Energy Sources for Contraction of the Rat Ventricle in Phosphate Media

By David A. Behman, Ph.D. and Paul R. Saunders, Ph.D.

Various substrates were tested for their ability to restore the amplitude of contraction of hypodynamic electrically-stimulated rat ventricle strips suspended in a phosphate-buffered medium. Greatest recovery of the contractile activity was obtained with pyruvate; lactate, β-hydroxybutyrate and acetate were more effective than succinate and glucose. The finding that glucose, in contrast to pyruvate, was relatively ineffective as an energy source for contraction, when a phosphate-buffered medium was employed, suggests that the defect was due to a rate-limiting step in the conversion of glucose to pyruvate, rather than to an impairment in the functioning of the Krebs cycle.

PHOSPHATE-BUFFERED media are commonly employed in the investigation of the positive inotropic action of the cardiac glycosides. However, evidence has recently been presented which indicates that important alterations in cellular function occur when phosphate is substituted for bicarbonate in physiologic media. The present investigation on the energy sources for the rat ventricle preparation was undertaken in order to provide data that may be pertinent to the elucidation of the sequence of events in isolated ventricle preparations that lead to the hypodynamic state and the reversal of this state by the cardiac glycosides. The data may also be of value in the interpretation of results obtained in other investigations where phosphate-buffered media are employed.

Methods

Male albino rats (150 to 200 Gm.) were killed by decapitation. A strip was prepared from the right ventricle according to the technic of Feigen and associates. The contractions were recorded under conditions described previously. The reference phosphate medium contained 0.9 per cent NaCl, 0.042 per cent KCl, 0.036 per cent CaCl₂, and 1 mM sodium phosphate buffer (pH 7.4), and was adjusted to pH 7.4 with 0.1 N sodium hydroxide.

The tissue was suspended in 150 cc. of substrate-free medium at 27 C. which was continuously aerated with oxygen. The strip was stimulated at a constant rate of 100 per minute. In the absence of added substrate, the amplitude of contraction gradually increased to a maximum value during the equilibration period and then steadily declined over a period of hours. The initial amplitude refers to the maximum amplitude of contraction attained at the end of the equilibration period. The mean initial amplitude in 81 experiments with the reference phosphate medium was 41 mm. (optical magnification of 65). Substances were added when the amplitude had declined to approximately 50 per cent of the initial amplitude (average time = 4 hours).

Results

Abilities of Substrates to Increase the Contractility of Substrate-Depleted Ventricle Strips. It is evident from figure 1 that the greatest effect was produced by pyruvate, while lactate, β-hydroxybutyrate, and acetate were more effective than succinate and glucose at the concentrations tested. The addition of glucose produced a rapid depression of the amplitude (7 to 19 per cent, 5 minutes after addition of glucose) which was followed by a slow increase; one hour after addition of glucose the amplitude was usually about 10 per cent greater than that observed prior to the addition of the substrate.

The results obtained with pyruvate, lactate and acetate are in general agreement with those previously reported for a bicarbonate medium at pH 7.4–7.6. The findings with glucose and succinate are similar to those obtained previously with a bicarbonate medium at pH 6.2, but are in contrast to those obtained...
in a bicarbonate medium at pH 7.4–7.6, as in the latter case excellent recovery of the amplitude was produced by glucose and no recovery by succinate.

**Effect of Bicarbonate upon Action of Glucose and Succinate in Phosphate Medium.**

Experiments were performed in a bicarbonate-phosphate medium in order to determine whether the effect of glucose and succinate upon the recovery of the amplitude of the substrate-depleted ventricle strip would be modified by the presence of bicarbonate. The bicarbonate-phosphate medium was identical with the reference phosphate medium except for the presence of 3 mM NaHCO₃ and gassing with a 99 per cent O₂-1 per cent CO₂ mixture to give a final pH of 7.4. The effect of glucose in bicarbonate medium was also studied. The composition of this medium differed from the reference phosphate medium only by the replacement of phosphate by 3 mM NaHCO₃ and gassing with 99 per cent O₂-1 per cent CO₂.

Figure 2 shows that the response to glucose in the bicarbonate-phosphate medium is indistinguishable from that obtained in the bicarbonate medium and is in contrast to that obtained in the phosphate medium. Figure 3 shows that the recovery of the amplitude obtained with succinate in a phosphate medium is antagonized by bicarbonate.

