The results of a number of earlier investigations (1–5) have shown that glucose utilization and glycogen synthesis in the isolated rat diaphragm are increased by the addition of insulin to the incubation medium. Insulin also increases the amount of glucose carbon metabolized by mammalian muscle to CO₂, both in vitro (5) and in vivo (6). The rates of these three reactions are increased by the removal of the adrenals (4, 5), the pituitary (7, 5), or both (5, 8). The utilization of pyruvate and the metabolism of pyruvate carbons to CO₂ are significantly less in diabetic muscle than in normal muscle and are returned to normal by the addition of insulin in vitro, although there is no observed insulin effect on pyruvate metabolism in normal muscle or in muscle from adrenalectomized rats (9). The amount of acetate carbon metabolized to CO₂ is also less in diabetic muscle than in normal muscle, but, in contrast to pyruvate metabolism, this is not brought back to normal by the addition, in vitro, of insulin (9). In the experiments reported here, carbohydrate metabolism in muscle was studied with both glucose and pyruvate present as substrates. This was accomplished by the method, introduced by Teng, Sinex, and Hastings (10) in experiments with liver slices, of using duplicate vessels containing identical amounts of glucose and pyruvate, but with labeled glucose and unlabeled pyruvate in one vessel and labeled pyruvate and unlabeled glucose in the other. By this method, the effect of insulin on the metabolism of glucose and pyruvate was studied in muscle from normal, diabetic, and adrenalectomized rats. In other experiments reported here, the ability of diaphragm muscle to metabolize α-labeled C¹⁴-glycerol was determined.

**Materials and Methods**

The strain of rats and methods of adrenalectomy and of inducing diabetes were identical with those used previously (5, 9). Rats were fasted...
288 METABOLISM OF GLUCOSE AND PYRUVATE

24 hours before being used in an experiment, and only those rats with a fasting blood glucose of 300 mg. per cent or more were included in the diabetic series.

The pyruvate-2-C\(^{14}\) and \(\alpha\)-labeled C\(^{14}\)-glycerol used in these experiments were synthesized by Dr. Manfred L. Karnovsky. The uniformly labeled glucose-C\(^{14}\) was prepared by Dr. Martin Gibbs by the photosynthetic production of starch from C\(^{14}\)O\(_2\) and the hydrolysis of the starch to yield glucose.

The incubations were carried out in Warburg vessels containing 3.0 ml. of incubation medium composed of 0.04 M sodium phosphate, 0.005 M MgCl\(_2\), 0.08 M NaCl, 0.01 M pyruvate, and 0.011 M glucose (200 mg. per cent). In paired vessels, either the glucose or the pyruvate was labeled. The initial pH of the medium was 6.8 and the pH after incubation was 6.8 ± 0.1 (determined by the glass electrode). The center wells contained a slip of hard filter paper and 0.2 ml. of CO\(_2\)-free 5 per cent NaOH to absorb the CO\(_2\) produced. A hemidiaphragm weighing about 100 mg. was placed in each vessel and the vessels were incubated for 2 hours at 37.5° after an initial 8 minutes gassing with 100 per cent oxygen.

At the end of the incubation period, the CO\(_2\) from the center well was precipitated as BaCO\(_3\) and plated on steel planchets, and its radioactivity was determined with a windowless proportional flow counter. The diaphragm was removed and digested in boiling 30 per cent KOH; the glycogen was precipitated by alcohol, purified, hydrolyzed, and an aliquot was used for a glucose determination by the method of Nelson (11). The rest of the glucose, plus 10 mg. of carrier glucose, was converted to glucosazone, washed, recrystallized, and washed again before being plated. Its radioactivity was then measured with the proportional flow counter. Aliquots of the medium were analyzed before and after incubation for pyruvate by the method of Friedemann and Haugen (12), for glucose by the method of Nelson (11), and for lactate by the method of Barker and Summerson (13). From other aliquots of the medium, glucose was isolated, after the addition of carrier glucose, as the glucosazone. This was washed, recrystallized, washed again, and then plated for a determination of its radioactivity. Pyruvate was isolated from the medium, after the addition of carrier pyruvate, as the 2,4-dinitrophenylhydrazone. This was washed a total of seven times with alcohol and water before being plated and analyzed for its radioactivity by the proportional flow counter. Blood glucose determinations were made at the time of sacrifice by the method of Nelson (11).

