TAURINE IN DYSTROPHIC MOUSE MUSCLE

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ABSTRACT. — Taurine concentrations in the rear leg muscles of eight-week-old male mice were compared for normal and hereditarily dystrophic animals. Normal concentrations were determined and found to be higher than previously reported for rats. The taurine concentration found in the dystrophic animal was not significantly different from that found in the normal animal, in contrast with similar experiments involving chickens.

A remarkable increase in the concentration of free taurine (2-aminoethanesulfonic) acid has been found in the dystrophic muscles of chickens (Peterson, 1963). Furthermore, a correlation between severity of the dystrophy and the free taurine concentration has been demonstrated (Peterson, 1965). Because its concentration in normal tissues has an extensive range (Jacobsen, 1968) and muscle injury usually results in excessive elimination (Goyer, 1967) without appreciable alteration of the tissue concentration (Abe, 1968), taurine accumulation in muscle tissue is not considered to be directly responsible for muscular dystrophy. However, if one assumes that the taurine was produced in abnormal amounts from a vital metabolite, such as Coenzyme A (CoA), an interesting relationship can be developed.

The concentration of CoA has been shown to be decreased in cases of both nutritionally induced (Bieri, 1963) and hereditary muscular dystrophy (Radu, 1968). Decreased levels of CoA would be expected to have far reaching effects on muscle metabolism, among which would be altered lipid and energy metabolism. Both have been shown to be factors in muscular dystrophy (Meier, 1967; Olson, 1968). If taurine were produced by an oxidation of the functional portion of CoA, then loss of CoA would produce an increase in taurine. In itself, this has important ramifications for muscle intergrity. Welty and Read (1964) have shown that taurine alters membrane permeability, long recognized as characteristic of muscular dystrophy (Hazelwood, 1968).

Complicating factors in studies of muscular dystrophy are its diversity of form and its variation from species to species. While taurine concentrations are increased and CoA decreased (unpublished results) in dystrophic chicken muscle, a step preliminary to testing a general hypothesis requires comparison of normal and dystrophic mouse muscle to determine if taurine accumulation is characteristic of muscular dystrophy in this animal. Free taurine in normal mouse muscle, not previously reported, must also be determined.

Methods and Materials

A total of sixteen mice obtained from Jackson Laboratories, Bar Harbor, Maine, were used in this study. Six dystrophic animals of the strain 129B6F$_1$/J-dy were compared to six normal littermates and four normal animals of the closedly related strain, C57BL/6J. The rear leg musculature was obtained from the frozen carcass after ether anesthesia, decapitation, and quick freezing on a block of dry ice. The tissue was homogenized by a Model S125 Bronson Sonifier at half maximum power in short bursts for a total period of five minutes. Free amino acids were extracted from the deprotenized homogenate by the method of Awapara (1953). The final extract was passed through a 62 x 1.3 cm column of Dowex 50W-X2 (acid form), using a 0.2 N citrate buffer at pH 3.1 as eluant to affect a separation of amino acids (Moore and Stein, 1954). Taurine was monitored in the column fractions by thin-layer chromatography on cellulose plates using a solvent of methanol:water:pyridine (80:20:4 v/v/v). Detection was by ninhydrin spray. Fractions containing taurine were assayed by the colorimetric ninhydrin method of Rosen (1957). Parts of the extracts were then pooled and submitted for analysis on an automatic amino acid analyser in the Northern Utilization Research and Development Laboratories of the U. S. Department of Agriculture.

Results and Discussion

Taurine concentration determined in our laboratories, reported in Table 1, is expressed as μ moles per gram wet tissue. Because the amount of fat in the animals appeared to be minimal, it seemed unnecessary to report on a fat-free basis. The large increases in taurine previously reported for dystrophic muscle appears not to occur in dystrophic mouse muscle. On the contrary, small but insignificant decreases in the free taurine of dystrophic mouse muscle are observed. Data from automatic amino acid analyses are tabulated in Table 2. Again, the taurine concentration of the dystrophic muscle is slightly lower than that of the normal litter-

Table 1.—Taurine Concentration in Normal and Dystrophic Leg Muscle of Mice[1] (μ mole/g wet tissue).

Tissue	*Trial 1*	*Trial 2*	
Normal - 1	30.46	33.49	
Normal - 2	26.86	25.17	
Normal - 3	24.28	25.21	
Average	27.20	27.96	
Overall Average			27.58
Dystrophic - 1	20.89	21.06	
Dystrophic - 2	26.45	27.52	
Dystrophic - 3	20.62	17.90	
Average	22.65	22.16	
Overall Average			22.41

[1] Each sample represents two animals

Table 2.—Taurine in Leg Muscle of Normal and Dystrophic Mice Determined By Automatic Amino Acid Analyser (μ moles/g wet tissue).

Sample	Description	Taurine
I	Four Normal Animals C57BL/6J	23.88
II	Combination of Normal 1 & 2	28.65
III	Combination of Normal 2 & 3	20.31
IV	Dystrophic 1	19.32
V	Dystrophic 2	22.32
VI	Dystrophic 3	17.79

mates. In addition, the strain C57-BL/6J produces muscle taurine concentrations comparable to those observed in littermates of the dystrophic animals. Thus, it would appear that the reported concentrations are not peculiar to the strain of mice which carry the mutant gene. In any case, the taurine concentrations are higher than had been anticipated from reports of taurine in the rat and rabbit (Jacobsen, 1968). Normal concentrations of free taurine in the muscle of eight-week-old male mice must be in the range of 20-30 μmoles taurine per gram wet tissue.

It has been demonstrated that rat muscle maintains its normal compliment of free taurine even in the face of severe trauma (Abe, 1968). Consequently, measurement of taurine excretion must be determined prior to drawing conclusions about taurine production in these mice. Published reports (Hurley, 1955; Berger, 1962) are somewhat contradictory, but in humans, increases in excreted taurine are reported to accompany some cases of muscular dystrophy.

The accumulation of taurine in the muscle of dystrophic chicken does not occur in the muscle of the dystrophic mouse. Therefore, this is not a parameter characteristic of the diseased state in all species, and increased concentrations of taurine cannot be responsible for muscular dystrophy in the mouse. An involvement of CoA in this disease cannot be eliminated by these experiments since CoA can be decreased in either of two ways—by possible oxidation to taurine and by inhibited biosynthesis. That is, nutritional and hereditary dystrophies producing large amounts of taurine could result from excessive oxidation of CoA, whereas, hereditary dystrophy not yielding large amounts of taurine could result from insufficient CoA systhesis.

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