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Effects of Monosaccharides, Acetate, Butyrate, Lactate, and Pyruvate on the Developed Tension of Isolated Rat Atria* (33714)

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In previous investigations we studied the importance of glucose for the contraction of isolated rat atria (1, 2). We found that the removal of glucose from the medium as well as the addition of 2-deoxy-D-glucose (2-DG) resulted in a rapid decrease in the isometric developed tension and ATP levels of rat atria. The addition of pyruvate or lactate to this hypodynamic preparation restored ATP levels but failed to completely restore the contractile tension to the control levels (1, 2). Furthermore, we have also demonstrated that high concentrations of pyruvate depressed the developed tension of atria suspended in glucose medium. The depression occurred even when the entry of pyruvate into the tricarboxylate cycle was blocked by arsenite (2) or when the conversion of pyruvate to lactate was inhibited by oxamate (3). Inasmuch as high concentrations of pyruvate can inhibit the uptake of glucose by the heart as well as several steps of the glycolitic pathway (4–7) we suggested that such metabolic influences could possibly explain the negative inotropic effect observed after pyruvate.

All the above evidence indicated that glucose plays a definite role for the maintenance of atrial developed tension. The present study was undertaken to determine whether other metabolizable and nonmetabolizable monosaccharides have a similar inotropic influence on the isolated rat atria; in addition the effects of butyrate, acetate, and pyruvate were also studied. It was found that only metabolizable monosaccharides were able to

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TABLE I. Initial Developed Tension Magnitude and Stability of the Developed Tension of Isolated Rat Atria in Glucose, Mannose, Fructose, or 3-*O*-Methylglucose.[a]

Substrate	Conc (m*M*)	Initial developed tension magnitude (mg)	Stability of the developed tension (% change) with time				
			10 min	30 min	60 min	90 min	120 min
Glucose	5.5	438 ± 28.9 (22)	−1.2 ± 0.7 (14)	−2.8 ± 0.9 (14)	−6.4 ± 1.1 (12)	−6.3 ± 1.7 (8)	−7.0 ± 1.8 (5)
	11.0	471 ± 30.7 (20)	0.0 ± 0.1 (6)	−0.3 ± 0.1 (6)	−1.0 ± 0.2 (6)	0.0 ± 0.1 (6)	+0.5 ± 0.09 (4)
Mannose	5.5	521 ± 38.2 (8)	−0.8 ± 0.06 (8)	−1.8 ± 1.6 (8)	−5.8 ± 1.9 (8)	−6.9 ± 1.0 (8)	−8.6 ± 1.5 (4)
	11.0	453 ± 32.1 (6)	−3.1 ± 1.3 (6)	−5.5 ± 1.4 (6)	−10.9 ± 1.7 (6)	—	—
Fructose	5.5	304 ± 37.1 (6)	−4.0 ± 1.3 (6)	−11.4 ± 2.2 (6)	−24.5 ± 3.0 (6)	—	—
	11.0	287 ± 26.4 (6)	−8.7 ± 1.8 (4)	−16.4 ± 1.5 (4)	−25.2 ± 3.2 (4)	—	—
3-*O*-Methyl-glucose	5.5	186 ± 18.7 (4)	−8.7 ± 0.8 (4)	−23.5 ± 2.0 (4)	−40.1 ± 2.9 (4)	—	—
	11.0	150 ± 20.6 (4)	−13.3 ± 1.5 (4)	−34.2 ± 0.6 (4)	−46.0 ± 2.5 (4)	—	—
None	—	196 ± 34.8 (9)	−7.5 ± 1.3 (5)	−22.0 ± 2.8 (5)	−40.7 ± 3.6 (5)	—	—

[a] Stability of the developed tension with time is expressed as percentage of change from the initial control values. In all cases the values are mean ± SEM; the number of atria given in parentheses.

stimulate atrial developed tension in a sustained form, glucose being the most effective. Furthermore, increasing concentrations of 3-*O*-methylglucose, acetate, butyrate, and pyruvate depressed the developed tension magnitude of isolated rat atria.

