Studies of Tissue Permeability

VIII. THE EFFECT OF ANAEROBIOSIS ON GLUCOSE UPTAKE IN FROG SARTORIUS MUSCLE

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Previous investigations of the effect of anaerobiosis on the metabolism of glucose have been carried out with mammalian tissues. Walaaas and Walaaas (1) found no difference in the rate of glucose uptake by rat hemidiaphragms incubated in Ringer-phosphate solution with either oxygen or nitrogen as the gas phase. The addition of insulin exerted an effect on glucose uptake only during the first few minutes of anaerobic incubation. After 10 to 15 minutes of anaerobic incubation, phosphocreatine and adenosine triphosphate both fell to very low levels. Randle and Smith (2) reported that anaerobiosis did cause an increase in rate of glucose uptake if rat hemidiaphragms were incubated in bicarbonate rather than in phosphate buffer, at pH 7.4. In the perfused rat heart, which quickly stops contracting when deprived of oxygen, Morgan, Randle, and Regen (3) observed that anoxia increased the rate of penetration and phosphorylation of glucose.

Frog muscle, in contrast to mammalian muscle, is known to retain its functional integrity after many hours of anaerobiosis (cf. Hill and Kupalov (4)). As part of this adaptation to anaerobic conditions, one finds that the adenosine triphosphate concentration remains unchanged even after 4 hours of anaerobic incubation at 29°C. The characteristics of permeability of frog sartorius muscle to glucose in oxygenated bicarbonate-Ringer’s solution have been described (5); the muscle membrane has a very low permeability to glucose unless insulin is added. In this paper it is shown that this is also the case under anaerobic conditions, at least during the first 2 hours of incubation. Thereafter, there is a pronounced increase in permeability to glucose.

On addition of insulin, the rates of glucose penetration and phosphorylation in isolated frog muscle were found to be faster anaerobically than aerobically. The increase in rate of phosphorylation occurred despite a rise in the concentration of glucose 6-phosphate to levels which greatly exceeded the Kₜ value of glucose 6-phosphate that was determined for hexokinase in a frog muscle extract.

**EXPERIMENTAL PROCEDURE**

**Enzyme Preparations**—Hexokinase was prepared from yeast (6) and was free from interfering levels of phosphoglucomutase, glutathione reductase, invertase, maltase, amylose, myokinase, and adenosine triphosphatase.

<table>
<thead>
<tr>
<th>Enzyme Preparation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-phosphofructokinase</td>
<td>Prepared from yeast (6) and was free from interfering levels of phosphoglucomutase, glutathione reductase, invertase, maltase, amylose, myokinase, and adenosine triphosphatase.</td>
</tr>
</tbody>
</table>

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† Fellow of the Rockefeller Foundation.
The authors wish to thank Drs. R. A. Darrow and S. P. Colowick for a description of their method before publication.

+ The authors are grateful to Dr. O. K. Behrens of Eli Lilly and Company for a gift of this crystalline insulin.
hydroxide and zinc sulfate, and these impurities did not cause a significant error in the estimation of tissue glucose. However, when sucrose was present in the extract to be analyzed, or when particularly low concentrations of glucose were expected in the tissue extract, glucose was determined with hexokinase plus glucose 6-phosphate dehydrogenase (11).

Frog Muscle Hexokinase—A homogenate of frog leg muscle in 0.3 m sucrose-0.005 m EDTA at pH 7 was subjected to differential centrifugation in the cold in order to determine the distribution of the enzyme in the subcellular fractions (15).

For kinetic studies, a muscle extract was prepared with 0.01 m Tris-0.005 m EDTA at pH 8 by grinding with sand in a mortar. The extract was centrifuged for 1 hour at 100,000 X g, and the supernatant fluid was dialyzed for 15 hours against 0.01 m Tris-0.005 m EDTA at pH 6.5. The dialysis removed most of the endogenous glucose and glucose 6-phosphate, and led to loss of phosphofructokinase activity.

