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# Effect of Starvation on Substrate Utilization of Isolated Rat Atria

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## ABSTRACT

The abilities of metabolic substrates, glucose, pyruvate, and acetate to produce a maximal increase in the force of contraction of substrate-depleted atria from fed rats were compared to those from starved rats, in order to observe the effect of starvation on substrate utilization of the myocardium. Starvation results in a marked loss of body weight in rats. In contrast to the starved rats, the body weight of fed rats increased with time. When placed in substrate-free medium, atria from fed rats showed marked decline in contractile force. In contrast to the atria from fed rats, the substrate-depleted atria from starved rats showed much less decline of the force of contraction. In the substrate-free medium, abilities of glucose, pyruvate, and acetate to produce a maximal increase in the force of contraction of atria from fed rats were much greater than those from starved rats. The data from these studies indicate that in the substrate-free medium atria from starved rats utilize much less exogenous substrates than those from fed rats.

These results suggest that starvation has no deleterious effect on contractile activity of the myocardium, and the starvation increase the storage of readily metabolizable endogenous substrstes useful for the functional activity of the isolated heart.

**Key Words:** Starvation, Heart, Contractility, Endogenous substrates

## INTRODUCTION

That the heart can utilize carbohydrates, fatty acids, and triglycerides as sources of energy for the myocardial contraction has been well documented (Bing, 1981), and the relative abilies of various substrates to maintain the functional activity of myocardium have been extensively investigated (Berman and Saunders, 1995; Gimeno *et al.*, 1969, Gimeno *et al.*, 1966; Ko and Paradise, 1970a, 1971b, 1971f; Ko *et al.*, 1969; Masuoka *et al.*, 1952; Nakamura *et al.*, 1949; Webb *et al.*, 1949; Webb, 1959; Webb and Hollander, 1956). Although numerous studies on heart metabolism involving the use of cardiac tissue slices (Pearson *et al.*, 1949; Webb *et al.*, 1949), isolated hearts from animals (Ko, 1989; Ko and Paradise, 1969, 1970a, 1970b, 1971a, 1971b, 1971c, 1972a, 1973a, 1975, 1979, 1989; Ko, 1977; Paradise and Ko, 1970; Pearson *et al.*, 1949), and human (Ko and Paradise, 1970c), and heart contracting in situ (Goodale and Hackel, 1953) have attempted to relate the effects of substrate on the contractile activity of the myocardium, less information is available concerning the relationship between the utilization of endogenous substrates in the myocardium and the ability of the heart to contract.

Biochemical data indicates that during starvation the heart accumulates lipid which apparently is used to support various energy-requiring processes in vitro (Evans, 1964). It has been also dem-

onstrated that starvation produces marked alterations in cardiac metabolism (Gimeno *et al.*, 1967), for example, decreased glucose, lactate and pyruvate metabolism accompanied by accelerated metabolism of lipid and a raised conentration of glycogen and glucose-6-phosphate. It is of interest to observe the ability of individual substrate to sustain a maximal increase in the force of contraction of isolated atria in which endogenous substrates had been accumulated.

In the previous demonstration with the functional experiments, isolated atria from 24 hour starved rats show a smaller rate of decline in the force of contraction with time, when incubated in the absence of exogenous substrate than atria from fed rats (Ko and Paradise, 1971d, 1971f). Later it was also demonstrated that the lowest rate of decline of developed tension was seen in atria from rats starved for two days. This may indicate that two days of starvation is the optimum time period for accumulation of readily utilizable substrates by the atria and suggest a useful starvation period for future experiments (Ko, 1977).

The purpose of present investigation was first to assess the nature and importance of endogenous substrate, acquired during starvation, for the contractile process, and next to compare the ability of metabolic substrates glucose, pyruvate and acetate to produce a maximal increase in the force of contraction of isolated atria obtained from fed rats and atria from starved rats. In order to determine the relationship between the effect of individual substrate and contractile behavior of isolated atria, one can introduce the substrate after the force of contraction has declined due to the prolonged activity in substrate-free medium, and determine the effect of individual substrate on the mechanical performance of the myocardium. The authors have carried out such experiments with glucose, and isolated atria obtained from fed and starved rats, inasmuch as such exogenous substrates have served as useful tools for the cardiac studies (Ko and Paradise, 1970a, 1970b, 1970c; Ko *et al.*, 1969).

