Regulation of Fructose Diphosphate Aldolase Concentrations in Skeletal Muscles of Normal and Dystrophic Chickens*

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We are using the glycolytic enzyme aldolase as a marker to investigate the molecular basis for reduced levels of specific enzyme activities associated with muscular dystrophy. Inbred strains of normal and dystrophic chickens were used as models to study the disease. In the present paper we show that: 1) aldolase $A_4$ was the only isoenzyme present in normal and dystrophic muscles derived from 1- to 12-week-old birds; 2) aldolase $A_4$ isolated from normal and dystrophic breast ("fast-twitch") muscles were identical molecules on the basis of their electrophoretic, immunological, and catalytic properties; 3) the steady state level of aldolase activity in dystrophic breast muscle was only 40 to 50% of normal; 4) the reduced level of enzyme activity was associated with a corresponding reduction in the level of catalytically active aldolase molecules as determined in immunotitration experiments; 5) the reduced steady state level of aldolase molecules was associated with a corresponding reduction in the relative rate of synthesis of the enzyme in dystrophic breast muscle fibers as determined by short term isotope incorporation experiments conducted in vivo and with muscle explants in culture; 6) large increases in synthesis of collagen or other non-muscle proteins by dystrophic muscle samples were not detected by electrophoretic and fluorographic analysis of the radioactive protein in the initial phases of our analysis since the chicken breast muscle fibers to accurately perform the transition from one enzyme synthetic program to another which normally occurs around the time of hatching.

Much attention has been focused on the biochemical manifestations of muscular dystrophy. However, considerable confusion in the area is apparent from a survey of the literature and much of it may be attributed to the fact that different laboratories use organisms inflicted with different dystrophic conditions as models to investigate the disease (1-3). In addition, some workers have chosen to use relatively old animals as experimental subjects and, as such, are most likely studying effects of muscular dystrophy which are far removed from the primary biochemical manifestations of the disease.

Many observations of decreased levels of specific enzymes in dystrophic skeletal muscles have been published (4-11), but less is known about the detailed events responsible for these decreases. Explanations may include leakage of enzymes from the damaged tissue (12-15), modification of amino acid residues which are essential for enzymatic activity (10), destruction of enzymes by increased levels of lysosomal or other proteases in diseased muscle (16-18), and alterations in relative rates of synthesis or turnover of proteins, or both, in dystrophic muscle cells (19-29).

We have chosen inbred strains of New Hampshire chickens to investigate, in detail, the biochemical basis for reduced activities of specific enzymes associated with muscular dystrophy. The disease is genetically carried by a single recessive autosomal locus (3). Birds homozygous at this locus are characterized by an early onset of the disease which includes extensive hypertrophy of "fast-twitch" (white) muscles while "slow-twitch" (red) muscles remain relatively unaffected (9, 30).

We chose the glycolytic enzyme aldolase as a "marker" protein in the initial phases of our analysis since the chicken enzyme is well characterized (31, 32), is easy to isolate by affinity chromatography or immunoprecipitation (33, 34), is found in high concentrations in skeletal muscles (34), and has previously been used by us as a marker to study the regulation of synthesis and turnover of specific proteins in normal animal cells (34-39). In the present paper we: 1) present evidence that aldolase molecules derived from normal and dystrophic muscles are identical; 2) show that, by 2 weeks of age, the level of aldolase activity in dystrophic breast muscle was only 40 to 50% of normal and that this reduced level of enzymatic activity was a direct reflection of a reduced number of catalytically active aldolase molecules; 3) show that the reduced content of aldolase molecules was associated with a corresponding reduction in the relative rate of synthesis of the enzyme in diseased muscle; 4) give evidence that the lowered content of aldolase protein in dystrophic breast muscle results from failure of the diseased tissue to accurately perform the transition to the enzyme synthetic program characteristic of mature fast-twitch muscles which commences around the time of hatching; and 5) show that in contrast to the situation with fast-twitch (breast) muscle, aldolase content and rate of synthesis in a slow-twitch (red) muscle of chickens inflicted with muscular dystrophy appeared to be normal.

EXPERIMENTAL PROCEDURES

Organisms—Newly hatched New Hampshire female chicks of line 412 (normal) and line 413 (dystrophic) were obtained from the Department of Avian Sciences, University of California, Davis. The chicks were reared on Startina (Purina Co.) food until used.