Methods. Atria from decapitated male rats were suspended in a tissue chamber filled with a modified Krebs–Ringer–bicarbonate solution (KRB) composed as follows (m*M*): Na^+, 145; K^+, 6.00; Ca^{2+}, 1.68; Mg^{2+}, 1.33; Cl^-, 126; CO_4^{2-}, 1.33; HCO^-, 25.30; and PO_4^{2-}, 1.20. The substrate for this solution was glucose, mannose, fructose, 3-*O*-methyl-glucose, lactate, acetate, butyrate, or pyruvate, at 5.5 or 11.0 m*M*. The solution was always aerated with a gas mixture of 95% O_2–5% CO_2 (at a flow rate of 150 ml/min) and maintained at pH 7.4 and 30°. Lactate determinations by an enzymatic method (8) were made to ensure adequacy of the oxygenation. The average lactate concentration found in 10 untreated atria incubated during 60 min in KRB medium with 5.5 m*M* glucose was 2.17 ± 0.18 μmoles/g (w/w) (mean ± SEM). A constant resting tension of 750 mg was applied to the atria by means of a micrometer device and the isometric atrial developed tension was determined with the aid of a strain gauge connected to a direct writing oscillograph. Atria were stimulated with square waves of 5–7 V and 1 msec duration at a constant rate of 200/min. The equilibration period was 60 min. The recorded tension at the end of the equilibration period is referred in the text as the "initial developed tension." The effects of increased osmolarity of the medium resulting from the addition of substrates were compared with those elicited by sucrose (the determination of osmotic pressure being made by cryoscopy). The effects of increasing Na^+ concen-

TABLE II. Initial Developed Tension Magnitude and Stability of Developed Tension of Isolated Rat Atria in Pyruvate, Lactate, Acetate, or Butyrate.[a]

Substrate	Conc (m*M*)	Initial developed tension magnitude (mg)	Stability of developed tension (% change) with time			
			10 min	30 min	60 min	90 min
Pyruvate	5.5	454 ± 28.0 (16)	−0.4 ± 0.08 (5)	−2.8 ± 0.5 (5)	−3.7 ± 0.6 (5)	−6.5 ± 1.5 (5)
	11.0	460 ± 44.9 (9)	−0.3 ± 0.1 (7)	−1.3 ± 0.2 (7)	−3.9 ± 0.3 (4)	−5.1 ± 0.9 (4)
Lactate	5.5	415 ± 29.6 (8)	−0.6 ± 0.5 (6)	−1.7 ± 1.0 (6)	−4.8 ± 1.0 (6)	−6.1 ± 1.3 (6)
	11.0	433 ± 32.2 (7)	−1.1 ± 0.4 (5)	−2.2 ± 0.8 (5)	−4.0 ± 1.1 (5)	−5.8 ± 1.4 (5)
Acetate	5.5	455 ± 50.3 (8)	+0.1 ± 0.2 (8)	−2.2 ± 1.8 (8)	−4.4 ± 3.6 (8)	−9.1 ± 4.3 (8)
	11.0	413 ± 41.5 (6)	−0.2 ± 0.2 (6)	−3.6 ± 2.1 (6)	−5.1 ± 1.6 (6)	−9.9 ± 1.0 (6)
Butyrate	5.5	403 ± 22.8 (8)	−0.5 ± 0.4 (8)	−2.2 ± 1.2 (8)	−4.6 ± 2.2 (8)	−10.4 ± 3.5 (8)
	11.0	408 ± 37.6 (7)	−0.7 ± 0.6 (7)	−0.2 ± 0.5 (7)	−4.7 ± 3.2 (7)	−5.6 ± 4.1 (7)

[a] Stability of the developed tension with time is expressed as percentage of change from the initial control values. In all cases the figures are mean ± SEM; number of atria given in parentheses.

tration upon adding the sodium salts of the substrates were determined with equivalent additions of NaCl (2). The addition of substrates did not produce significant changes in the pH of the medium (>0.1 pH unit). Mean values of developed tension under different experimental conditions were compared using Student's *t* test. Differences were considered significant if $p = 0.05$ or less.

Results. Initial developed tension magnitude and stability of developed tension of isolated rat atria suspended in different substrates. Rat atria were set up in a medium containing glucose, mannose, fructose, 3-*O*-methylglucose, lactate, acetate, butyrate, or pyruvate as the substrate; the initial developed tension, immediately after the 60 min of equilibration period, was determined (Tables I and II). It is evident that when glucose, mannose, acetate, lactate, butyrate, or pyruvate were present the developed tension was significantly greater than when fructose or 3-*O*-methylglucose was the substrate. On the other hand, atrial developed tension in fructose was significantly greater than in 3-*O*-methylglucose or in the substrate-free condition. Increasing the substrate concentration from 5.5 to 11.0 m*M* had no significant effect on the magnitude of the initial developed tension.

