Effect of Epinephrine on Permeability to Sugar and on the Production of Free Glucose in Skeletal Muscle*

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SUMMARY

Epinephrine increases the permeability of isolated frog sartorius muscles to 3-O-methyl-D-glucose. This effect resembles the action of insulin in that, at 19°, there is an absolute lag of 30 min preceding a rise in permeability, while at 0° no alteration of permeability is seen during several hours of incubation. The delayed effect of epinephrine on permeability contrasts with its prompt glycogenolytic effect at 19°; the hormone also causes a distinct rise in the concentration of glucose 6-phosphate at 0°. The effect of epinephrine on permeability can be prevented completely by the addition of pronethalol, an adrenergic β-receptor blocking agent, to the medium. Moreover, when muscles that have been incubated with epinephrine are subsequently incubated with pronethalol, the hormonal effect on permeability is readily reversed.

Adrenochrome does not affect permeability of sartorius muscles to sugar.

Of the concentrations of epinephrine tested, the lowest one that causes a change in permeability is 10⁻⁷ M. A supramaximal concentration, 10⁻⁵ M, produces an increase in permeability that is equivalent to that of a submaximal concentration of insulin, 50 units per ml.

The effects of supramaximal concentrations of epinephrine and insulin on permeability are not additive; thus, the two hormones appear to act on the same transport system for sugar. However, epinephrine causes the formation of free glucose within the cells, and this glucose in turn induces counterflow transport when the movement of 3-O-methyl-D-glucose across the cell membrane is studied.

The fact that epinephrine accelerates the formation of free glucose in muscle cells should be taken into consideration when the effects of epinephrine on utilization of sugars in muscle are evaluated.

The principal effects of epinephrine on carbohydrate metabolism in skeletal muscle are generally considered to be an acceleration of glycogenolysis (1, 2) and an inhibition of glucose utilization (3, 4). The present paper deals with two other effects of epinephrine in muscle cells which, although not as well recognized in the past, are of considerable interest: (a) enhancement of permeability to sugar, and (b) stimulation of the production of glucose.

Previous studies have shown no clear-cut effect of epinephrine, at a concentration of 10⁻⁵ to 10⁻⁴ M, on permeability of isolated rat diaphragm muscle to sugars (5, 6). However, Wohltmann, Narahara, and Wesley (7) have recently found that epinephrine at a concentration of 10⁻⁵ M significantly increases the permeability of isolated frog sartorius muscles to 3-O-methylglucose. Since kinetic data indicate that 3-O-methylglucose enters frog muscle cells by the same hormonally responsive transport system as glucose (8), and since radioactively labeled 3-O-methylglucose affords a sensitive means of measuring graded changes in permeability, the effects of epinephrine on the transport of 3-O-methylglucose have been investigated in greater detail, and the responses to epinephrine and insulin have been compared.

In the course of earlier studies on the regulation of glycolysis in skeletal muscle (9), it was found† that epinephrine caused the formation and accumulation of free intracellular glucose in frog sartorius muscles that were incubated without the addition of glucose to the medium. This observation has been confirmed and extended in the present studies. Cori and Larner (10) have shown that amylo-1,6-glucosidase can release free glucose from the limit dextrin that is produced by phosphorylase. Thus, when phosphorylase is more active, there will be more substrate for the debranching enzyme. Free glucose that is produced within muscle cells has been found to affect the transport of other sugars across the cell membrane and presumably can compete with exogenously supplied substrates for phosphorylation by hexokinase.

EXPERIMENTAL PROCEDURE

†-Epinephrine bitartrate was a product of Winthrop Laboratories. Crystalline bovine insulin was a gift from Dr. Otto K.

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1 P. Ózand and H. T. Narahara, unpublished observations.
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Fig. 1. Time course of effect of epinephrine on permeability at 19°.

Muscles were first incubated with 10^{-6} M epinephrine for varying periods of time and then, the initial rate of penetration of 3-O-methylglucose, was measured during a brief subsequent incubation. The period of effective exposure to epinephrine was taken to be the period of initial exposure to the hormone alone plus half the period of exposure to the hormone while sugar uptake was being measured. There were six muscles per point; vertical bars indicate twice the standard error of the mean.