Preparation of Tissue Extracts and Isolation of Aldolase—Muscle tissue was homogenized in 10 or 20 volumes of 50 mm potassium phosphate, 1 mm EDTA, 1 mm MgCl$_2$, 1 mm 2-mercaptoethanol, pH 7.0.
Aldolase Synthesis in Normal and Dystrophic Muscles

7.5, in the Sorvall Omninixor, for small amounts of tissue, in a glass-glass hand homogenizer. Under these extraction conditions, greater than 95% of the total aldolase activity was extracted from particulate muscle proteins. Twenty volumes of extraction buffer were used when quantifying aldolase levels and 10 volumes of buffer were used to extract muscle tissue in synthesis studies. Soluble muscle protein is defined as the supernatant fraction after centrifuging crude homogenates at 12,000 g for 15 min and particulate muscle protein is referred to as material which sedimented during centrifugation. 

Aldolase was isolated from the soluble fraction by collecting those proteins which precipitated at 4 and 75% ammonium sulfate saturation following affinity chromatography on phosphocellulose columns as previously described (34). Alternatively, aldolase was isolated from the soluble muscle fraction by immunoprecipitation with monospecific antiserum as described below.

Enzyme Activity and Protein Determinations—Aldolase activity was determined by measuring absorbance at 280 nm using an extinction coefficient of ε280 = 0.9 (42) to convert absorbance to protein concentration. 

Electrophoretic Methods—Cellulose polyacetate electrophoresis followed by staining the strips specifically for aldolase activity was performed as previously described (43). Polyacrylamide gel electrophoresis either in basic 5% cylindrical gels (44, 45) or in 9% slab gels containing sodium dodecyl sulfate (46, 47) was carried out as previously described. Afterward, the gels were fixed, stained with Coomasie blue, and destained as previously described (48). Fluorographic analysis of stained slab gels was accomplished essentially as described by Bonner and Laskey (49) using Kodak XR-5 Omat film which was sensitized by flash pre-exposure as described by Laskey and Mills (50).

Labeling of Muscle Proteins with Radioactive Leucine—Muscle proteins were labeled in vivo with L-[4,5-3H]leucine (Amersham, 50 to 60 Ci/mmole) or with L-[U-14C]leucine (Amersham, 330 mCi/mmol) by intraperitoneal injection as previously described (35). Proteins were labeled in culture by incubating slices of muscle in the presence of [3H]- or [14C]leucine at 37°C using the medium and conditions recently described (47).

Measurements of Leucine Incorporation into Protein—Incorporation of labeled leucine into hot trichloroacetic acid-precipitable material was measured as follows. Samples were adjusted to 10% trichloroacetic acid by addition of an equal volume of 1 NaOH base to dissolve particulate proteins before performing the assays. Protein concentrations of pure aldolase preparations were determined by measuring absorbance at 280 nm using an extinction coefficient of ε280 = 0.9 (42) to convert absorbance to protein concentration.

Immunological Experiments—Preparation of monospecific antisera towards pure chicken muscle aldolase and the method for Ouchterlony double diffusion tests have been previously described (34, 35). Methods for immunoprecipitation were also described earlier (34, 35) with the following modification. After isolation of 'H-labeled aldolase by immunoprecipitation, the washed precipitates were boiled in 5% trichloroacetic acid and, thereafter, the samples were treated as described above for other labeled protein fractions.

RESULTS

Characterization of Aldolase Derived from Normal and Dystrophic Skeletal Muscles—Before investigating the regulation of aldolase concentrations in normal and dystrophic muscles, we compared some characteristics of the enzymes present in the muscle types. Fig. 1 (top) shows that aldolase A, was the only isoenzyme detected in crude homogenates of the pectoralis major (a fast-twitch muscle) and the lateral abductor (a slow-twitch muscle) of normal and dystrophic birds. Similar electrophoretic patterns were obtained for muscles derived from one to 12-week-old chickens. Fig. 1 (center) shows that the aldolases present in crude extracts of the muscles derived from normal and dystrophic chickens are

