Serum Aldolase Activity in Neuromuscular Disorders. 2. Experimental Application.* (22945)

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Extensive clinical application of the serum aldolase determination has demonstrated its value in the serologic diagnosis of progressive muscular dystrophy(1,2,3). Muscle atrophies secondary to inanition, disuse or paralysis of cerebral origin have not caused any serum elevation of this enzyme. Muscle atrophy consequent to a lower motor neuron deficit (*e.g.*, recently convalescent poliomyelitis or infantile progressive spinal muscular atrophy) appears to result in a moderate, occasionally evanescent, rise in the serum aldolase, never however to the degree representative of progressive muscular dystrophy. These clinical observations suggest that serum aldolase may reflect atrophic changes due to neuromuscular junction deficit as well as those caused by primary myopathies.

In an effort to substantiate experimentally these findings a series of 33 dogs were submitted to various procedures resulting in muscle atrophy.

Materials and methods. A. 13 mongrel dogs, weighing from 15 to 20 kg each, were subjected to a femoral nerve section, performed in the upper inguinal canal. B. Ten dogs were subjected to an achilles tendon section or suprapatellar tenotomy. C. Four dogs were submitted to a lumbar laminectomy following which the cauda equina roots and the sacral cord were surgically transsected. D. In 3 dogs, the left femoral artery was surgically constricted in the upper inguinal canal. E. Three dogs were submitted to a sham surgical procedure in which the inguinal canal was entered but in which neither the traversing femoral nerve nor artery were violated. Pre-operative serum was withdrawn on all dogs to establish the normal levels of serum aldolase, determined by the technic of Sibley and Lehninger(4) and SGO-transaminase, using the procedure described by Karmen, Wroblewski and La Due(5). At intervals of 3 to 7 days following surgical intervention, serum for these evaluations was withdrawn from all dogs for periods up to 210 days. In addition, periodic biopsies of the involved musculature as well as normal muscle tissue derived from an uninvolved limb were carried out. Resected muscle biopsies were studied histologically to determine the degree of observable atrophy, quantitated by means of measurement of the average muscle fiber diameter microscopically, and the remaining fresh tissues used to determine the level of tissue aldolase and phosphorylase. Tissue aldolase levels were derived by the method described by Sibley and Lehninger (4). The technic described by Cori and associates(6) for the determination of tissue phosphorylase was used. The values of phosphorylase b were utilized in accordance with the greater reliability of this fraction(6). At the termination of all experiments the dogs were autopsied for verification of the procedures employed.

Results. A. *Histologic studies*: An unremittant atrophy of the involved skeletal musculature was noted following neurotomy, tenotomy, cauda equina transsection or vascular occlusion. Muscle fibers displayed a significant diminution in cross sectional diameter, assumed a slightly basophilic appearance and showed a relative increase in sarcolemmal cells. Cross striations, however, were invariably preserved and no fatty infiltration, significant fibrosis or necrosis was observed. The most extensive atrophic alterations were associated with animals subjected to cauda equina transsection (Group C) (Fig. 1).

B. *Serum aldolase and SGO-transaminase*: In all categories, including the animals comprising the sham surgery group, a variable degree of elevation in serum aldolase was re-

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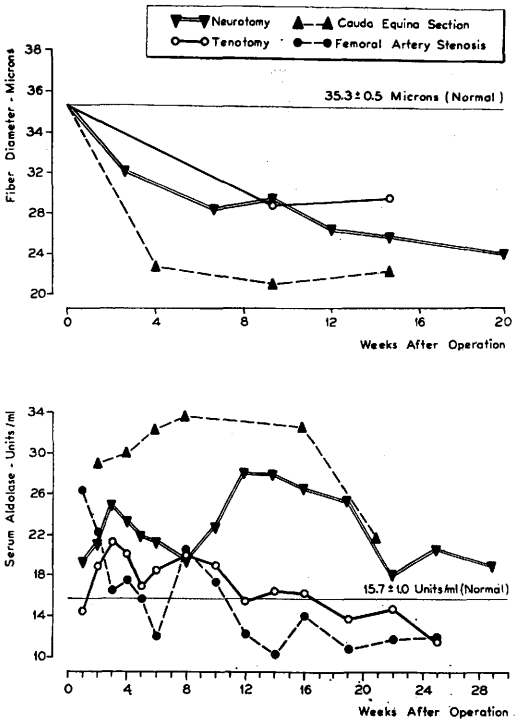