Dehrens of Eli Lilly and Company, and pronethalol was donated by Dr. J. W. Black of Imperial Chemical Industries, Ltd., Cheshire, England. Crystalline yeast hexokinase and glucose-6-P dehydrogenase were obtained from the Boehringer Mannheim Corporation. 3-O-Methyl-^{14}C-n-glucose (2.7 μC per μmole) and mannitol-1-^{3}H (224 μC per μmole) were obtained from the New England Nuclear Corporation.

The composition of frog Ringer's solution, general procedures for handling the animals and tissues, and the method of counting radioactivity in muscles and media have been described elsewhere (8, 11). The frogs were obtained from September through March, unless otherwise specified, and sartorius muscles weighing 90 to 140 mg were used. Muscles were generally incubated with 2 ml of medium; each milliliter of incubation medium contained 2 mg of crystalline bovine plasma albumin (Armour Laboratories), to help stabilize insulin, and 0.1 mg of ascorbic acid (adjusted to pH 7.4 with NaOH) for the stabilization of epinephrine. This concentration of ascrobate prevented darkening and autoxidation of a buffered solution of epinephrine for at least 12 hours at room temperature; 1 mg of ascrobate per ml prevented autoxidation for at least 48 hours. Ascorbate did not alter the permeability of muscles to sugar.

After prior treatment of the muscles with epinephrine or insulin, permeability was measured by determining the initial rate of penetration of 3-O-methyl-n-glucose-^{3}H (8); the medium contained radioactive and nonradioactive 3-O-methylglucose at a concentration of 5 μm (0.25 μC per ml). Nonradioactive mannitol was present in media during prior incubation of muscles with hormones, and mannitol-^{3}H was present during incubation with 3-O-methylglucose-^{14}C. The concentration of mannitol was adjusted so that the total osmolarity for mannitol plus 3-O-methylglucose was always 10 milliosmos per liter.

For measurement of glucose and glucose-6-P, muscles were frozen in Freon 12 (dichlorodifluoromethane) that had been chilled to its freezing point in liquid nitrogen, and each muscle was ground at 0° with 0.8 ml of 0.3 N perchloric acid. Glucose and glucose-6-P were measured spectrophotometrically in neutralized extracts with the use of glucose-6-P dehydrogenase and hexokinase (12, 13). Media were treated with barium hydroxide and zinc sulfate (14) to remove dehydroascorbate, a product of incubation that interfered with the enzymatic assays.

RESULTS

Time Course of Effect of Epinephrine on Permeability—When muscles were incubated in the presence of 10^{-4} M epinephrine at 19°, permeability to 3-O-methylglucose began to increase after an absolute lag period of approximately 30 min (Fig. 1). Permeability continued to rise for 2 to 3 hours, and then little further change occurred during longer incubation. The duration of the absolute lag period was influenced by the temperature of incubation with epinephrine; no effect on permeability was observed in several hours at 0°. For six muscles that had been incubated with 10^{-6} M epinephrine at 0° for 5 hours, the initial rate of penetration of 3-O-methylglucose was 1.03 ± 0.21 μmoles per ml of cell water per hour. For paired control muscles that had been incubated in the absence of epinephrine, the value was 1.04 ± 0.11.

Reversibility of the increase in permeability caused by epinephrine at 19° was investigated. It was found that the effect of 10^{-5} M epinephrine on permeability was completely prevented by 10^{-5} M pronethalol, an adrenergic β-receptor blocking agent (15). Therefore, this antagonist was used to help terminate the effect of epinephrine in the tissue. After incubation of five muscles with 10^{-6} M epinephrine for 2 hours at 19°, the initial rate of penetration of 3-O-methylglucose was elevated to a value of 2.15 ± 0.35 μmoles per ml of cell water per hour. When paired muscles that had been similarly incubated with epinephrine were subsequently incubated for 24 hours with 10^{-5} M pronethalol in the absence of epinephrine, the rate of penetration of sugar fell to 1.36 ± 0.14, a level only slightly above the basal value.

It has been reported (16) that pronethalol at a concentration of 10^{-5} M inhibits the effect of insulin, 100 μunits per ml, on glucose uptake by rat epididymal adipose tissue and on the incorporation of glucose-^{14}C into glycogen of rat hemidiaphragms. However, pronethalol did not interfere with the effect of insulin in augmenting the permeability of frog sartorius muscles. Muscles from six summer frogs were incubated with insulin, 2000 μunits per ml, for 2 hours at 19°; the rate of penetration of 3-O-methylglucose was 3.02 ± 0.19 μmoles per ml of cell water per hour. Paired muscles incubated with insulin plus 10^{-5} M pronethalol had a value of 3.03 ± 0.27.