Tables I and II also shows the effects of substrates on the stability of the developed tension magnitude with time. It is evident that the stability was influenced not only by the substrates, but also with glucose by the concentration. Comparison of the stability at 5.5 and 11.0 m*M* glucose shows that the decrement with time was significantly less when the concentration of this substrate was 11.0 m*M*. On the other hand, increasing concentrations of mannose, 3-*O*-methylglucose, fructose, acetate, lactate, butyrate, or pyruvate from 5.5 to 11.0 m*M* did not prevent the decrement of developed tension with time. Although fructose was significantly better than 3-*O*-methylglucose or the substrate-free control, it was not as effective as glucose, mannose, lactate, acetate, butyrate, or py-

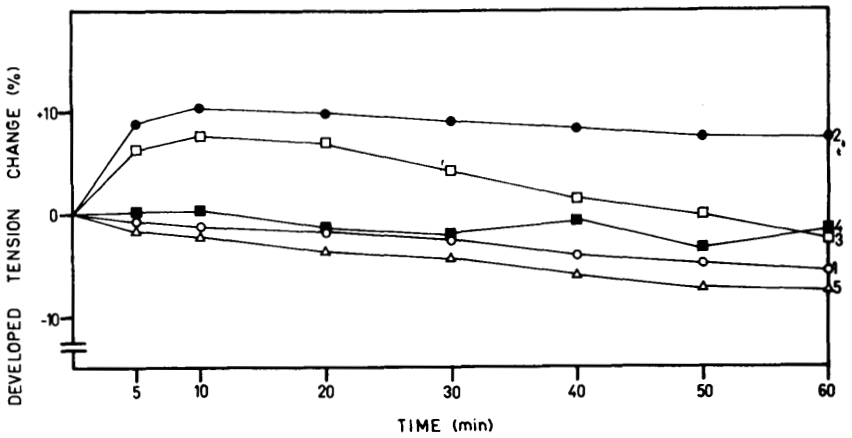

FIG. 1. Effects of 5.5 m*M* glucose, mannose, fructose or 3-*O*-methylglucose on the developed tension of atria suspended in glucose medium. Atria were equilibrated in 5.5 m*M* glucose and the initial developed tension was recorded; 5.5 m*M* of the different substrates were added immediately after (0 time). Each point represents the mean of 5–14 different experiments: Curve 1, controls in 5.5 m*M* glucose with no additions (14 atria); curve 2, glucose (13 atria); curve 3, mannose (7 atria); curve 4, fructose (5 atria); curve 5, 3-*O*-methylglucose (6 atria).

ruvate in maintaining the magnitude of atrial developed tension.

The effects of glucose, mannose, fructose, 3-O-methylglucose, pyruvate, acetate, and butyrate on the developed tension of isolated rat atria suspended in Krebs–Ringer–bicarbonate–glucose medium. The ability of several substrates to modify the isometric contractile tension of isolated rat atria suspended in KRB medium containing 5.5 m*M* glucose