FIG. 1. Effect of various forms of experimentally induced muscle atrophies upon muscle fiber diameter (above) and serum aldolase (below).

corded in the 2 weeks following surgery. A plateau within normal limits (15.7 units/ml ± 1.0 S.E.) was then achieved in the tenotomy, femoral artery constriction and sham-operated dogs (Groups B, D and E). In the animals which have undergone neurotomy (Group A) a second elevation of serum aldolase was seen commencing about 9 weeks after nerve section reaching levels of 28.0 units/ml (± 2.7), finally reverting to normal approximately 21 weeks after the experimental procedure. This delayed elevation was noted in 10 of the 13 dogs constituting this group. Weekly serum aldolase levels of the dogs in which multiple motor roots were divided within the vertebral canal (Group C) showed a sustained rise beginning immediately after the operation and reaching the highest concentrations of this series, reaching levels of 33.7 units/ml (± 3.3) (Fig. 1). Some regression of the hyperaldolasemia was seen in this group by the 18th week after surgery. No elevations of SGO-transaminase were observed except for an insignificant and transient post-operative rise in some dogs.

C. *Tissue phosphorylase and aldolase*: A wide fluctuation in tissue enzyme levels was noted. Nevertheless a progressive but slight decrease in the tissue phosphorylase b from normal mean values of 20,267 units/g of non-collagenous protein (± 1,725) was seen in the 7 to 8 weeks following neurotomy (Group A). In the succeeding 7 weeks a sharp fall in tissue phosphorylase levels to a mean of 8,584 units/g of non-collagenous protein was demonstrable. The range, however, was still wide, varying from 2,620-28,500 units/g. A roughly similar pattern of decreased enzyme activity was noted in the determined levels of tissue aldolase in animals subjected to neurotomy. The normal mean value was 23.1 units/g of non-collagenous protein $x10^4$ (± 1.1). Twenty weeks after operation a mean of 15.3 units (range: 13.6-18.2) was obtained. In contrast, no depression of either the phosphorylase b or the aldolase in involved tissues was seen in animals in which tenotomy (Group B) was performed. Animals submitted to the more extensive neuromuscular atrophy secondary to cauda equina section (Group C) showed a depression of both tissue enzymes. Five weeks after operation the mean tissue aldolase was 19.2 units/g of non-collagenous protein $x10^4$ (range: 18.7-19.8 units) and the phosphorylase b, 14,048 units/g of non-collagenous protein (range: 2,145–31,600 units).

Discussion. The progress of various forms of experimental muscle atrophy was surveyed in terms of serial histologic and enzymatic assay. No significant distinction between the atrophy induced by neurotomy, cauda equina section, tenotomy or vascular constriction could be established on the basis of microscopic appearance alone. In all instances, a progressive decrease in muscle cell diameter was noted, with preservation of cross striations. No singular morphologic feature emerged to distinguish one form of atrophy from another.

In dogs submitted to femoral neurotomy

(Group A) or the more extensive denervation resulting from transsection of the cauda equina nerve roots (Group C), the levels of skeletal muscle aldolase and phosphorylase b diminished in accordance with previous observations of others(7). Two rises in serum aldolase were seen in this series of dogs. The first immediately following the surgical procedures was attributed to the operative intervention and resulting tissue damage, and was noted also in dogs subjected to sham-operation without compromise of the motor nerve supply. The initial post-operative elevation was slightly higher in the neurotomy group as compared to the animals subjected to tenotomy, possibly because of the more extensive tissue trauma incident to the former procedure. The second, delayed elevation occurred about 9 weeks after neurotomy, persisted for about 13 weeks and then reverted to normal. Previous investigators have surmised that elevated serum aldolase, in the absence of hepatic disease or extensive tissue necrosis, may be due to liberation of this enzyme from degenerative muscle(8,9). A delayed rise of this serum enzyme, exhibited by dogs in which motor nerves have been severed, would therefore be anticipated, if an inverse correspondence between serum and tissue aldolase is accepted. Further, the quantitative extent of neuromuscular atrophy seems to bear some relationship to the serum aldolase rise. Animals in which the cauda equina roots were sectioned showed the greatest topographic degree of muscular atrophy and the most pronounced and prolonged incrementation of this serum enzyme. It is to be emphasized, however, that the transient serum aldolase elevation seen in these nerve-section dogs is not as sustained nor as marked as the hyperaldolasemia noted in human cases of muscular dystrophy(3).