Although autoxidation of epinephrine in oxygenated Ringer's solution was minimized by the addition of ascorbate to the media, it was desirable to rule out the possibility that small amounts of adrenochrome, a principal oxidation product of epinephrine, might account for the observed alterations in permeability. Adrenochrome at a concentration of 10^{-7} M did not augment permeability to 3-O-methylglucose in 34 hours of incubation at 19°.

Effect of Different Concentrations of Epinephrine on Permeability of Muscles to Sugar—Epinephrine at a concentration of 10^{-3} M did not affect permeability to 3-O-methylglucose, but a definite effect was observed at 10^{-5} M, and a maximal effect was attained at levels of 10^{-3} to 10^{-2} M (Table I, Experiment A). The re-
spontaneous efflux of 3-o-methylglucose, could be measured. Penetration is expressed as micromoles of 3-o-methylglucose per ml of cell water per hour. The mean and standard error of the mean are given for each group of muscles; the number of muscles is indicated in parentheses.

**Table I**

**Effect of epinephrine and insulin on permeability of muscle to 3-O-methylglucose**

Muscles were incubated for 3½ hours at 19° in the absence of added hormone, or in the presence of epinephrine or insulin alone. However, when permeability had been increased by exposing the muscles to a supramaximal concentration of insulin, the addition of epinephrine caused a slight decrease rather than increase in efflux. In Experiment C the decrease was small but statistically significant. Although a somewhat lesser effect was seen in Experiment D, a decrease was consistently observed in each pair of muscles.

The findings described above have revealed that, in the presence of a supramaximal concentration of insulin, epinephrine causes a small increase in the rate of efflux of 3-O-methylglucose and a slight decrease in the rate of efflux. These changes might be expected to increase the final equilibrium level of accumulation of 3-O-methylglucose within the cells after a long incubation.
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muscels with 3-O-methylglucose and insulin for 4 hours at 19°, the apparent intracellular concentration of 3-O-methylglucose attained a final value that was 88% of the external concentration. It is reasonable to assume that the intracellular concentration was actually even closer to the external concentration than it appeared to be, because some of the intracellular water was probably tightly associated with macromolecules and not freely available to the sugar. To test the effect of epinephrine on the equilibrium distribution of 3-O-methylglucose across the cell membrane, six muscles were incubated with 3-O-methylglucose and insulin, 6000 units per ml, for 6 hours at 19°. Paired muscles were incubated in a similar medium that also included 10 \textsuperscript{-8} M epinephrine. Since 3-O-methylglucose is not metabolized by frog muscle, its concentration in the medium did not change appreciably during incubation; the concentration of 3-O-methylglucose in the medium at the end of incubation was used as a 5 mM reference standard for calculating the tissue content of 3-O-methylglucose. The apparent intracellular concentration of 3-O-methylglucose was 4.48 ± 0.12 mM (89.6% of the external concentration) for muscles that had been incubated with insulin alone, and was 4.93 ± 0.11 mM (98.6% of the external concentration) for muscles that had been incubated with insulin plus epinephrine. There was little variation in the final intracellular level of sugar attained within each group, and the small difference between the means was statistically significant (p < 0.02). If one assumes that only 90% of the intracellular water was available to the sugar, then the actual intracellular concentration of 3-O-methylglucose in the epinephrine-treated group was 5.48 mM, a value higher than the final external concentration. Observations that will be described below suggest that this phenomenon represents countertransport (18, 19). This finding, therefore, supports the concept that a mobile carrier is involved in the movement of sugars across the frog muscle cell membrane.

Effect of Epinephrine on Formation of Free Glucose in Muscle—When frog sartorius muscles were incubated in Ringer's solution with 10\textsuperscript{-6} M epinephrine at 19°, free glucose appeared within the cells and rose to a level of 0.6 nM or more in 34 hours (Table III). A significant increase in intracellular glucose was detectable after \( \frac{1}{2} \) hour of incubation. Glucose-6-P rose promptly during incubation with epinephrine at 19° (Table III), in keeping with the finding (20, 21) that conversion of phosphorylase \( b \) to \( a \) occurs within a few minutes during incubation of frog skeletal muscle with epinephrine. The increase in concentration of glucose-6-P and fructose-6-P that occurs in sartorius muscles in the presence of epinephrine has been attributed to the fact that the marked acceleration of glycogenolysis is not accompanied by a corresponding activation of phosphofructokinase, and the rate of the latter step becomes limiting for glycolysis (1, 9). In a separate experiment, not included in Table III, it was found that epinephrine caused a significant increase in the glucose-6-P content of sartorius muscles at 0°; an average value of 0.32 + 0.05 mM was observed in six muscles after 34 hours of incubation with 10\textsuperscript{-4} M epinephrine, compared with a value of 0.05 ± 0.01 in control muscles.

