Influence of Fasting and Alloxan Diabetes on Rat Cardiac Actomyosin and Subcellular Phosphorus Levels[1] (35632)

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It is well documented that the main substrates for cardiac metabolism during fasting and experimental diabetes are derived from noncarbohydrates—fatty acids, phospholipids, cholesterol, and ketone bodies (*e.g.*, 1, 2). Although cardiac metabolism is affected in fasting and diabetes, it has not been determined whether the contractile proteins and/or associated phosphate compounds linked with myocardial contractility are likewise altered. Several investigators have found alterations in soluble actomyosin as a result of abnormal cardiac effort (1, 3–5); other workers have reported normal physicochemical properties of this myocardial protein (6).

Protein synthesis at the myocardial ribosome level is impaired in very early (4d) experimental diabetes (7, 8); however, it is yet to be determined whether such alterations also deleteriously affect myocardial contractility. It was the purpose of this investigation to determine whether or not the levels (or activity) of rat cardiac actomyosin and the levels of phosphate-containing compounds were altered by fasting or alloxan diabetes in rats.

Materials and Methods. Adult male rats (Long-Evans strain) were housed in individual cages at a temperature of 75 ± 2°F and fed Purina Laboratory Chow and water *ad libitum*.

Fasting periods were 1, 2, 3, or 5d, and the duration of alloxan diabetes was terminated at 3, 7, 14, 30, 60, 90, 120, or 210 days. Preparation of diabetic rats as well as determination of blood and urinary glucose were carried out as reported earlier (2).

Rats were sacrificed by decapitation, the hearts excised, trimmed of atria, blotted, and weighed quickly on a Roller-Smith torsion balance to the nearest 0.2 mg. One portion of the heart was employed for actomyosin extraction, while the remaining portion was employed for total phosphorus determination (9) in subcellular fractions. The ventricular tissue was homogenized with 7 vol (w/v) of 0.1 *M* KCl, 0.03 *M* borate buffer, pH 7.1, and the homogenate then was fractionated by differential centrifugation (10, 11) into supernatant, nuclei–myofibril, mitochondrial, and microsomal fractions.

Actomyosin was extracted by the method of Benson *et al.* (12) as modified by Grimm *et al.* (4). Total ventricular protein concentration and the actomyosin concentration were determined by the Lowry *et al.* method (13). The actomyosin solution was characterized using modified Ostwald viscosimeters at 24° wherein relative viscosity was determined before and after the addition of ATP. Viscosity data are expressed in terms of viscosity number and "ATP sensitivity" as described in detail by Portzehl *et al.* (14) and Grimm *et al.* (4) and the validity of which established by several laboratories (*e.g.*, 3, 15, 16).

Ventricular water content was determined by drying in an oven at 105° until successive constant dry weights were obtained. Cardiac actomyosin solutions were subjected to polyacrylamide electrophoresis (17) and ultracentrifugal analysis (Model E Spinco) to determine the purity of the isolated protein solution.

Results. Blood glucose levels and percentage loss of body weight data (Table I) indicate the physiological impact of the various fasting periods. Total myocardial protein content was significantly decreased at day 3 of fasting ($p < .05$) when compared with non-

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TABLE I. Influence of Fasting on Rat Cardiac Actomyosin Levels.

Days of fasting	Blood glucose (mg/100 ml)	Ventricle wt/body wt (mg/g × 10)	Body wt loss (%)	Cardiac protein total (%)	Cardiac actomyosin		
					% Total protein	mg per g protein	ATP sensitivity (%)
Fed normal controls (15)[c]	118[a] ±1	2.61 ±0.01	—[b]	19.1 ±0.8	13.1 ±0.5	18.0 ±0.5	85.97 ±4.88
1 day (8)	78 ±3	2.79 ±0.01	10.78 ±1.09	18.4 ±0.9	13.2 ±0.6	17.9 ±0.8	73.93 ±6.28
2 day (8)	80 ±4	2.95 ±0.01	15.16 ±0.57	20.6 ±1.4	12.3 ±0.7	19.9 ±0.5	73.09 ±4.42
3 day (8)	85 ±3	2.72 ±0.01	16.85 ±0.52	17.2[d] ±0.4	14.9[d] ±0.6	18.8 ±0.5	82.00 ±3.51
5 day (7)	100 ±4	2.72 ±0.01	23.09 ±1.69	18.8 ±0.9	12.0 ±0.9	19.8 ±0.9	80.85 ±2.81

[a] Means ± SE.