![Fig. 1. Properties of aldolase molecules derived from normal and dystrophic skeletal muscles. Top, aldolase isoenzymes present in crude preparation of breast (left) and lateral abductor (right) muscles of normal (N) and dystrophic (D) chickens. Samples of muscle extracts were electrophoresed on cellulose polyacetate strips and the strips were stained for aldolase activity as described under “Experimental Procedures.” Similar results were obtained for muscles obtained from 1- to 12-week-old chickens. Middle, Ouchterlony double diffusion tests of aldolase in extracts of breast and lateral abductor muscles of normal and dystrophic chickens. Center well, antiserum; Well 1, normal breast; Well 2, dystrophic breast; Well 3, mixture of I and 2; Well 4, normal lateral abductor; Well 5, dystrophic lateral abductor; Well 6, mixture of 4 and 5. Bottom, electrophoresis of aldolase isolated from normal and dystrophic breast muscles. The aldolases were isolated by affinity chromatography on phosphocellulose columns. Left, electrophoresis in basic 5% polyacrylamide gels in the absence of sodium dodecyl sulfate; right, electrophoresis in 9% gels containing sodium dodecyl sulfate. n, normal; d, dystrophic. See “Experimental Procedures” for details on methodology.](http://www.jbc.org/)

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immunologically identical as judged by Ouchterlony double diffusion tests. Further, aldolase isolated from normal and dystrophic breast muscles by affinity chromatography on phosphocellulose columns behaved identically upon electrophoresis in basic polyacrylamide gels and in gels which contained sodium dodecyl sulfate (Fig. 1, bottom). The specific catalytic activities of aldolases isolated from normal (15 units/mg) and dystrophic (16 units/mg) breast muscles were also found to be essentially identical. Finally, aldolase activity present in crude homogenates of normal and dystrophic breast muscles exhibited comparable stabilities upon incubation at 4°C for 1 week; in both cases, greater than 90% of the initial enzymatic activity was recovered after the incubation period. The data presented in this section suggest that aldolase molecules in normal and dystrophic skeletal muscles are identical. 

Aldolase Content in Normal and Dystrophic Breast Muscles—The steady state level of aldolase activity in breast muscles of dystrophic chickens (5 to 12 weeks old) was found to be only 40 to 50% of normal when enzyme activity was normalized to tissue wet weight (204 ± 6.4 units/g for normal and 88 ± 1.3 units/g for dystrophic) or to milligrams of total muscle protein (1.28 ± 0.08 units/mg for normal and 0.57 ± 0.015 units/mg for dystrophic). Total protein content/g of tissue was more similar (182 ± 9 mg/g wet weight for normal and 162 ± 8 mg/gm wet weight for dystrophic). Samples containing equal volumes of the two extracts exhibited catalytic activities exactly intermediate between those obtained for each extract alone. Finally, the immunotitration data presented in Fig. 2 show that aldolase in extracts of normal and dystrophic breast muscles exhibited the same degree of antigenicity; that is, the two enzymes expressed the same relationship between catalytic activity and immunological potency. These data show that the reduced amount of aldolase activity in dystrophic breast muscle is a direct reflection of a reduced number of catalytically active enzyme molecules.

Relative Rates of Synthesis of Aldolase in Normal and Dystrophic Breast Muscles—The reduced number of functional aldolase molecules in dystrophic breast muscle fibers could result from a reduced rate of synthesis or an increased rate of degradation of the enzyme, or both, in diseased tissue. The experiments presented in this section were performed to clarify the mechanism(s) involved. We demonstrated a reduced relative rate of synthesis of aldolase in dystrophic breast muscle in a double isotope incorporation experiment conducted in vivo. Normal and dystrophic 5-week-old chickens received injections of [15N]- or [14C]-leucine, respectively, and, 2.5 h later, the chickens were killed. The muscle tissues were extracted in the phosphate-sodium dodecyl sulfate (Fig. 1, bottom). The specific catalytic activities of aldolase in extracts of normal breast muscles were also found to be essentially identical. Finally, aldolase activity present in crude homogenates of normal and dystrophic breast muscles was found for total muscle protein and for the particulate and soluble fractions.

