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## Localization of Proteolytic Activity in Muscle Protein Fractions. (19753)

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In the past most of the studies on muscle have been done in an attempt to learn more about the enzymes involved in the important carbohydrate energy cycle. Consequently, little is known about the proteolytic enzymes in muscle.

The following investigation was undertaken in an attempt to identify some of the proteolytic enzymes and localize them in muscle protein fractions.

*Materials and methods.* Muscles from the legs of 10 specimens of frog, *Rana pipiens,* were removed and placed in a mixture of ice and water (0 to 2°C) to inhibit bacterial growth and autodigestion. As much connective tissue and as many blood vessels as possible were excluded.

These muscles were then used as a source of muscle protein fractions. Myosin was extracted according to the method of Greenstein and Edsall(1). The procedure of Baranowski(2) was adapted for the extraction of myogen. The procedures outlined by Bate-Smith(3) and Meyer and Weber(4) yielded protein fractions rich in myoalbumin and globulin x, respectively.

A modified method of Anson(5) was employed to obtain some quantitative data concerning the proteolytic enzymes pepsin, trypsin, and cathepsin. Because absolute values could not be ascertained, the amount of proteolytic digestion by increasing quantities of muscle protein during a 10 minute period, was compared with that by standard solutions of pepsin, trypsin, and tyrosine. Standard tyrosine solutions were prepared for comparison with cathepsin because cathepsin is not available commercially.

*Results.* Very few, small crystals of myogen appeared. They were too small and few in number to separate from the myoalbumin. For that reason and because myogen cannot be extracted from myoalbumin in the native state(3), the myogen and myoalbumin fractions were analyzed together. When preliminary investigations revealed that no proteolytic activity was present in the globulin x fraction, analyses were made only on myosin and myosin-free fractions.

Cathepsin and pepsin were localized in the myosin fraction and trypsin in the myosin-free fraction. No enzyme appeared in both the myosin and myosin-free fractions.

Fig. 1 shows curves resulting from digestion of hemoglobin by increasing quantities of muscle protein fractions and of standard enzyme solutions during a 10 minute period. The curves of digestion by muscle proteins are almost parallel to each other.

Maximum digestion by trypsin in the myosin-free fraction occurred after 6 cc of myosin-

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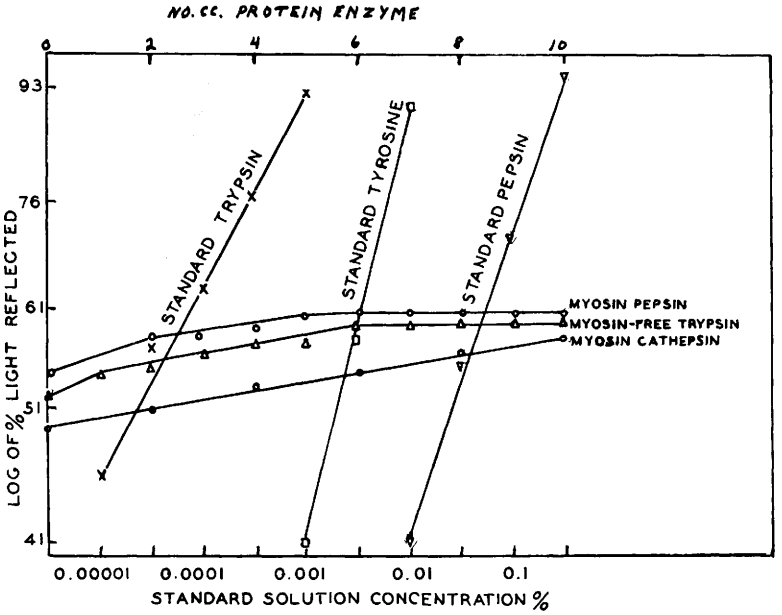


FIG. 1. Amount of digestion by increasing quantities of muscle protein-enzymes during a 10 minute period.