**Influence of Malonate upon Recovery with Glucose.**

Preliminary experiments have shown that ouabain at a concentration which inhibited recovery with succinate in reference phosphate medium stimulated recovery with glucose. Experiments were performed to determine whether malonate had a similar action on recovery with glucose. A positive inotropic response occurred upon the addition of malonate as has been reported previously to occur in bicarbonate medium at pH 6.2. Glucose was added after this positive inotropic effect had...
disappeared and figure 4 shows clearly that substantial recovery occurred by comparison with the controls to which no malonate was added.

In order to eliminate the possibility that the recovery obtained with glucose following the addition of malonate was due to a decrease in ionized calcium, control experiments were run in a medium containing one-half the normal calcium concentration. It was found in four experiments that one hour after the addition of glucose the amplitude was 47 per cent of the initial value (range = 32 to 68 per cent). The fact that the maximum recovery obtained with glucose in this low calcium medium was within the range of the control experiments (fig. 4) indicates that the potentiation of glucose recovery by malonate is not due to a decrease in the calcium ion concentration.

Table 1 shows that malonate inhibited slightly the recovery produced by pyruvate but produced no significant depression of the recovery with succinate. The finding that malonate did not produce a marked depression of recovery of the amplitude with pyruvate and succinate was unexpected. Webb and associates demonstrated with rat heart slices that 20 mM malonate inhibited pyruvate oxidation 70 per cent and succinate oxidation 92 per cent.

Influence of Ammonium Chloride upon Recovery with Glucose. In a previous study with ventricle strips made hypodynamic by prolonged activity in substrate-free bicarbonate medium at pH 6.2, it was found that glucose produced only slight recovery of activity, but succinate produced marked recovery. These results are similar to those reported in the present work with a phosphate medium at pH 7.4, but are in contrast to the results obtained in bicarbonate medium at pH 7.4-7.6. The similarity in response in bicarbonate at pH 6.2 and in phosphate at pH 7.4 suggested the possibility that these effects may be related to the intracellular pH, in view of the proposal of Creese that the intracellular pH decreases when a muscle is suspended in a phosphate-buffered medium.

Experiments were performed in a modified phosphate medium at pH 8.0 in the absence and presence of ammonium chloride (which has been shown to increase intracellular pH).

Table 1.—Effect of Malonate upon Recovery of Amplitude of Contraction with Pyruvate and Succinate

<table>
<thead>
<tr>
<th>Additions</th>
<th>No. of Expts</th>
<th>Per Cent of Initial Amplitude</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyruvate (2 mM)</td>
<td>6</td>
<td>49 (45-52)</td>
</tr>
<tr>
<td>Pyruvate (2 mM) after malonate (10 mM)</td>
<td>5</td>
<td>52 (50-53)</td>
</tr>
<tr>
<td>Succinate (10 mM)</td>
<td>8</td>
<td>47 (30-61)</td>
</tr>
<tr>
<td>Malonate (10 mM) control</td>
<td>5</td>
<td>51 (46-57)</td>
</tr>
<tr>
<td>Substrate-free control</td>
<td>4</td>
<td>50 (50-51)</td>
</tr>
</tbody>
</table>

* Mean value with range in parenthesis.
† Difference from pyruvate control significant at p = 0.05.

![Figure 4: Effect of malonate on recovery of the amplitude with glucose. Malonate (10 mM) added as indicated by the arrows. M. Glucose added at zero time. Shaded, half-shaded, and open circles represent individual experiments with malonate. Curve represented by dotted circles is an average curve from 12 experiments with glucose in absence of malonate. Vertical lines show range of values. Ordinate: percent of initial amplitude; abscissa: time in minutes.](image)
FIG. 5. Effect of ammonium chloride on recovery with glucose of the amplitude of contraction of substrate-depleted rat ventricle strips. Open circles represent an average curve from three experiments on recovery with glucose following the addition of ammonium chloride. Solid circles represent average curve from 3 control experiments with glucose. Vertical lines show the range of values. Ordinate: percent of initial amplitude; abscissa: time in minutes.