## MATERIALS AND METHODS

Male rats weighing 150 to 200 g were decapitated, and the atria were removed and suspended in modified Krebs-Ringer bicarbonate glucose medium (Gimeno *et al.*, 1965).

The medium was gassed with 95% $O_2$ and 5% $CO_2$ at pH 7.4 and 30℃. A constant tension of 750 mg was maintained throughout the experiments. The mechanical activity of rat atria electrically stimulated at a rate of 200 per minute in the medium was determined using a sensitive strain gage as previously described (Ko and Paradise, 1973b; Park *et al.*, 1992).

In the experiments with the metabolic substrates, glucose, sodium pyruvate and sodium acetate at a concentration of 5 mM were employed. In the experiments with substrate-free medium, the normal Krebs-Ringer bicarbonate glucose medium was changed to substrate-free medium (free of glucose) following the one-hour equilibration period (Ko *et al.*, 1969).

## RESULTS

### Effect of starvation on body weight of rats

Figure 1 shows that the body weight of starved rats decreased markedly with time. In contrast to the starved rats, the body weight of fed rats increased with time. It is also evident from the Fig. 1 that the decreased rate in body weight of starved rats was more than two times greater than that of the increased body weight of fed rats.

### Effect of starvation on depression rate of atrial contractility in substrate-free medium

The experiments were performed to determine the importance of endogenous substrates for the contractile force of starved atria by comparing the rate of contractile depression of starved atria in substrate-free medium to those of fed atria. After the one-hour equilibration period, the normal Krebs-Ringer bicarbonate glucose (5.5 mM) medium was replaced with substrate-free medium (free of glucose). The results are summarized in the Fig. 2. The Fig. 2 shows the effects of omission of exogenous substrate from the medium on the tension developed by atria from fed and starved rats following the one-hour equilibration period in the normal medium. The Fig. 2 also shows that when the atria from starved rats are suspended in

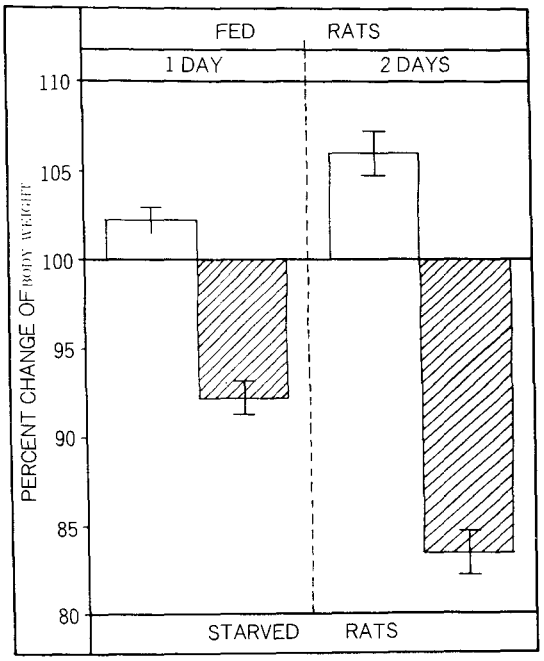


**Fig. 1.** Percent changes in body weight of fed and starved rats. Vertical lines represent standard error (10 cases).

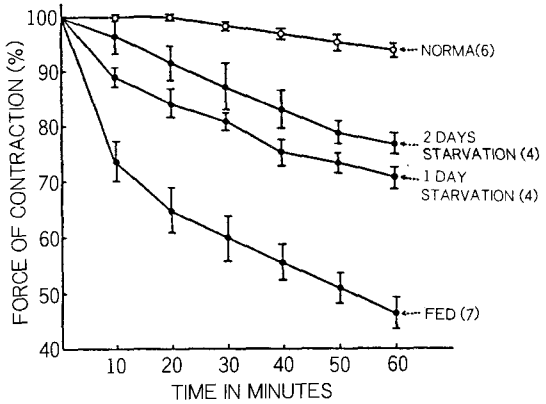


**Fig. 2.** Effect of starvation on force of contraction in substrate-free medium.
Normal represents force of contraction of atria from fed rats in the normal glucose-containing medium. All other curves represent force of atrial contractilities from fed or starved rats incubated in the normal medium for one hour, then exposed to glucose-free medium for an additional 60 minutes. Values in parenthese represent number of experiments. Vertical bars indicate ± one standard error of the mean.

substrate-free medeum, they have a significantly smaller reduction in contractility than those of fed atria.