Results

Glucose-6-phosphatase Activity—From observations made on intact animals, it has long been inferred and accepted that muscle does not contain
glucose-6-phosphatase activity. For example, the administration of epi-
nephrine produces a rise in blood glucose in normal dogs but not in hepa-
tectomized dogs (14). The present in vitro experiments with rat dia-
phragm gave us an opportunity to provide further more direct chemical
evidence on this point. The glucosazones isolated from the medium after
incubation in experiments with labeled pyruvate and unlabeled glucose
contained no C\textsuperscript{4}, although glycogen synthesized by the muscle during the
incubation period did contain C\textsuperscript{14}. Since glycogen was presumably formed
from the pyruvate by a reversal of the glycolytic cycle and via glucose-6-
phosphate, the fact that there was no radioactivity in the glucose of the
medium is confirmatory evidence that diaphragm muscle has no phospha-
tase to convert glucose-6-phosphate to glucose. Additional evidence comes
from experiments in which labeled glucose and unlabeled pyruvate were
used. The specific activity of the glucosazones isolated from the medium
after incubation was the same as that of the initial glucose; thus, the
muscle did not secrete any unlabeled glucose to dilute that of the medium.

Production of Pyruvate—The amount of pyruvate produced from all
sources during the incubation can be calculated from the decrease in the
specific activity of the radiopyruvate of the medium and from the total
amounts of pyruvate present initially and at the end of the incubation.
For example, in an experiment with normal muscle without added insulin,
the initial specific activity of the pyruvate was 44.5 × 10\textsuperscript{3} c.p.m. per \textmu M,
its final specific activity was 33.6 × 10\textsuperscript{3} c.p.m. per \textmu M, and there were
30 \textmu M of pyruvate per vessel initially and 20.0 \textmu M of pyruvate per vessel
at the end of the incubation. Then 33.6/44.5 × 20 = 15.1 \textmu M of pyruvate
present at the end of the incubation which was derived from the original
labeled pyruvate of the medium and 20 - 15.1 = 4.9 \textmu M of pyruvate,
the apparent amount of final pyruvate derived from sources other than
the original labeled pyruvate of the medium. But since this pyruvate
production continued throughout the incubation period, and some of
the pyruvate produced was utilized during the incubation, this figure must be
corrected for the amount of pyruvate that was produced and then utilized:
4.9 + (4.9 × (30 - 20)/30) = 6.5 \textmu M, the total amount of pyruvate
produced during the 2 hour incubation. This figure multiplied by 1000
over the weight of the muscle used (114 mg. in this experiment) and
multiplied by \frac{1}{2} = 28.5 \textmu M of pyruvate produced per hour per gm. of
muscle. In this calculation, a linear relation between time and pyruvate
disappearance is assumed.

From the duplicate vessel, which contained radioglucose and unlabeled
pyruvate initially, one can calculate the amount of pyruvate produced
which was derived from glucose. In the example given, the initial specific
activity of the labeled glucose was 38.5 × 10\textsuperscript{3} c.p.m. per \textmu M, the specific
activity of the pyruvate isolated from that vessel at the end of incubation
was $1.6 \times 10^3$ c.p.m. per mM, and there were $17.4 \, \mu\text{M}$ of pyruvate present at the end. Then $1.6/38.5 \times 17.4 \times 2 = 1.44 \, \mu\text{M}$, the apparent amount of pyruvate at the end derived from the initial glucose. (The figure 2 is used because each mole of glucose is equivalent to 2 moles of pyruvate.) This figure, too, must be corrected for the amount of pyruvate produced and then utilized during the course of the incubation period: $1.44 + (1.44 \times (30 - 17.4)/30) = 2.03 \, \mu\text{M}$, the total amount of pyruvate produced from glucose in 2 hours. And $2.03 \times 1000/131.5$ (the weight of the muscle in mg.) $\times \frac{1}{2} = 7.72 \, \mu\text{M}$ of pyruvate per gm. per hour produced from glucose.

The averages of results with normal, diabetic, and adrenalectomized muscle are given in Table I, Lines 10 and 11. It is clear that in normal and adrenalectomized muscle insulin did not increase the total production of pyruvate from all sources, but it did increase the proportion of pyruvate made from glucose. Both the total production of pyruvate and the production of pyruvate from glucose were less in muscle from diabetic rats than in muscle from normal or adrenalectomized rats, and both were increased by the addition of insulin.

