**Effect of Denervation on the Levels and Rates of Synthesis of Specific Enzymes in "Fast-twitch" (Breast) Muscle Fibers of the Chicken**

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It has been well documented that neural information, or the consequences of it, is required for the full phenotypic expression of different skeletal muscle fiber types. In the present work, we investigate the effect of removal of neural information, via surgical denervation, on the levels and rates of synthesis of several enzymes in mature breast ("fast-twitch") white muscle fibers of the chicken. Denervation of these muscles resulted in reductions in the concentrations of several glycolytic enzymes to new steady state levels which were only about 50% of normal, and these decreases in enzyme levels were completed within 2 weeks after severing the nerves. In contrast, denervation for as long as 6 weeks did not have a significant effect on the levels of creatine-P kinase molecules in this muscle type. The decreased level of the skeletal muscle-specific aldolase A1 isoenzyme in denervated breast muscle fibers was associated with a 2- to 3-fold reduction in the relative rate of synthesis of this enzyme following denervation. As expected, denervation had no appreciable effect on the relative rate of synthesis of the muscle-specific MM isoenzyme of creatine-P kinase in this muscle. Our results show that neural information, or the consequences of it, is required to maintain the levels and rates of synthesis of glycolytic enzymes but not of creatine-P kinase in immature and mature fibers of both types. When mature "fast-twitch" muscles are cross-innervated with "slow" motor neurons, they begin to contract like slow muscles (6), to synthesize the myosin molecules characteristic of slow muscles (7-9), and to resemble slow muscles in terms of the levels of glycolytic and oxidative enzyme activities they contain (10-12). Opposite changes appear to occur when slow-twitch muscles are innervated with fast motor neurons. The specific identities of the neural signals which so profoundly influence the phenotypic expressions of muscle fibers have been the subject of considerable debate. Some workers believe that "chemical" signals, in the form of neural "tropic factors," are of paramount importance in governing the phenotypic expressions of muscle fibers (13-19). Other workers have presented evidence that "electrical" signals derived from innervating motor neurons are primarily responsible for the distinctive contractile behavior and biochemical expressions exhibited by fast-twitch and slow-twitch muscle fibers (20-23). Numerous studies have been performed on denervated muscles in the hope of gaining a better understanding of the influence of neural information on the levels and rates of synthesis of specific enzymes in muscle fibers. Chicken breast muscle was chosen for analysis because this tissue is composed predominantly of a single fiber type (fast-twitch), and since it contains high concentrations of a number of well-characterized enzymes which lend themselves as convenient "markers" in regulation studies. We will show that denervation causes an approximate 2-fold reduction in the content and relative rate of synthesis of glycolytic enzymes in breast muscle fibers, but that denervation has no appreciable effect on the content or relative rate of synthesis of the muscle-specific MM isoenzyme of creatine-P kinase levels and rates of synthesis of specific enzymes in "fast-twitch" (breast) muscle fibers of the chicken. Denervation of these muscles was associated with a 2- to 3-fold reduction in the levels and rates of synthesis of several enzymes in mature breast ("fast-twitch") white muscle fibers of the chicken.

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P kinase in this fiber type. Our results suggest that removal of neural information, or the consequences of it, results in a partial "dedifferentiation" of white muscle fibers.

**EXPERIMENTAL PROCEDURES**

**Organisms**—Newly hatched male chicks of the New Hampshire strain were obtained from Irvine's Hatchery, Downey, CA, and were maintained on chick starter mash (Harrison-Riedy Co.) until used. Under sodium pentobarbital anesthesia the pectoralis (breast) muscle on one side of 1-month-old chickens (3 weeks to 2 months of age) was surgically denervated at the brachial plexus.

