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Myocardial Contractile Protein: Dependence of Viscosimetric Properties on Organ Size.* (30179)

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Solutions of the contractile protein actomyosin characteristically undergo a marked reduction of viscosity upon the addition of ATP. It is generally believed that this response is the result of dissociation of the actomyosin into its components, actin and myosin(1). The magnitude of this change may be conveniently expressed as the "ATP sensitivity"(2) calculated in the following manner:

$$\text{ATP sensitivity} = \left(\frac{Z\eta - Z\eta_{ATP}}{Z\eta_{ATP}} \right) \times 100$$

where $Z\eta$ the viscosity number $= \dfrac{2.3 \log \eta \text{ rel}}{\text{C (protein conc) in g per l}}$

and η rel = the ratio of the outflow time of the protein solution to that of the solvent.

The subscript ATP refers to values obtained after addition of the nucleotide.

Determination of ATP sensitivity has been widely used in identification and characterization of contractile proteins. In the case of the heart it has been used to characterize myocardial contractile proteins extracted following a variety of experimental procedures concerned with the growth and function of that organ(3,4,5,6). In the course of such experiments conducted in this laboratory during the past several years, it became apparent that a significant relationship exists between ATP sensitivity and ventricular size(5) or age(6). This report is concerned with this relationship.

The experimental animals were male or female albino rats of the Sprague-Dawley strain. Actomyosin (Myosin B) was extracted by a modification of the method of Benson *et al*(7). Homogenized ventricular muscle was extracted in Weber's solution for 24 hours at 4°C in the presence of excess ATP. The soluble fraction containing the contractile proteins was separated by centrifugation at 12,000 *g* for 20 minutes. Actomyosin was precipitated by dilution to a molarity of 0.1 at pH 6.8 and separated by centrifugation at 10,000 *g* for 20 minutes. The pellet was then redissolved in 0.6 M KCl and the solution centrifuged again. All operations were carried out at 0-4°C. Nitrogen content of the supernatant was determined by micro-Kjeldahl technique and viscosimetric properties were studied using Ostwald viscosimeters at a temperature of 24°C and a pH of 7.0. The concentration of the protein solution was about 1.0 mg per ml. Care was taken to insure a constant concentration of ventricular myocardium in the extraction procedures and all determinations were based on aliquot samples. Pooling of hearts was carried out when necessary to achieve these requirements. Values for actomyosin content of rat ventricle obtained by this method are lower than those reported for the dog(3,4) or the rabbit(8). It is not clear whether

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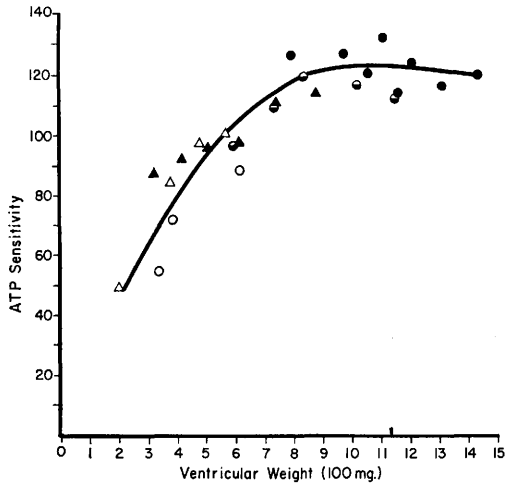

FIG. 1. Relation of ATP sensitivity to ventricular weight. △—Group A, normal animals; variable age series. ▲—Group B, normal animals; variable age series. ◒—Group C, normal animals, exercised animals, and aortic constricted animals. ●—Group D, normal animals, growth hormone treated animals, and aortic constricted animals. ○—Group E, normal animals, hypophysectomized animals, and growth hormone treated animals.

these differences are indicative of species variation or are due to technical factors such as those suggested by Inchiosa(8).

Other determinations included body and ventricular weight, total ventricular protein concentration and myocardial water content. In some cases parallel determinations of active and passive length-tension curves and other physiological parameters were carried out on surviving papillary muscles.

