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Studies on the Fragmented Sarcoplasmic Reticulum and "Natural" Tropomyosin of Normal and Dystrophic Chickens[1] (38808)

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Sreter *et al.* (1) observed that the Ca^{2+} transport function of the FSR[2] was impaired in the muscles of dystrophic animals. Previous work in this laboratory (2, 3) confirmed the discovery of Sreter and further showed that the lipid composition of the FSR obtained from the dystrophic chickens was different from FSR of normal controls. In this study experimental evidence has been obtained that the Ca^{2+}-activated ATPase enzyme in the FSR of the dystrophic chickens is also different from that of the normal control. On the other hand, no alterations were found in the activity of the natural tropomyosin (tropomyosin plus troponin) in the muscles of the dystrophic chickens.

Materials and Methods. All reagents used were of analytical grade. Chickens inflicted with genetically controlled muscular dystrophy were obtained from the Department of Avian Sciences of the University of California, Davis, CA. The FSR was prepared using the method of Martonosi and Feretos (4), and the natural tropomyosin was isolated by the method of Ebashi and Ebashi (5). The yield of natural tropomyosin from the breast muscles of both the normal and the dystrophic chicken (2 mg natural tropomyosin per g of muscle protein) was approximately the same. Troponin-free actin was prepared according to the method of Bailin and Barany (6), and myosin A and myosin B were prepared as described by Mommaerts (7). The ATPase enzyme of the FSR was solubilized by the method of Ikemoto *et al.* (8) except that we omitted glycerol from the mixtures. The ATPase activity was measured as described previously (3) except that the incubation time was 1 min. The composition of each assay mixture will be specified in the text.

Results. The Ca^{2+}-activated "extra" ATPase activity was studied first using the FSR of the normal and the dystrophic

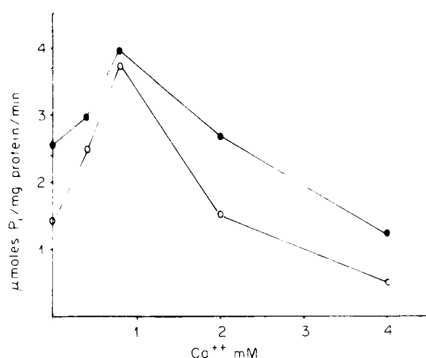

FIG. 1. Effect of Ca^{2+} on the ATPase activity of the FSR of normal and dystrophic chickens. Each incubation mixture contained 5.0 µmole ATP, 5.0 µmole Mg^{2+}, 50.0 µmole Tris buffer, pH 7.5, to which one of the following was added: ● 0.3 mg FSR protein of the dystrophic chickens; ○ 0.3 mg FSR protein of the normal chickens. The total volume of the incubation mixture was 1 ml. The reaction was carried out at 37°C. The Ca^{2+} concentration was assumed to be "zero" in the presence of 0.5 µmole/ml EGTA. Four to six experimental animals were used to determine the individual values presented on the graph. The cross bars indicate standard deviations.

chickens as the source of the enzyme. It may be seen from Fig. 1 that in the presence of 0.5 µmole/mg EGTA, the Mg^{2+}-activated ATPase of the FSR obtained from the dystrophic chickenes was 80% higher than that of the normal controls. The maximal ATPase activity of the FSR was found in the presence of 8×10^{-4} Ca^{2+} concentration. ATPase of FSR from the dystrophic

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[2] Abbreviations: FSR; Fragmented sarcoplasmic reticulum; EGTA; Ethylene glycol bis (βaminoethyl ether)-*N*,*N*′ tetraacetic acid; ATPase; adenosine triphosphatase; µmole P_i/mg/prot/min; µmoles of inorganic phosphate liberated per minute.