[b] Control body weight at sacrifice: 248 ± 5 g; preexperimental body weights averaged 260 ± 4 g.

[c] Numbers in parenthesis indicate number of observations.

[d] $p < 0.05$.

TABLE II. Influence of Alloxan Diabetes on Rat Cardiac Actomyosin Levels.

Days after alloxan	Observations	Blood glucose (mg/100 ml)	Ventricle wt/body wt (mg/g × 10)	Cardiac protein total (%)	Cardiac actomyosin		
					% Total protein	mg per g protein	ATP sensitivity (%)
Normal controls	31[a]	124[b] ±3	2.64[c] ±0.01	18.4 ±0.4	14.3 ±0.4	19.1 ±0.4	87.82 ±2.51
3 day	8	478 ±40	2.79 ±0.01	21.4[d] ±1.3	12.5 ±1.1	20.1 ±1.2	73.60[e] ±4.83
7 day	12	520 ±20	2.63 ±0.01	20.2 ±1.0	12.9 ±0.6	20.2 ±0.7	69.68[f] ±5.10
14 day	8	467 ±30	2.71 ±0.01	17.7 ±0.5	14.6 ±0.6	20.2 ±1.0	84.85 ±3.61
30 day	12	446 ±20	2.63 ±0.01	19.1 ±0.7	13.8 ±0.6	19.9 ±0.4	79.91 ±4.09
60 day	7	498 ±35	2.96 ±0.01	17.1[d] ±0.4	16.8[d] ±0.3	20.8 ±0.9	83.54 ±1.76
90 day	8	458 ±26	2.80 ±0.01	18.6 ±0.5	15.4[d] ±0.3	18.5 ±1.0	84.36 ±4.39
120 day	6	414 ±23	2.72 ±0.01	18.2 ±0.4	14.9 ±0.8	17.5 ±1.3	89.13 ±9.49
210 day	5	413 ±7	2.72 ±0.01	18.8 ±0.8	16.1 ±0.8	21.1[e] ±0.7	81.16 ±6.02

[a] All observations made on nonfasted rats.
[b] Mean ± SE.
[c] Body weight at sacrifice: Controls—242 ± 5 g; diabetic—234 ± 5 g.
[d] $p < .05$.
[e] $p < .02$.
[f] $p < .01$.

fasted control rats. This decrease was not due to an increase in ventricular water content but rather was reflected in the significant increase in actomyosin concentration (expressed as percentage of total protein) after 3 days of fasting ($p<.05$). Neither the actomyosin concentration (when expressed as milligrams actomyosin per gram protein) nor the ATPase activity of the actomyosin solution (expressed as ATP sensitivity) were reduced significantly at any time during the fasting periods ($p>.05$).

Subcellular phosphorus analyses were determined on the myocardial fractions of the rats employed for the above (fasting) study, and the range of values expressed as milligrams per gram wet weight of tissue were: total homogenate, 2.432 ± 0.071–2.654 ± 0.039; supernatant, 1.298 ± 0.036–1.385 ± 0.026; nuclei–myofibril, 0.793 ± 0.028–0.874 ± 0.021; mitochondrial, 0.115 ± 0.008–0.128 ± 0.005; and microsomal, 0.087 ± 0.005–0.093 ± 0.005. A significant reduction in phosphorus occurred only in the total homogenate fraction at day 3 of fasting ($p<.05$).