A typical reaction mixture contained 0.05 to 2 μmoles of glucose, 4 to 12 μmoles of ATP, 8 μmoles of MgCl2, 1 μmole of EDTA, 1 mg of crystalline bovine serum albumin, and 100 μmoles of Tris per ml at pH 7. Hexokinase activity was measured by following either the disappearance of glucose or the production of glucose 6-phosphate during incubation. In control experiments, the amount of glucose that disappeared was accounted for by the amount of glucose 6-phosphate formed during incubation when a correction was applied for the equilibrium established by the phosphoglucose isomerase reaction (3 glucose 6-phosphate and 3 fructose 6-phosphate). In the determination of $K_m$, the disappearance of glucose was measured under conditions in which glucose 6-phosphate could not accumulate, so that rates remained linear with time. This was accomplished by adding to the reaction mixture glucose 6-phosphate dehydrogenase and TPN, and in some cases phosphofructokinase as well.

In other experiments, glucose 6-phosphate was added and also allowed to accumulate during the reaction. By relating the increase in concentration of glucose 6-phosphate to the decrease in the rate of glucose disappearance, the $K_i$ of glucose 6-phosphate could be evaluated.

RESULTS

Effects of Anaerobiosis and Insulin on Penetration, Accumulation, and Utilization of Glucose—It has been shown in previous experiments (5) that when frog sartorius muscle is incubated aerobically at 29° in a medium containing glucose but no insulin, there is no detectable intracellular accumulation of glucose even during 6 hours of incubation. The reason for this is a very slow rate of penetration, with which the rate of utilization is able to keep pace. In the presence of insulin, penetration exceeds utilization so that intracellular glucose does accumulate. In Fig. 1 it is shown that under anaerobic conditions and in the absence of insulin there was no appreciable accumulation of glucose within the cell during the first 2 hours of incubation. This result duplicates the findings under aerobic conditions and suggests that the permeability characteristics of the cell membrane were not markedly altered by the lack of oxygen.

In contrast to the aerobic situation, however, glucose began to accumulate intracellularly between 2 and 3 hours of anaerobic incubation, and continued to rise during the next hour. This result suggests that prolonged anaerobiosis made the cell membrane more permeable to glucose. Fig. 1 reveals that this was the case; after 2 hours of incubation anaerobically there was a steep rise in the rate of penetration and a somewhat smaller increase in the rate of utilization.

An experiment with insulin is presented in Fig. 1 for comparison. It demonstrates that the cell membrane can undergo rapid changes in permeability during the initial period of incubation when anaerobiosis alone has no effect.

In the foregoing experiments, the glucose concentration in the medium was 10 μmoles per ml. It seemed desirable to repeat the measurements with an external glucose concentration within the range of the glucose level in frog blood. In 17 nonhibernating female frogs that had been maintained at a room temperature of approximately 25° and pithed, the average blood sugar determined by the glucose oxidase method was 30.6 ± 2.3 mg per 100 ml (1.7 μmoles per ml; cf (16)).

The results obtained at an external glucose concentration of 2 μmoles per ml (Table I) confirm those obtained at a higher concentration. During the first 2½ hours of anaerobic incubation without insulin, penetration proceeded at a barely measurable rate similar to the rate found under aerobic conditions.3 Thereafter, the rate of penetration anaerobically greatly exceeded the aerobic rate. Insulin produced its characteristic acceleration of glucose entry anaerobically during the first 2½ hours of incubation, but with more prolonged incubation, after anoxia per se had increased the rate of penetration (a change of 1.6 μmoles per ml in 2 hours), insulin had relatively little further enhancing effect (a change of 5.8 μmoles per ml in 2 hours for insulin plus

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3 The reason for the negative value for utilization in the anaerobic experiment will be discussed in a later section.
Winter frogs were used. In the anaerobic experiments, one muscle from each frog was incubated for 2 hours, and the paired muscle for 4 hours. The medium contained 2 μmoles of glucose per ml, and insulin as indicated. Each value is the average for four muscles, and the standard error of the mean is given. Δ represents the difference between the values obtained for 4 and 2 hours. Data from an aerobic experiment are included for comparison.

