Regulation of Glucose Uptake in Muscle

VI. EFFECTS OF HYPOPHYSECTOMY, ADRENALECTOMY, GROWTH HORMONE, HYDROCORTISONE, AND INSULIN ON GLUCOSE TRANSPORT AND PHOSPHORYLATION IN THE PERFUSED RAT HEART

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It was concluded in earlier papers (1–7) that the transport of glucose through the cell membrane and the phosphorylation of free glucose inside the cell are the major rate-controlling steps for glucose uptake by the heart. In the muscle from severely diabetic animals, glucose uptake was very low. This could be attributed to a low rate of transport, which is principally limiting for uptake under these conditions. When insulin was added, transport was strongly stimulated but glucose uptake increased only moderately and the rate remained much below that reached in a normal heart similarly treated. The poor response to insulin could be explained by a marked depression of phosphorylation, which imposes the major limitation on the rate of uptake after transport acceleration. The phosphorylation step seemed to be insensitive to insulin in vitro.

The low transport activity in the heart muscle of the diabetic rat was found by Morgan et al. (8) to be caused by a deficiency of insulin and not by a direct inhibition by pituitary and adrenal cortical factors. A similar conclusion was reached by Kipnis (9) and Kipnis and Cori (10), who measured the penetration of 2-deoxyglucose in the rat diaphragm. Morgan et al. (5), Park and Morgan (7), and Henderson et al. (11, 12) concluded, however, that pituitary and adrenal factors may affect transport indirectly by interfering with the action of insulin.

The depression of phosphorylation in the diabetic muscle was attributed to pituitary and adrenal cortical activity (8–10). Morgan et al. (8) showed that growth hormone and hydrocortisone were among the hormones involved.

The present paper gives a further exposition of how pituitary and adrenal cortical secretions, including the growth hormone and hydrocortisone, influence glucose uptake in the perfused heart. The effect of these factors and of insulin on the transport and phosphorylation steps individually is considered. From these observations, some general concepts are advanced concerning the regulation of peripheral glucose utilization in the intact animal under a variety of conditions.

EXPERIMENTAL PROCEDURE

Methods and Materials

The preparation of the heart, the technique and apparatus for perfusion, and the methods of calculation have been described previously (4). All perfusions were carried out for 30 minutes at 37° with a bicarbonate-buffered medium.

Diabetes was induced by the intravenous injection of 6 mg of alloxan per 100 g of rat. Hearts were removed for testing 48 to 72 hours later. Hypophysectomized rats were purchased from Hormone Assay Laboratories, Chicago, and were used 2 to 4 weeks after operation. They were made diabetic as described above. Hypophysectomized rats tolerated alloxan administration very well and lived indefinitely thereafter, whereas normal animals rarely survived beyond 72 hours. High fasting blood glucose concentrations also pointed to a severe degree of insulin deficiency. Adrenalectomized-diabetic rats were prepared by alloxan injection as described above and were adrenalectomized 24 hours later. The immediate mortality after operation was about 30%. The rats were used 3 to 4 days after operation.

All rats, regardless of preparation, were fasted about 12 hours before they were killed.

The growth hormone was a highly purified preparation of bovine origin. It was kindly supplied by the Endocrinology Study Section of the National Institutes of Health (lot No.: R50109). Hydrocortisone was the sodium succinate derivative obtained from The Upjohn Company.

The insulin preparation was kindly supplied by Eli Lilly and Company (lot no. 496368) and had been treated to remove glucagon.

RESULTS

General Considerations—Transport and phosphorylation activities could be assessed as follows. Details are given elsewhere (4–6).

The uptake of glucose from the medium could be equated to net transport into the cell, inasmuch as any change in the extracellular glucose of the tissue during the perfusion was negligibly small. When no insulin was added, the intracellular free glucose concentration remained very low. Under these conditions, uptake provided an approximate measure of the rate of unidirectional transport inward. Transport activity could then be assessed by relating this rate to substrate concentration, that is, to the extracellular or perfusate concentration of glucose. By the use of several perfusate concentrations, a curve of transport activity for any given condition of the heart was obtained which could be compared to a similar curve obtained in hearts from normal animals.