**Preparation of Muscle Extracts and Isolation of Aldolase and Creatine-P Kinase**—Muscle specimens were homogenized in 10 or 20 volumes of 50 mM potassium phosphate, 1 mM EDTA, 1 mM magnesium acetate, 1 mM mercaptoethanol, pH 7.5, or in Hanks' physiological media (24), either in a glass-glass hand homogenizer or in the Sorvall Omni-Mixer at full speed. Homogenates were centrifuged at 12,000 \( \times g \) for 10 min, and the supernatant fractions were taken for analysis. Under these conditions, greater than 95% of the total amounts of each enzyme under study was recovered in the supernatant.

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**Enzyme Activity Measurements and Protein Determinations**—Extractions were assayed for aldolase (27), enolase (28), and glyceraldehyde-3-P dehydrogenase (29), and creatine-P kinase (30) activities at 25 °C by the spectrophotometric procedures previously described. Assays of creatine-P kinase activity were performed both in the presence and absence of substrate to correct for "background" activity which was present in crude extracts. Protein concentrations of crude extracts were determined by the method of Lowry et al. (31) using bovine serum albumin as a standard.

**Electrophoretic Methods**—Electrophoresis of crude muscle extracts on cellulose polyacetate strips (32) and staining of the strips specifically for aldolase (32) and creatine-P kinase (33) activities were performed as previously described. Electrophoresis of crude muscle extracts and purified enzymes in 5% polyacrylamide slab gels containing 0.1% sodium dodecyl sulfate was performed essentially as described by Laemmli (34). The 9-cm long and 1.5-mm thick gels were stained for protein with Coomassie blue and were subjected to densitometric analysis as previously described. Fluorographic analysis of gels containing \( ^3 \)H]leucine-labeled proteins was accomplished essentially as described by Bonner and Laskey (35), using Kodak XR 5 Omat film which was sensitized with flash pre-exposure (36). Gel slices containing labeled proteins were treated and counted as recently described by us. See appropriate figure and table legends for any additional experimental details.

**RESULTS**

**Levels of Specific Enzymes in Innervated and Denervated Breast Muscle Fibers**—Preliminary experiments showed that denervation of one side of chicken breast muscles for as long as 6 weeks had no effect on the other side, in terms of the levels of specific enzymes in the muscle specimens. In contrast, as shown below, breast muscles denervated for 2 to 6 weeks contained approximately 50% lower levels of the activities of three glycolytic enzymes (aldolase, enolase, and glyceraldehyde-3-P dehydrogenase). However, denervation for as long as 6 weeks had no significant effect on the level of creatine-P kinase activity in muscle fibers.

The decreased catalytic activity of the glycolytic enzymes in denervated muscle fibers was due to corresponding reductions in the levels of these enzyme molecules, rather than due to decreases in the catalytic activities of the enzymes themselves, as shown by electrophoretic analysis of crude muscle homogenates. Quantitation of these enzymes by the electrophoretic method is possible, since the glycolytic enzymes and creatine-P kinase comprise a very large percentage of total muscle protein. As shown in Fig. 1, similar amounts of myosin heavy chain, actin, and creatine-P kinase polypeptides/g of muscle were detected in innervated and denervated fibers, but the levels of glycolytic enzyme subunits were considerably lower in denervated as compared with innervated muscle. Particularly note the reduced levels of glycolytic enzymes relative to creatine-P kinase in the denervated sample. Moreover, as shown in Fig. 2, similar time courses for the decreases in glycolytic enzyme content in breast muscle fibers following denervation were apparent when enzyme levels were determined by direct catalytic activity measurements, or by electrophoretic and densiometric analysis of the 12,000 \( \times g \) soluble fraction of muscle homogenates. Homogenization of muscle specimens in isotonic Hanks' solution (24), rather than in the hypotonic phosphate buffer, had no effect on the enzyme levels calculated for innervated and denervated muscles, and all enzyme activities remained fully stable when crude muscle extracts were stored at 4 °C for as long as 1 week. These observations further demonstrate that inactivation of glycolytic enzymes by the action of lysosomal or other proteases was not responsible for the reduced levels of these molecules in homogenates of denervated muscle specimens.