Table I

<table>
<thead>
<tr>
<th>Condition</th>
<th>Insulin</th>
<th>Incubation</th>
<th>Intracellular Glucose</th>
<th>Utilization</th>
<th>Δ</th>
<th>Penetration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic</td>
<td>0</td>
<td>2.5 hours</td>
<td>0.7 ± 0.1</td>
<td>-0.5 ± 0.2</td>
<td>4.6</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>3.5 hours</td>
<td>0.7 ± 0.1</td>
<td>4.1 ± 0.3</td>
<td>4.8</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>4.5 hours</td>
<td>1.1 ± 0.2</td>
<td>7.6 ± 0.2</td>
<td>5.9</td>
<td>8.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>5.5 hours</td>
<td>1.0 ± 0.2</td>
<td>13.5 ± 0.7</td>
<td>5.8</td>
<td>14.5 ± 0.8</td>
</tr>
<tr>
<td>Aerobic</td>
<td>0</td>
<td>4 hours</td>
<td>0.04 ± 0.0</td>
<td>0.3 ± 0.2</td>
<td>4.6</td>
<td>0.4 ± 0.2</td>
</tr>
</tbody>
</table>

Table II

Effect of anaerobiosis on glucose penetration and utilization in presence of insulin

Winter frogs were used. One muscle from each frog was incubated aerobically, and the other anaerobically. The incubation medium contained 0.4 unit of insulin per ml, and glucose at the concentration indicated. Each value is the average for four muscles, and the standard error of the mean is given. Aerobic conditions is 2.0 hour⁻¹. The corresponding rate constant for anaerobic conditions is 2.0 hour⁻¹.

Changes in the ratio, Vh/k', in the above equation should be a measure of the relative effects of an experimental procedure, in this case anaerobiosis, on utilization and penetration. This ratio was 4.0 μmoles per ml in the aerobic experiment in Table II at an external glucose concentration of 11.1 μmoles of glucose per ml yield a value for k' of 1.7 hour⁻¹, which compares well with a k' of 1.4 hour⁻¹ obtained previously at the same external concentration of glucose (5). The corresponding rate constant for anaerobic conditions is 2.0 hour⁻¹.

The rate constant of penetration, is given by (5),

$$k'(a - x) = V_h$$

where a and x are the external and internal concentrations of glucose, respectively, and V_h is the rate of utilization of glucose per hour. Calculations based on the data in Table II for the steady state during aerobic incubation at 11.1 μmoles of glucose per ml yield a value for k' of 1.7 hour⁻¹, which compares well with a k' of 1.4 hour⁻¹ obtained previously at the same external concentration of glucose (5). The corresponding rate constant for anaerobic conditions is 2.0 hour⁻¹.

**Anaerobic Glucose Production**—Frog sartorius muscle, when incubated anaerobically at 29° without glucose in the medium,
produces lactate from glycogen at a linear rate of approximately 6 μmoles per ml of intracellular water per hour. About 8% of the glycogen that is broken down is converted to glucose owing to the action of α-amylase-glucosidase on the 1,6-linked glucose.

**TABLE III**

Rate of glucose utilization anaerobically at different steady state levels of intracellular glucose

One muscle from each frog was incubated for the shorter, and the other for the longer, time period. Winter frogs were used. The incubation medium contained 0.4 unit of insulin per ml, and the other for the longer, time period. Winter frogs were used.

The apparent $K_m$ for glucose phosphorylation in the experiments on intact muscles is approximately $2 \times 10^{-3} \text{ M}$; the apparent $K_m$ for hexokinase in the extract is $9 \times 10^{-8} \text{ M}$.