The phosphorylation rate was estimated by the uptake of glucose from the perfusate corrected for any change in the free,
intracellular glucose content of the tissue. This approach was based on the generally accepted concept that the hexokinase reaction is an obligatory step for the utilization of the free hexose. Phosphorylation (hexokinase) activity could be estimated by relating the rate to substrate concentration, which in this case was the concentration of free, intracellular glucose. Experiments were carried out with various concentrations of glucose in the perfusate, with insulin added to accelerate transport. In this way, phosphorylation rates at several readily measurable levels of intracellular glucose were obtained. It was then possible to draw curves of phosphorylation activity in the heart as a function of substrate concentration under various experimental conditions which could be compared to a normal control curve.

The rates and activities reported here are values for the steady state. Earlier work (4, 5, 11) had shown that glucose uptake was stable beyond the present test period. Furthermore, the distribution of free glucose in the tissue and the concentration of hexose phosphates reached virtually constant values within 2 to 3 minutes after starting a perfusion (15).

Effect of Hypophysectomy of Diabetic Rat on Glucose Uptake and Transport—Glucose uptake by the heart from diabetic animals in the absence of added insulin is shown by the curve (open squares) in the upper panel of Fig. 1. In general, the curve shows about a 70% inhibition when compared to a similar curve for hearts from normal rats (4, 6). Hypophysectomy did not alter the diabetic curve appreciably when tests were made at three different perfusate concentrations (open circles). As seen in the lower panels, the concentrations of intracellular glucose remained low. The uptake curves, therefore, could be taken to indicate transport inward as a function of extracellular concentration. It was thus apparent that hypophysectomy in the diabetic did not increase transport activity. The effect of hypophysectomy on phosphorylation activity in the absence of added insulin could not be assessed because the levels of intracellular glucose were below the level of reliable estimation.

Effect of Hypophysectomy in Diabetic Rat on Glucose Phosphorylation—Glucose uptake by the diabetic heart was only moderately accelerated by the addition of insulin (Fig. 1, upper panel, solid squares). On the other hand, insulin caused a relatively large stimulation of uptake in the heart from hypophysectomized-diabetic animals (upper panel, solid circles). The rates were nearly as fast as those seen in hearts from normal (4) or hypophysectomized rats (11). In the lower panel, it is seen that the insulin effect was accompanied by a substantial accumulation of intracellular free glucose, the usual finding with a marked stimulation of transport. Under these conditions it was possible to evaluate phosphorylation activity.

Phosphorylation as a function of the intracellular glucose concentration is shown in Fig. 2. It can be seen that hypophysectomy restored phosphorylation activity in the diabetic to near the normal level. In comparison to the diabetic curve, the apparent \( K_m \) was substantially reduced and the \( V_{max} \) increased.

Effect of Adrenalectomy in Diabetic on Glucose Uptake—It was of interest to observe whether adrenalectomy of the diabetic animal would have the same effect as hypophysectomy. The preparation of adrenalectomized-diabetic rats was relatively difficult and studies were carried out at a single perfusate glucose concentration. The results are shown in Table I.