**Effects of substrates on contractility of substrate-depleted atria from fed rats**

The normal atria were suspended in the normal Krebs-Ringer bicarbonate medium containing 5. 5 mM glucose, and allowed for the one-hour equilibration period before the experiments began. Immediately after the equilibration period, the normal Krebs-Ringer bicarbonate medium was changed to substrate-free medium. Glucose, sodium acetate, and sodium pyruvate at concentration of 5 mM were added to the bathing medium at 30 minutes after the atria were incubated in the substrate-free medium. At that time, the force of contraction of the atria were depressed about 40% in fed atria. The results are summarized in Fig. 3. It is evident from the Fig. 3 that the addition of glucose (5 mM) restored the contractile activity of

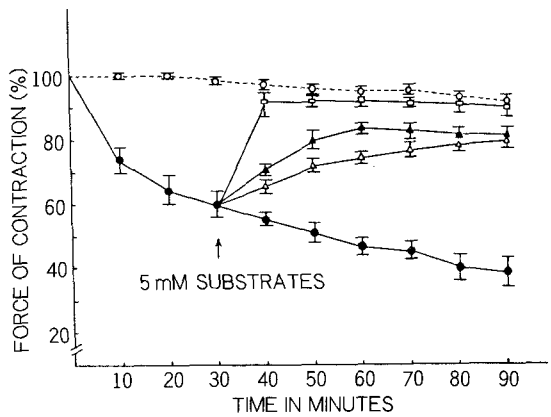


**Fig. 3.** Effect of substrates on contractility of substrate-depleted atria from fed rats.
The normal medium was changed to one free of glucose (substrate-free) at zero time, and substrates were added at 30 minutes. Vertical lines represent standard error.
○−○: Normal control (6); ●−●: Substrate-free control (7)
□−□: Substrate-free+glucose (4)
▲−▲: Substrate-free+pyruvate (4)
△−△: Substrate-free+acetate (5)

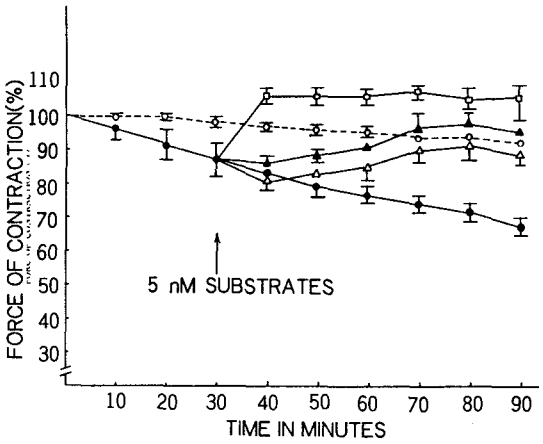


**Fig. 4.** Effect of substrates on contractility of substrate-depleted atria from rats starved two days.
The normal medium was changed to one free of glucose (substrate-free) at zero time, and substrates were added at 30 minutes. Vertical lines represent standard error.
○−○: Normal control (6); ●−●: Substrate-free control (7)
□−□: Substrate-free+glucose (4)
▲−▲: Substrate-free+pyruvate (4)
△−△: Substrate-free+acetate (5)

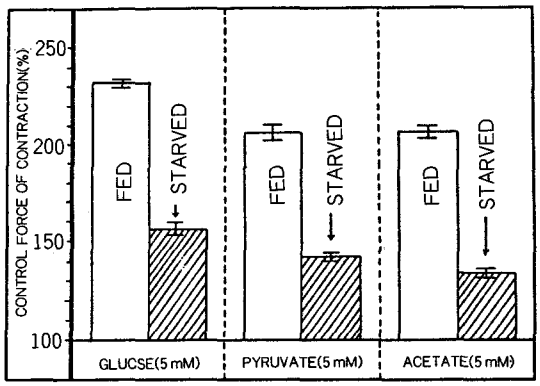


**Fig. 5.** Ability of substrates (5 mM) to produce a maximal increase in the force of contraction of substrate-depleted atria from normal (fed) and 2-days starved rats.
Bars indicate the mean increase in the force of contraction produced by substrates, at 60 minutes after their additions. Vertical lines represent ± one standard error of the man.

depressed atria completely to the control levels. However, it is also evident from the Fig. 3 that the addition of pyruvate and acetate to the depressed atria in the substrate-free medium produced gradual and partial recovery of the contractility from the depression.