Production of glucose within muscle cells exposed to epinephrine leads to a net efflux of glucose (Table III). Since insulin exerts a greater maximal effect on permeability than does epinephrine (Table I), it was of interest to ascertain whether or not insulin would facilitate efflux of glucose that had been generated intracellularly under the influence of epinephrine. The data of Experiment A of Table IV suggest that this is the case. However, the effect of insulin is complicated by the finding, more clearly discerned at 29° (Experiment B), that it decreases the net rate of glucose production. Nevertheless, the finding that the rate of efflux was sustained despite a lower intracellular concentration of glucose indicates that insulin does enhance the efflux of endogenously generated glucose.

Effect of Epinephrine on Utilization of Glucose Added to Incubation Medium—When frog sartorius muscles are incubated with glucose at 29°, insulin enhances penetration of glucose to an extent that phosphorylation becomes the rate-limiting step for utilization, and free glucose accumulates in the cells (13, 22). In the present experiments, when muscles were incubated for 2 hours at 29° with insulin and 2 mM glucose, intracellular glucose reached a level that was slightly greater than half the external concentration (Table V). Previous studies have shown that this situation represents a steady state in which the rate of influx of glucose is balanced by its rate of utilization. Exposure of the muscles to a supramaximal concentration of epinephrine resulted in a change from a condition of net utilization to one of net production of glucose, and the intracellular concentration rose accordingly. Although there was an influx of glucose from the medium in the epinephrine-treated muscles, much of this occurred during the early stages of incubation when the intracellular concentration of glucose was still low; in other experiments not recorded here, the concentration of glucose within the

### Table III

Formation of glucose and glucose-6-P in muscles incubated with epinephrine in absence of added external glucose

Muscles were incubated in Ringer's solution at 19° with or without the addition of epinephrine, 10\textsuperscript{-6} M. The number of muscles in each group is indicated in parentheses. The appearance of any glucose in excess of 0.01 \( \text{\mu mol} \) per ml of cell water was considered to represent net production.