FIG. 1. Glucose uptake and intracellular free glucose concentration at various extracellular (perfusate) glucose concentrations in the isolated heart from diabetic and hypophysectomized-diabetic rats. Each point is the mean value for 6 to 12 hearts. The vertical line through the points shows 2 standard errors of the mean. (It has been omitted when the value is so small that it impinges on the symbol itself.) Insulin was added when indicated in a concentration of 3 \( \mu \)g per ml. The intra- and extracellular glucose concentrations are mean values for the experimental period determined as described earlier (4). Blood plasma glucose concentrations at the time the rats were killed, after about 12 hours of fasting, were as follows (mean ± standard error): diabetic (controls), 690 ± 25; diabetic (insulin), 700 ± 38; hypophysectomized-diabetic (controls), 494 ± 37; hypophysectomized-diabetic (insulin), 498 ± 31. The values for the diabetic hearts have been reported earlier (5) and are shown here for reference.
The effects of hypophysectomy, adrenalectomy, growth hormone, and hydrocortisone on the kinetics of glucose phosphorylation in the perfused heart. Hearts were perfused with 3 μg per ml of insulin in the medium in all cases. The data for normal and diabetic hearts have been reported earlier (4, 5) with 3 pg per ml of insulin in the medium in all cases. The data when indicated, growth hormone (GH) and hydrocortisone (cort.) were injected over a 24-hour period before removal of the hearts for testing. The dosages used are those described in footnotes d and e of Table II. The concentrations of blood plasma glucose at the time of killing, after about 12 hours of fasting, were as follows (mean ± standard error): hypophysectomized-diabetic, 468 ± 31; diabetic, 607 ± 29; hypophysectomized-diabetic treated with growth hormone and cortisone, 624 ± 32 mg per 100 ml; normal, not determined.

The results of various schedules of pretreatment are shown in Table II. In the first experiments (upper panel), a moderately high dose of growth hormone by itself caused a modest depression of glucose uptake and phosphorylation activity when compared with rates obtained in hearts from untreated controls (see Figs. 1 and 2) or from normal animals at comparable substrate concentrations. A high dosage of hydrocortisone alone reduced the rate to one-half that of the controls. The effect of both substances together was additive, or synergistic, and phosphorylation activity was depressed to about 15% of normal. The effect of combined treatment at other intracellular glucose concentrations is shown by the lowest curve of Fig. 2. It is apparent that these agents suppressed phosphorylation to a level substantially below that in the heart from the diabetic animal.

As shown in the lower panel of Table II, the hormone was tested at one-tenth the above dose level. Under these conditions, growth hormone alone was ineffective, whereas hydrocortisone by itself was still active. In combination, an additive effect was not observed with the low dose of growth hormone but was evident with the high dosage.

A limited number of experiments were carried out in which hypophysectomized or normal rats were pretreated with growth hormone for 48 hours before phosphorylation was tested (Table III). Assays were carried out with insulin in the medium as before. In the muscle from hypophysectomized rats, the treatment led to a slight lowering of uptake and phosphorylation, the significance of which is doubtful. In the tissue of normal animals, no effect was seen after administration of growth hormone in vivo although a normal level of adrenal cortical secretion was presumably present.

The effect of growth hormone in vitro on uptake and phosphorylation was also tested with hearts from normal rats in the presence of insulin. No inhibition was observed. Bronk and Fisher (17) have reported a depression of uptake under these conditions at a substrate concentration of 100 mg per 100 ml. The number of hearts tested is given by the figure in parentheses.

### Table I

**Glucose uptake and phosphorylation by perfused heart of adrenalectomized-diabetic rats**

<table>
<thead>
<tr>
<th>Insulin</th>
<th>Glucose uptake</th>
<th>Mean intracellular glucose</th>
<th>Glucose phosphorylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>μg/ml</td>
<td>mg g⁻¹ hr⁻¹</td>
<td>mg/g 100 ml</td>
<td>mg/g⁻¹ hr⁻¹</td>
</tr>
<tr>
<td>0</td>
<td>1.8 ± 0.3   (11)</td>
<td>77</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>+ 5.4 ± 0.7 (13)</td>
<td>140</td>
<td>20.2 ± 9 b</td>
</tr>
<tr>
<td>3</td>
<td>1.8 ± 0.3  b</td>
<td>140</td>
<td>5.3 ± 0.7 e</td>
</tr>
</tbody>
</table>

- Comparisons have been made with values obtained with the diabetic heart at the corresponding perfusate concentration in the case of uptake and corresponding intracellular glucose concentration in the case of phosphorylation (see Figs. 1 and 2).
- Standard error of the mean.
- Abbreviation for none detected. It was estimated that concentrations below 10% of the perfusate concentration might escape detection by the present methods (see footnote 4).
- p < 0.01 versus 0.
- p < 0.01 versus corresponding diabetic control.