<table>
<thead>
<tr>
<th>Glucose in medium (μmoles/ml)</th>
<th>Time Interval (hrs)</th>
<th>Intracellular glucose (μmoles/ml)</th>
<th>Utilization (μmoles/ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>2-4</td>
<td>6.3 ± 0.2</td>
<td>7.5 ± 0.4</td>
</tr>
<tr>
<td>10</td>
<td>2-4</td>
<td>5.9 ± 0.2</td>
<td>7.2 ± 0.3</td>
</tr>
<tr>
<td>5</td>
<td>2-4</td>
<td>2.3 ± 0.2</td>
<td>4.5 ± 0.6</td>
</tr>
<tr>
<td>2</td>
<td>2-4</td>
<td>1.0 ± 0.2</td>
<td>2.9 ± 0.5</td>
</tr>
<tr>
<td>2</td>
<td>2-4</td>
<td>1.0 ± 0.1</td>
<td>2.9 ± 0.3</td>
</tr>
</tbody>
</table>

* Value for maximal phosphorylating capacity of frog sartorius muscle extract (100,000 X g supernatant fraction).

**TABLE IV**

Effect of anaerobiosis upon glucose 6-phosphate levels in muscle

Winter frogs were used for Experiment I, and summer frogs were used for the other three experiments. Results are expressed as micromoles of glucose 6-phosphate per ml of intracellular water, with the standard error of the mean. Each value is the average for four muscles.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Condition</th>
<th>Glucose in medium (μmoles/ml)</th>
<th>Insulin (units/ml)</th>
<th>Incubation (hrs)</th>
<th>Glucose 6-phosphate (μmoles/ml/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Aerobic</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>I</td>
<td>Aerobic</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0.96 ± 0.02</td>
</tr>
<tr>
<td>I</td>
<td>Aerobic</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0.98 ± 0.03</td>
</tr>
<tr>
<td>I</td>
<td>Anaerobic</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0.44 ± 0.07</td>
</tr>
<tr>
<td>I</td>
<td>Anaerobic</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0.61 ± 0.04</td>
</tr>
<tr>
<td>II</td>
<td>Anaerobic</td>
<td>11.1</td>
<td>0</td>
<td>0</td>
<td>0.54 ± 0.07</td>
</tr>
<tr>
<td>II</td>
<td>Anaerobic</td>
<td>11.1</td>
<td>0</td>
<td>4</td>
<td>1.11 ± 0.03</td>
</tr>
<tr>
<td>III</td>
<td>Aerobic</td>
<td>11.1</td>
<td>0.4</td>
<td>2</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td>III</td>
<td>Aerobic</td>
<td>11.1</td>
<td>0.4</td>
<td>4</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>IV</td>
<td>Anaerobic</td>
<td>11.1</td>
<td>0.4</td>
<td>4</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>IV</td>
<td>Anaerobic</td>
<td>11.1</td>
<td>0.4</td>
<td>1</td>
<td>0.49 ± 0.03</td>
</tr>
<tr>
<td>IV</td>
<td>Anaerobic</td>
<td>11.1</td>
<td>0.4</td>
<td>2</td>
<td>0.56 ± 0.01</td>
</tr>
<tr>
<td>IV</td>
<td>Anaerobic</td>
<td>11.1</td>
<td>0.4</td>
<td>3</td>
<td>0.87 ± 0.06</td>
</tr>
<tr>
<td>IV</td>
<td>Anaerobic</td>
<td>11.1</td>
<td>0.4</td>
<td>4</td>
<td>0.98 ± 0.13</td>
</tr>
<tr>
<td>IV</td>
<td>Anaerobic</td>
<td>11.1</td>
<td>0.4</td>
<td>5</td>
<td>0.96 ± 0.12</td>
</tr>
</tbody>
</table>

* Through an error, a value of 0.005 μmole per ml was given for the concentration of glucose-6-P in unincubated muscles in Table VI of a previous report (5), whereas the correct value is 0.055 μmole per ml.
Glucose Phosphorylation in Frog Muscle Extract—After differential centrifugation of a frog muscle homogenate prepared in 0.3 M sucrose, 80% of the extractable hexokinase activity was in the 100,000 $\times$ g supernatant fraction and 20% in the mitochondrial fraction. The microsomal fraction had an insignificant activity.

