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Received June 2, 1966. P.S.E.B.M., 1966, v123.

Effects of Glucose, Pyruvate, Lactate and Starvation on Contractility of Isolated Rat Atria.* (31629)

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(Introduced by P. R. Saunders)
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The utilization of substrates has been extensively studied in cardiac tissue slices(1-5), isolated hearts(6-11), and hearts *in situ*(12-14), and it has been demonstrated that glucose, pyruvate, lactate, acetoacetate, and fatty acids can be oxidized by the myocardium. However, the functional importance of the different substrates for cardiac contractility has not been fully clarified. Recent work on the regulation of glucose metabolism has shown that glycolysis can be inhibited by pyruvate, acetoacetate, and fatty acids(15-17), and that high concentrations of pyruvate suppress endogenous respiration(18) and glucose uptake(19) in the isolated heart. A reduction of pyruvate uptake in the presence of glucose and insulin was also observed. In addition, lactate and glucose compete for utilization by the rat heart(9). Current interpretations emphasize the phosphofructokinase reaction as an important regulatory step in glycolysis(16); it is proposed that during the accelerated oxidation of pyruvate, fatty acids, or ketone bodies this enzyme is inhibited, leading to the accumulation of hexose phosphates, which in turn decreases hexokinase activity and glucose uptake. These inhibitions may be mediated by elevated levels of citrate and ATP(20-24). More distal enzymes in the Embden-Meyerhof pathway, such as phosphoglyceraldehyde dehydrogenase or pyruvate kinase, may also be involved in this regulation(19).

Results obtained with rat atria(25) and rabbit atria(26) suggest that although the glycolytic energy yield is comparatively small, either the uptake of glucose or the operation of the glycolytic pathway are important for a fraction of the contractile activity, inasmuch as pyruvate is only partially effective in restoring the developed tension in the absence of glucose or during block with 2-deoxy-glucose. The present work examines the effects of glucose, pyruvate, and lactate under different conditions in order to evaluate their capacities for maintenance of atrial contractility.

Methods. Atria were removed from decapitated rats and suspended in a modified Krebs-Ringer bicarbonate medium with the following compositions: Na^+ 145 mM, K^+ 6 mM, Ca^{++} 1.22 mM, Mg^{++} 1.33 mM, Cl^- 126 mM, $SO^{=}_4$ 1.33 mM, bicarbonate 25.3 mM, phosphate 1.2 mM, and glucose 5.5 mM. The medium was gassed with 95% O_2-5% CO_2 at a rate of 200 ml/min and maintained at pH 7.4 and 30°C. Cryoscopic determination gave a value of 286 milliosmolar for this medium. A constant resting tension of 750 mg was exerted on the atria with a micrometer head, the developed tension was recorded through a strain gauge, and the atria were electrically stimulated at a rate of 200/min. An equilibration period of 60 minutes was

* Supported in parts by grants 1441 and HE-994801 from Conejo Nacional de Investigaciones de Investigaciones de la Republica Argentina, and from Nat. Inst. Health, respectively.

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allowed before readings were taken. The experimental values of contractility (peak tension) were compared with those of the control records obtained at 0 time, and expressed as per cent changes in developed tension. The increase in medium osmotic pressure up to 316 milliosmolar from addition of substrates was shown not to affect atrial behavior by adding equivalent concentrations of sucrose, and the effects of increasing Na^+ concentration upon adding the salts of pyruvate and lactate were determined with equivalent additions of NaCl. Pyruvate free of parapyruvate was obtained from Sigma Chemical Co. and DL sodium lactate from Fluka A. G.

Results. Stability of the atria and effects of starvation. To determine the contractile behavior of untreated atria over intervals corresponding to the experimental periods, 10 atria from normal rats were followed for 90 minutes after equilibration (Table I). The developed tension remains constant for 30-40 minutes and then slightly declines to a level of 7-8% below the initial tension, which is maintained for at least 120 minutes (—6.9% depression for 2 atria). Such minor but definite spontaneous fluctuations in the contractility must be considered in interpreting the late effects of substances on the atria. The stability of 6 atria from rats starved for 48 hours is very similar (Table I). The significance of the somewhat lower initial tension developed is doubtful. It is rather surprising in view of the changes demonstrated in the metabolic properties of hearts from starved animals that both stability and initial tension are not altered; possibly the hour in contact with glucose during equilibration allows the atria to return towards normal.