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TABLE II
Effect of growth hormone and hydrocortisone on uptake and phosphorylation of glucose by isolated heart from hypophysectomized-diabetic rats

All perfusions were carried out with 3 µg per ml of insulin and 100 mg per 100 ml of glucose in the medium. The number of hearts tested is given by the figure in parentheses.

<table>
<thead>
<tr>
<th>Treatment of heart donor rats</th>
<th>Blood plasma glucose</th>
<th>Glucose uptake</th>
<th>Mean intracellular glucose</th>
<th>Glucose phosphorylation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/100 ml</td>
<td>mg g⁻¹ hr⁻¹</td>
<td>mg/100 ml</td>
<td>mg g⁻¹ hr⁻¹ % normal²</td>
</tr>
<tr>
<td>None</td>
<td>379 ± 57</td>
<td>7.2 ± 0.5 (11)</td>
<td>27 ± 0.5</td>
<td>7.0 ± 0.0 (5) 61 ⁶</td>
</tr>
<tr>
<td>+ GH⁴</td>
<td>282 ± 43</td>
<td>5.3 ± 0.4 (9)</td>
<td>44 ± 5</td>
<td>5.3 ± 0.4 45 ⁴</td>
</tr>
<tr>
<td>+ Cort ¹</td>
<td>700 ± 53</td>
<td>4.1 ± 0.5 (9)</td>
<td>47 ± 6</td>
<td>3.8 ± 0.5 32 ⁴</td>
</tr>
<tr>
<td>+ GH⁴ + cort ¹</td>
<td>606 ± 32</td>
<td>2.0 ± 0.2 (8)</td>
<td>66 ± 5</td>
<td>2.0 ± 0.2 10 ⁵</td>
</tr>
<tr>
<td>None</td>
<td>495 ± 69</td>
<td>9.3 ± 0.4 (9)</td>
<td>48 ± 6</td>
<td>9.0 ± 0.4 75 ⁶</td>
</tr>
<tr>
<td>+ GH¹</td>
<td>362 ± 19</td>
<td>10.2 ± 1.0 (4)</td>
<td>36 ± 14</td>
<td>10.0 ± 1.0 88</td>
</tr>
<tr>
<td>+ Cort ²</td>
<td>525 ± 71</td>
<td>6.1 ± 0.5 (9)</td>
<td>40 ± 5</td>
<td>5.8 ± 0.5 54 ⁶</td>
</tr>
<tr>
<td>+ GH¹ + Cort ²</td>
<td>500 ± 39</td>
<td>6.5 ± 0.6 (9)</td>
<td>47 ± 4</td>
<td>6.5 ± 0.6 50 ⁶</td>
</tr>
<tr>
<td>+ GH⁴ + cort ²</td>
<td>821 ± 45</td>
<td>3.4 ± 0.4 (7)</td>
<td>47 ± 4</td>
<td>3.1 ± 0.4 26 ⁴</td>
</tr>
<tr>
<td>+ GH⁴ + cort ²</td>
<td>785 ± 46</td>
<td>2.0 ± 0.2 (4)</td>
<td>48 ± 3</td>
<td>2.3 ± 0.2 19 ⁵</td>
</tr>
</tbody>
</table>

a Comparisons have been made with values obtained in normal hearts at the corresponding intracellular glucose concentrations (see Fig. 2).

b Standard error.

c p < 0.01 versus corresponding normal control.

d 0.1 mg of growth hormone (GH) per 100 g of body weight was injected intraperitoneally at 24 and again at 12 hours before killing.

e 2.5 mg of hydrocortisone (cort.) per 100 g was injected subcutaneously at 24 hours followed by 1.25 mg per 100 g at 12 and 4 hours before killing.

f 0.01 mg of growth hormone per 100 g was administered as above.

g 0.25 mg of hydrocortisone per 100 g was injected at 24 hours followed by 0.125 mg per 100 g at 12 and 4 hours before killing.