As shown in Fig. 3, considerable atrophy of denervated muscle fibers and increased proportions of fibroblast-like cells

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**Fig. 1. Electrophoretic analysis of proteins present in innervated (I) and denervated (D) breast muscle fibers.** The left side of the breast muscle of a 1-month-old chicken was surgically denervated. Two weeks later samples of breast muscle derived from the innervated and denervated sides were extracted in 20 volumes of buffer as described under "Experimental Procedures." Equal volumes (8 \( \mu l \)) of these homogenates were electrophoresed in 1.5-mm thick, 9-cm long sodium dodecyl sulfate polyacrylamide gels as described under "Experimental Procedures." myo, myosin heavy chain; act, actin; phos, phosphorylase b; eno, enolase; cpk, creatine-P kinase; ald, aldolase; g3pdh, glyceraldehyde-3-P dehydrogenase. Identities of the indicated proteins were determined by comparisons of the electrophoretic mobilities of the stained protein bands with those exhibited by pure protein preparations.

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preferential expression of these non-muscle cells in the denervated muscle specimens. However, this conclusion is based on the following observations. 1) Chickens were killed and muscle specimens derived from innervated and denervated sides were taken for analysis. The soluble fractions of muscle homogenates were prepared and assayed for the enzymatic activities of interest. Samples were also analyzed by the electrophoretic method described under “Experimental Procedures.” Since the levels of creatine-P kinase (CPK) did not change following denervation, the amounts of glycolytic enzymes were expressed relative to that of creatine-P kinase. Ratios based on catalytic activity measurements and electrophoretic analysis are presented by \(\bullet\) and \(\bigcirc\), respectively. ALD, aldolase; G-3-PDH, glyceraldehyde-3-P dehydrogenase; ENO, enolase.

were detected in denervated muscle specimens. However, preferential expression of these non-muscle cells in the denervated musculature did not appear to influence the levels of the abundant enzymes detected in denervated specimens. This conclusion is based on the following observations. 1) As shown in Fig. 1, denervation did not result in appreciable reductions in the amounts of contractile proteins (myosin heavy chains and actin) in muscle specimens; 2) the MM isoenzyme of creatine-P kinase and the \(A_1\) isoenzyme of aldolase may be considered to be specific for skeletal muscle fibers, since all other tissues of the chicken which have been investigated, including fibroblasts, contain other, or additional, isoenzymic forms of these molecules (37, 38). As shown by the data in Fig. 4, these muscle-specific isoenzymes of aldolase and creatine-P kinase were the only ones observed in crude extracts of muscles denervated for as long as 6 weeks. In addition, our results do not reflect muscle fiber “death,” since the content of the muscle-specific MM isoenzyme of creatine-P kinase was not altered following denervation of breast muscle fibers.

The data presented in this section demonstrate that denervation of mature breast muscle fibers results in the establishment of new steady state levels of several glycolytic enzymes which are only about 50% of normal, and that establishment of these new levels is accomplished by 2 weeks after severing the nerves.

Synthesis of Specific Enzymes in Innervated and Denervated Breast Muscle Fibers—Since intracellular proteins are continuously being turned over (3), the reduced content of glycolytic enzymes in denervated breast muscle fibers could reflect increases in the rates of turnover and/or decreases in the rates of synthesis of these molecules in the denervated fibers. We investigated this question in short term amino acid incorporation experiments conducted in vivo and with muscle explants in vitro. The explant system is a valid alternative to the more cumbersome in vivo system for measuring relative rates of synthesis of muscle proteins, since we recently showed...
that fibers in these explants express the same protein synthetic programs during short term incubation as they do in vivo (26).2 We first showed that denervation caused pronounced changes in the pattern of synthesis of cytosolic proteins by electrophoretic and fluorographic analysis of the [3H]leucine-labeled proteins synthesized by innervated and denervated muscle fibers in vitro. As shown by the fluorographs presented in Fig. 5, large reductions in the rates of synthesis of a number of glycolytic enzymes relative to other cytosolic proteins was visually apparent in explants derived from denervated as compared with innervated muscles. Particularly note the reduced film exposure elicited by aldolase subunits relative to that elicited by creatine-P kinase subunits in the denervated sample.