A reduced relative rate of aldolase synthesis by dystrophic breast muscle was also demonstrated in double isotope experiments conducted in culture. Slices of normal and dystrophic breast muscle were incubated in the presence of [14C]- or [15N]-leucine, respectively, for 3 h. Afterward, the tissue samples were homogenized in the culture medium which was adjusted to 50 mM potassium phosphate to ensure complete solubilization of the enzyme from myofibrils. Then, aliquots of the homogenates were mixed and aldolase was isolated by affinity chromatography. The data in Table II show that the relative rate of aldolase synthesis in breast muscle from 7-week-old dystrophic chickens was about one-half of the normal value. Since the muscle tissues were extracted in the culture medium, it cannot be argued that the low normalized ratios found for aldolase are due to preferential leakage of the enzyme from dystrophic muscle into the surrounding medium. This is an important point since considerable leakage of some enzymes into the culture has been demonstrated in some dystrophic disorders and this phenomenon has been used as a diagnostic tool in studies on muscular dystrophy.
We further demonstrated a reduced relative rate of aldolase synthesis in single isotope incorporation experiments utilizing immunoprecipitation of aldolase with monospecific antiserum to isolate the radioactive enzymes. Slices of normal and dystrophic breast muscle were incubated in the presence of [\(^{3}H\)]leucine for 3 h. Afterward, the tissues were extracted as described above and the soluble protein fractions were obtained by centrifugation. Then, aldolase was precipitated from the soluble fraction with excess amounts of antiserum. The data in Table III show that approximately 18 and 8.4% of the total labeled soluble proteins in normal and dystrophic muscle extracts, respectively, could be precipitated with the antiserum. That the labeled enzymes were completely precipitated was shown by the similar amounts of \(^{3}H\) precipitated whether 50 or 100 \(\mu\)l of antiserum was used for immunoprecipitation. Also, essentially all of the aldolase activity was precipitated by both volumes of antiserum. From these immunoprecipitation experiments, it can be calculated that the rate of aldolase synthesis relative to total soluble protein synthesis by dystrophic breast muscle was only 46% of normal. This value is close to those obtained in our double isotope incorporation experiments conducted in vivo and in culture (Tables I and II).

Finally we demonstrated a reduced relative rate of aldolase synthesis by electrophoretic and fluorographic analysis of the \([\(^{3}H\)]leucine-labeled proteins synthesized by muscle slices in culture (Fig. 3); notice the considerable reduction in film exposure by radiolabeled aldolase relative to that produced by other proteins in the dystrophic muscle sample.

Since there was a close correspondence between the reduced aldolase content and reduced relative rate of aldolase synthesis in dystrophic breast muscle, we did not find it necessary to investigate the relative rates of turnover of the enzyme in normal and diseased muscle; measurements of rates of protein turnover are complicated by problems resulting from isotope reutilization (51).

Levels and Rates of Aldolase Synthesis during Maturation of Normal and Dystrophic Breast Muscles—In normal breast muscle, large accumulations of aldolase and other glycolytic enzymes occur during postembryonic maturation of the chicken (6, 9, 34, 52). We measured aldolase content in breast muscles during maturation of normal and dystrophic chicks. Aldolase content expressed as activity/g wet weight or as activity/mg of muscle protein was fairly similar in breast muscles of 1-day-old normal and dystrophic chicks (Fig. 4). By about 10 days of age, steady state levels of the enzyme

| Table III |
| Rates of aldolase synthesis in normal and dystrophic breast muscles in culture as determined by the immunoprecipitation method |

Slices of normal (6-week-old) and dystrophic (9-week-old) breast muscles were incubated in the presence of \([\(^{3}H\)]leucine under the conditions described in Table II. After 3 h, soluble proteins were obtained by homogenization and centrifugation as described in Table II. Fifty-microliter aliquots of the soluble protein fractions were incubated with the indicated amounts of antiserum and the resulting immunoprecipitates were washed as described under “Experimental Procedures.” The percentages of total hot trichloroacetic acid-precipitable counts which appeared in the immunoprecipitates are given as “% total cpm.”

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Volume of antiserum added</th>
<th>50 (\mu)l</th>
<th>100 (\mu)l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm</td>
<td>% total cpm</td>
<td>cpm</td>
</tr>
<tr>
<td>Normal</td>
<td>4920</td>
<td>17.9</td>
<td>5000</td>
</tr>
<tr>
<td>Dystrophic</td>
<td>1184</td>
<td>8.3</td>
<td>1209</td>
</tr>
</tbody>
</table>

Fig. 3. Spectrum of proteins synthesized by slices of normal and dystrophic breast muscles in culture. Muscle slices (0.5 g each) from 9-week-old normal and dystrophic chickens were incubated in the presence of 400 \(\mu\)Ci of \([\(^{3}H\)]leucine for 3 h. The soluble proteins were obtained as described under “Experimental Procedures” and aliquots were subjected to electrophoresis in sodium dodecyl sulfate-polyacrylamide gels. Radioactive proteins were visualized by fluorographic analysis of the gels. See “Experimental Procedures” for details on methodology. ald, aldolase; N, normal; D, dystrophic.