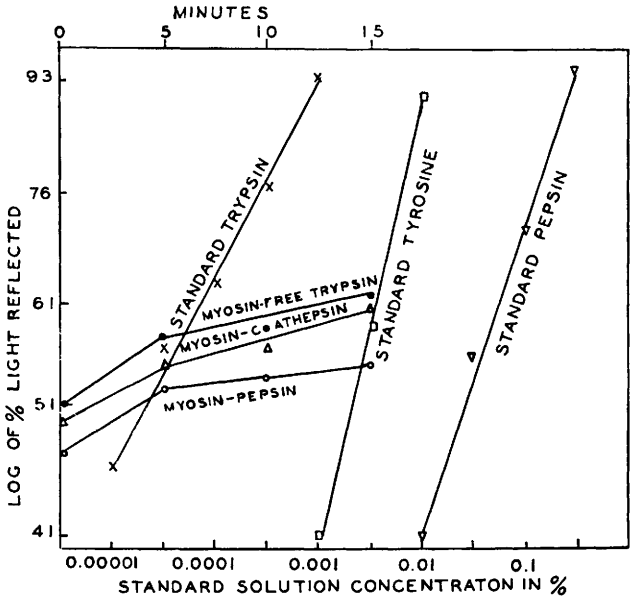


FIG. 2. Amount of digestion by 1 cc of muscle protein-enzymes during varied time intervals.

free solution were added. The total colorimetric change produced (from 52% to 60% light reflected) was comparable to a change produced by increasing the concentration of standard trypsin solution from 0.0004% to 0.0009%. Maximum digestion by pepsin occurred after 5 cc of myosin solution were added. The total colorimetric change (from 55% to 61% light reflected) was comparable to a change produced by increasing the concentration of a standard pepsin solution from 0.05% to 0.075%. There was a straight line

increase in the amount of catheptic digestion by increasing quantities of myosin solution. The overall colorimetric change due to cathepsin digestion (from 40% to 50% light absorbed) was comparable to an increase in concentration of a standard tyrosine solution from 0.0025% to 0.005%. A leveling off phase, indicative of equilibrium, was recorded with myosin-pepsin and myosin-free trypsin solution, but not with myosin-cathepsin solutions.

Curves showing the amount of digestion by proteolytic enzymes in 1 cc of muscle protein solution during different time intervals, are shown in Fig. 2. In all instances there was a rapid increase in the amount of digestion during the first 5 minutes. This was followed by a slower, but steady, increase in the rate of digestion.

*Summary.* 1. An investigation was made to obtain information regarding the presence and localization of proteolytic enzymes in muscle protein fractions from frog leg muscles. 2. Pepsin and cathepsin were localized in the myosin fraction while trypsin was localized in the myosin-free fraction. No proteolytic activity was displayed by the myoalbumin fraction. 3. The myogen content of the frog muscles is considerably lower than that reported for mammals.

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## Effect of Sodium Monofluoroacetate on Multiplication of Eastern Equine Encephalomyelitis Virus.* (19754)

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Sodium monofluoroacetate has been shown to affect the citrate metabolism of tissues from animals treated with this compound. Potter (1) has found that the citrate level of rat brain after treatment of the animal with this drug was approximately 6 times that of normal. Tricarboxylic acid fractions isolated from tissues of fluoroacetate-treated animals(2) have shown the presence of a fluorine-containing compound ("inhibitor fraction") that would inhibit the oxidation of citrate. Recently it has been found(3) that aconitase activity is inhibited by this "inhibitor fraction". The inhibitory effect is thought to be produced by a fluorine-containing compound other than fluoroacetate and which has been shown to be chromatographically inseparable from the tricarboxylic acid preparations. It has been suggested that the inhibitory substance is fluorocitrate$_2$(4).

Ackermann(5) has found that fluoroacetate will cause an initial depression in the rate of influenza virus production in experimentally infected mouse lung. However, the virus concentration in the treated lung tissue was found to be similar to that of the non-treated lung tissue at a later period in the virus incubation time.

Ainslie(6) has investigated the effect of this compound on the rate of virus production in Lansing poliomyelitis virus infected mice and has noted a significant delay in the virus growth which he was able to relate to an increased survival time of the infected animals treated with fluoroacetate, but the natural course of the infection was not otherwise altered.

*Materials and methods.* Swiss albino mice

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