More quantitative estimates of the relative rates of synthesis of aldolase and creatine-P kinase in innervated and denervated muscle fibers were determined in two ways. First, explants of the muscle types were labeled with [3H]leucine, and the newly synthesized cytosolic proteins were resolved by electrophoresis in sodium dodecyl sulfate polyacrylamide gels. The gels were stained for protein and the regions of the gels corresponding to the locations of aldolase and creatine-P kinase were cut out, and the radioactivity in the gel slices was determined. Ratios of counts per min in aldolase/counts per min in creatine-P kinase were calculated in each comparison.

The left sides of the breast muscle of 3-week-old chickens were surgically denervated as described under “Experimental Procedures.” Three weeks later, the chickens were killed and 0.5 g of muscle from innervated and denervated sides of the breasts were incubated separately in the presence of 400 µCi of [3H]leucine for 3 h. The samples were homogenized and soluble proteins were collected as described under “Experimental Procedures.” Labeled extracts containing about 40 µg of protein were electrophoresed in sodium dodecyl sulfate polyacrylamide slab gels (1.5 mm x 9 cm, Experiment A; 1.5 mm x 27 cm, Experiment B). The gels were stained for protein, regions of the gels were cut out, and the radioactivity in the gel slices was determined. Ratios of counts per min in aldolase/counts per min in creatine-P kinase were calculated in each comparison.

| Muscle       | Aldolase | Creatine-P kinase | Aldolase:Creatine-P kinase D:1 |
|--------------|----------|------------------|--------------------------------|
| Innervated   | 4.9 x 10^3 | 1.5 x 10^3 | 3.3 |
| Denervated   | 2.7 x 10^3 | 2.6 x 10^3 | 1.04 |
| Innervated   | 3.1 x 10^4 | 0.68 x 10^5 | 4.56 |
| Denervated   | 2.6 x 10^4 | 1.8 x 10^4 | 1.44 |

"The ratios of values obtained for denervated versus innervated fibers.

Fig. 5. Fluorographic analysis of the [3H]leucine-labeled cytosolic proteins synthesized by explants of innervated and denervated breast muscle fibers in vitro. Muscle explants derived from innervated and denervated (3 weeks) sides of chicken breasts were labeled as described under “Experimental Procedures.” The explants were homogenized and aliquots of the 12,000 x g soluble fractions were subjected to electrophoretic analysis in sodium dodecyl sulfate polyacrylamide gels. Labeled proteins were revealed by fluorographic analysis as described under “Experimental Procedures.” I, innervated; D, denervated; phos, phosphorylase; eno, enolase; cpk, creatine-P kinase; ald, aldolase; gapdh, glyceraldehyde-3-P dehydrogenase.

3 Unpublished observation.

of the data in this fashion allows us to neglect possible differences in amino acid precursor pools or in general protein synthetic capabilities which may exist between innervated and denervated muscle fibers. The results of two different experiments of this kind are presented in Table I. In both experiments, the rate of synthesis of aldolase relative to the rate of synthesis of creatine-P kinase was found to be about 3-fold lower in denervated muscle fibers than it was in innervated fibers.