Effects of changes in NaCl concentration. The addition of excess NaCl (5.5-40 mM) causes an immediate depression which is maximal around 1 minute (Fig. 1). Partial recovery rapidly occurs and this is followed by a slow progressive decline from 10 to 60 minutes (and probably beyond judged from the few atria examined after this time). Concentrations of 5.5-11 mM certainly produce an initial depression but the later behavior is not very significantly different from untreated atria; at 60 minutes control atria are —7.6% ± 1.0; with 5.5 mM excess NaCl they are —9.1% ± 2.4, and with 11 mM excess NaCl they are —10.0% ± 1.9. It is likely that addition of 0.5-11 mM Na-pyruvate or Na-lactate would not affect the atria significantly after 5 minutes due to the Na^+, although higher concentrations undoubtedly would exert a small depression. One does not know the contribution made by Cl^- when NaCl is added but it is likely to be negligible since various Na salts produce the same response as to NaCl. As stated in the *Methods,* these effects are not due to hyperosmolarity since sucrose additions produce no effects at all.

TABLE I. Spontaneous Contractile Changes in Atria from Fed and Starved Rats.

Time (min)	% Change in developed tension	
	Fed	Starved
1	— .6 ± .6 (4)	—
10	— .8 ± .6 (10)	— .9 ± 1.0 (6)
20	— .9 ± .8 (10)	—1.9 ± 1.1 (6)
40	—3.1 ± 1.0 (10)	—2.2 ± 2.4 (6)
60	—7.6 ± 1.0 (10)	—6.3 ± 1.7 (6)
90	—7.2 ± 1.9 (5)	—

Initial developed tension of atria from fed rats was 446 ± 15.5 mg (12) and from starved rats was 417 ± 5.3 mg (12). Mean ± standard error of mean. Numbers in parentheses refer to number of atria used.

Effects of pyruvate and lactate in glucose-free medium. Atrial developed tension progressively decreases following removal of glucose from the medium in agreement with previous work(25) (Fig. 2). Replacing the glucose with equivalent sucrose does not alter the failure indicating it is not a hypoosmotic effect. Addition of glucose at 30 minutes (Fig. 2, curve 2) causes an immediate stimulation and recovery is essentially complete by 20-30 minutes (in all cases comparison with the control results in Table I is made).

Pyruvate and lactate at 2.75-5.5 mM also rapidly stimulate atria depressed by 30 minutes lack of glucose but recovery occurs to a level 20% below the control (Fig. 2). Addition of glucose after 60 minutes with either pyruvate or lactate (Fig. 2, curves 3 and 4) brings about complete recovery when compared with the control results in Table I. On the other hand, 11 mM pyruvate is not as effective as the lower concentrations; if added simultaneously with the removal of glucose

it does not prevent the initial fall in contractility, although after 10 minutes the decline is slowed (Fig. 3, curve 2), and if added 30 minutes after glucose removal the recovery is slower and less maintained than with the lower concentrations (Fig. 3, curve 3). Furthermore, addition of glucose does not produce recovery in the presence of 11 mM pyruvate. While the recovery of glucose-free depressed atria with 11 mM lactate is to the same degree as with 2.75-5.5 mM, and more complete than obtained with 11 mM pyruvate, the subsequent addition of glucose (Fig. 3, curve 4) is able to stimulate the atria to a smaller extent than in the presence of 2.75 mM lactate (Fig. 2, curve 3).

Effects of pyruvate and lactate in the presence of glucose. The inability of 11 mM pyruvate to support contractility as well as the lower concentrations and its inhibition of the effect of glucose might be due to some inhibitory action superimposed on the stimulation presumably related to ATP generation. The effects of pyruvate and lactate at different concentrations on atria in normal medium were thus studied (Fig. 4 and 5). The initial transient depressions are similar to those produced by comparable concentrations of NaCl (Fig. 1) and are probably unrelated to any metabolic effects of pyruvate or lactate. Py-