TABLE III
Lack of any large effect of growth hormone on glucose uptake and phosphorylation in hearts from hypophysectomized and normal rats

All perfusions were carried out with 3 µg per ml of insulin in the perfusion medium. Growth hormone (GH) was injected in a dosage of 0.1 mg per 100 g at 48 and again at 24 hours before killing. In the experiment in which growth hormone was added in vitro, a preparation (B168) kindly supplied by Dr. A. Wilhelmi was used in 8 of the hearts tested.

<table>
<thead>
<tr>
<th>Heart donor rats</th>
<th>Glucose concentration</th>
<th>Glucose uptake</th>
<th>Mean intracellular glucose</th>
<th>Glucose phosphorylation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/100 ml</td>
<td>mg g⁻¹ hr⁻¹</td>
<td>mg/100 ml</td>
<td>mg g⁻¹ hr⁻¹</td>
</tr>
<tr>
<td>Hypex</td>
<td>300 14.3 ± 0.6 e (7)</td>
<td>120 ± 8 e</td>
<td>13.6 ± 0.6 e</td>
<td></td>
</tr>
<tr>
<td>Hypex, GH injected, 48 hrs.</td>
<td>300 11.8 ± 0.6 e (8)</td>
<td>190 ± 9 e</td>
<td>10.8 ± 0.6 e</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>300 14.1 ± 0.7 e (6)</td>
<td>90 ± 6 e</td>
<td>13.2 ± 0.7 e</td>
<td></td>
</tr>
<tr>
<td>Normal, GH injected, 48 hrs.</td>
<td>300 12.9 ± 0.4 e (8)</td>
<td>73 ± 9 e</td>
<td>12.4 ± 0.4 e</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>300 11.1 ± 0.5 e (16)</td>
<td>30 ± 6 e</td>
<td>10.9 ± 0.5 e</td>
<td></td>
</tr>
<tr>
<td>Normal, GH added in vitro, 6 µg/ml</td>
<td>200 11.0 ± 0.5 e (16)</td>
<td>32 ± 5 e</td>
<td>10.8 ± 0.5 e</td>
<td></td>
</tr>
</tbody>
</table>

a Standard error.

4 This material was assayed by Dr. A. Wilhelmi just before sending it to us and was found to have full growth activity.

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the 24-hour experiments in which protamine zinc insulin was injected in divided doses 24 and 4 hours before sacrifice. All rats had indicated. Insulin was injected subcutaneously in a single dose the number of hours indicated before removal of the heart except in phosphorylation showed a normal activity.

These conditions led to substantial levels of intracellular glucose and the assay of phosphorylation activity was not possible. The residual impairment of phosphorylation. The intracellular concentration in the assay medium. These conditions led to substantial levels of intracellular glucose and the assay of phosphorylation activity was approximately doubled.

In the third experiments, pretreatment was carried out for 24 hours. Uptake was now completely normal with no obvious restoration of phosphorylation activity.

In the third experiments, pretreatment was carried out for 24 hours. Uptake was now completely normal with no obvious restoration of phosphorylation activity. The intracellular glucose concentrations were so low, however, that reliable assessment of phosphorylation activity was not possible. The experiments were, therefore, repeated with a high glucose concentration in the assay medium. These conditions led to substantial levels of intracellular glucose and the assay of phosphorylation showed a normal activity.

The possibility was considered that the repair of phosphorylation in vivo was secondary to suppression of growth hormone and hydrocortisone secretion. This seemed unlikely, however, as shown by the final experiment of this series in which the phosphorylation defect was fully corrected by insulin, despite simultaneous treatment of the animals with growth hormone and hydrocortisone in amounts which had caused an extreme depression of phosphorylation in the insulin-deficient animal as noted in Fig. 2 and Table II. The possibility was considered that the repair of phosphorylation in vivo was secondary to suppression of growth hormone and hydrocortisone secretion. This seemed unlikely, however, as shown by the final experiment of this series in which the phosphorylation defect was fully corrected by insulin, despite simultaneous treatment of the animals with growth hormone and hydrocortisone in amounts which had caused an extreme depression of phosphorylation in the insulin-deficient animal as noted in Fig. 2 and Table II.