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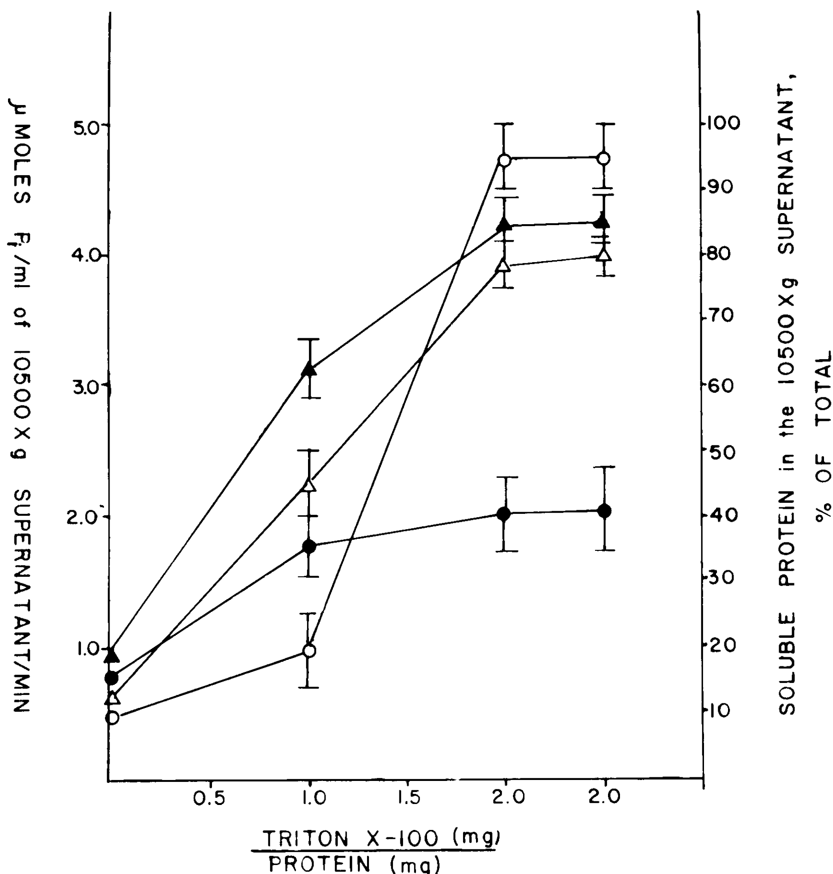

FIG. 2. Effect of Triton X-100 on the protein concentration and ATPase activity of the 105,000*g* supernatant extract of FSR obtained from normal and dystrophic chickens. Each incubation mixture contained about 0.1–0.5 mg protein (105,000*g* extract from the FSR of normal or dystrophic chickens), 5.0 μmole ATP, 5.0 μmole Mg^{2+}, 0.05 μmole Ca^{2+}, and 50.0 μmole Tris buffer, pH 7.5. ○ ATPase activity in the FSR extracts of normal chickens; ● ATPase activity in the FSR extracts of dystrophic chickens; △ Protein concn. of the extract of normal chickens as percentage of total protein; ▲ Protein concn. in the extract of dystrophic chickens as percentage of total protein. An average of five experimental animals were used to determine the individual values presented on the graph. The cross bars indicate standard deviations. (For details of the extraction procedure see Ref. 8.)

chicken increased by 46% while ATPase of the control FSR increased by 159%.

In order to obtain further evidence to decide whether or not a significant difference existed between the ATPase of FSR of normal and dystrophic chickens, we solubilized the enzymes and determined their maximal activity in the presence of 2×10^{-5} *M* Ca^{2+}. It may be seen from Fig. 2 that with 2 mg Triton X-100/1 mg sarcoplasmic protein about 80–85% of the total sarcoplasmic protein was solubilized in 10 min (8). At the same time the ATPase activity of the 105,000*g* supernatant extract of the normal FSR was more than twice as high as the supernatant extract of sarcoplasmic reticulum of the dystrophic chickens. It has been published (8, 9) that low concentrations of Triton X-100 increase the ATPase activity of FSR 3.5 times. Since the results of experiments compiled in Fig. 2 showed a great difference between the ATPase activities of the Triton X-100 "treated" supernates of the FSR of dystrophic and normal