A simulation of disuse atrophy was induced in dogs by severing the tendons of major lower limb muscle groups. Atrophy, as exemplified by morphologic study of serial muscle biopsies, advanced progressively, but no alteration in serum aldolase was noted during the prolonged period of observation. Tissue phosphorylase b remained essentially unaltered in the face of this continuing atrophy and tissue aldolase was not diminished, in contrast to the changes of these enzymes seen in animals in which nerve section was performed. In some instances, the tissue aldolase levels appeared to be actually elevated *above* normal. This finding was interpreted as indicating only a relative increase of enzyme content attributable to the decrease of tissue bulk during the course of inactivity.

Atrophy consequent to vascular anoxia did not appear to affect the level of the serum aldolase, during the period of observation, except during the initial post-operative period.

SGO-transaminase determinations in all groups failed to show any significant elevation other than some occasional transient rises immediately after surgery.

Summary. Thirty-three mongrel dogs were submitted to a variety of experimental procedures instituted for the purpose of creating various forms of experimental muscle atrophies. Dogs in which either the femoral nerve was sectioned or the entire cauda equina root system was severed showed a delayed and erratic depression of tissue phosphorylase b and aldolase, and in parallel fashion, a delayed but transient rise in the serum aldolase. Dogs in which the atrophy was caused by tendon section or compromise of the arterial blood supply, showed no depression of the tissue enzymes or commensurate elevation of the aldolase level within serum. The degree of atrophy, as determined by histologic measurement of the implicated muscle fibers, however, was approximately equivalent in all groups. These findings are in concurrence with the clinical data accumulated in the application of the serum aldolase test and appear to indicate that atrophy consequent to denervation may be distinguished from atrophy of other causes by the moderate and transient rise in the serum aldolase.

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In vivo Effect of CoA and Other Factors on Lipide Phosphorylation.* (22946)

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Considerable progress has been made in possible pathways of phospholipide synthesis in liver for partial purified enzyme systems. Recent work has provided evidence that CoA (1), ATP(2,3), and maintenance of oxidative phosphorylation(4) are essential for phospholipide synthesis in liver mitochondria. Recently Kennedy has shown that liver mitochondria synthesize lecithins, involving phosphorylcholine as an intermediate and incorporate choline directly into the lipide molecule, requiring ATP and CTP(4,5). Phospholipide synthesis in the liver of animals maintained on stock diet as measured from P^{32} turnover studies is already at a maximum rate(6) and even under nutritional stress or liver cell death this is not altered. In choline deficiency the total liver lecithin content decreases and total lipide concentration increases(7) resulting in increased need in the liver cell for choline-containing phospholipides(8). Thus, compounds which are involved directly in phospholipide synthesis should affect the rate of turnover in fatty liver cell since there is an increased demand for lecithins. In view of these observations and the experimental work on partial purified enzyme systems, the *in vivo* effects of CoA, ATP, AMP, oxalacetate and CMP on lipide phosphorylation in liver of rats have been investigated in choline deficient animals.

Methods. Male albino rats of Sprague-Dawley strain weighing 100-110 g were divided into 5 series. The animals in Series I, II, and III were maintained on 5% Casein-5% Fat Diet(9), for 4 weeks. In Series IV, rats were maintained on pantothenic acid deficient diet(10) for 10 weeks. Control animals for this group received similar diet containing the vitamin. In each series, 4 to 6 rats were housed in individual cages with raised screen bottoms. Animals were fed *ad libitum*. Intraperitoneal injection of 4 μc of P^{32} as NaH_2PO_4 was administered one minute following injection of above compounds listed in Series I-IV. Six hours later the animals were killed by decapitation. The liver was removed, rapidly weighed and divided into 2 fractions. On one fraction acid-soluble phosphorus was removed by extracting with 10% TCA containing 0.4 M $MgCl_2$(11) and radioactivity and total P(12) were determined. The other fraction was covered with alcohol and lipides extracted with alcohol, alcohol-ether and purified with chloroform (13). On aliquots of the chloroform solution, radioactivity(13) and phosphorus(12) were determined. As a measure of phospholipide synthesis specific and relative specific activity (14) have been calculated. Twelve animals in Series V were maintained on Diet 26(9) for 8 weeks. At the time of dietary regime they were divided into 3 groups and intraperitoneal injection of compounds listed in Series II(5) was given. One minute later 4 μc of P^{32} was administered and in 6 hours the animals were killed by decapitation, liver was

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