**Effects of Added Insulin**—From the initial and final concentrations of glucose and pyruvate in the incubation medium, the amounts of these substances disappearing during the course of the incubation were calculated and are recorded in Table I as utilization. There was no significant difference in glucose utilization by muscle from normal, diabetic, or adrenalectomized animals. However, when glucose alone is present in the incubation medium, there is a significant difference in glucose utilization by the three types of muscle (4, 5). In both the former and the present experiments, there was a marked increase in glucose utilization when insulin was added to the medium.

The utilization of pyruvate was less in diabetic muscle than in normal or adrenalectomized muscle but was brought back to normal by the addition, *in vitro*, of insulin. There was no increase in pyruvate utilization by normal or adrenalectomized muscle when insulin was added. The rate of pyruvate utilization was the same in normal and adrenalectomized muscle. These results confirm those obtained previously (9) with pyruvate alone as the substrate.

Muscle from diabetic rats synthesized less glycogen than that from normal rats, and muscle from adrenalectomized rats synthesized even less glycogen than did diabetic muscle. This same low rate of glycogen synthesis in adrenalectomized muscle was noted previously (5) in experiments with glucose alone as the substrate. In all three types of muscle, the addition of insulin *in vitro* produced a marked increase in glycogen synthesis, 410 per cent in normal, 227 per cent in diabetic, and 355 per cent in adrenalectomized muscle.
The addition of insulin did not increase the amount of lactic acid accumulated during the incubation; this confirms the earlier observations (9) with pyruvate alone as the substrate. Adrenalectomy resulted in a decreased production of lactic acid by the muscle and in no significant difference in the amount of pyruvate carbon metabolized to CO₂. This same effect of adrenalectomy was found in the previous experiments (9) with pyruvate alone as the substrate. It appears that one effect of some adrenal hormone is to favor the conversion of pyruvate to lactic acid.

The amount of glucose or pyruvate metabolized to CO₂ was calculated as described previously (5). The amount of pyruvate carbon metabolized to CO₂ is less in diabetic than in normal muscle. The addition of insulin increased the metabolism of pyruvate carbon to CO₂ in diabetic muscle

### Table 1

**Effect of Insulin on Metabolism of Glucose and Pyruvate by Rat Diaphragm Muscle**

The figures given are the mean, ± the standard error, in micromoles per gm. of wet weight per hour.

<table>
<thead>
<tr>
<th>Line No.</th>
<th>Normal rats</th>
<th>Diabetic rats</th>
<th>Adrenalectomized rats</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Glucose utilization</td>
<td>11.1 ± 2.6</td>
<td>24.3 ± 1.8</td>
<td>9.6 ± 1.7</td>
<td>30.3 ± 2.3</td>
</tr>
<tr>
<td>2 Pyruvate</td>
<td>52.4 ± 1.6</td>
<td>35.9 ± 1.9</td>
<td>35.9 ± 4.5</td>
<td>51.8 ± 5.1</td>
</tr>
<tr>
<td>3 Net glycogen synthesized</td>
<td>4.0 ± 1.0</td>
<td>16.4 ± 1.1</td>
<td>2.2 ± 0.5</td>
<td>5.0 ± 0.7</td>
</tr>
<tr>
<td>4 Lactate produced</td>
<td>41.8 ± 2.7</td>
<td>35.5 ± 2.0</td>
<td>29.8 ± 1.8</td>
<td>34.3 ± 1.9</td>
</tr>
<tr>
<td>5 Pyruvate metabolized to CO₂</td>
<td>12.0 ± 1.0</td>
<td>12.3 ± 1.1</td>
<td>8.9 ± 1.3</td>
<td>10.5 ± 1.1</td>
</tr>
<tr>
<td>6 Glucose accounted for</td>
<td>0.82 ± 0.07</td>
<td>0.88 ± 0.13</td>
<td>0.45 ± 0.11</td>
<td>0.63 ± 0.15</td>
</tr>
<tr>
<td>7 Total carbons utilized</td>
<td>223.8 ± 12.0</td>
<td>307.5 ± 247.2</td>
<td>165.3 ± 123.0</td>
<td>337.2 ± 168.0</td>
</tr>
<tr>
<td>8 Carbons accounted for, %</td>
<td>86 ± 30.6</td>
<td>80 ± 29.2</td>
<td>80 ± 25.6</td>
<td>50 ± 33.8</td>
</tr>
<tr>
<td>9 Total pyruvate produced</td>
<td>30.6 ± 1.0</td>
<td>29.2 ± 1.6</td>
<td>25.6 ± 1.5</td>
<td>33.8 ± 2.1</td>
</tr>
<tr>
<td>10 Pyruvate produced from glucose</td>
<td>7.7 ± 0.3</td>
<td>10.1 ± 0.5</td>
<td>4.9 ± 0.3</td>
<td>11.8 ± 0.6</td>
</tr>
<tr>
<td>11 from glucose, %</td>
<td>25 ± 7.7</td>
<td>35 ± 10.1</td>
<td>19 ± 4.9</td>
<td>35 ± 11.8</td>
</tr>
</tbody>
</table>