The medium was identical to the reference phosphate medium except that the pH was adjusted to 8.0 instead of 7.4, and the calcium chloride concentration was reduced to 0.027 per cent in order to prevent precipitation of calcium phosphate. A pH of 8.0 was selected because at this pH approximately 5.6 per cent of the ammonium chloride added is in the form of ammonia, whereas at pH 7.4 only about 1.4 per cent is in the form of ammonia. Figure 5 shows that a markedly greater recovery occurred with glucose in the presence of ammonium chloride than in its absence. It is possible that a decrease in intracellular pH occurs in a phosphate medium and is responsible by an unknown mechanism for the observed difference in glucose recovery in phosphate as compared to a bicarbonate medium of the same pH. Such a decrease in intracellular pH would presumably be minimized by ammonium chloride at pH 8.0, due to the entry of ammonia into the cells. However, the finding of Recknagel and Potter indicating that the reductive amination of α-ketoglutarate by ammonium chloride in rat liver homogenates resulted in the inhibition of the Krebs cycle presents the possibility that the recovery with glucose in the present experiments might be related to a metabolic action of ammonium chloride rather than to an effect on intracellular pH.

DISCUSSION

The finding in the present study that glucose was relatively ineffective as an energy source for contraction in phosphate medium, whereas it was very effective in bicarbonate medium at pH 7.4, may have bearing on the problem of the action of the cardiac glycosides. In most of the studies of the positive inotropic action of the cardiac glycosides in which isolated ventricle preparations have been employed, the tissues have been suspended in phosphate media. White and Salter, in their study of the response of the cat papillary muscle to cardiac glycosides, state that the muscle contracted for long periods without appreciable decline in amplitude in a bicarbonate medium, and that under such circumstances the muscle may fail to respond well to cardiac glycosides. They found that in a phosphate medium, on the other hand, a rapid decrement in the amplitude occurred, and that the cardiac glycosides greatly increased the contractility of such hypodynamic muscles. Therefore, it would appear that a knowledge of the nature of the hypodynamic state would be of value to the understanding of the mechanism of action of the cardiac glycosides.

It has been shown in the present work that pyruvate, in contrast to glucose, is an effective energy source for contraction in a phosphate medium. It has also been demonstrated that the addition of pyruvate to ventricle preparations suspended in a glucose-containing phosphate medium produced an increase in the amplitude of contraction. Webb and associates demonstrated that glucose was relatively ineffective in increasing the rate of oxygen consumption of rat heart slices, whereas pyruvate produced a marked increase. These findings suggest that the relative inability of glucose to increase the force of contraction of ventricle strips or the respiration of cardiac slices suspended in phosphate media is not due to an impairment of the Krebs cycle, but rather to a defect in the conversion of glucose to pyruvate.

It has been shown that the cardiac glycosides
increase the oxygen consumption of heart slices (suspended in phosphate media) in substrate-free and glucose-containing media, but not when pyruvate was used as a substrate. Furthermore, Wollenberger demonstrated that ouabain increased the rate of oxidation of C14-labeled glucose by dog heart slices suspended in a modified Krebs-Ringer phosphate medium, but had no effect on the utilization of labeled pyruvate.

The findings in the present study pose these questions: (1) is there a metabolic defect in cardiac tissue, suspended in phosphate media, limiting the rate of conversion of glucose and glycogen to pyruvate, with a resultant development of the hypodynamic state and (2) is one of the mechanisms for the reversal of the hypodynamic state in isolated ventricle preparations by the cardiac glycosides, the removal of such a rate limiting step?

**SUMMARY**

The ability of various substrates to restore the amplitude of contraction of electrically-stimulated rat ventricle strips suspended in a phosphate medium was investigated. The greatest recovery of the contractile activity was produced by pyruvate, while lactate, \( \beta \)-hydroxybutyrate and acetate were more effective than succinate and glucose.

The findings with glucose and succinate in a phosphate medium were in contrast to the results obtained with a bicarbonate medium; in the latter glucose produced a marked restoration of the amplitude of contraction and succinate was ineffective.

Malonate and ammonium chloride potentiated the action of glucose on the restoration of the amplitude of contraction of substrate-depleted rat ventricle strips.

**ACKNOWLEDGMENT**

Preliminary experiments on the effect of glucose and succinate on the contractile activity of substrate-depleted rat ventricle strips suspended in a phosphate-buffered medium were carried out in this laboratory by Dr. David T. Masuoka.

The authors wish to thank the Allan Hancock Foundation for the use of facilities during this investigation.

**REFERENCES**

Energy Sources for Contraction of the Rat Ventricle in Phosphate Media
DAVID A. BERMAN and PAUL R. SAUNDERS

Circ Res. 1955;3:559-563
doi: 10.1161/01.RES.3.6.559

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1955 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/3/6/559

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/