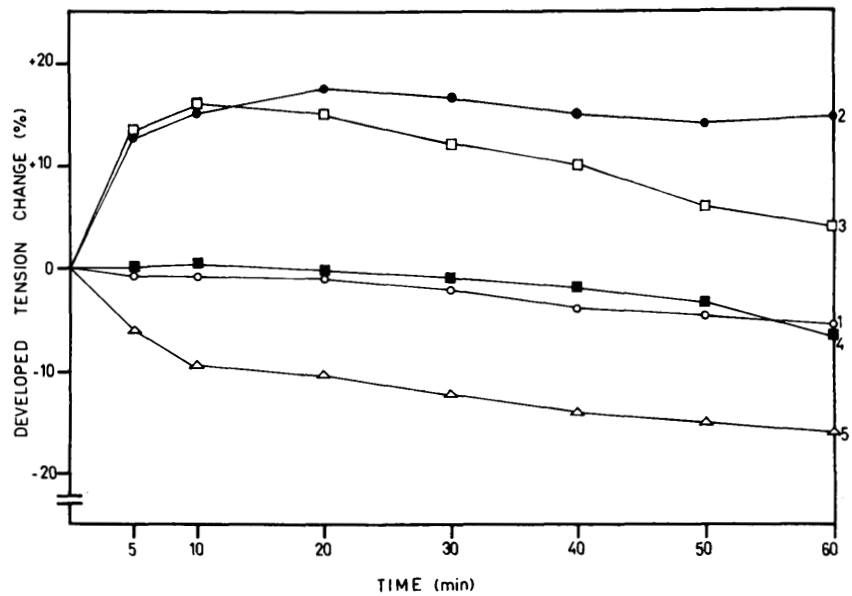

FIG. 2. Effects of 11.0 m*M* glucose, mannose, fructose or 3-*O*-methylglucose on the developed tension of atria suspended in glucose medium. Conditions and details as described in Fig. 1. Curve 1, controls in 5.5 m*M* glucose with no additions (14 atria); curve 2, glucose (7 atria); curve 3, mannose (5 atria); curve 4, fructose (5 atria); curve 5, 3-*O*-methylglucose (5 atria).

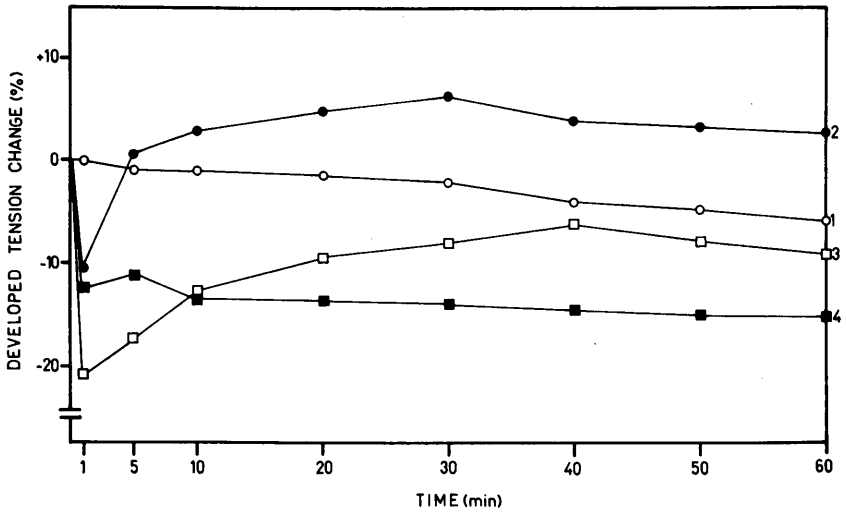

FIG. 3. Effects of 2.75 m*M* pyruvate, acetate or butyrate on the developed tension of atria suspended in glucose medium. Conditions and details as described in Fig. 1. Curve 1, controls in 5.5 m*M* glucose with no additions (14 atria); curve 2, pyruvate (7 atria); curve 3, acetate (8 atria); and curve 4, butyrate (8 atria).

was investigated (Figs. 1–5). As shown in the previous section the developed tension of atria suspended in KRB medium with 5.5 m*M* glucose, exhibited a slight and progressive decrement with time. The addition at 0 time of 5.5 or 11.0 mM glucose, to make a final concentration in the medium of 11.0 or 16.5 m*M*, respectively, resulted in a sustained positive inotropic effect (Figs. 1 and 2, curves 2). Although 5.5 or 11.0 m*M* mannose also stimulated the atria (Figs. 1 and 2, curve 3) the effect was less sustained than with identical concentrations of glucose; this being particularly evident 40 min after the addition of mannose (at the time when the changes of atrial contractile tension were significantly smaller than those after the addition of glucose). On the other hand 5.5 or 11.0 m*M* fructose (Figs. 1 and 2, curve 4) as well as 5.5 m*M* 3-*O*-methylglucose (Fig. 1,