The figures given are the mean, ± the standard error, in micromoles per gm. of wet weight per hour.
by 24 per cent, but produced no significant change in normal or adrenalectomized muscle. The amount of glucose carbon metabolized to CO₂ is less in diabetic than in normal muscle and is increased by the addition of insulin, as in previous experiments (5).

Calculation of Carbon Balances—The total micromoles of glucose and pyruvate carbons utilized were calculated by multiplying the glucose utilization by 6 and the pyruvate utilization by 3 (Line 7, Table I). The total micromoles of carbon accounted for as glycogen, lactate, and CO₂ were similarly calculated (Line 8, Table I). The percentage of pyruvate and glucose carbons which could be accounted for in this way (Line 9, Table I) varied from 85 per cent in the normal to 50 per cent in the adrenalectomized or diabetic muscle with insulin. The carbons unaccounted for are presumably present in the medium at the end as other intermediates which were not tested for. In other experiments (15), it was found that about 5 per cent of the pyruvate carbons that disappeared was incorporated into lipides and proteins during the 2 hour incubation period.

Effect of Substrate on Production of Glycogen and CO₂—The production of CO₂ and glycogen by diaphragm muscle from glucose (11.1 mM per liter), pyruvate (10 mM per liter), and from the two combined is summarized in Table II. The figures for glucose alone and pyruvate alone were derived in part from data reported previously (5, 9, 16). It is clear that when glucose and pyruvate are both present much less CO₂ is formed from glucose than when glucose alone is the substrate. This is to be expected, since pyruvate is an intermediate in the metabolism of glucose to CO₂. The percentage of CO₂ derived from pyruvate carbon is not decreased, but is the same or slightly increased when the substrate is glucose and pyruvate rather than pyruvate alone.

The percentage of glycogen made from glucose appears to be independent of the presence or absence of pyruvate in the medium in muscle from normal or adrenalectomized rats and the percentage of glycogen made from pyruvate appears to be independent of the presence or absence of glucose in the medium in normal muscle.

When both glucose and pyruvate are present in the medium, the addition of insulin increases markedly the percentage of glycogen made from glucose and decreases the percentage of glycogen made from pyruvate. However, when one calculates the actual amount of glycogen made from pyruvate (Table II), it is evident that the addition of insulin has little or no effect on the rate of synthesis of glycogen from pyruvate in normal muscle and actually increases it in muscle from adrenalectomized rats.

The effects of varying the substrate on the utilization of glucose and pyruvate and on the synthesis of glycogen are summarized in Table III. The differences in glucose utilization by normal, diabetic, and adrenalecto-
### Table II