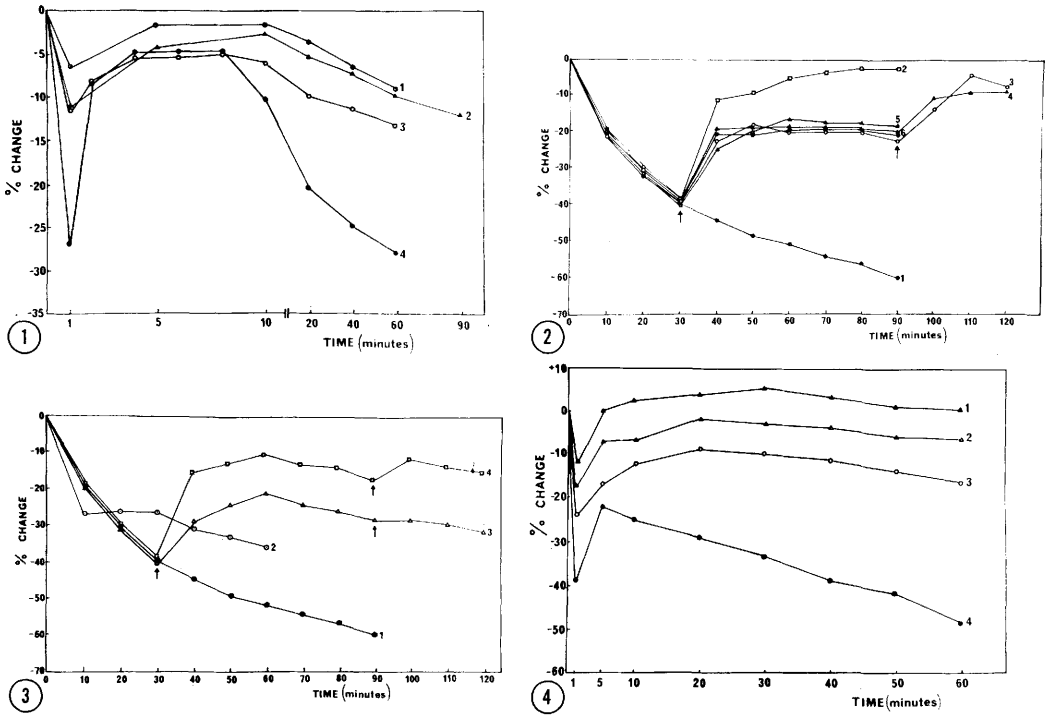
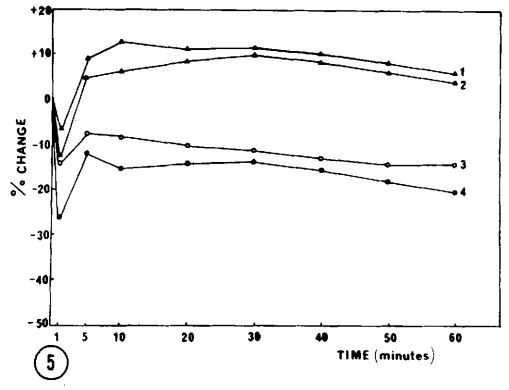


FIG. 1. Effects of increased NaCl on atrial developed tension. Concentrations of excess NaCl added: curve 1, 5.5 mM (5 atria); curve 2, 11 mM (5 atria); curve 3, 22 mM (5 atria); curve 4, 40 mM (3 atria).

FIG. 2. Effects of substrates on atria depressed by omission of glucose. Atria washed 3 times with glucose-free medium at 0 time. Curve 1, control in glucose-free medium (6 atria); curve 2, 5.5 mM glucose at 30 min (8 atria); curve 3, 2.75 mM lactate at 30 min, 5.5 mM glucose at 90 min (5 atria); curve 4, 2.75 mM pyruvate at 30 min, 5.5 mM glucose at 90 min (9 atria); curve 5, 5.5 mM lactate at 30 min (5 atria); curve 6, 5.5 mM pyruvate at 30 min (6 atria).

FIG. 3. Effects of higher pyruvate and lactate concentrations on atria in absence of glucose. Atria washed 3 times with glucose-free medium at 0 time. Curve 1, control in glucose-free medium (6 atria); curve 2, 11 mM pyruvate at 0 time (5 atria); curve 3, 11 mM pyruvate at 30 min, 5.5 mM glucose at 90 min (10 atria); curve 4, 11 mM lactate at 30 min, 5.5 mM glucose at 90 min (7 atria).

FIG. 4. Effects of pyruvate on atria in glucose medium. Concentrations of sodium pyruvate added at 0 time: curve 1, 2.75 mM (5 atria); curve 2, 5.5 mM (12 atria); curve 3, 11 mM (7 atria); curve 4, 22 mM (6 atria).