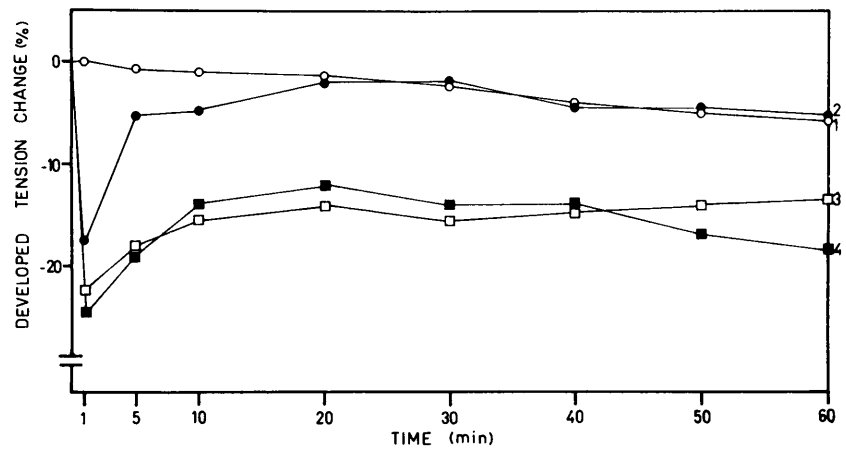

FIG. 4. Effects of 5.5 m*M* pyruvate, acetate or butyrate on the developed tension of atria suspended in glucose medium. Conditions and details as described in Fig. 1. Curve 1, controls in 5.5 m*M* glucose with no additions (14 atria); curve 2, pyruvate (12 atria); curve 3, acetate (12 atria); curve 4, butyrate (9 atria).

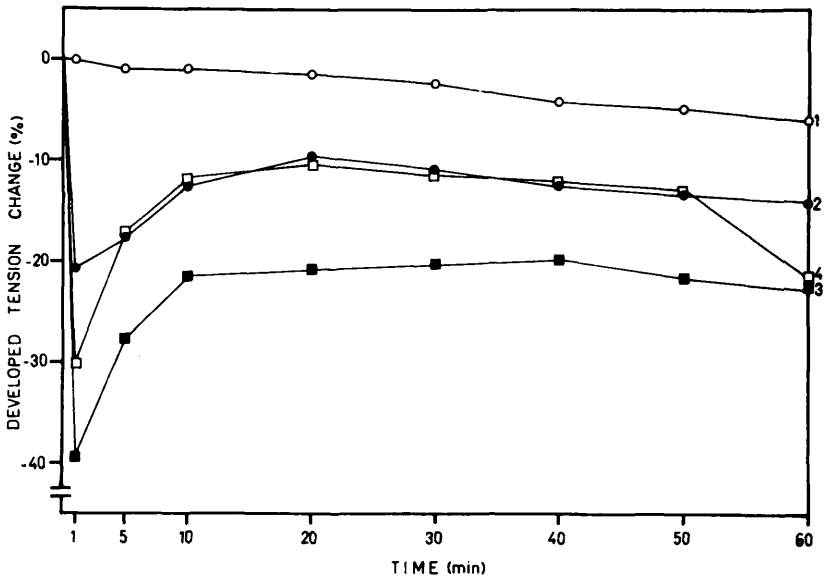

FIG. 5. Effects of 11.0 m*M* pyruvate, acetate or butyrate on the developed tension of atria suspended in glucose medium. Conditions and details as described in Fig. 1. Curve 1, controls in 5.5 m*M* glucose with no additions (14 atria); curve 2, pyruvate (9 atria); curve 3, acetate (10 atria); and curve 4, butyrate (8 atria).

curve 5), a nonmetabolizable monosaccharide, had no significant effect on the magnitude of atrial developed tension. Similar results were obtained with 5.5 or 11.0 m*M* sucrose. However 11.0 m*M* 3-*O*-methylglucose, resulted in a significant negative inotropic response (Fig. 2, curve 5).

The positive inotropic effect produced by the addition of glucose does not appear to be related to a release of catecholamines. The stimulation of developed tension by glucose occurred in atria isolated from reserpinized animals (5 mg/kg/24 hr) as well as in atria exposed to tyramine to the point of tachyphylaxis. Also, a reduction of cholinergic activity is not the mechanism for the positive inotropic effect for the stimulation of developed tension by glucose occurred in atropinized atria (0.01 m*M* atropine). Inasmuch, as 5.5–11.0 m*M* sucrose did not produce a significant positive inotropic response it is unlikely that the effect of glucose on atrial developed tension resulted from an elevation of the osmolarity of the medium.