<table>
<thead>
<tr>
<th>Epinephrine</th>
<th>Incubation</th>
<th>Intracellular glucose</th>
<th>Efflux of glucose</th>
<th>Net production of glucose</th>
<th>Glucose-6-P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hrs</td>
<td>( \text{mM} )</td>
<td>( \text{\mu mol/ml cell water} )</td>
<td>( \text{mM} )</td>
<td>( \text{\mu mol/ml cell water} )</td>
</tr>
<tr>
<td>-</td>
<td>1/2</td>
<td>0.01 ± 0.004 (3)</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.03 ± 0.009</td>
</tr>
<tr>
<td>-</td>
<td>1</td>
<td>0.01 ± 0.002 (3)</td>
<td>0.02 ± 0.005</td>
<td>0.02 ± 0.005</td>
<td>0.04 ± 0.007</td>
</tr>
<tr>
<td>-</td>
<td>2</td>
<td>0.01 ± 0.004 (3)</td>
<td>0.02 ± 0.005</td>
<td>0.02 ± 0.005</td>
<td>0.04 ± 0.007</td>
</tr>
<tr>
<td>+</td>
<td>1/2</td>
<td>0.08 ± 0.01 (3)</td>
<td>0.04 ± 0.02</td>
<td>0.11 ± 0.01</td>
<td>1.06 ± 0.22</td>
</tr>
<tr>
<td>+</td>
<td>1</td>
<td>0.30 ± 0.03 (3)</td>
<td>0.08 ± 0.02</td>
<td>0.37 ± 0.05</td>
<td>1.95 ± 0.14</td>
</tr>
<tr>
<td>+</td>
<td>2</td>
<td>0.51 ± 0.05 (6)</td>
<td>0.88 ± 0.05</td>
<td>1.13 ± 0.10</td>
<td>2.00 ± 0.21</td>
</tr>
<tr>
<td>+</td>
<td>3/4</td>
<td>0.62 ± 0.06 (3)</td>
<td>1.81 ± 0.18</td>
<td>2.42 ± 0.20</td>
<td>3.07 ± 0.22</td>
</tr>
</tbody>
</table>
concentration, and an efflux of glucose was seen. The cell eventually rose to levels that actually exceeded the external concentration of 3-0-methylglucose in frog sartorius muscles (Tables I and II). Epinephrine increases the concentration of adenosine 3',5'-monophosphate, which is believed to mediate the changes in permeability produced by epinephrine. The phenomenon has been found to exhibit several striking similarities to the action of insulin. Studies of the action of insulin and electrical stimulation on isolated frog muscles (8, 23) have indicated that there are at least two different mechanisms whereby permeability to sugar can be enhanced. In contrast to electrical stimulation, insulin affects permeability only after an absolute lag period of 30 min at 19° and does not act for several hours at 0°. Furthermore, the effect of insulin can be reversed to a considerable degree by washing exposed muscles with Ringer's solution from which calcium has been omitted. The absence of contracture makes it seem unlikely that these agents act by releasing appreciable amounts of calcium ions from the sarcoplasmic reticulum; release of calcium ions within the cells has not been elucidated, but the studies of Rodbell (24) suggest that these ions are released by the action of the hormone insulin. In accord with these findings, the final equilibrium concentration of 3-O-methylglucose in insulin-treated muscle cells is elevated by epinephrine. This apparent paradox is resolved by the observation that epinephrine promotes the formation of free glucose within the cells. Glucose competes with 3-0-methylglucose for transport across the membrane of sartorius muscle cells (8) and induces countertransport of 3-0-methylglucose. The earlier observation (7) that epinephrine increases the permeability to sugar that are associated with muscle contraction. Since the duration and temperature dependence of the lag are similar for insulin and epinephrine, it is tempting to speculate that these hormones both trigger the same process that is involved in altering permeability. Epinephrine, like insulin, facilitates efflux as well as influx of 3-0-methylglucose in frog sartorius muscles (Tables I and II). In contrast, in the presence of a supramaximal concentration of insulin, although epinephrine causes a small increase in the rate of influx of 3-O-methylglucose, the rate of efflux is slightly diminished. In accord with these findings, the final equilibrium concentration of 3-O-methylglucose in insulin-treated muscle cells is elevated by epinephrine. This apparent paradox is resolved by the observation that epinephrine promotes the formation of free glucose within the cells. Glucose competes with 3-O-methylglucose for transport across the membrane of sartorius muscle cells (8) and induces countertransport of 3-O-methylglucose. Thus, in the presence of a supramaximal concentration of insulin, epinephrine does not appear to exert an additional effect on membrane permeability per se; this interpretation of the results suggests that the two hormones act on the same system for sugar transport in skeletal muscle cells. The chemical nature of the changes produced in the membrane by insulin and epinephrine has not been elucidated, but the studies of Rodbell (24) suggest that an alteration of the composition and arrangement of membrane phospholipids may be involved.

Epinephrine and insulin can each augment permeability to sugar in their usual manner during incubation of muscles in Ringer's solution from which calcium has been omitted. The absence of contracture makes it seem unlikely that these agents act by releasing appreciable amounts of calcium ions from the sarcoplasmic reticulum; release of calcium ions within the cells has been postulated (11, 25) to mediate the changes in permeability to sugar that are associated with muscle contraction. Epinephrine increases the concentration of adenosine 3',5'-monophosphate, which is believed to mediate the changes in permeability produced by epinephrine. The phenomenon has been found to exhibit several striking similarities to the action of insulin. Studies of the action of insulin and electrical stimulation on isolated frog muscles (8, 23) have indicated that there are at least two different mechanisms whereby permeability to sugar can be enhanced. In contrast to electrical stimulation, insulin affects permeability only after an absolute lag period of 30 min at 19° and does not act for several hours at 0°. Furthermore, the effect of insulin can be reversed to a considerable degree by washing exposed muscles with Ringer's solution from which calcium has been omitted. The absence of contracture makes it seem unlikely that these agents act by releasing appreciable amounts of calcium ions from the sarcoplasmic reticulum; release of calcium ions within the cells has not been elucidated, but the studies of Rodbell (24) suggest that an alteration of the composition and arrangement of membrane phospholipids may be involved.

