Effect of Starvation on Contractile Response of Isolated Rat Atria to Citrate and Bicarbonate-Free Medium[1] (35738)

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Biochemical data indicates that during starvation the heart accumulates lipid which apparently is used to support various energy-requiring processes *in vitro* (1). This study attempted to assess the nature and importance of endogenous substrate, acquired during starvation, for the contractile process. The technique employed involves the use of the glycolytic inhibitors citrate and bicarbonate-free medium. These inhibitors block the phosphofructokinase (PFK) reaction (2–6). This is apparently responsible for their negative inotropic effects (7–9). Addition of these inhibitors to isolated rat atria, incubated in the absence of exogenous substrate, should have effects on the force of contraction predicated on the nature of endogenous substrate used to support contractility. If the atria are primarily dependent on substrates above the PFK step the inhibitors should produce marked negative inotropic effects. Conversely, little effect should be seen if substrates below the PFK step are the primary sources of energy. The present experiments suggest that endogenous substrates above and below the PFK step are used to support contractility of atria from fed or 24-hr starved rats in the absence of exogenously supplied substrate. Atria from starved rats contain more utilizable endogenous substrate, some of which is not metabolized via PFK. These functional studies are thus consistent with reported biochemical data which indicates that starvation increases the lipid content of the heart and further suggest that this lipid is utilized for the contractile process.

Methods. Male Sprague-Dawley rats, weighing 180–200 g, which either had *ad libitum* access to food and water (fed) or were without food for 24 hr (starved) were employed. Atria were removed from decapitated rats and suspended in 50 ml of modified Krebs–Ringer bicarbonate glucose medium (10) of the following composition (m*M*): NaCl, 120; KCl, 4.8; $CaCl_2$, 1.22; $MgSO_4 \cdot 7H_2O$, 1.33; KH_2PO_4, 1.2; $NaHCO_3$, 25.3; glucose, 5.55. The atria were electrically stimulated at 200/min in this medium and aerated with 95% O_2 and 5% CO_2 to maintain a pH of 7.4 at 30°. The developed tension of atria was determined as previously described by Ko and Paradise (7).

1. Bicarbonate-free experiments. The procedures were conducted by means of techniques previously described by Ko *et al.* (11). The bicarbonate-free medium was prepared by replacing sodium bicarbonate from the Krebs–Ringer bicarbonate medium with an equivalent concentration of sodium chloride and bubbling with 100% O_2. The pH of the bicarbonate-free medium was initially adjusted with dilute sodium hydroxide to 7.4 just prior to the experimental procedure. Electrodes, placed in the tissue bath to monitor the medium pH, demonstrated no significant change from 7.4 throughout the course of the bicarbonate-free experiments. After a 1-hr equilibration period in the normal Krebs–Ringer bicarbonate glucose medium, the medium was changed to one free of glucose, then to the bicarbonate-free medium 15 min later.

2. Citrate experiments. After a 60-min

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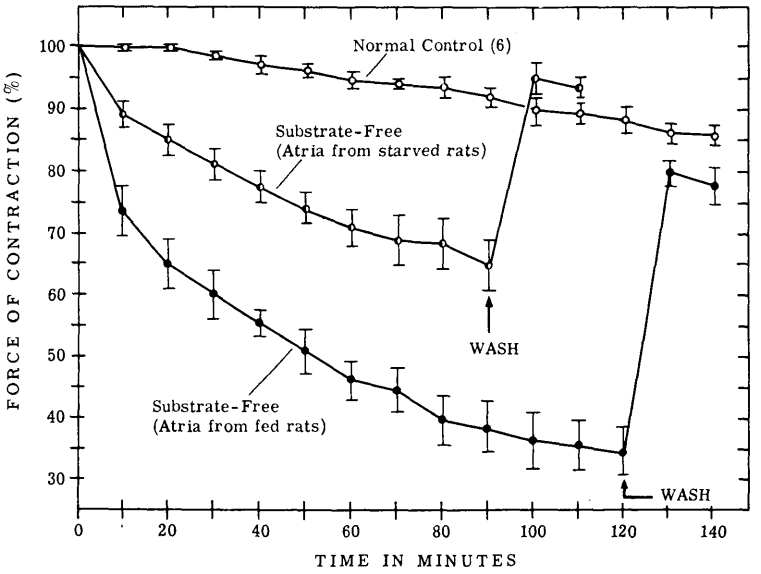

FIG. 1. Contractility of substrate-depleted atria from normal (fed) and 24-hr starved rats. Zero time represents a 1-hr equilibration period in the normal Krebs–Ringer bicarbonate glucose medium. Normal control represents force of contraction of atria from fed rats for an additional 140 min in this medium. In the other two experiments, the medium was changed to one free of glucose (substrate-free) at zero time. Four atria from starved rats and six from fed rats were used in the substrate-free experiments. Wash represents a return to the normal medium following 3 rinses in normal medium. Vertical bars indicate ± one standard error.

equilibration period in the normal Krebs–Ringer bicarbonate glucose medium, and a further 15 min period in glucose-free medium, 1.5 m*M* sodium citrate was added to the bathing medium.