Figures 3–5 summarize the inotropic effects of various concentrations of pyruvate, acetate, and butyrate. All of these substrates produced an initial transient depression of the magnitude of developed tension; this effect was followed by a secondary increase of developed tension, the magnitude of this increase differed with each substrate as well as with each concentration. Pyruvate at 2.75 m*M* produced a small but significant positive inotropic effect (Fig. 3, curve 2); at 5.5 m*M* had no significant effect (Fig. 4, curve 2) whereas at 11.0 m*M* a definite progressive decrement occurred (Fig. 5, curve 2). With acetate or butyrate no stimulation above the control levels was observed (Figs. 3–5, curves 3 and 4). It is evident that the effects of pyruvate, acetate, and butyrate differ from those obtained with increasing concentrations of glucose or mannose. It is also clear that the initial transient depression produced by acetate (Figs. 3–5, curve 3) or butyrate (Fig. 3–5, curve 4) was significantly greater than that elicited by pyruvate; hence the anionic part of these molecules appears to have a more specific influence on this initial depression than the accompanying cation, Na^+ (2).

The possibility that the negative inotropic effect observed after the addition of these

substrates were due to an elevation of the osmolarity of the medium was tested with isosmolar concentrations of sucrose. It was found that sucrose at these concentrations had no significant effects on the magnitude of atrial developed tension. Furthermore, the participation of cholinergic mechanisms do not appear to be significantly involved in these phenomena, for the negative inotropic effects of these substrates were also present in atropinized preparations (0.01 m*M* atropine).

Discussion. The results of this investigation demonstrate that the magnitude and stability of developed tension of isolated rat atria suspended in substrate-free medium or in the presence of 3-*O*-methylglucose, was significantly less than when glucose, mannose, fructose, lactate, acetate, butyrate, or pyruvate was the substrate. The rapid negative inotropic response observed in substrate-free medium as well as in the presence of the nonmetabolizable monosaccharide 3-*O*-methylglucose, suggest that the endogenous energy stores of rat atria are not adequate for the support of the processes involved in the maintenance of the developed tension magnitude.

Furthermore, glucose 11.0 m*M* was able to support the developed tension of a prolonged period of time without significant decrement. On the other hand, with 5.5 m*M* glucose as well as with 5.5–11.0 m*M* mannose, pyruvate, lactate, acetate, or butyrate a small but definite decrement occurred. It was also observed that increasing developed tension magnitude followed the addition of increasing concentrations of glucose. The addition of mannose also stimulated the developed tension but significantly less sustained than glucose. The 3-*O*-methyglucose not only failed to support atrial contractile tension but also did not produce a positive inotropic effect when 5.5 m*M* were added to a medium containing glucose. Furthermore the addition of higher concentrations (11.0 m*M*) were clearly depressant. This is in contrast to the contractile stimulation observed after the addition of metabolizable monosaccharides (9–11), *i.e.,* glucose and mannose. Inasmuch as 3-*O*-methylglucose is transported across the membrane of muscle cells with a similar kinetics as glucose but is not metabolized (12–13), it would appear that transport per se is not the primary mechanism by which the metabolizable monosaccharides can either maintain atrial developed tension or produce a positive inotropic effect.

As indicated above the stability of developed tension varied with the concentration of glucose. At 5.5 m*M* a small but definite decrement was observed, whereas at 11.0 m*M* the decrement was significantly less. Furthermore, the addition of increasing concentrations of glucose resulted in a sustained positive inotropic effect. Opie *et al.* (14) demonstrated in the perfused rat heart that when the concentration of glucose was elevated from 5 to 10 m*M* the rate of metabolism of this substrate was almost doubled. Therefore, it is likely that the effects of increasing concentrations of glucose on the magnitude and stability of myocardial developed tension, as presently observed on isolated rat atria, could be associated with an increased metabolic rate. Inasmuch as mannose is also transported (15) and metabolized (10–11) by muscle cells, it is reasonable that the inotropic effects of mannose are also associated to a metabolic mechanism simular to that of glucose.