Fig. 2. Inhibition of hexokinase by glucose-6-P in frog muscle extract. Each milliliter of assay mixture contained 0.6 $\mu$ mole of glucose, 4 $\mu$ moles of ATP, 1 $\mu$ mole of EDTA, 8 $\mu$ moles of Mg++, 1 mg of bovine plasma albumin, 100 $\mu$ moles of Tris buffer at pH 7, and 0.4 ml of dialyzed supernatant fraction obtained by centrifuging a frog muscle extract at 100,000 $\times$ g for 1 hour. In the control tubes glucose-6-P was removed during incubation by the action of glucose-6-P dehydrogenase and TPN, and the final concentration of glucose-6-P was less than 0.002 $\mu$ mole per ml. In the other tubes, glucose-6-P was added at the beginning of incubation and was also allowed to accumulate, and the amount present after varying intervals of incubation was measured enzymatically in a perchloric acid filtrate. Incubation was carried out at 30°C. Results are expressed as the amount of glucose phosphorylated during the period of incubation, relative to the amount phosphorylated in control tubes incubated for 60 minutes.

Fig. 3. Muscle content of ATP, creatine phosphate, and inorganic phosphate during aerobic incubation. Summer frogs were used. The medium contained 2 $\mu$ moles of glucose and 0.4 unit of insulin per ml. Results are expressed as micromoles of compound per ml of intracellular water, and each point is the mean for four muscles. Vertical bars represent two standard errors of the mean.

In order to insulate linearity of rate during the period of measurement, glucose 6-phosphate dehydrogenase and TPN, and at times phosphofructokinase, were added to remove glucose 6-phosphate as rapidly as it was formed. The effective glucose concentration was taken as the median amount during the period of incubation. By choosing different amounts of enzyme and periods of incubation, a large decrease in the glucose concentration was avoided during measurements of $K_m$. A total of 40 experimental points, covering a range of concentrations from $4 \times 10^{-5}$ to $1 \times 10^{-2}$ M glucose, was used for a Lineweaver-Burk plot (20) fitted by the method of least squares. The $K_m$ value for glucose was found to be $9 \times 10^{-5}$ M at pH 7. A similar set of data at pH 8 gave a $K_m$ value of $6 \times 10^{-5}$ M.

In agreement with the findings for mammalian hexokinases (18, 21) glucose 6-phosphate caused a noncompetitive inhibition of glucose phosphorylation in frog muscle extract. Fig. 2 shows that if the glucose 6-phosphate formed in the frog muscle system was allowed to accumulate during incubation, the rate of phosphorylation decreased. From these and other similar experiments the $K_i$ for glucose 6-phosphate was found to be $4.4 \times 10^{-4}$ M at pH 7. Changing the pH from 5.5 to 8 did not alter $K_i$ significantly. The $K_i$ determined for hexokinase in a frog muscle mitochondrial preparation was $4 \times 10^{-5}$ M. This value is approximately one-tenth of that reported for particulate mammalian brain hexokinase (18) and about one-half of that reported for a heart muscle hexokinase preparation (19).

ATP, Creatine Phosphate, and Inorganic Phosphate Content of Muscle—Fig. 3 shows that during 4 hours of incubation in oxygen there was a slight rise in creatine phosphate and a slight fall in inorganic phosphate, but no change in ATP. Under anaerobic conditions, the concentration of ATP within the cell also remained unchanged, whether or not glucose and insulin were present in the medium (Fig. 4, A and B). The steady rate of formation of lactic acid from glycogen observed in sartorii incubated anaerobically (Fig. 4B) could account for the production of approximately 9 $\mu$ moles of ATP per ml of intracellular water per hour. There was a pronounced fall in the concentration of creatine phosphate within the first 2 hours of anaerobic incubation; this breakdown of creatine phosphate, together with the rapid rate of glycolysis, could account for the constancy of the ATP level anaerobically. After 2 hours of incubation, creatine phosphate fell much more slowly and appeared to approach a steady state concentration equal to approximately one-third of the initial level. This result suggests that the breakdown of ATP was gradually decreasing to a rate which could be balanced by glycolysis.