The question of whether insulin in vivo would elevate phosphorylation in the heart from normal rats was examined in a few experiments shown at the bottom of Table IV. Despite high dosage with protamine zinc insulin, phosphorylation activity was the same as found in hearts from untreated rats. A high perfusate glucose concentration was used in some experiments to ensure a high enough intracellular glucose concentration for reliable assay.

## DISCUSSION

Membrane transport is the major rate-limiting step for glucose uptake in hearts from normal (4), diabetic (5), hypophysectomized (11), hypophysectomized-diabetic, and adrenalectomized-diabetic rate in the absence of added insulin. Insulin is the only hormone which has a strong and rapid acceleratory effect on the process, as described earlier in some detail (4–6). Henderson et al. (12) have observed a weak stimulatory effect of high concentrations of growth hormone in vitro in the muscle from hypophysectomized animals, but it is doubtful that this aspect of growth hormone activity has any physiological importance.

Pituitary and adrenal cortical factors do not seem to inhibit transport directly. This is illustrated in the present work, in which neither hypophysectomy nor adrenalectomy led to any rise in the transport activity of muscle from the diabetic rat. The low transport rate in the diabetic muscle is thus most simply explained by a deficiency of insulin. These conclusions have also

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**Table IV**

**Effect of insulin in vivo on glucose phosphorylation by perfused heart from diabetic and normal rats**

All experiments were carried out with 3 μg per ml of insulin and 100 mg per 100 ml of glucose in the perfusion medium except as indicated. Insulin was injected subcutaneously in a single dose the number of hours indicated before removal of the heart except in the 24-hour experiments in which protamine zinc insulin was injected in divided doses 24 and 4 hours before sacrifice. All rats had free access to food during the time of insulin treatment. The dosage schedule for growth hormone and cortisone has been given in footnotes 1 and 2 of Table II.

<table>
<thead>
<tr>
<th>Heart donor rats</th>
<th>Insulin in vivo</th>
<th>Blood plasma glucose</th>
<th>Glucose uptake</th>
<th>Mean Intracellular glucose</th>
<th>Glucose phosphorylation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>units</td>
<td>mg/100 ml</td>
<td>mg.g⁻¹.hr⁻¹</td>
<td>mg/100 ml</td>
<td>% normal²</td>
</tr>
<tr>
<td>Diabetic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Perfusate glucose, 600 mg/100 ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(+ GH and cortisone, 24 hrs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>24</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Perfusate glucose, 600 mg/100 ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **a** Comparisons are made with values taken from the curve of phosphorylation for the normal heart at the appropriate intracellular glucose concentration.
- **b** Standard error. The number of hearts tested is given by the figure in parentheses.
- **c** p < 0.01 versus 0.
- **d** p < 0.01 versus corresponding normal control value.
- **e** Abbreviation for none detected.

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5 Blood plasma glucose concentrations in the normal rat after 18 hours of fasting, average about 90 mg per 100 ml.
been reached by Kipnis and Cori (10), who measured the penetration and phosphorylation of 2-deoxyglucose in the isolated rat diaphragm.

Pituitary and adrenal cortical factors secreted by the animal or administered in vitro influence transport indirectly by retarding the action of insulin (12). In the rat with an intact pancreas, however, no depression of uptake may occur, even with large dosages of growth hormone, because of a compensatory increase in insulin secretion. Randle (18, 19) has shown a rise in blood insulin-like activity after growth hormone treatment, and hyperplasia of the islets of Langerhans is well documented after either growth hormone (20) or cortisone treatment (21). An anti-insulin activity of growth hormone in vitro was not observed in the present work, and Fisher (22) has recently questioned his earlier positive findings.