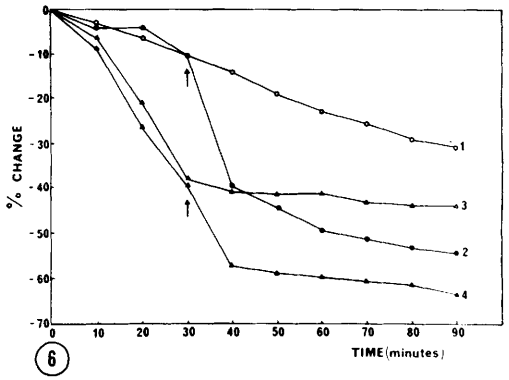


FIG. 5. Effects of lactate on atria in glucose medium. Concentrations of sodium lactate added at 0 time: curve 1, 2.75 mM (12 atria); curve 2, 5.5 mM (5 atria); curve 3, 11 mM (7 atria); curve 4, 22 mM (5 atria).

FIG. 6. Effect of arsenite on atrial response to pyruvate. Curve 1, 1 mM arsenite at 0 time (7 atria); curve 2, 1 mM arsenite at 0 time, 11 mM pyruvate at 30 min (7 atria); curve 3, 0.5 mM arsenite at 0 time (9 atria); curve 4, 0.5 mM arsenite at 0 time, 11 mM pyruvate at 30 min (8 atria).

ruvate at 2.75 mM causes a small but consistent stimulation of contractility, while 11-22 mM pyruvate definitely exerts a depressant action. Pyruvate at 5.5 mM has no apparent effect on the atrial behavior after 20 minutes, presumably because of a balancing of the stimulatory and inhibitory actions. Lactate produces greater stimulation than pyruvate and higher concentrations do not depress as readily. Since it has been found that certain β-halogenated pyruvates have cholinergic activity(27-29), the atria were first exposed to 0.01 mM atropine for 15 minutes and then treated with 22 mM pyruvate or lactate; an identical depression was observed and thus the effect is not through cholinergic mechanisms. In all cases the effects of pyruvate and lactate are rapidly and completely reversible upon replacement with normal medium. It is true that the initial transient depression produced by pyruvate or lactate is generally somewhat greater than with equivalent concentrations of NaCl, so it is possible that the anions exert some effect, but the data do not allow any interpretation of this phenomenon. The effects of substrates were not related to pH changes.

Effect of arsenite on response to pyruvate in glucose medium. Is the oxidation of pyruvate through the tricarboxylate cycle necessary for the effects upon atria? Arsenite inhibits the entry of pyruvate into the cycle in myocardium(30-31) and thus should prevent the effects of pyruvate on the atria if these are mediated through the cycle. Arsenite at 0.1-0.5 mM causes a progressive depression of the atria (Fig. 6, curve 1 and 3), as observed in the same tissue by Webb and Hollander(32). Addition of 11 mM pyruvate after 30 minutes in arsenite depresses more than in normal atria (Fig. 6, curve 2 and 4). The pyruvate effect is apparently not dependent on its entry into the cycle.

Discussion. The stability of rat atria under the conditions used is satisfactory for evaluation of the effects of metabolic changes over 1-2 hours. The hearts from starved rats have been shown to be deficient in the utilization of glucose, pyruvate, and lactate, with increased glycogen levels and a shift towards the utilization of lipids(9-11,23,33), but such changes are not reflected in the behavior of isolated atria in medium containing glucose with respect to the tension developed or stability. Whatever the explanation, it would seem that atrial developed tension is not closely related to the acute nutritional state of the animal. Excess NaCl (5.5-40 mM) causes a rapid transient contractile depression, which may be explained on the basis of the antagonism between Na^+ and Ca^{++} proposed by Lüttgau and Niedergerke(34), or on the shortening of the action potential duration(35) since this favors contractile depression(36).