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### Table IV

**Effect of epinephrine and insulin on net production of glucose**

In Experiment A, muscles were given a prior incubation without or with the addition of insulin for 3½ hours at 19° in order to obtain a full effect of insulin in the experimental muscles; then the muscles were incubated at 19° in 2 ml of fresh medium of the same composition for another 2 hours, and glucose production was measured. In Experiment B, muscles were incubated for 2 hours at 29° without prior incubation. Each value is the mean for six muscles.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Incubation conditions</th>
<th>Temperature</th>
<th>Epinephrine</th>
<th>Insulin</th>
<th>Intracellular glucose</th>
<th>Efflux of glucose</th>
<th>Net production of glucose</th>
<th>Glucose-6-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>19°</td>
<td>10⁻⁶</td>
<td>0</td>
<td>0.45 ± 0.05</td>
<td>0.53 ± 0.10</td>
<td>0.98 ± 0.12</td>
<td>2.19 ± 0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19°</td>
<td>10⁻⁴</td>
<td>6000</td>
<td>0.21 ± 0.03</td>
<td>0.65 ± 0.10</td>
<td>0.86 ± 0.12</td>
<td>2.06 ± 0.32</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>29°</td>
<td>3 × 10⁻⁷</td>
<td>0</td>
<td>0.62 ± 0.13</td>
<td>1.02 ± 0.10</td>
<td>1.64 ± 0.07</td>
<td>2.06 ± 0.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20°</td>
<td>3 × 10⁻⁷</td>
<td>6000</td>
<td>0.16 ± 0.05</td>
<td>0.92 ± 0.12</td>
<td>1.08 ± 0.16</td>
<td>2.06 ± 0.32</td>
</tr>
</tbody>
</table>

### Table V

**Effect of insulin and epinephrine on net production of glucose added to medium**

Muscles were given a prior incubation for 3½ hours at 19° with insulin. They were then incubated for 2 more hours at 29° in 2 ml of fresh medium that contained insulin and 2 mM glucose; the media for half of the muscles also contained epinephrine. Penetration refers to the amount of glucose taken up from the medium, corrected for the small amount that remained in the extracellular space. Net utilization indicates the amount of glucose that disappeared from the system (tissue + medium) during incubation. Each value is a mean for 10 muscles.

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>Glucose-6-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin/Epinephrine</td>
<td>mM</td>
</tr>
<tr>
<td>µM/ml</td>
<td>µM/ml cell water</td>
</tr>
<tr>
<td>6000</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>10⁻⁵</td>
<td>2.19 ± 0.01</td>
</tr>
</tbody>
</table>
phosphate (cyclic AMP) in cells (26), and Edelman and Schwartz (27) have found that cyclic AMP enhances glucose uptake by rat hemidiaphragms in vitro. Although epinephrine itself usually tends to decrease glucose utilization, these observations suggest the possibility that cyclic AMP may mediate the special effect of epinephrine on permeability to sugar. In frog sartorius muscles, 10^{-4} M cyclic AMP did not alter permeability to 3-O-methylglucose in 3 hours of incubation at 19°, but it is conceivable that the nucleotide penetrates less readily into the intact fibers of this preparation than into the cut fibers (28) of rat hemidiaphragms.

It is of interest to consider the effects of epinephrine on the uptake and utilization of glucose by frog muscle in relation to the finding that epinephrine promotes the formation of glucose in this tissue. It should be emphasized that the experimental measurements reported in this paper refer to net glucose production and utilization rather than the absolute amounts involved in these reactions. The absolute quantity of glucose produced or utilized includes a moiety which presumably undergoes cyclic formation and rephosphorylation without leaving the cell.

The absolute rate of glucose formation can be estimated if it is assumed (a) that an average of 8% of the glucosyl residues in glycogen are susceptible to the action of amylo-1,6-glucosidase (10, 29), and (b) that the rate of conversion of glucose-1-P to UDP-glucose and glycerone is slow relative to the rate of its conversion to glucose-6-P and lactate. The first assumption appears to provide a reasonable value in most cases, but the degree of error involved in the second assumption is uncertain. From the rate of lactate production in sartorius muscles incubated anaerobically at 19° in the presence of insulin, but in the absence of glucose and epinephrine (9), it can be calculated that the absolute rate of glucose production under these conditions is approximately 0.3 µmole per ml of cell water per hour. Muscles that were incubated aerobically with insulin and 2 mM glucose exhibited a net rate of glucose utilization of 2.9 µmole per ml of cell water per hour (Table V). Glycogenolysis is slower aerobically than anaerobically, so that the formation of glucose from glycogen, and its rephosphorylation, is relatively slight. Thus, the absolute rate of utilization of glucose is probably close to 3 µmole per ml of cell water per hour in the absence of epinephrine.