The phosphorylation of glucose has the lesser rate-controlling function in the absence of substantial insulin concentrations under all conditions we have tested. With transport acceleration, however, phosphorylation becomes increasingly limiting and eventually assumes the greater role in uptake control. A quantitative analysis of control by transport and phosphorylation has been presented (6).

It seems that phosphorylation in the diabetic animal is inhibited by the pituitary and adrenal cortex because removal of either gland restores activity to normal. The present studies in this regard extend our earlier studies (8), those of Kipnis (9), and those of Kipnis and Cori (10), who observed that adrenalectomy of the diabetic animal improved phosphorylation of 2-deoxyglucose in the isolated diaphragm. Growth hormone and hydrocortisone are both inhibitory when administered to an insulin-deficient animal over a period of several hours. The protein is active only in high dosage in the hypophysectomized-diabetic animal in which adrenal cortical secretion is much reduced. Whether or not any effect would be obtained in the complete absence of cortical secretion has not been tested. It is apparent that the largest effect is obtained when both growth hormone and hydrocortisone are present together. Significant effects are obtained under these conditions with dosages which are probably within the physiological range. It seems likely that hydrocortisone (or related steroids) and growth hormone are the substances which cause the inhibition of phosphorylation in the diabetic tissue but the participation of other factors has not been excluded. It is also clear that insulin in vitro can, in time, counteract the effect of these hormones. Increased endogenous insulin secretion could thus account for our failure to see inhibition by these substances injected into normal or hypophysectomized animals. It is also possible that the dosages used were not optimal.

The addition of insulin in vitro does not reverse the inhibition of phosphorylation in the heart from the diabetic or the hypophysectomized-diabetic rat pretreated with growth hormone and cortisone. Henderson (7) has exposed the diabetic heart to insulin in vitro for 2 hours with a medium fortified with protein and red blood cells which maintains the heart in excellent condition for long periods of time. No effect of insulin on phosphorylation could be detected, although a substantial improvement in phosphorylation was seen with insulin in vivo in this period. The lack of effect in vitro cannot, therefore, be ascribed simply to slow penetration into the cell. At present, it may be suggested that the repair of phosphorylation in vivo is not a direct insulin effect but is caused by suppression or release of other hormones. Suppression of growth hormone and hydrocortisone specifically seems unlikely as noted earlier. Morgan, Randle, and Henderson (8) have shown recently that glucagon in vitro improves phosphorylation in the diabetic heart but the conditions for secretion are not known and its role in the improvement of phosphorylation in vitro remains uncertain.

The perfused heart has unique advantages (4) for studying the control of glucose uptake. It permits a far greater experimental flexibility, control, and precision than do experiments with muscle in situ. When comparisons have been possible, findings in the heart and in skeletal muscle have been qualitatively in agreement. Furthermore, a number of parallel studies with the isolated rat diaphragm, (9, 10), and with the isolated fat pad (26), suggest a wide applicability of the control mechanisms found in the heart.

With the above in mind, it may be justified to advance some proposals of a general nature regarding the control of peripheral glucose utilization in the whole animal. These concepts fit well with studies of de Bodo and Altszuler (27) on the turnover of glucose in the whole animal in various states of hormonal balance.

1. Normal Animal—In the postabsorptive state, glucose uptake is limited by transport, the rate of which is intermediate because it is partially activated by a low level of circulating insulin. The sensitivity of transport to insulin is also intermediate owing to the presence of pituitary and adrenal cortical secretions, including growth hormone and hydrocortisone. Phosphorylation is fully active; thus glucose uptake can be strongly and rapidly accelerated by insulin if the concentration is sufficient. The tissues are resistant to inhibition of uptake by pituitary and adrenal secretions because their effect is counteracted by increased insulin secretion.