**Effects of substrates on contractility of substrate-depleted atria from starved rats**

Experiments were performed to determine the ability of substrates to produce a maximal increase in the force of contraction of atria from starved rats in comparison with the effect of substrates on atria from fed rats. In this study the atria were obtained from the rats of two days starvation, because it is suggested that two days of starvation is the optimum time period for the accumulation of readily utilizable endogenous substrates by the atria (Ko, 1977), as seen in Fig. 2. After 30 minutes incubation from starved rats in substrate-free medium, glucose, sodium pyruvate, and sodium acetate, at concentration of 5 mM as a same amount in the experiments with the atria from fed rats, were added. The results are shown in Fig. 4. It is evident from Fig. 4 that the addition of glucose (5 mM) produced marked increase in the force of contraction, despite the fact that 5 mM of glucose produced the elevation of contraction of substrate-depleted atria from fed rats to the control levels (Fig. 3). It is also evident from Fig. 4 that pyruvate and acetate restored the depressed atria from starved rats to control levels, although the same concentration (5 mM) of pyruvate and acetate produced only partial recovery of the depressed atria from fed rats (Fig. 3).

**Relative abilities of substrates on maximal increase in contractile force of atria from fed and starved rats**

In this series of study, the abilities of metabolic substrates glucose, pyruvate, and acetate to produce a maximal increase in the force of contraction of substrate-depleted atria from fed and starved rats were compared in Fig. 5 and 6. It is evident from Fig. 5 that all of substrates tested

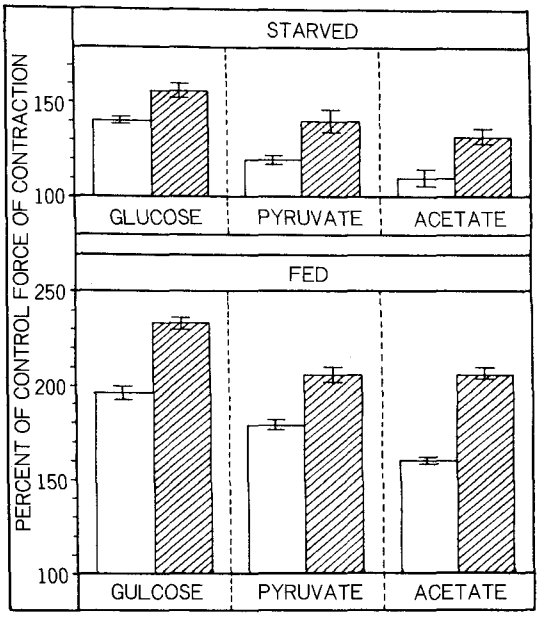


**Fig. 6.** Ability of substrates (5 mM) to produce a maximal increase in the force of contraction of substrate-depleted atria from normal (fed) and 2-days starved rats.
Bars indicate the mean increase in the force of contraction produced by substrates, at 30 and 60 minutes after their additions. Vertical lines represent ± one standard error of the man.
□: 30 minutes, ▩: 60 minutes

here produced much more increase in the force of contraction of substrate-depleted atria from fed rats than those from starved rats, indicating apparently that the substrate-depleted atria obtained from fed rats utilize more exogenous substrates than those from starved rats. It is also evident from Fig. 6 that the abilities of substrates, glucose, pyruvate, and acetate to increase the contractile force of substrate-depleted atria from fed and starved rats were much greater at 60 minutes after administration of the sutstrates than those at 30 minutes after the administration.