3. Substrate-free experiments. The normal medium was changed to substrate-free medium (*i.e.*, free of glucose) following the 1-hr equilibration period.

Results. Contractility of substrate-depleted atria from fed and starved rats. Experiments were performed to determine the importance of endogenous substrates for the force of contraction of atria from starved rats by comparing the rate of contractile depression of these atria in substrate-free medium to that from fed rats. Figure 1 shows the effects of omission of exogenous substrate from the medium (free of exogenous glucose) on the tension developed by atria from starved and fed rats, following a 1-hr equilibration period in the normal Krebs–Ringer bicarbonate glucose medium. Figure 1 shows that when the atria from starved rats are suspended in substrate-free medium, they have a significantly smaller reduction in contractility than do those from fed rats.

Effect of starvation on contractile response of isolated rat atria to citrate and bicarbonate-free medium. Following a 1-hr equilibration period in the normal Krebs–Ringer bicarbonate glucose medium, atria from fed or starved rats were placed in glucose-free medium (zero time in Fig. 2). Fifteen min later, this medium was replaced by one deficient in both bicarbonate and glucose (bicarbonate-free medium) or 1.5 m*M* citrate was added. The results indicate that bicarbonate-free medium produced marked negative inotropic effects on atria from both fed and starved rats although the extent to which the contractility had decreased was greater in the fed group. Citrate effects in the fed series were similar to bicarbonate-free medium. In the starvation experiments, however, citrate produced a much smaller effect than bicarbonate-free medium.

Effect of glucose on contractility of substrate-depleted atria from starved and fed rats. After 30-min incubation of atria from

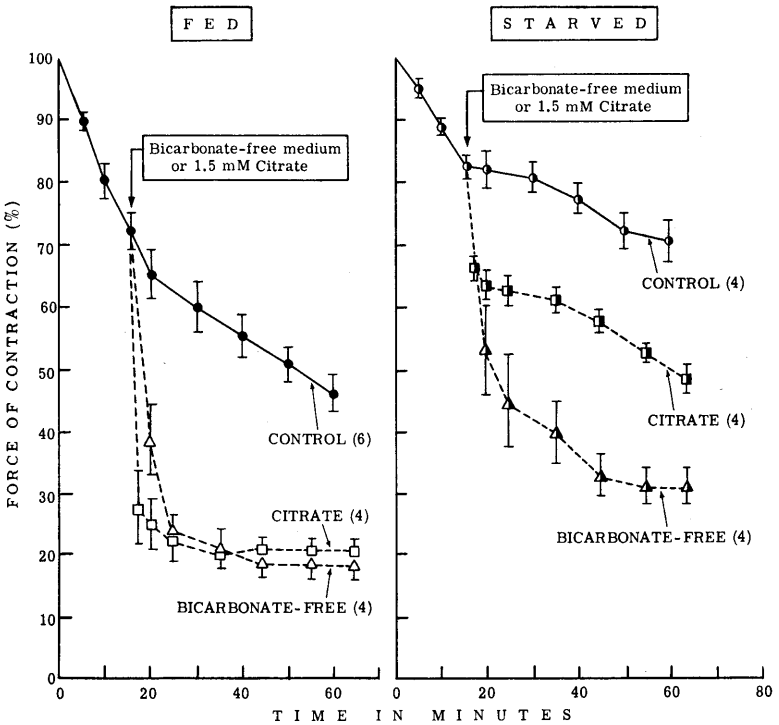

FIG. 2. Contractile depression of substrate-depleted atria from fed and starved rats by citrate or bicarbonate-free medium. At zero time, normal medium was changed to one free of glucose. At 15 min, medium was changed to one free of glucose and bicarbonate (bicarbonate-free) or 1.5 m*M* citrate was added.

starved and fed rats in substrate-free medium, 20 m*M* glucose was added to both atria (Fig. 3). Figure 3 shows that glucose produced a marked increase in force of contraction in both cases.

Discussion. The slower rate of decline of contractile activity of isolated atria, in substrate-free medium, from 24-hr starved rats compared to fed rats (Fig. 1) was also observed by Gimeno *et al.* (12) and suggests a greater availability of endogenous substrate, accumulated during the starvation period, for the contractile process. Glycogen and lipid are known to accumulate in the heart during starvation (1, 12, 13) and these endogenous substrates may account for the better maintenance of contractility in isolated atria incubated in substrate-free medium.

Bicarbonate-free medium produced a marked depression of contractility in atria from fed and starved rats (Fig. 2). Since the negative inotropic action of this medium is probably due to a complete and insurmountable block of the glycolytic enzyme, phosphofructokinase, PFK (2, 3, 7, 8) this implies a block in utilization of substrates, probably glycogen, above the PFK step for the contractile process in atria from fed and starved rats. In the absence of exogenous substrate and bicarbonate, contractility was better maintained in atria from starved rats (Fig. 2). This may indicate that substrates below the PFK step, probably lipid, accumulated in the heart during starvation and was used as an energy source for contraction during these conditions. This is consistent with biochemical data indicating an accumulation of lipid by the heart during starvation (1).