2. Insulin-Deficient, Diabetic Animal—Peripheral uptake is mainly limited by transport which is low or very low depending on the degree of insulin deficiency. The response of transport to insulin in a physiological concentration is poor owing to the presence of pituitary-adrenal cortical secretions which are usually excessive in amount, at least in the case of the adrenal, and have been unbalanced by a normal output of insulin. Phosphorylation is depressed because of the excessive and uncompensated adrenal cortical and pituitary activity. This depression also contributes to the low uptake in the absence of insulin and is particularly important in delaying the rise in glucose utilization after insulin administration. Thus the tissues of the severely diabetic animal are relatively insensitive to insulin at both the transport and phosphorylation levels. The mild diabetic, with limited insulin reserves for compensatory output, is very sensitive to inhibition of uptake and readily develops insulin-insensitivity after increased pituitary or adrenal cortical secretion such as that induced by stress.

6. As discussed elsewhere (15, 23), the inhibition may not be on hexokinase directly. Activity of the enzyme is probably regulated in part, at least, by the intracellular concentration of the inhibitor (24, 25), glucose 6-phosphate, through hormone effects at the level of the phosphofructokinase reaction.

7. M. J. Henderson, unpublished observations from this laboratory.


9. This refers to the aerobic state. Phosphorylation activity is increased above this level by anoxia (3, 4).
3. Hypophysectomized-Diabetic and Adrenalectomized-Diabetic Animal—Peripheral glucose uptake will be low if the insulin deficiency is severe enough to result in a low transport rate. Transport is very sensitive to insulin, however, and uptake will be strongly stimulated even by low levels of the hormone because phosphorylation activity is not depressed. This is one important aspect of the well known extreme sensitivity of these animals to insulin. In the total picture, however, reduced glycogen stores and impaired gluconeogenesis are also of great importance. The tissues will also be very sensitive to inhibition of uptake by pituitary and adrenal factors at the transport and phosphorylation levels because of insufficient insulin reserves. The well known marked instability of glucose metabolism in these animals can thus be explained in part by an absence of the buffering effect of normal levels of inhibitors and accelerators of peripheral glucose uptake.

4. Hypophysectomized Animal—In the postabsorptive state, peripheral glucose uptake is limited by transport which is low because of a diminished insulin secretion. The same high sensitivity to insulin is seen as in the diabetic-hypophysectomized preparation. This accounts for the rapid and excessive rise in glucose utilization and respiratory quotient with subsequent hypoglycaemia when insulin is secreted in response to food ingestion. The tissues are relatively resistant, on the other hand, to the diabeticogenic activity of the pituitary and adrenal cortical factors because the animal retains an essentially normal capacity for compensatory insulin secretion.

5. Adrenalectomized Animal—The muscle has not been studied directly by us but a variety of considerations suggest that the peripheral tissues would react similarly to those of the hypophysectomized animal.

Some other factors affecting peripheral glucose utilization have been discussed elsewhere (23).

**SUMMARY**

The isolated rat heart has been used to test the effects of pituitary, adrenal cortical secretions and insulin on glucose transport and phosphorylation. The following observations and conclusions have been made.

1. The low transport activity in hearts from diabetic animals is not raised by hypophysectomy or adrenalectomy. This suggests that neither pituitary nor adrenal factors inactivate the transport process directly. The low activity is ascribed simply to insulin deficiency.

2. The depression of glucose phosphorylation activity in the diabetic muscle is relieved by hypophysectomy or adrenalectomy, indicating an inhibitory effect of these glands on this step.

3. Treatment of the hypophysectomized-diabetic animal with either growth hormone or hydrocortisone depresses glucose phosphorylation. The hormones injected together show an additive (or synergistic) effect which can reduce phosphorylation to 15% of normal. It is probable that the inhibition of phosphorylation in the diabetic muscle is caused by endogenous secretion of these substances.

4. The addition of insulin in vitro accelerates glucose trans-

5. Treatment of the diabetic rat with insulin in vitro fully repairs the phosphorylation defect in the heart after a time lapse of about 4 hours. Inhibition of phosphorylation by growth hormone and hydrocortisone in vivo does not occur in the presence of sufficient insulin.

The observations in this and earlier papers are used to develop general concepts of how peripheral glucose utilization is controlled by insulin and pituitary and adrenal cortical factors under various conditions.

**REFERENCES**
