Phosphorylase in Skeletal Muscle of Normal and Selected Lines of Dystrophic Chickens[1] (35728)

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(Introduced by H. H. Cole)

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Hereditary muscular dystrophy in the chick is characterized by progressive hypertrophy of affected muscle fibers, necrosis, and atrophy. These characteristics are variable in degree of expression in different lines of dystrophic chicks (1). Identification of the initial insult to the muscle fiber in time or place has not been established. It does, however, initiate a large number of biochemical and morphological alterations which culminate in loss of fiber function. Progression of these events in dystrophic chick muscle occurs during the time that the muscle fiber is attempting to complete a normal developmental transition in terms of growth and energy-producing enzyme patterns (2, 3). This confounds the identification of the primary gene defect. It seems reasonable to assume that pleiotropic effects of the mutation could be variably expressed due to different modifier alleles. Therefore, a useful tool for identification of at least some of the secondary alterations would be evaluation of selected lines of dystrophic chicks. Although such lines exist (1), comparative studies have largely been confined to anatomical measurements (1, 4). In a study using histochemical techniques (2), we found that phosphorylase activity is initially higher in dystrophic muscles than in normal muscles, and suggested that progressive loss of the enzyme is secondarily due to degeneration of the muscle rather than a block in fiber maturation as had been previously proposed (5). However, the histochemical assay of phosphorylase in skeletal muscle is at least in part dependent upon native glycogen in the muscle fiber (6, 7) so that caution must be exercised in interpretation of observations made using this technique. We report below the results of the biochemical assay of phosphorylase activity in the pectoralis and adductor muscles of normal and selected lines of dystrophic chicks.

In the newly hatched chick, two fiber types are seen histochemically (2). Both of these initially are equipped for aerobic metabolism and have been designated αR and βR according to their differential reaction for myosin ATPase activity and equivalent reaction for succinic dehydrogenase activity (SDH). αR fibers have the capacity to transform from the aerobic state to an anerobic state of metabolism, and in this event have been termed αW. The pectoralis of the chick is composed entirely of α fibers, nearly all of which convert rapidly during the first 3 weeks from αR to αW. The adductor is composed of αR and βR fibers, and in this muscle only a small proportion of the αR fibers convert to αW. Conversion of αR fibers to αW fibers is accompanied by a rapid increase in phosphorylase activity, and a decrease in mitochondria and oxidative enzymes. βR fibers, on the other hand, maintain a high capacity for aerobic metabolism, and are phosphorylase negative.

Materials and Methods. Lines of New Hampshire chicks used in this study have been previously described in detail (1). Briefly, line 304 is selected for early clinical onset of the myopathy (1) and exhibits early and extended hypertrophy of the pectoralis muscle. Line 307 is selected for high fat con-

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tent in the pectoralis muscle and although it exhibits a later clinical onset than line 304 chicks, biochemical and morphological alterations in line 307 chicks precede those in line 304 chicks. Line 307 chicks are characterized by early and rapid fiber atrophy and fiber splitting. Line 308 is selected for low pectoralis fat content and exhibits a later onset than the other two lines. Fiber hypertrophy is slower than in line 304 chicks, and at any given age muscle abnormalities are least severe in line 308 chicks. Line 200 is the control line.

Pectoralis and adductor muscles were removed from chicks of each line at 1, 2, 4, and 6 weeks of age, quickly weighed, cut into small pieces, and frozen. For assay (within 3 days of taking the muscle) the samples were thawed at 2–4° and homogenized (1:8, w/v) in a Thomas Teflon tissue homogenizer. The homogenizing medium consisted of 0.18 *M* KCl and 0.05 *M* Tris–HCl, pH 7.4. Total phosphorylase (*a* and *b*) was assayed at 25° by following the reduction of TPN at 340 mμ. Assay medium contained (final concentrations) 0.05 *M* Tris–HCl, pH 7.4; 10.6 m*M* cysteine; 9.4 m*M* $MgCl_2$; 2.2 mg/ml of glycogen; 0.04 m*M* TPN; 6 m*M* AMP; 10 m*M* PO_4; 2.5 m*M* EDTA; and excess glucose-6-phosphate dehydrogenase and phosphoglucomutase. Assay was started by addition of homogenate. Preliminary experiments showed that activity increased with dilution of the homogenate. Therefore, assays were performed at dilutions which provided maximal activity. Adductor muscles were not assayed at 1 week of age.

Results and Discussion. Phosphorylase activity is higher in the pectoralis, a typical fast muscle, than in the adductor, a typical slow muscle. In normal pectoralis muscle, activity nearly doubles between 1 and 2 weeks of age (Fig. 1), but there is little change in activity after 2 weeks of age. Bass *et al.* (3), using a similar assay procedure, obtained a similar pattern of activity for the posterior latissimus dorsi, also a fast muscle. Their values were somewhat higher than ours (Fig. 1), but in addition to using a different muscle, they used the White Leghorn breed of chick.