The concentration of inorganic phosphate changed in an inverse manner from that of creatine phosphate (Fig. 4), as might be expected if the reaction of creatine phosphate and ADP were...
The changes in permeability and glucose utilization that occur in mammalian tissues when they are made anaerobic have been attributed to concomitant changes in the concentration of ATP and glucose 6-phosphate. The general validity of these interpretations is not supported by the experiments with frog sartorius muscle.

According to Randle and Smith (2), a restraint on the entry of glucose into the muscle cell, dependent upon a supply of energy-rich phosphate, would be removed by agents such as anaerobiosis, cyanide, and 2,4-dinitrophenol, which lower the concentration of ATP. In both the isolated diaphragm and the perfused rat heart there is a drastic fall in the concentration of ATP during short periods of anaerobiosis, but this is not the case in frog sartorius muscle, which withstands anaerobiosis for long periods of time. In particular, the rather sharp increase in glucose penetration that occurs after 2 hours of anaerobic incubation in the absence of insulin (cf. Fig. 1) is not accompanied by any change in the concentration of ATP.

Newsholme and Randle (23) and Regen, Davis, and Morgan (24) attribute the increased utilization of glucose to the decrease in the concentration of glucose 6-phosphate that occurs when perfused rat hearts are made anaerobic. The argument is based on the fact that in a cell-free system glucose 6-phosphate acts as a noncompetitive inhibitor of the hexokinase reaction, with a $K_i$ of approximately $1 \times 10^{-4}$ M for heart muscle hexokinase (19).

The question that is being examined here is whether or not observations made on cell-free systems can be applied directly to the intact cell. In frog sartorius muscles incubated with glucose and insulin, anaerobiosis increased the rate of phosphorylation by 50 to 100%; at the same time, the steady state level of intracellular glucose was lowered and the ATP concentration remained unchanged. This increase in utilization was accompanied by a 5-fold rise in glucose 6-phosphate to a steady state concentration of $9 \times 10^{-4}$ M. If the data obtained with the cell-free system were directly applicable, glucose utilization should have been inhibited 95%.

Results obtained with isolated lymph node cells by Helmreich and Eisen (26) are particularly illustrative because these cells do not contain any glycogen that could serve as a source of glucose 6-phosphate. The steady state intracellular concentration of hexose monophosphate was 0.4 µmole per ml aerobically as well as anaerobically, but the glucose uptake and lactate production were 4 times higher in the latter than in the former case. This increase in utilization occurred at a glucose 6-phosphate concentration sufficiently high to inhibit hexokinase strongly in vitro.

A similar discrepancy arises when the effect of the intracellular glucose concentration on the rate of utilization is considered. The $K_m$ of hexokinase for glucose in a frog muscle extract was found to be $9 \times 10^{-5}$ M. When sartorii were incubated anaerobically with 2 and 10 µmoles of glucose per ml, the intracellular glucose reached levels of approximately 1 and 6 µmoles per ml, respectively (Table III). At the higher concentration, the rate of glucose utilization was 2.5 times as great as at the lower.

In the dog heart with interrupted coronary circulation, the immediate effect of anaerobiosis is a rise in the concentration of hexose monophosphate and a decrease in ATP (25). Reperfusion of the heart with oxygen-free, buffered salt solution causes a decrease in the concentration of hexose monophosphate, presumably connected with the sweeping out of lactate.