Citrate, 1.5 m*M*, another inhibitor of PFK (4–8) produced the same degree of depression of contractility as bicarbonate-free medium in atria from fed rats but less depression than bicarbonate-free medium in atria from starved rats (Fig. 2). Since the negative ino-

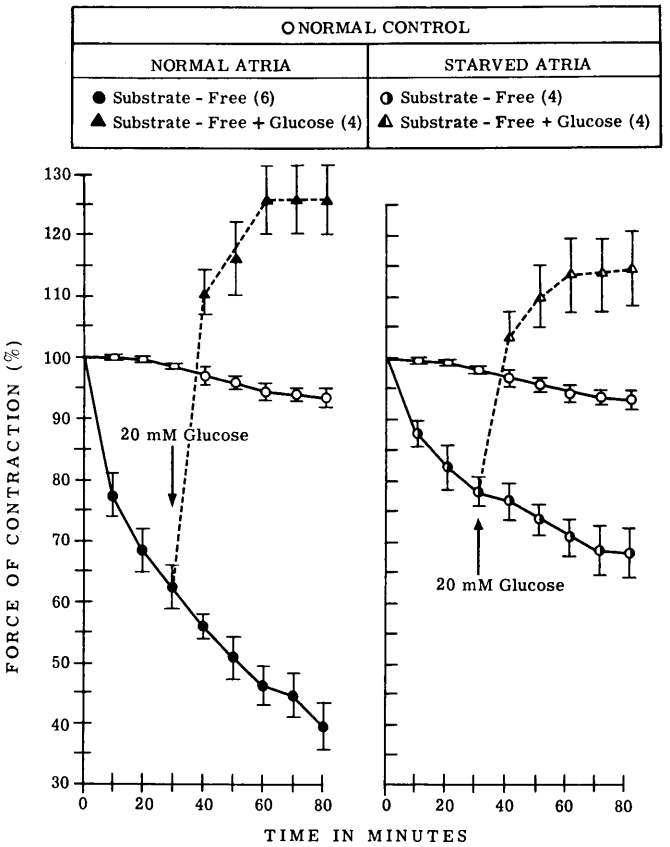

FIG. 3. Effect of glucose on contractility of substrate-depleted atria from normal rats and starved rats. At zero time, normal medium was changed to one free of glucose (substrate-free); glucose (20 m*M*) was added at 30 min.

tropic action of citrate can be partially overcome with glucose (7, 8) this implies that the blockade of PFK is surmountable by glucose and may also be surmountable by any additional glycogen that may have accumulated during starvation. The latter point is not clear, however, since Gimeno *et al.* (12), who indeed found an increase in glycogen in atria from freshly killed 24-hr starved rats compared to fed rats, could show no statistically significant difference following 1-hr incubation in glucose-containing medium. Since our atria were incubated in the same medium as those of Gimeno *et al.* for 1 hr prior to all experiments, the glycogen concentrations in "fed and starved" atria should have been similar at zero time.

That glycogen can serve as an adequate substrate for the contractile process in atria from starved rats is implied by Fig. 3 which shows the marked positive inotropic action of 20 m*M* glucose in substrate-free treated atria from starved rats. Glucose, of course, shares the same metabolic pathway as glycogen beyond the glucose-6-phosphate step. Although lipid is primarily used by the heart *in vivo* during starvation (1), Fig. 3 clearly shows that the enzymes necessary for the glycolytic process are still present after 24 hours of starvation.

Summary. Isolated atria from 24-hr starved rats show a smaller rate of decline of contractility with time, when incubated in the absence of exogenous substrate than atria from fed rats. During starvation, endogenous substrate apparently accumulates and can

be used by the heart to maintain contractility *in vitro*. The nature of this endogenous substrate was elucidated by the use of bicarbonate-free medium and 1.5 m*M* citrate. These agents apparently depress contractility by an inhibition of the phosphofructokinase (PFK) reaction in the glycolytic cycle. Both inhibitors produced marked, but not complete, contractile depression of atria from starved or fed rats incubated in the absence of exogenous substrate. This depression is likely due to an interference with the utilization of endogenous substrate, probably glycogen, normally metabolized via PFK. The maintenance of some degree of contractility in the presence of these inhibitors indicates that endogenous substrates, not metabolized via PFK, are utilized. Addition of 20 m*M* glucose to atria from fed or starved rats incubated in substrate-free medium resulted in a marked positive inotropic effect in both cases. This may indicate that glycolytic enzymes are present and functional after 24 hr of starvation.

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Vol. 136, No. 4 (1971), "Inotropic Effects of Prostaglandin E_2 on Isolated Cardiac Tissue," by Joseph V. Levy and E. Killebrew, pp. 1227-1231:

Page 1228, second column, line 16 from the bottom, ". . . negative and positive chronotropic . . ." should read: ". . . negative and positive inotropic . . ."