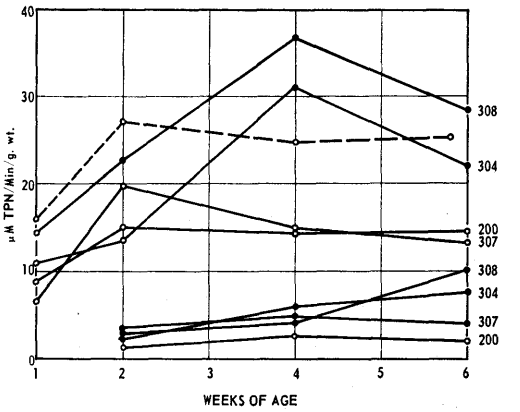

FIG. 1. Phosphorylase activity in crude homogenates of pectoralis (upper values) and adductor (lower values) muscles of normal and selected lines of dystrophic New Hampshire chicks. Lines of chicks are normal, 200; dystrophic, 304, 307, 308. (- - -) data from Bass *et al.* (3), obtained on posterior latissimus dorsi muscle of White Leghorn chicks. (–●–) comparison with 200, $p<0.05$. Each value is the mean of 6-15 muscles.

In normal adductor muscle, activity is relatively constant from 2 to 6 weeks of age, and is only about 15% of the activity in the pectoralis muscle. In the adductor, only a small percentage of the αR fibers convert to αW fibers (2).

At 2 weeks of age, phosphorylase activity is significantly higher than normal only in line 308 chicks. By 4 weeks of age, activity in line 308 and 304 is more than twice that of normal; whereas activity in line 307 (earliest onset) is declining. By 6 weeks of age, activity has significantly declined in lines 308 and 304. Histological examinations have shown that at 4 weeks of age, whereas the average fiber diameter of line 308 and 304 chicks is nearly twice that of normal chicks (8), fiber atrophy and splitting has progressed to a marked degree in the pectoralis of line 307 chicks. By 6 weeks of age, necrosis is also visible in many fibers of line 308 chicks, and to a greater extent in the pectoralis of line 304 chicks.

Phosphorylase activity is significantly higher in the adductor muscles of all lines of dystrophic chicks at every age examined (Fig. 1). Red muscle fiber types (αR and βR) do not appear to be susceptible to dystrophic alterations in the chick (2). Since the

adductor is composed mostly of αR and βR fibers, the muscle as a whole is little affected by the myopathy. There are some degenerative changes in αW fibers of adductor muscles in line 307 chicks by 5 weeks of age (2). The lower enzyme activity at 6 weeks of age in the adductor of this line relative to the other dystrophic lines (Fig. 1), is likely a reflection of these changes.

These data varify histochemical observations previously reported (2) but are not in agreement with data reported earlier by Cosmos (5). Although the latter author found comparable levels of phosphorylase in normal and dystrophic muscle through 1 week of age, her data show that after 1 week, activity continues to increase in normal muscle and decreases in dystrophic muscle. This was interpreted to indicate a prolonged embryonic state, or block in maturation of the dystrophic fiber (conversion from αR to αW).

The reason for the difference between data reported here and those of Cosmos (5) is unknown. Although we have used a different assay system, we have analyzed some samples for inorganic phosphate released (5) and obtained results similar to those reported here. It seems possible that the source of dystrophic chickens may be a factor. Lines of dystrophic and normal chicks used in our study are inbred New Hampshires derived from the same stock. Dystrophic chicks maintained at the University of Connecticut were originally obtained from this station, but later outcrossed onto White Leghorn stock. White Leghorn chicks are used as a control line, and may not be completely comparable with the dystrophic line. Differences in enzyme activity due to breed, especially during early growth stages, could mask differences of enzyme activities between normal and dystrophic genotypes. In addition, dystrophic stock maintained at the Connecticut station is continually selected for early onset of the myopathy which, according to our data, would hasten the decline of enzyme activity.

The physiological significance of increased phosphorylase activity in dystrophic chick muscle is not known. In normal muscle increase in phosphorylase activity is accompanied by a decrease in mitochondria and SDH activity as αR fibers convert to αW fibers (2). In dystrophic muscle, in addition to an abnormally rapid increase in phosphorylase activity, mitochondria and mitochondrial enzymes do not decline (8). Qualitatively, however, mitochondria in dystrophic muscle assume enzyme patterns like mitochondria from normal αW fibers (8). We interpret these observations to indicate that the normal conversion process is occurring in dystrophic muscle in terms of gene activity patterns, but the quantitative control mechanisms are altered. Rapid growth rate of dystrophic fibers may be a reflection of these high enzyme activities. These same characteristics may then quickly render supply/demand ratios inadequate.

Summary. Phosphorylase activity was assayed in pectoralis (white) and adductor (red) muscles of normal chicks and three selected lines of chicks with hereditary muscular dystrophy. Activity is higher than normal in the adductor of all lines of dystrophic chicks. The adductor is relatively spared by the dystrophic process. In the pectoralis muscle activity is also higher than in normal muscle prior to the onset of degenerative changes, but falls with progression of the myopathy. It is suggested that quantitatively higher levels of energy-producing enzymes in dystrophic muscle may promote fiber hypertrophy and ultimately initiate fiber degeneration.

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