Opie *et al.* (14) demonstrated in the perfused rat heart that the uptake and metabolism of fructose was significantly less than that of glucose and a similar finding has been made by Danforth and co-workers on the human heart (16). From these studies they concluded that it is unlikely that fructose could serve directly as a fuel for myocardial contraction. Our observation that fructose was not as effective as glucose, mannose, lactate, acetate, butyrate, or pyruvate for the maintenance of atrial developed tension magnitude is in agreement with the above findings. However, the fact that the contractile tension of rat atria suspended in the presence of fructose was higher and better sustained than in substrate-free medium or in the presence of the nonmetabolizable monosaccharide 3-*O*-methylglucose, indicate that fructose, even though less effective than glu-

cose, mannose, lactate, acetate, butyrate, or pyruvate, can support a definite level of atrial contraction. On the other hand the fact that increasing concentrations of fructose failed to stimulate atrial contraction as glucose or mannose, could also be associated with its comparatively reduced metabolism by the myocardium.

When the concentration–action relation of glucose was compared to those of 3-*O*-methylglucose, pyruvate, acetate, and butyrate, it was found that increasing concentrations of glucose resulted in a positive inotropic effect; whereas increasing concentrations of 3-*O*-methylglucose, pyruvate, acetate, and butyrate depressed the contractile tension. It is well known that pyruvate, acetate, and butyrate are readily oxidized by the perfused rat and guinea pig heart (5, 17–20) and we have demonstrated that pyruvate can elevate ATP levels of isolated rat atria (1). On the other hand it has also been shown that acetate, butyrate, and other souces of acetyl–CoA markedly reduce the oxidation of exogenous glucose by the isolated heart and the suggestion was made that this resulted from an inhibition of pyruvate oxidation with no influence upon glucose uptake or glycolysis (20). Indeed, the finding that fatty acids as well as acetate increase the heart levels of acetyl–CoA which in turn can inhibit heart pyruvate dehydrogenase (21) is in favor of such a mechanism as the reason for the reduction of glucose metabolism. However, it was also observed that fatty acids, acetate, and pyruvate at high concentrations inhibited not only the oxidation of glucose but reduced its uptake by the perfused rat heart (5, 7). Suggestions were made that these influences are mediated by changes in the tissue levels of citrate as well as by changes in the ATP/ADP ratio which regulate the activity of the enzyme phosphofructokinase in the glycolytic sequence (4–7, 22–24). Therefore, it is reasonable that the negative inotropic effect observed with increasing concentrations of pyruvate, acetate, or butyrate could be the consequence of an inhibition of early steps in the metabolism of glucose. Also, it would appear from the present data that the stimulation of contractile tension produced by increasing concentrations of glucose or by the addition of mannose, was not merely the consequence of an increased formation of oxidizable substrates for the tricarboxylate cycle. On the other hand, inasmuch as the nonmetabolizable monosaccharide 3-*O*-methylglucose compete with glucose for transport across muscle cell membranes (11, 25), it is possible that the negative inotropic effect observed with 11.0 m*M* 3-*O*-methylglucose is also associated to a reduction in the uptake and metabolism of glucose by the atria.

In previous studies we found that the developed tension of isolated rat atria is particularly sensitive to the removal of glucose, decreasing very rapidly in glucose-free medium. The readmission of glucose is followed by a complete recovery of the control levels of developed tension (2, 3). Similarly, 2-DG, an inhibitor of glycolysis, exerted a negative inotropic effect; this was accompanied by a fall in the ATP content of the atria (1). The addition of increasing concentrations of glucose counteracted to some extent the contractile depression by 2-DG and partially restored atrial ATP levels. Pyruvate, on the other hand, completely restored the ATP concentration of atria made hypodynamic by incubation in glucose-free medium or by exposure to 2-DG, however it was significantly less effective than glucose in restoring the magnitude of developed tension. Similar results were obtained with lactate (2). From these studies it was suggested that the magnitude of atrial developed tension is influenced by some process that occurs during the initial phases of glucose metabolism. The findings of the present study are also in accord with this interpretation and again suggest that glucose and the operation of the Embden-Meyerhof pathway chain exerts a unique role in the regulation of the magnitude and stability of the developed tension of isolated rat atria.