Fig. 4. Accumulation of aldolase activity during postembryonic maturation of normal and dystrophic breast muscle. Top, aldolase activity/g wet weight as a function of age; center, aldolase activity/mg of total muscle protein as a function of age; bottom, ratio of aldolase content (units/g wet weight) in dystrophic (D) versus normal (N) breast muscle as a function of age. See “Experimental Procedures” for details.
were accumulated by both muscle types; however, the extent of aldolase accumulation in dystrophic muscle was only 40 to 50% of normal. The defective accumulation of enzyme in dystrophic breast muscle is more vividly illustrated in Fig. 4 (bottom) where the ratio of aldolase content in dystrophic versus normal muscle is plotted as a function of age. These data show that the lowered content of aldolase protein in dystrophic breast muscle results largely from a reduced accumulation of the enzyme during maturation.

Relative rates of aldolase synthesis in breast muscles of 5- to 62-day-old normal and dystrophic chickens were measured in double isotope incorporation experiments conducted in culture. In all instances, the relative rate of aldolase synthesis in dystrophic muscle was only 40 to 50% of normal (Fig. 5).

Content and Synthesis of Aldolase in Lateral Abductor Muscles of Normal and Dystrophic Chickens—Slow-twitch (red) muscles of the chicken, such as the lateral abductor, appear to be relatively unaffected by muscular dystrophy (9, 30). In view of these observations and the data presented above on the fast-twitch pectoralis muscle, we compared the content and rate of aldolase synthesis in lateral abductor muscles of normal and dystrophic chickens. The content of aldolase based on gram wet weight and on milligrams of total muscle protein was found to be essentially identical for muscles obtained from normal (about 67 units/g, 0.6 units/mg of protein) and dystrophic (about 70 units/g, 0.6 units/mg of protein) 10-week-old chickens.

The rate of synthesis of aldolase by lateral abductor muscles of dystrophic chickens was found to be essentially normal in isotope incorporation experiments conducted in culture. Slices of muscle obtained from normal and dystrophic 10-week-old chickens were labeled with [3H]- or [14C]leucine, respectively, for 3 h. Afterward, the aldolases were co-isolated and the 14C/3H ratio of the purified enzyme preparation was compared

<table>
<thead>
<tr>
<th>Muscle</th>
<th>cpm</th>
<th>% total cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3906</td>
<td>6.3</td>
</tr>
<tr>
<td>Dystrophic</td>
<td>1726</td>
<td>7.3</td>
</tr>
</tbody>
</table>

The percentage of total hot trichloroacetic acid-precipitable counts which appeared in the immunoprecipitates is given as 100% total cpm.

Fig. 6. Spectrum of proteins synthesized by slices of lateral abductor muscles obtained from normal (N) and dystrophic (D) 10-week-old chickens. The experiment was performed as described in Fig. 3. ald, aldolase.
DISCUSSION

The present work was initiated to investigate the molecular basis for alterations in the levels of enzyme activities which are associated with avian muscular dystrophy. The lines of chickens used here have been extensively inbred to ensure minimal differences between them. We restricted our investigations to relatively young animals so that the experimental data would not be influenced by effects which may be far removed from the primary manifestations of the disease. The glycolytic enzyme aldolase was used as a marker enzyme for the reasons given in the Introduction.

The present work confirms previous reports that the activity levels of glycolytic enzymes are reduced in fast-twitch (breast) muscles of dystrophic chickens (6, 9). However, our demonstration that aldolase A, was the only isoenzyme detected in muscles of 1- to 12-week-old dystrophic birds is in conflict with the report by Shapira et al. (53) that dystrophic breast muscles contain considerable levels of isoenzymes containing aldolase C subunits. The reason for this discrepancy is not known, but the present work demonstrates that a partial reversal of the aldolase C to A subunit transition which occurs during normal muscle development (34, 37, 54, 55) is not associated with the early phases of muscular dystrophy in the chickens used here.

The aldolase A, molecules isolated from normal and dystrophic muscles were judged to be identical on the basis of electrophoretic, catalytic, and immunological properties. Thus, we conclude that the reduced level of aldolase activity in dystrophic muscles was due to a corresponding reduction in the number of catalytically active molecules rather than being due to the production of partially or totally inactivated molecules. These observations together with our finding that enzyme activity in crude muscle extracts was not inactivated during storage are consistent with our view that increased levels of lysosomal or other proteases which have been observed in some dystrophic disorders (16-18) are not responsible for the reduced levels of enzyme activities in the early stages of avian muscular dystrophy.

