Technical Papers

Propagation of Group A Coxsackie Viruses in Denervated Adult Mouse Muscle

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Evidence in the literature (*1, 2*) indicates that denervation of adult skeletal muscle results in decrease in activity of two enzymes: phosphorylase and phosphoglucomutase, toward the low levels found in embryonic and infant muscle. The concept that denervated muscle may resemble immature muscle in some aspects of its metabolism suggested that the metabolic change in denervated muscle might result in a return to the ability of the tissue to support the multiplication of the Group A Coxsackie viruses. These viruses previously have not been demonstrated to undergo multiplication in adult mice; Syverton *et al.* (*3*) have reported that following the simultaneous administration of cortisone and x-irradiation, a Group A, Type 4, Coxsackie virus was lethal for adult mice; however, no evidence was presented to prove that viral multiplication had occurred or to indicate that muscle was affected.

Five- to six-week-old white Swiss mice were subjected to unilateral sciatic nerve section in the thigh, and approximately 2 weeks later were inoculated intramuscularly into the calf of the denervated leg with a dilution of a Group A, Type 2, Coxsackie virus (N.I.H. strain 93). Three days after inoculation the gastrocnemius muscle was removed and the infectivity titer determined in 2-day-old mice. It has been found repeatedly that following inoculation of 10^3–10^4 LD_{50} (determined in 2-day-old mice) the infectivity titer of the muscle reached 10^{-5}–10^{-6}. In controls, consisting of normal adult mice, or of adult animals subjected to a sham operation, only traces or no virus remained on the third day. Strain 93 has been carried without difficulty through 50 serial passages in denervated adult mouse muscle, each passage being made by the injection of 0.02 ml of a 2.5% muscle suspension into the denervated calf. The infectivity titer of the 50th passage muscle was $10^{-6.1}$. Material from the 50th adult passage was typed serologically as strain 93.

For initiation of infection by the intramuscular route, an interval of at least 7 days after denervation and an inoculum of at least 3 logs of virus (LD_{50}'s in suckling mice) are required. Preliminary experiments indicate that the infection is confined to the denervated limb. Infection of the denervated muscle is occasionally initiated when large doses of virus are given intraperitoneally, but not when given orally.

Strains representing 7 additional types of Group A Coxsackie viruses have been tested, and evidence of multiplication has been obtained in all; no conclusive evidence of growth in controls has been found, and attempts to pass the viruses serially in sham-operated mice have been consistently negative. Two strains, representing Albany Type 3 and Type H_3, have been carried successfully through 7 serial passages in denervated adult mouse muscle, with virus present at theoretical dilutions of the original inoculum of 10^{-23}.

Although the hypothesis of the reversion of the muscle metabolism to that of immature muscle is attractive in regard to the ability of the denervated muscle to support the growth of the Group A Coxsackie viruses, the available data are not sufficient to warrant any conclusions on the mechanism of the phenomenon.

References

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Catalase Assay with Special Reference to Manometric Methods[1]

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The Michaelis-Menten concept of the mechanism of enzyme action has been very fruitful in correlating the rate of a catalyzed reaction with the concentration or "activity" of the enzyme (*1*). In the reaction

$$E + S \rightleftarrows ES \rightarrow E + \text{products}, \qquad (1)$$

where E is the free enzyme and S the substrate, the rate of breakdown of the intermediate complex, ES, is assumed to be rate-determining. Since in the presence of excess substrate all the enzyme is in the form of ES, the monomolecular breakdown of ES is linearly proportional to the total enzyme concentration. The concentration of the intermediate complex under these conditions is constant, and therefore the rate of the reaction obeys a zero order equation with respect to substrate concentration. These properties of a Michaelis-Menten type of enzyme provide the basis for the rationale for computing enzyme concentrations from a zero order slope.

The same rationale cannot be used for catalase,

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