Fig. 1 presents the relationship between ventricular weight and ATP sensitivity of myocardial actomyosin in 5 groups of experiments. Each point represents the mean of 6 to 10 determinations. In Groups A and B cardiac size varied with age alone. In Groups C, D, and E cardiac size was modified by exercise, aortic constriction or endocrine manipulation. The data indicate a progressive increase in ATP sensitivity as ventricular weight increased to a value of 800 mg. Beyond this point maximum values for ATP sensitivity obtain. The solid line is a best visual fit to the data. The data of Group E are of particular interest in providing some differentiation between cardiac age and cardiac size as factors in the relationship observed. The small hearts in this group were obtained from hypophysectomized animals (5) whose chronological age was the same as that of the larger controls. It is clear that in this instance ATP sensitivity varied with degree of cardiac growth rather than with chronological age. Although ATP sensitivity varied with ventricular size, the concentration of actomyosin, as determined on the redissolved precipitate by micro-Kjeldahl technique did not vary whether expressed as a percentage of wet ventricular weight or of total ventricular protein. Table I presents data of Group A and is illustrative.

In the case of Groups C, D, and E the maximum developed isometric tension was determined on isolated surviving papillary muscles. There was no significant change in tension production associated with differences in ATP sensitivity.

Modification of the viscosimetric response of skeletal contractile proteins in relation to embryonic and post-embryonic development has been reported(9,10). Changes in ATP sensitivity have also been shown to occur in uterine actomyosin in relation to stimulation of growth by estrogens(11). The effects reported here for cardiac muscle are probably of a related nature and are compatible with the suggestion that cardiac growth involves a stage in which the muscle contains a reduced concentration of actin. It seems remarkable, however, that if such is the case some other material is extracted in an amount

TABLE I. Myocardial Protein Content and Characteristics.
Group A: Normal animals, variable age.

Age groups, mean body wt (g)	No. of animals	Actomyosin concentrations: % Wet ventricular wt	% Total ventricular protein	ATP sensitivity
50	15	2.76 ± .55	13.3 ± .5	49 ± 11
100	18	2.69 ± .26	13.8 ± .8	84 ± 10
150	18	2.75 ± .37	13.2 ± .6	97 ± 5
200	18	2.73 ± .25	13.5 ± .4	100 ± 9

which keeps the total isolated contractile protein concentration unchanged. It is also notable that no measureable effects on the ability of the muscle to develop tension were associated with these changes in chemical characteristics. These matters deserve further consideration.

Summary. The viscosimetric response of rat myocardial actomyosin solutions to addition of ATP (ATP sensitivity) varies with ventricular weight. This suggests quantitative or qualitative changes in the components of the contractile system with cardiac growth. Total contractile protein concentration is unchanged and maximum isometric tension development by surviving muscles did not vary.

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Effect of Estradiol on Albumin-I^{131} in the Skin of Mice Following Intravenous Injection.* (30180)

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It was previously observed that clearance of albumin-I^{131} from plasma and apparent transvascular passage of this substance into the myocardium of male dogs decreased with age(1). It was suggested that age changes in the connective tissue may play a role in this phenomenon(2). To gain further insight into this matter the passage of albumin-I^{131} into connective tissue-rich organs was investigated. During this study it was observed that estradiol treatment resulted in increased bound I^{131} in the skin after the latter was injected intravenously.

Method. Male mice weighing 25-30 g were obtained from commercial sources. Hormones were injected daily in 0.1 cc sesame oil or aqueous suspension subcutaneously for 5 days. Control animals received only oil. Following the last injection 0.1 cc (original activity 1 μc/0.1 cc) of human albumin-I^{131}† was injected into a tail vein. Eight hours after intravenous injection the animals were killed by cervical fracture. The tail, head, and limbs were removed, the hair was shaved off, and the fat was scraped from the underside of the pelt. The skin was cut into small pieces and put into 15 ml of 90% formic acid in a screw-cap tube. The tubes were autoclaved at 15 lb pressure, 250°F for exactly one hour. After cooling, the contents of the tube were shaken and 5 ml were placed over a "silver saddle" overnight. Triplicate samples of 1 ml were placed in plastic planchettes, the formic acid was evaporated to dryness and I^{131} was counted in a gas-flow apparatus. The recovery of bound I^{131} exceeded 90% by this procedure.

Results. Results are shown in Table I. Four minutes after injection the cpm in the skin of the estradiol-dipropionate-treated mice did not differ from that of the controls.

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† Volk Radiochemical Co., Skokie, Ill.