From the rate of formation of lactate and the extent of accumulation of hexose monophosphates during anaerobic incubation of sartorius muscles at 29° in the presence of insulin and epinephrine, but in the absence of added glucose (9), it can be calculated that the absolute rate of glucose production is approximately 0.8 µmole per ml of cell water per hour. This value is probably close to the rate of aerobic formation of glucose in the presence of 10^{-4} M epinephrine, the conditions of the present experiments. As there was a net production of only 0.2 µmole of free glucose per ml of cell water per hour in muscles incubated aerobically with epinephrine and insulin and 2 mM glucose (Table V), the absolute rate of utilization must have been approximately 0.6 µmole per ml per hour.

The assumptions and calculations described above indicate that the absolute rate of phosphorylation of glucose was diminished from a value of approximately 3 to a value of 0.6 µmole per ml of cell water per hour in the presence of epinephrine (Table V), a decrease of 80%. Concomitantly, the total glucose-6-P content of the cell rose 0.20 to 1.82 nm (22). Glucose-6-P inhibits the hexokinase activity of frog muscle extracts noncompetitively with respect to glucose, and the K_i is 0.04 mm (22); this observation is in agreement with measurements on mammalian heart muscle hexokinase (30). The increase in glucose-6-P concentration that was observed would decrease the activity of hexokinase in a muscle extract by 87%.

Although it appears likely that a rise in glucose-6-P in the epinephrine-treated cell accounts for the decreased rate of phosphorylation, the exact relationship of the rate of phosphorylation to the total glucose-6-P content of the cell requires further elucidation. Direct measurements of the absolute rate of phosphorylation under aerobic conditions would be desirable, but it should be borne in mind that glucose produced within the cell in the presence of epinephrine would compete with any externally supplied substrate for the hexokinase reaction. An interesting question that remains to be resolved is the possibility that the glucose-6-P of muscle cells may exist in separate pools that differ in their accessibility to various cellular enzymes (9, 22, 31).

The data of Table IV reveal that insulin decreased the net rate of glucose production in epinephrine-treated muscles. This interesting finding may reflect an increased rate of rephosphorylation of endogenously generated glucose. Insulin lowered the concentration of glucose-6-P in these cells, in agreement with earlier observations (9); the fall may be attributable in part to an increased rate of glycolysis (9). Insulin stimulates glycogen synthesis in rat diaphragm muscle (32), but this effect of the hormone has not yet been demonstrated in frog skeletal muscle. Insulin does not appear to cause an appreciable decrease of phosphorylase activity in rat diaphragm muscle (33). The injection of epinephrine into rats raises the concentration of free glucose in skeletal muscle (34, 35), and this increase has generally been ascribed to an inhibition of the hexokinase reaction by glucose-6-P (35, 36). The observation of a similar effect of epinephrine in isolated frog sartorius muscles during incubation in the absence of added glucose (Table IV) indicates that, in addition to bringing about an inhibition of phosphorylation, epinephrine also stimulates the formation of glucose. The fact that the concentration of free glucose can rise to readily detectable levels in muscle cells is of interest, in view of the recent finding of Holmes and Manoeur (37) that glucose, at a concentration of approximately 0.5 mM, can increase the phosphorylase activity of muscle by inhibiting phosphorylase phosphatase. Direct effects of glucose on the activity of phosphorylase have been reported, but they require much higher concentrations of glucose (35, 39).

Epinephrine increases glucose uptake in isolated perfused rat heart preparations (40, 41), but interpretation of this observation is difficult because there is a concomitant increase in the force of cardiac contraction, and this factor can independently alter glucose utilization (42). Permeability of the cell membrane is believed to be a limiting factor for glucose uptake in adipose tissue (43, 44), and epinephrine augments the uptake and utilization of glucose by this tissue in vitro (45). Thus, epinephrine appears to enhance the permeability of adipose tissue cells to sugar. This observation suggests the interesting possibility that, under special circumstances, epinephrine might be able to enhance rather than inhibit the uptake and utilization of glucose by skeletal muscle as well.

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Effect of Epinephrine on Permeability to Sugar and on the Production of Free Glucose in Skeletal Muscle

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