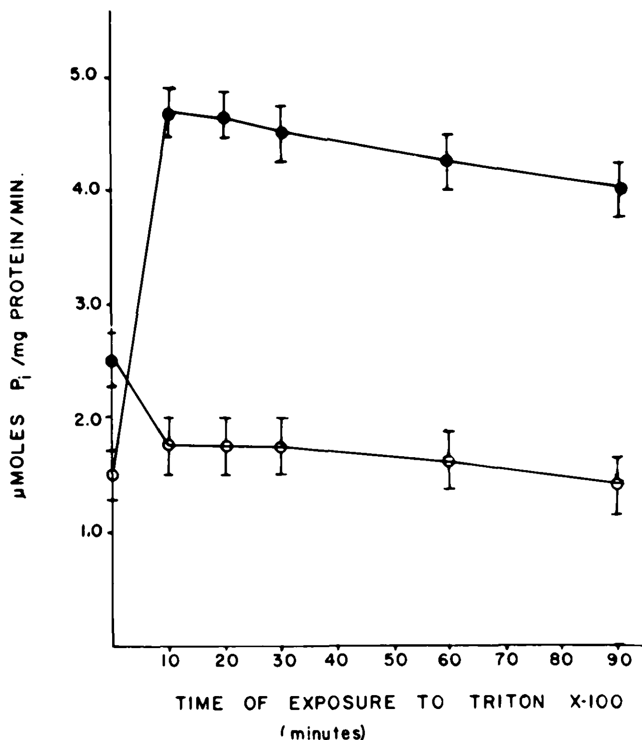

FIG. 3. Effect of the duration of Triton X-100 exposure (mg Triton X-100/mg protein) on ATPase activity of FSR from normal and dystrophic chickens. The composition of the incubation mixtures and the symbols used are the same in Fig. 2. Without exposure to Triton X-100 the ATPase activity of the intact FSR was measured and recorded in zero minutes. ATPase activities in the 105,000*g* supernatant after different amounts of exposure to Triton X-100. Assay was run for 1 min.

chickens, we decided to compare the activating effect of Triton X-100 on the ATPase activities of the FSR obtained from normal and dystrophic chickens. As the results in Fig. 3 show, the ATPase activity of the FSR obtained from the dystrophic chickens was actually inhibited by 20–25% after a 10- to 90-min incubation with Triton X-100. Under similar experimental conditions the ATPase activity of the FSR from the normal controls was activated by 300%. This 300% activation is in agreement with the results of previous authors (8, 9).

In the subsequently described experiments we compared the activity of the natural tropomyosins obtained from the breast muscle of normal and dystrophic chickens. As the results in Fig. 4 show, the EGTA sensitivity of the myosin B enzymes from normal and dystrophic chickens is similar. The results of experiments depicted in Fig. 5 show that in the absence of Ca^{2+}, natural tropomyosin from dystrophic chickens inhibited the ATPase activity of the synthetic actomyosins regardless of whether these proteins were prepared from normal or alternatively from dystrophic chickens. The results did not change when otherwise under the same experimental conditions the natural tropomyosin prepared from normal chickens was substituted for the natural tropomyosin isolated from the dystrophic chickens.

Discussion. Previous work in this laboratory (2, 3) and in other laboratories (10, 11) have shown that the lipid composition of the FSR of the dystrophic chickens was different from that of the normal controls. The results of the present work have shown that the ATPase activity of the FSR of the