Second, we measured the relative rates of synthesis of the muscle-specific isoenzymes of aldolase (A4) and creatine-P kinase (MM) in innervated and denervated muscle fibers in short term double isotope incorporation experiments. In the in vivo experiment, a denervated chicken received [3H]leucine and an innervated one received [14C]leucine. One hour later, the chickens were killed and innervated and denervated breast muscle specimens were mixed. The muscle tissue was homogenized and aliquots were taken to determine the [3H]/[14C] ratios of bulk and 12,000 X g soluble protein. Then, the two isoenzymes were purified to homogeneity by the method referred to under “Experimental Procedures.” The isoenzymes of these molecules which are found in fibroblasts (aldolase C4, ACs, and BB creatine-P kinase (33)) do not copurify with these muscle-specific forms of these enzymes (39). The isotope ratios of the pure muscle-specific isoenzymes were determined. Note that the [3H]/[14C] ratios obtained for the pure enzymes when normalized to that calculated for bulk muscle protein give direct estimates of the relative rates of synthesis of aldolase and creatine-P kinase in denervated as compared with innervated muscle fibers. The data obtained in this experiment are presented in Table II. Since different amounts of the protein fractions were counted, the actual counts per min in the samples are not to be compared. The important values here are the normalized isotope ratios calculated for the various protein fractions. These ratios show that the synthesis of aldolase A4 relative to the synthesis of bulk muscle protein was about 2-fold lower in denervated muscle fibers as compared with innervated fibers. In contrast, the
The left side of the breast muscle of a 1-month-old chicken was denervated. Two weeks later this chicken received 1 mCi of \[^{14}C\]leucine by intraperitoneal injection. A normal chicken received 100 pCi of \[^{14}C\]leucine instead. One hour later the chickens were killed and \[^{1}H\] and \[^{14}C\]-labeled denervated and \[^{1}H\] and \[^{14}C\]-labeled innervated muscles were mixed. The tissues were homogenized and an aliquot of the homogenate was taken to determine the \[^{1}H]/[^{14}C\] ratio of bulk and 12,000 \times g soluble muscle protein. Then, the muscle-specific isoenzymes of aldolase and creatine-P kinase were isolated to homogeneity. The \[^{1}H]/[^{14}C\] ratios of the pure enzymes were determined. Normalized ratios refer to those obtained when the \[^{1}H]/[^{14}C\] ratio found for bulk protein was set equal to 1.0.

The normalized ratio calculated for the MM isoenzyme of creatine-P kinase (1.24) suggests that the relative rate of synthesis of this enzyme was more similar in innervated and denervated muscle fibers.

For the in vitro experiment, explants obtained from innervated and denervated muscles were labeled with \[^{1}H\] and \[^{14}C\]leucine, respectively. Then, the explants were homogenized and aliquots of the homogenates were mixed. Protein fractions and purified enzymes were prepared and their isotope ratios were determined. The normalized isotope ratios presented in Table III again show that denervation caused a marked reduction in the relative rate of synthesis of aldolase, while the normalized isotope ratio calculated for the MM isoenzyme of creatine-P kinase (1.19) suggests that denervation had little effect on the relative rate of synthesis of this enzyme in breast muscle fibers.

**Table II**

| Protein                  | \[^{1}H\] (cpm) | \[^{14}C\] (cpm) | \[^{1}H\]/[^{14}C\] Normalized ratio |
|--------------------------|-----------------|-----------------|------------------------------------|
| Total                    | 6.6 \times 10^6 | 3.7 \times 10^6 | 1.78                               |
| Soluble                  | 4.4 \times 10^5 | 2.8 \times 10^6 | 1.57                               |
| Aldolase                 | 12.2 \times 10^5| 13.9 \times 10^5| 0.88                               |
| Creatine-P kinase        | 13.5 \times 10^5| 6.2 \times 10^6 | 2.18                               |