Table II presents data relative to the influence of alloxan diabetes on cardiac actomyosin. The severity of diabetes was evident by the nonfasted blood glucose values; the mean urinary glucose level for all diabetic groups employed was 11–12 g/24 hr. The heart wt/body wt ratio was significantly increased at 3, 14, 60, 90, 120, and 210 days of alloxan diabetes ($p<.01$ for each group). Compared with normal rats the actomyosin concentration expressed as percentage of total protein was increased significantly after 60 and 90 days of alloxan diabetes ($p<.05$) and expressed as milligrams actomyosin per gram protein was significantly increased at day 210 ($p<.02$). The ATP sensitivity was reduced significantly at 3 and 7 days of alloxan diabetes ($p<.02$ and $p<.01$, respectively), returning to normal levels by day 14.

Analysis of myocardial subcellular phosphorus levels from the alloxan diabetic rats resulted in values which did not differ appreciably from those given above for the fasting study. However, unlike the fasting study, there was a significant decrease in total phosphorus in the total homogenate fraction at 30, 60, 90, and 120 days of alloxan diabetes ($p<.05$, $<.01$, $<.02$, $<.02$, respectively) when compared with nondiabetic control rats. While other statistically significant changes in phosphorus levels occurred in various subcellular fractions no consistent pattern of phosphorus translocation between or within compartments could be determined.

Disc electrophoretic patterns obtained on the final actomyosin preparation (used for ATPase sensitivity evaluation) from normal rat hearts did not differ from those patterns obtained from preparations made of diabetic hearts. Ultracentrifugation patterns of both diabetic and nondiabetic rat hearts also were indistinguishable from each other; sedimentation coefficients were 5.6 and 5.4 for normal and diabetic cardiac actomyosin, respectively.

Discussion. The increase in the heart wt/body wt at day 3 of alloxan diabetes probably was due to the decrease in body weight immediately prior to (*e.g.*, 18) and after (*e.g.*, 19) alloxan administration. Increases in heart wt/body wt ratios at days 7, 14, 60, 90, 120, and 210 probably are due to hypertrophy of the myocardium as is observed frequently in clinical diabetic studies.

Although ventricular actomyosin concentrations were altered little or none at all during alloxan diabetes the significant reduction of ATP sensitivity at days 3 and 7 are of interest because ATP sensitivity is a means of characterizing actomyosin solutions with respect to actin content or activity (5, 14). Possible explanations for these alterations include the influence of fasting, the diabetic state, and/or the pharmacologic (toxic) effects of alloxan. It is unlikely that fasting per se was the basis for the observed reduction in myocardial ATP sensitivity because no alterations in this variable were detected throughout a 5-day fasting period (Table I). It is also improbable that the diabetic state is a causative factor because the ATP sensitivity was not altered significantly from day 14 through the completion of the diabetic period 7 months later (Table II). Alloxan has been reported to produce lesions in organs such as the kidney, liver, and various nonpancreatic

endocrine tissues during the first weeks following administration (20, 21) as well as interfering with intestinal transport in rats (18). These deleterious effects are transitory and usually disappear within 2–3 weeks after alloxan administration. It has not been reported that alloxan administration directly affects cardiac muscle. Since the ATP sensitivity was reduced only at days 3 and 7 and returned to normal levels by day 14 (and remained so for 210 days) the deleterious effect of alloxan on heart muscle appears to be transitory in duration and, therefore, similar to the adverse effects of this agent observed in other rat tissues. From the foregoing it would appear that a distinction should be made between the pharmacological impact of alloxan and the physiological impact of the induced diabetes; also, cognizance should be made of the multiple short-term tissue effects from those of established chronic pancreatic deficiency.

The results presented in this investigation indicate again that alloxan is more than merely a beta cytotoxic agent.

Summary. Actomyosin concentration, its viscosity response to ATP, and total phosphorus levels in subcellular fractions were determined in fasted (1–5d) and alloxan diabetic (3–210d) rats. No alterations in these parameters were detected as a result of fasting. However, during alloxan diabetes cardiac actomyosin ATPase activity was reduced for at least 7 days following alloxan administration and returned to normal levels 14 days after alloxan injection. These transitory toxic effects on cardiac muscle indicate that alloxan is not specifically beta cytotoxic.

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