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**DISCUSSION**

Substituted in part for oxidative phosphorylation as a means of regenerating ATP. The inorganic phosphate content of sartorii rose from a resting value of 6.4 ± 0.2 µmoles to approximately 19 µmoles per ml after 2 hours of anoxia, and then leveled off. The concentration of phosphate in the medium was 1 µmole per ml and did not contribute appreciably to the total tissue values.

Glucose 6-phosphate concentrations have been included in Fig. 4 in order to show that this intermediate of glycolysis does not play a quantitatively important role in the over-all phosphate balance.

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4 Recent studies of Seraydarian et al. (22) indicate that the true level of inorganic phosphate in resting frog sartorius muscle may be about 1.2 µmoles per g or 2 mM, which is lower than the values found here.
whereas an effect of less than 10% would have been expected on the basis of the $K_{m}$ of hexokinase for glucose. Data that yield a relatively high $K_{m}$ for the intracellular phosphorylation of glucose have also been reported for the perfused rat heart (27) and the chick embryo heart (28).

Although only a rough comparison is possible, values calculated for $V_{\text{max}}$ suggest that most of the hexokinase present in intact muscle can be made available for utilization. From the data in Table III, one can estimate that $V_{\text{max}}$ in anaerobic sartorius muscle would be approximately 10 μmoles of glucose per ml of intracellular water per hour, as compared with a value of 14 μmoles obtained under conditions for optimal hexokinase activity in a sartorius muscle extract.

The problem remains, however, why such a discrepancy exists between the effective concentrations of glucose found in vitro and in vivo. A definite answer cannot be given at this time. One possible explanation is that intracellular permeability barriers exist between glucose and hexokinase and that the enzyme is in contact with only a small portion of the total measurable pool of tissue glucose. In the case of glucose 6-phosphate, it would seem possible that the portion formed from glycogen remains in a different compartment of the cell, but this could hardly apply to the portion formed directly by hexokinase. Alterations in the properties of the enzyme itself may conceivably occur in the cellular environment. The results reported in this paper emphasize the difficulties which arise when it is assumed that conditions for enzyme activity in vitro and in vivo are exactly equivalent.

SUMMARY

When isolated frog sartorii were incubated anaerobically in Ringer's solution containing glucose but no insulin, the rates of glucose penetration and utilization remained low for approximately 120 minutes and then increased markedly. Free intracellular glucose accumulated during the phase of rapid penetration. Aerobically, the muscles remained relatively impermeable to glucose during 6 hours of incubation. The addition of insulin to muscles incubated anaerobically produced a large acceleration of the rates of penetration and utilization during the first 120 minutes of incubation. During the following 2 hours of anaerobic incubation, insulin exerted only a slight additional effect over that produced by anaerobiosis alone.

There was a pronounced fall in the level of creatine phosphate and a rise in inorganic phosphate during the first 2 hours of anaerobic incubation, followed by an approach to a steady state. No change was observed in the total adenosine triphosphate content of the muscle during 4 hours of anaerobic incubation with or without glucose and insulin. During later periods of anaerobic incubation, glycoglycine alone appeared to be sufficient to maintain a steady concentration of adenosine triphosphate.

The concentration of glucose 6-phosphate was more than 4 times higher anaerobically than aerobically when sartorii were incubated in the presence or absence of glucose and insulin. Examination of the kinetic properties of hexokinase in frog muscle extracts showed that the enzyme was strongly, and non-competitively, inhibited by glucose 6-phosphate at the concentration found intracellularly during anaerobic incubation. Nevertheless, in muscles incubated with glucose plus insulin, the rate of glucose phosphorylation was greater anaerobically than aerobically.

Another discrepancy was noted when the relation of intracellular glucose concentration to the rate of phosphorylation was studied in sartorii. Much higher concentrations were required for a half-maximal rate of phosphorylation in intact cells than in cell-free preparations, although the maximal rate was not markedly different. These results emphasize the fact that data that describe the kinetic behavior of an enzyme in a cell-free system are not of necessity directly applicable to the more complex conditions existing within the cell.

REFERENCES

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