Comparisons between the Relative Rates of Synthesis of Aldolase and Creatine-P Kinase in Denervated Breast and Innervated Lateral Adductor Muscles—We have recently demonstrated that the relative rates of synthesis of several abundant glycolytic enzymes in presumptive red (lateral adductor) and white (breast) muscles of newly hatched chicks are very similar, and that the relative rates of synthesis of the glycolytic enzymes increase considerably during maturation of white fibers but not during maturation of red ones. Further, we found that the relative rates of synthesis of creatine-P kinase in red and white fibers were similar. These observations, together with the results presented in the above section, suggest that denervation of mature breast muscle results in a partial dedifferentiation of this tissue in terms of the enzyme synthetic programs expressed by the muscle fibers. We investigated the extent of this dedifferentiation by comparing the relative rates of synthesis of aldolase and creatine-P kinase in explants derived from denervated breast and innervated lateral adductor muscles. This was accomplished in a double isotope incorporation experiment using the experimental protocol employed in the above section. As shown by the normalized isotope ratios presented in Table IV, the relative rate of synthesis of aldolase \(A_1\) in fibers of the lateral adductor muscle was about 2-fold lower than it was in denervated breast muscle fibers, while the relative rate of synthesis of creatine-P kinase was fairly similar in the two muscle preparations. These data suggest that only a partial white to red transformation occurred in breast muscle fibers following denervation, since the relative rates of synthesis of aldolase and other glycolytic enzymes by mature innervated red fibers in vivo (4) or in explants are about 4-fold lower than those expressed by mature innervated white fibers.

**Table III**

| Protein                  | \[^{1}H\] (cpm) | \[^{14}C\] (cpm) | \[^{1}H\]/[^{14}C\] Normalized ratio |
|--------------------------|-----------------|-----------------|------------------------------------|
| Total                    | 4.12 \times 10^1| 7.47 \times 10^1| 1.00                               |
| Soluble                  | 21.9 \times 10^1| 4.99 \times 10^1| 0.68                               |
| Aldolase                 | 6.27 \times 10^1| 3.66 \times 10^1| 1.70                               |
| Creatine-P kinase        | 7.50 \times 10^1| 1.14 \times 10^1| 6.58                               |

**Table IV**

| Protein                  | \[^{1}H\] (cpm) | \[^{14}C\] (cpm) | \[^{1}H\]/[^{14}C\] Normalized ratio |
|--------------------------|-----------------|-----------------|------------------------------------|
| Total                    | 25.7 \times 10^1| 23.7 \times 10^1| 1.08                               |
| Soluble                  | 60.1 \times 10^1| 62.7 \times 10^1| 0.96                               |
| Aldolase                 | 22.0 \times 10^1| 36.4 \times 10^1| 0.60                               |
| Creatine-P kinase        | 8.1 \times 10^1 | 8.5 \times 10^1 | 0.95                               |

**Discussion**

As mentioned in the introduction, the functional and biochemical expressions of different fiber types of vertebrate skeletal muscles are largely determined by information these fibers receive from innervating motor neurons. Recent studies from our laboratory (4) showed that red and white muscles synthesize abundant glycolytic enzymes at different relative rates, and that the synthesis of creatine-P kinase molecules is independently regulated with respect to the synthesis of the glycolytic enzymes in these fibers. These studies further suggested that the enzyme synthetic programs expressed by presumptive red and white fibers in immature chicks resemble that expressed by mature red fibers, and that establishment of the white phenotype is associated with large increases in the relative rates of synthesis of the glycolytic enzymes during maturation of white muscle fibers. The present work was initiated to determine the effect of neural information, or the consequences of it, "maintaining" the characteristic levels and relative rates of synthesis of abundant glycolytic enzymes and creatine-P kinase in mature white fibers. Our results clearly show that denervation causes approximate 2-fold reductions in the levels of three abundant glycolytic enzymes in mature white fibers, but apparently has little effect on the
levels of creatine-P kinase molecules in this tissue. Although increased populations of fibroblast-like cells were evident in denervated musculature by microscopic examination, we ruled out the possibility that the reduced content of the glycolytic enzymes in the denervated musculature reflected the preferential expression of these, or possibly other, non-muscle cells in this tissue (see "Results").