Pyruvate is unable to completely restore or maintain developed tension in the absence

of glucose, despite the fact that pyruvate is effectively metabolized and can contribute up to 50-100% of the respiration of isolated rat heart(7,9), and that pyruvate can elevate the atrial ATP levels above normal(25). Pyruvate and lactate can apparently penetrate satisfactorily and generate ATP, but without glucose the atria develop contractile tension 15-20% below that when glucose is present. The uptake or utilization of glucose through the Embden-Meyerhof pathway, over and above the production of substrates for the Krebs cycle, is thus important to maintain a fraction of contractile activity. Such influence is not easily explained, mainly due to the difficulties in understanding how the sole inhibition of glycolysis or glucose uptake can result in a rather marked alteration of atrial force of contraction, particularly when the tissue is provided with external substrates which are known to be oxidizable metabolites for the myocardium. One possible and presumably partial explanation could be that the initial steps of glucose breakdown or even its transport across the membrane, might serve to regulate certain electrical properties of the cell, such as the amplitude and duration of action potentials, which in turn can exert some control on the auricular contractility. Higher pyruvate concentrations are not only less effective in supporting contractions but tend to depress the atria and prevent the recovery produced by glucose. This cannot be due to an elevation of the NAD: NADH ratio inasmuch as lactate exerts similar effects, nor does it seem to be due to the increase in citrate levels because arsenite does not reduce the depression by pyruvate. Part of the depression might be related to the partial suppression of glucose uptake and glycolysis by pyruvate, on the basis that glucose uptake or utilization is related to a fraction of the contractility, and some might be due to the increase in the ATP:ADP ratio, but that this is not the whole explanation is indicated by the fact that arsenite is not able to block the pyruvate effect. The possibility remains that pyruvate and lactate are able to exert some direct suppression of a process which is involved in controlling the developed tension, and such actions are being examined.

Summary. Atria from fed and starved rats developed essentially the same tension and are equally stable in glucose medium. Contraction is depressed in glucose-free medium and pyruvate or lactate (2.75-5.5 mM) partially restore the tension developed, but higher concentrations of pyruvate (11 mM) produce slower and less sustained recovery. Readmittance of glucose fully restores developed tension but has little effect in the presence of 11 mM pyruvate. In glucose medium, 2.75 mM pyruvate stimulates contractility slightly, 5.5 mM pyruvate has no effect, and 11-22 mM pyruvate depresses. This depression occurs even when the entry of pyruvate into the tricarboxylate cycle is prevented by arsenite. Lactate (2.75-5.5 mM) produces stimulating effects somewhat more marked than those of pyruvate but at higher concentrations (11-22 mM) is not quite depressant. The uptake or utilization of glucose through the Embden-Meyerhof pathway seems to be important for a fraction of atrial contractility, and thus high concentrations of pyruvate or lactate might depress by altering some phase of the initial metabolism of glucose.

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Received June 13, 1966. P.S.E.B.M., 1966, v123.

Use of Xenogeneic (Heterologous) Sera for Cultivation of Rabbit Lymphocytes *in vitro*.* (31630)

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In vitro cultivation of the peripheral lymphocytes of many species, including the rabbit, usually requires media enriched with serum(1,2,3). Antisera to rabbit IgG or to whole rabbit serum prepared in other species can induce "blast transformation" of rabbit lymphocytes *in vitro,* while normal sera from these other species have very little or no blastogenic effect(3,4). In recent attempts to define better the induction of transformation with antisera, sheep were chosen for the preparation of more specific antisera to rabbit serum proteins, as sheep sera were found to be capable of supporting the viability of rabbit lymphocytes *in vitro* in our previous studies. However, normal sheep sera obtained from local sources were either toxic or did not satisfactorily maintain the viability of rabbit lymphocytes *in vitro*. This communication delineates the culture conditions under which the effect of the sheep antisera upon rabbit lymphocytes *in vitro* could be evaluated.

Materials and methods. Healthy adult rabbits with known immunoglobulin allotype were used as donors of lymphocytes for culture. Blood was obtained from the marginal ear vein and lymphocyte-rich suspensions were prepared as described previously, using 3% w/v pig skin gelatin(3). The cell concentration was determined by counting in a standard hemocytometer. The lymphocytes were washed once in Eagle's suspension medium (Microbiological Associates, Inc., Bethesda, Md.) containing 1/10th volume tryptose broth (Difco), 200 units/ml of penicillin, and 100 units/ml of streptomycin. The lymphocytes were suspended in the volume

* Supported by Grant AI-07125-01 from Nat. Inst. of Allergy and Infect. Dis., NIH.

† Research Development Awardee 1-K3-AI-23,308-01 of NIAID, NIH.