## DISCUSSION

In this study, it has been demonstrated that isolated rat atria from starved rats show a smaller rate of decline of the force of contraction with time, when incubated in the absence of exogenous substrate than atria from fed rats (Fig. 2). However, the body weight of starved rats decreased markedly with time. In contrast, the body weight of fed rats significantly increased (Fig. 1). It is in agreement with the previous report by Ko and Paradise (1972c, 1971e) that the starved rats effectively accumulate the endogenous substrates during the starvation period and actively utilize the endogenous substrates which are important for the contractile process (Fig. 2).

This was interpreted as indicating that starvation result in the accumulation of endogenous substrates by the atria useful for the contractile process when placed in a substrate-free environment. Such an interpretation is consistant with biochemical data indicating that during starvation the heart accumulates lipid which apparently is used to support various energy requiring processes in vitro (Evans, 1964).

In the substrate-free medium, abilities of exogenous substrates, glucose, pyruvate, and acetate to produce a maximal increase in the force of concentration of atria from fed rats were much greater than those from the starved rats (Fig. 3, 4, 5, and 6). In the previous report, to elucidate the nature of the positive inotropic effect of sodium pyruvate on atria depressed with the cardiac depressant action of general anesthetics experiments were designed in which sodium chloride was employed under the same conditions as pyruvate (Ko *et al.*, 1972b). And it has been demonstrated by Ko *et al.* (1972b) that sodium chloride at concentration of 5 mM did not produce any significant alterations in the force of contraction of atria depressed by the general anesthetics, whereas the sodium pyruvate at a concentration of 5 mM restored the depressed atria. It has been also reported by Webb *et al.* (1956) that no demonstrable effect of the sodium ion on the functional activity of the myocardium can be detected at concentration below 5 mM. Thus the effect of sodium pyruvate and sodium acetate on the depressed contractility of atria is pyruvate or acetate ion itself, not sodium effect.

The data from these experiments indicate that the substrate-depleted atria from starved rats utilize less exogenous substrates, because in the

absence of exogenous substrates, the atria from the starved rats utilize presumably the endogenous substrate of glycogen or lipids accumulated during the starvation period. These functional studies are consistent with the previously reported biochemical data by Evans (Evans, 1964) which inidcate that starvation increases the lipid content of the heart and further suggest that this lipid is utilized for the contractile process.

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**=국문초록=**

## 적출심장의 대사기질 이용에 대한 내인성 기질의 영향에 관한 연구

경희대학교 의과대학 약리학교실

**고계창 · 정주호 · 정지창 · 심명석**

심장에 작용하는 약물의 기전연구에 있어서 약물의 작용이 심근수축에 필요한 대사 기질과의 관계를 검토하고자, 심근 수축력 유지에 필요한 에너지원인 각종 대사기질의 정상쥐 적출 심방 수축력 유지능력과 굶긴 쥐 적출 심방의 수축력 유지 능력을 비교 관찰한 바 다음과 같은 실험결과를 얻었다.

1. 굶긴 쥐의 체중은 정상쥐에 비해 기아 (starvation) 시작 2일에서 약 15%의 체중감소를 나타냈다.

2. 정상 쥐의 적출 심방은 기질제거 용액에서 30분에 약 40%의 현저한 수축력의 감소를 보였다. 그러나 2일간 굶긴 쥐의 적출 심장은 기질제거액에서 30분에 약 13%의 수축력의 감소를 보여 정상 쥐에서의 수축력 저하보다 현저히 낮은 감소율을 나타냈다.

3. 기질제거 용액에서 glucose, pyruvate 및 acetate가 적출 심장의 수축력을 회복시키는 능력은 굶긴 쥐에서보다 정상쥐에서 현저히 높게 나타났다.

4. 이상의 data들은 기질제거 용액에서 굶긴 쥐 적출 심장은 정상 쥐 적출 심장에 비해 현저히 적게 외인성 기질을 쓰고 있음을 시사하고 있다.

이상의 연구 결과로 미루어 보아 기아가 흰쥐의 현저한 체중감소를 초래하나, 심근의 수축성에 대해서는 유해하지 않은 것 같이 보여지며, 오히려 기아 기간 중 흰쥐의 적출 심장의 수축 기능에 필요한 내인성 대사 기질의 축적을 증가시킨다는 사실을 시사하고 있다.