The decreased content of glycolytic enzymes in denervated breast muscle fibers could be caused by decreases in the relative rates of synthesis and/or increases in the relative rates of turnover of these molecules following denervation. Results of our short term amino acid incorporation experiments conducted both in vivo and with muscle explants in vitro clearly showed that reductions in relative rates of synthesis of these molecules was predominantly responsible for establishing the lower steady state levels of these enzymes in breast muscle fibers following denervation. Although other workers (40, 41) have reported that denervation may cause alterations in the synthesis and turnover of bulk muscle protein, possible changes in general protein metabolism following denervation should not influence our interpretations, since the amino acid incorporation data were normalized to bulk muscle protein. Since chicken breast muscle is composed essentially of a single fiber type (fast-twitch), and since our analysis centered on the skeletal muscle-specific isoenzymes of aldolase and creatine-P kinase, we suggest that our observations reflect the behavior of individual muscle fibers. This interpretation is supported by the recent observations by Rubinstein et al. (23) on the myosin isoenzyme transition which occurs during chronic electrical stimulation of rabbit muscle fibers.

The present observations, that denervation did not result in a decrease in the level or relative rate of synthesis of the skeletal muscle-specific MM isoenzyme of creatine-P kinase, support our recent contention that the synthesis of this kinase is independently regulated with respect to the synthesis of glycolytic enzymes in different muscle fiber types and during fiber maturation. In contrast to the situation with the glycolytic enzymes, neural information is apparently not important in the regulation of synthesis of creatine-P kinase molecules in breast muscle fibers.

The mode of fiber type differentiation and the role of neural information in effecting this differentiative process are subjects of considerable debate. For example, Rubinstein and Kelly (42) have concluded, on the basis of an analysis of the isomorphs of myosin light chains, that neural information is required for differentiation of red fibers but not for differentiation of white ones. Yet, the present work clearly shows that neural information, or the consequences of it, is required for maintaining the high relative rates of synthesis of the glycolytic enzymes in mature white fibers and that removal of it results in a partial dedifferentiation of these fibers. Also, the report by Keller and Emerson (43) that embryonic muscle cells synthesize both the red and white subsets of myosin light chains and the reports (44, 45) that embryonic myosins may be different from the adult forms suggest that the mode of fiber type differentiation and the role of neural innervation in establishing distinct fiber types may be more complex than previously thought.

Finally, the present work is of interest in view of our recent analysis of the levels and synthesis of aldolase in breast muscle fibers of dystrophic chickens (26). In both denervated normal and innervated dystrophic breast muscles we found that the content of the muscle-specific A₁ isoenzyme of aldolase was about 50% lower than normal, and in both conditions this reduction in enzyme content was associated with a 2- to 3-fold reduction in the relative rate of synthesis of this molecular species. Further analysis of the apparent parallels between these two conditions are being performed in our laboratory, and these studies may give further insights into the lesion in avian muscular dystrophy which alters the protein synthetic programs expressed by white muscle fibers.