Summary. The initial developed tension magnitude as well as the stability of the developed tension magnitude of isolated rat atria suspended in different substrates were

studied. The initial developed tension was significantly greater in the presence of glucose, mannose, lactate acetate, butyrate, or pyruvate than when fructose or the nonmetabolizable monosaccharide 3-*O*-methylglucose was the substrate. Atrial developed tension in fructose was significantly greater than in 3-*O*-methylglucose or in substrate-free medium. The stability of the developed tension was comparable in 5.5 m*M* glucose, mannose, lactate, acetate, butyrate, or pyruvate showing a small decrement with time (5–10% in 60–90 min period). Increasing concentrations of mannose, fructose, lactate, acetate, butyrate, or pyruvate up to 11.0 m*M* did not prevent the decrement of developed tension whereas it was completely abolished in the presence of 11.0 m*M* glucose. The ability of several substrates to modify the isometric contractile tension of rat atria suspended in KRB medium containing 5.5 m*M* glucose was also investigated. The addition of increasing concentrations of the metabolizable monosaccharides glucose and mannose, stimulate atrial developed tension, whereas fructose, had no effect. The 3-*O*-methylglucose had either no effect, or at higher concentrations resulted in a negative inotropic response. Increasing concentrations of butyrate, acetate, or pyruvate depressed the developed tension. It would appear that transport of sugars across the cell membrane or the increased formation of oxidizable substrates for the Krebs cycle are not "per se" the only mechanism by which the metabolizable monosaccharides can maintain atrial developed tension or produce a positive inotropic effect. The possibility that the magnitude of atrial developed tension is influenced by some process that occur during the initial phases of the glycolytic sequence is discussed.

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1. Gimeno, A. L., Lacuara, J. L., Gimeno, M. F., Ceretti, E., and Webb, J. L. Mol. Pharmacol. **2**, 67 (1966).

2. Gimeno, A. L., Gimeno, M. F., Savino, E. A., and Bedners, A. S., Proc. Soc. Exptl. Biol. Med. **123**, 875 (1966).

3. Savino, E. A., Gimeno, M. F., and Gimeno, A. L., Experientia **23**, 144 (1967).

4. Newsholme, E. E., Randle, P. J., and Manchester, K. L., Nature **193**, 270 (1962).

5. Williamson, J. R., Biochem. J. **93**, 97 (1964).

6. Garland, P. B., Randle, P. J., and Newsholme, E. A., Nature **200**, 169 (1963).

7. Williamson, J. R., J. Biol. Chem. **240**, 2308 (1965).

8. Hohorst, H. J., *in* "Methods of Enzymatic Analysis" (H. U. Bergmeyer, ed.), Academic Press, New York (1963).

9. Bing, R. J., Physiol. Rev. **45**, 171 (1965).

10. Park, C. R., Johnson, L. H., Wright, J. H., and Batsel, H., Am. J. Physiol. **191**, 13 (1957).

11. Slein, M. W., J. Biol. Chem. **186**, 753 (1950).

12. Morgan, H. E. and Park, C. R., Federation Proc. **17**, 278 (1958).

13. Narahara, H. T. and Ozand, P., J. Biol. Chem. **238**, 40 (1963).

14. Opie, L. H., Shipp, J. C., Evans, J. R., and Leboeuf, B., Am. J. Physiol. **203**, 839 (1963).

15. Levine, R., Goldstein, M. S., Huddlestun, B., and Klein, S. P., Am. J. Physiol. **163**, 70 (1950).

16. Danforth, W. H., Hogacamp, C. E., Ballard, F. B., and Bing, R. J., Am. J. Med. Sci. **238**, 477 (1959).

17. Williamson, J. R., Biochem. J. **83**, 377 (1962).

18. Evans, J. R., Opie, L. H., and Renold, A. E., Am. J. Physiol. **205**, 971 (1963).

19. Shipp, H. C., Matos, O. E., Knizley, H., and Crevasse, L. E., Am. J. Physiol. **207**, 1231 (1964).

20. Davis, E. J. and Quastel, J. H., Can. J. Biochem. **42**, 1605 (1964).

21. Garland, P. B. and Randle, P. J. Biochem. J. **91**, 6c (1964).

22. Passoneau, J. V. and Lowry, O. H., Biochem. Biophys. Res. Commun. **13**, 372 (1963).

23. Williamson, J. R., Jones, E. A., and Azzone, G. F., Biochem. Biophys. Res. Commun. **17**, 696 (1964).

24. Parmeggiana, A. and Bowman, R. H., Biochem. Biophys. Res. Commun. **12**, 268 (1963).

25. Randle, P. J., *in* "Membrane Transport and Metabolism," (A. Kleinzeller, and A. Kotyk, eds.), p. 431. Academic Press, New York (1961).

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