Results of short term amino acid incorporation experiments showed that the rate of aldolase synthesis relative to that of total protein synthesis in muscles derived from 5- to 62-day-old dystrophic chickens was only 40 to 50% of normal. The experiments conducted with muscle explants in culture ruled out the possibility that the low synthetic rates observed for aldolase in vivo experiments were obscured by preferential leakage of the labeled enzyme from diseased tissue.

The relative rate of aldolase synthesis in dystrophic breast muscle was found to be about one-half of normal whether the data were normalized to "total," "soluble," or "particulate" (myofibrillar) muscle protein. Further, electrophoretic and fluorographic analysis of the proteins synthesized by muscle explants did not reveal large increases in film exposure by "novel" radioactive polypeptides which confirms the report by Battelle and Florini (25) that increased collagen synthesis in dystrophic muscles is not associated with muscular dystrophy of the chicken. Thus, synthesis of proteins by non-muscle cells which are known to infiltrate the musculature in some dystrophic disorders did not appear to affect our synthesis data.

The present work shows that dystrophic breast muscle fibers accumulated aldolase molecules during postembryonic maturation but that the steady state level of these molecules was only about one-half of normal. Since the extent of reduction in aldolase concentration in dystrophic muscle was essentially identical with the reduction in relative rate of synthesis of the enzyme, it is not necessary to invoke significant changes in relative rates of aldolase turnover to explain our results. This is apparent from the relationship \( (P) = \frac{K_s}{K_d} \), where \( (P) \) is the steady state level of a given protein, \( K_s \) is the zero order rate constant for synthesis of the protein, and \( K_d \) is the first order rate constant for degradation of that protein (56). This conclusion is significant in view of the emphasis placed on alterations in rates of protein turnover as being a major manifestation of muscular dystrophy (19, 20, 28, 57). In contrast to the situation with fast-twitch pectoralis muscle, aldolase content and relative rate of synthesis in the slow-twitch lateral abductor muscle of dystrophic chickens were found to be normal. These observations are consistent with the reports that slow-twitch muscles are not severely affected by muscular dystrophy (9, 30).

The levels of aldolase in breast muscles of 1-day-old normal and dystrophic chicks were similar. Cosmos (6) and Ashmore and Doerr (58) noted that the differences in levels of phosphorylase and ATPase activities between normal and dystrophic breast muscles also became manifest after hatching. These observations are consistent with the view that one effect of muscular dystrophy may be a failure of presumptive fast-twitch muscle fibers to accurately make the transition to an enzyme synthetic program characteristic of mature fibers which normally commences around the time of hatching (34, 38, 39).

Since the intracellular concentrations of the glycolytic enzymes appear to be coordinately regulated (59), it might be expected that the dystrophic process in chickens would affect the rates of synthesis of glycolytic enzymes other than aldolase. We have preliminary data which suggest that the relative rate of synthesis of glyceraldehyde-3-P dehydrogenase\(^2\) in diseased muscle is also reduced and we are expanding this work to include other enzymes.

Alterations in rates of specific protein synthesis in dystrophic breast muscle fibers may be affected at a variety of points during the biogenesis of these molecules. Changes in transcriptional activities of structural genes or in the translational process itself, or both, may be envisioned. Alterations in the processing, transport, and/or turnover of mRNAs may be involved. Finally, differences in the translational machinery of normal and dystrophic muscles have been implicated by the work of others (21, 25, 26, 60, 61). We can now accomplish the complete synthesis of aldolase (45, 47, 62) and glyceraldehyde-3-P dehydrogenase\(^3\) in cell-free protein synthesizing systems using polysomes, mRNAs, and other components derived from a variety of sources. Consequently, detailed investigations on the molecular basis for alterations in rates of specific protein synthesis in dystrophic muscle fibers are now possible.

The primary lesion(s) which cause the multitude of clinical symptoms associated with muscular dystrophy is not known. Defects in the muscles (63-65), in the nerves (66, 67), in the vascular system (68, 69), and in general membrane structure (70-72) have been suggested to be of primary importance. Reductions in the rates of synthesis of aldolase and other glycolytic enzymes is obviously a secondary manifestation of muscular dystrophy. However, the fact that these defects become fully realized by the first week of life suggests that detailed analysis of these phenomena may be useful in studying early changes associated with the disease. Such detailed analyses are currently being performed in our laboratory and these investigations may give new insights into cause-effect relationships associated with avian muscular dystrophy.

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\(^3\) J. E. Shackelford and H. G. Lebherz, unpublished observation.
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