ml of mitochondrial suspension. Incubation was at room temperature for 15 min. The mitochondria were then sedimented at 8500*g* for

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[2] Abbott Laboratories, Chicago. Radioactivity 57.6 $\mu$Ci/$\mu$g of thyroxine at time of shipment.

TABLE I. L-($^{131}$I)Thyroxine-Binding by Mitochondria from Normal and Genetically Dystrophic Chicken Muscle.

| | Thyroxine prebound to serum protein (cpm/mg of mitochondrial protein) after: | | | |
|---|---|---|---|---|
| Wash: | 1 | 2 | 3 | 4 |
| Normal | 99 | 68 | 59 | 51 |
| Dystrophic | 127 | 94 | 77 | 64 |

| | *df* | Variance | *F* |
|---|---|---|---|
| Strain | 1 | 6847 | 18.91[b] |
| Wash | 3 | 9313 | 25.73[b] |
| Test | 3 | 8360 | 23.09[b] |
| Error | 56 | 362 | |

| | Thyroxine added directly to mitochondrial suspension (cpm/mg of mitochondrial protein) after: | | | |
|---|---|---|---|---|
| Wash: | 1 | 2 | 3 | 4 |
| Normal | 8857 | 6722 | 5533 | 4592 |
| Dystrophic | 9601 | 7302 | 5958 | 4891 |

| | *df* | Variance | *F* |
|---|---|---|---|
| Strain | 1 | 4189702 | 3.68[a] |
| Wash | 3 | 59949430 | 52.66[b] |
| Test | 3 | 8732279 | 7.67[b] |
| Error | 56 | 1138411 | |

[a] Required for significance at 5% level 4.02.
[b] Significant at 1% level.

10 min and washed four times with Chappell–Perry medium. Radioactivity of the mitochondrial pellet was measured after each washing.

In Expt. 2, thyroxine-binding was again determined in 4 separate tests using mitochondria from 2 normal and 2 dystrophic birds each time. In this experiment L-($^{131}$I) thyroxine was added in Chappell–Perry solution in free form to the mitochondrial suspension. Incubation, washing and counting procedures were as in Expt. 1. The relative concentrations of thyroxine and mitochondria were similar to those in Expt. 1. Incubation, washing, and counting procedures were also as in the first experiment.

*Results.* The affinity of the mitochondria for radiothyroxine is expressed as cpm per mg of mitochondrial protein in Table I. Although conditions under which the mitochondria were isolated were kept as constant as possible there were, nevertheless, highly significant differences in the thyroxine-binding of mitochondria isolated at different times. The values for each experiment were therefore subjected to analysis of variance as indicated in Table I. In both experiments the average degree of thyroxine-binding was greater for the mitochondria of the dystrophic muscle. The difference between the normal and dystrophic muscle was highly significant ($p<0.01$) in Expt. 1, but was not significant under the conditions of Expt. 2.

*Discussion.* The findings that mitochondria of normal and dystrophic muscle have a similar affinity for free thyroxine and that they exhibit a difference in affinity when the thyroxine is prebound to serum protein are not incompatible. Incubation of mitochondria with prebound thyroxine, whereby the mitochondria binding sites compete with serum protein for the thyroxine, provides a more sensitive comparison of relative affinity of mitochondria from the two sources. By this technique the competitive affinity of the mitochondria from the dystrophic muscle for thyroxine was significantly greater than normal. The possibility has to be acknowledged that the isolation process may affect the mitochondria of the dystrophic tissue differently than those of the normal. If, in the present experiments, for example, the membrane of mitochondria from dystrophic muscle were more prone to disruption during isolation, the thyroxine-binding capacity might differ from that of mitochondria isolated from normal muscle, even though the binding capacity of the mitochondria *in situ* could be similar in normal and dystrophic muscle. A higher level of mitochondrially-bound thyroxine in dystrophic muscle would be consonant with the swollen appearance and the higher rate of oxygen uptake of mitochondria incubated in the presence of ATP and $Mg^{2+}$. These characteristics of the mitochondria probably represent associated effects of a to-date unidentified primary lesion in inherited muscular dystrophy.

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