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REFERENCES

1. Leibherz, H. G., Petell, J. K., and Shackelford, J. E. (1979) Int. Cong. Biochem. Abstr. 11, 499
2. Leibherz, H. G., Shackelford, J. E., Petell, J. K., and Sardo, M. J. (1980) Abstracts Second International Congress on Cell Biology, Berlin, West Germany, p. 433
3. Schumke, R. T., and Doyle, D. (1970) Annu. Rev. Biochem. 39, 929-976
4. Leibherz, H. G., Sardo, M. J., Petell, J. K., and Shackelford, J. E. (1978) in Protein Turnover and Lysosomes Function (Segal, H., and Doyle, D., eds) pp. 655–685, Academic Press, New York
5. Deleted in proof.
6. Buller, A. J., Eccles, J. C., and Eccles, R. M. (1960) J. Physiol. 150, 417–439
7. Hoh, J. F. Y. (1975) Biochemistry 14, 742–747
8. Soret, F. A., Luff, A. R., and Gergely, J. (1975) J. Gen. Physiol. 66, 811–821
9. Weeds, A. G., Treenthal, D. R., Keas, C. J. C., and Buller, A. (1974) Nature 247, 135–139
10. Golisch, G., Pette, D., and Pichlmair, H. (1979) Eur. J. Biochem. 16, 110–116
11. Romand, F. C. A., and Vander Meulen, J. P. (1966) Nature (Lond.) 212, 1369–1370
12. Prewitt, M. A., and Salafsky, B. (1970) Am. J. Physiol. 218, 69–74
13. Younkin, S. G., Brett, R. S., Davey, B., and Younkin, L. H. (1978) Science 200, 1292–1296
14. Albuquerque, E. X., Warnick, J. E., Tasse, J., and Sansone, F. M. (1972) Exp. Neurol. 37, 607–634
15. Cangiano, A. (1973) Brain Res. 58, 255–259
16. Crain, S. M., and Peterson, E. R. (1974) Ann. N. Y. Acad. Sci. 228, 6–34
17. Oh, T. H., Johnson, D. D., and Kim, S. U. (1972) Science 178, 1298–1300
18. Oh, T. H. (1976) Exp. Neurol. 50, 373–386
19. Engelhardt, J. K., ishikawa, K., Mori, J., and Shimakakuro, Y. (1977) Brain Res. 128, 243–248
20. Salmons, S., and Vrbova, G. (1969) J. Physiol. (Lond.) 201, 535–549
21. Salmons S., and Sreter, F. (1976) Nature 263, 30–34
22. Salmons, S., Gale, D. R., and Sreter F. A. (1978) J. Anat. 127, 31–31
23. Rubinstein, N., Mabuchi, K., Pepe, F., Salmons S., Gergely, J., and Sreter, F. (1978) J. Cell. Biol. 79, 252–261
24. Hanks, J. H., and Wallace, R. E. (1949) Proc. Soc. Exp. Biol. Med. 71, 196–200
25. Deleted in proof.
26. Petell, J. K., and Leibherz, H. G. (1979) J. Biol. Chem. 254, 7911–7917
27. Leibherz, H. G., and Rutter, W. J. (1975) Methods Enzymol. 42C, 249–258
28. Wold, F. (1975) Methods Enzymol. 42C, 329–334
29. Allison, W. S., and Kaplan, N. O. (1964) J. Biol. Chem. 239, 2140–2146
30. Eppenberger, H. M., Dawson, D. M., and Kaplan, N. O. (1967) J. Biol. Chem. 242, 204–209
31. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
32. Sosor, W. A., Penhote, E., and Rutter, W. J. (1975) Methods Enzymol. 41, 66–72
33. Turner, D. C., Maier, V., and Eppenberger, H. M. (1974) Dev. Biol. 37, 63–89
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34. Laemmli, U. K. (1970) *Nature (Lond.)* 227, 680–685
35. Bonner, W. M., and Laskey, R. A. (1974) *Eur. J. Biochem.* 46, 83–88
36. Laskey, R. A., and Mills, A. D. (1975) *Eur. J. Biochem.* 56, 335–341
37. Eppenberger, H. M., Eppenberger, M., Richterich, R., and Aebi, H. (1964) *Dev. Biol.* 10, 1–16
38. Lebherz, H. G., and Rutter, W. J. (1969) *Biochemistry* 8, 109–121
39. Lebherz, H. G., and Shackelford, J. E. (1979) *J. Biol. Chem.* 254, 4227–4232
40. Goldberg, A. L. (1969) *J. Biol. Chem.* 244, 3223–3229
41. Goldspink, D. F. (1978) *Comp. Biochem. Physiol.* 61, 37–41
42. Rubinstein, N. A., and Kelly, A. M. (1978) *Dev. Biol.* 62, 473–485
43. Keller, L. R., and Emerson, C. P. (1980) *Proc. Natl. Acad. Sci. U. S. A.* 77, 1020–1024
44. Hoh, J. F. Y., and Yeoh, G. P. S. (1979) *Nature* 280, 321–322
45. Sreter, F. A., Balint, M., and Gergely, J. (1975) *Dev. Biol.* 46, 317–325