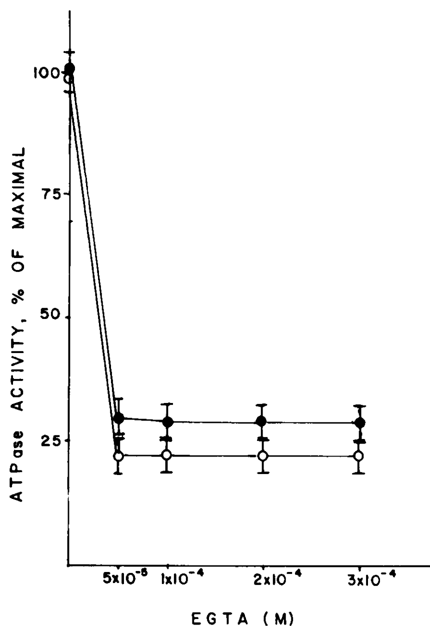

FIG. 4. Effect of EGTA on the Mg^{2+}-activated ATPase of myosin B obtained from normal and dystrophic chickens. Each incubation medium contained 5.0 μmole ATP, 5.0 μmole Mg^{2+}, 50.0 μmole KCl, and 25.0 μmole Tris buffer pH 7.5 with one of the following additions: ○ 1.0 mg myosin B, normal; ● 1.0 mg myosin B, dystrophic. The total volume of the individual mixtures was 1 ml. The incubation was performed at 37°C for 5 min.

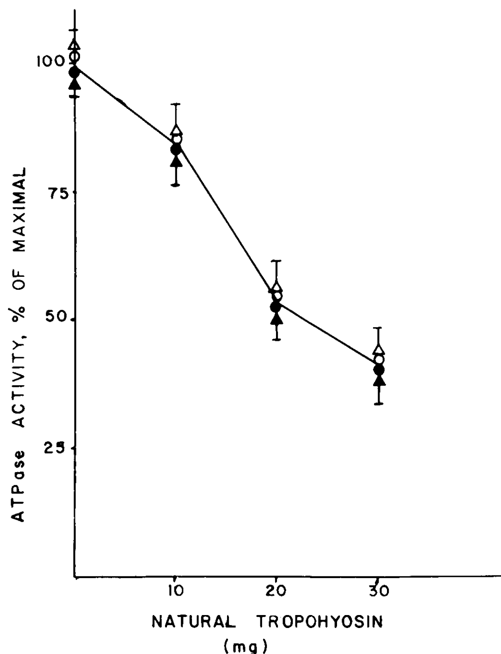

FIG. 5. The effect of natural tropomyosin on the ATPase activity of synthetic actomyosins assembled from the contractile proteins of normal and dystrophic chickens. Each incubation medium contained 5.0 μmole ATP, 5.0 μmole Mg^{2+}, 0.1 μmole EGTA, and 50.0 μmole KCl and 25.0 μmole Tris buffer, pH 7.5, to which the following were added: ○ 1 mg myosin A, normal 1 + 0.5 mg actin, normal + natural tropomyosin, normal, ● 1 mg myosin A, dystrophic + 0.5 mg actin, dystrophic + natural tropomyosin, dystrophic; △ 1 mg myosin A, normal + 0.5 mg actin normal + natural tropomyosin, dystrophic; ▲ 1 mg myosin A, dystrophic + 0.5 mg actin, dystrophic + natural tropomyosin, normal. The total volume of the mixtures was 1 ml. The experiments were performed at 37°C. An average of three experimental animals were used to determine the individual values presented in the graph. The cross bars indicate standard deviations.

dystrophic chickens is less sensitive to Ca^{2+} than that of the normal controls (Fig. 1). Studies on the solubilized sarcoplasmic enzymes show conclusively that the sarcoplasmic ATPase enzymes of the dystrophic and normal chickens are markedly different after the Triton X-100 solubilization in an aqueous environment (Figs. 2 and 3).

On the other hand there was no significant difference found between the natural tropomyosins obtained from the breast muscles of normal and dystrophic chickens (Figs. 4 and 5). Accordingly, the results of this study did not reveal any qualitative or quantitative alteration on part of the control proteins (troponin and tropomyosin). Previous studies in this laboratory have failed to show a major functional alteration on part of the contractile proteins (myosin and actin) in the case of the genetically controlled muscular dystrophy of the chickens (12). The results of this investigation lend further support to the theory that muscular dystrophy is primarily a disease of the various membranes of the inflicted organisms (2, 3, 10, 11).

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