**Production of Glycogen and CO₂ by Diaphragm Muscle under Varying Experimental Conditions**

<table>
<thead>
<tr>
<th>Condition of rats</th>
<th>Substrate</th>
<th>Per cent CO₂ from glucose</th>
<th>Per cent CO₂ from pyruvate</th>
<th>Per cent glycogen from glucose</th>
<th>Per cent glycogen from pyruvate</th>
<th>Glycogen made from pyruvate, µM per gm. per hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Glucose alone</td>
<td>10.4</td>
<td>16.3</td>
<td>7.5</td>
<td>15.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pyruvate &quot; alone&quot;</td>
<td>34.8</td>
<td>35.1</td>
<td>13.1</td>
<td>11.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glucose + pyruvate &quot; alone&quot;</td>
<td>2.1</td>
<td>2.5</td>
<td>48.0</td>
<td>51.0</td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>Pyruvate &quot; alone&quot;</td>
<td>8.1</td>
<td>9.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glucose + pyruvate &quot; alone&quot;</td>
<td>2.2</td>
<td>2.5</td>
<td>43.0</td>
<td>44.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pyruvate &quot; alone&quot;</td>
<td>15.0</td>
<td>25.3</td>
<td>35.4</td>
<td>41.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glucose + pyruvate</td>
<td>2.0</td>
<td>2.5</td>
<td>47.6</td>
<td>47.9</td>
<td></td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table III

**Utilization of Glucose and Pyruvate by Diaphragm Muscle under Varying Experimental Conditions**

The results are expressed in micromoles per gm. per hour.

<table>
<thead>
<tr>
<th>Condition of rats</th>
<th>Substrate</th>
<th>Glucose utilized</th>
<th>Pyruvate utilized</th>
<th>Glycogen made</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Per cent</td>
<td>Per cent</td>
<td>µM per gm. per hr.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal</td>
<td>Diabetic</td>
<td>Adrenalectomized</td>
</tr>
<tr>
<td>Normal</td>
<td>Glucose alone</td>
<td>8.4</td>
<td>15.8</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>Pyruvate &quot; alone&quot;</td>
<td>42.4</td>
<td>44.3</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td>Glucose + pyruvate &quot; alone&quot;</td>
<td>11.1</td>
<td>24.3</td>
<td>52.4</td>
</tr>
<tr>
<td></td>
<td>Pyruvate &quot; alone&quot;</td>
<td>4.3</td>
<td>12.0</td>
<td>2.77</td>
</tr>
<tr>
<td></td>
<td>Glucose + pyruvate &quot; alone&quot;</td>
<td>9.6</td>
<td>30.3</td>
<td>35.9</td>
</tr>
<tr>
<td></td>
<td>Pyruvate &quot; alone&quot;</td>
<td>12.6</td>
<td>21.6</td>
<td>39.1</td>
</tr>
<tr>
<td></td>
<td>Glucose + pyruvate</td>
<td>11.6</td>
<td>25.3</td>
<td>51.6</td>
</tr>
</tbody>
</table>

Adrenalectomized muscle are much less marked when both glucose and pyruvate are present than when glucose alone is the substrate. In both normal and diabetic muscle, more glucose is utilized when pyruvate is present than when it is not. Similarly, more pyruvate is utilized when glucose is
present than when it is not. The amount of glycogen synthesized when both glucose and pyruvate are present is generally greater than the sum of the amounts made when the two are present singly.

Non-Utilization of Glycerol by Diaphragm Muscle—Teng, Hastings, and Nesbett (17) had shown that glycerol is utilized rapidly by rat liver slices and metabolized to glycogen, glucose, and lipides and to CO₂. It was of interest to study the metabolism of glycerol by muscle and to compare the results with the data for liver. Normal rat diaphragms were incubated for 2 hours in the usual phosphate-saline buffer containing 20 mM per liter of α-labeled glycerol. In some experiments, the muscle, at the end of the incubation period, was hydrolyzed in boiling 30 per cent KOH for the isolation of glycogen and in others it was frozen by dipping in liquid air, ground, and extracted with cold 5 per cent trichloroacetic acid. The residue was then extracted with hot 3:1 alcohol-ether followed by 1:1 chloroform-methanol. The total lipide extract was concentrated, plated on steel planchets, and counted. Diaphragm muscle apparently lacks the enzyme for phosphorylating glycerol, for at the end of the incubation the glycerol was recovered quantitatively from the medium with its specific activity unchanged, and no C₁⁴ was present in the respiratory CO₂, in the glycogen or lipides isolated from the diaphragm, or in the pyruvate dinitrophenylhydrazones or glucosazones isolated from the medium. The experiments were repeated with glucose, 5.6 mM per liter, as well as glycerol, 20 mM per liter, present in the incubation medium, but the results were the same: no glycerol was utilized.

DISCUSSION

The results of these experiments, in which both glucose and pyruvate were present in the incubation medium, confirm and extend the results found previously when the two substances were used singly. The effects produced experimentally by the addition, in vitro, of insulin are in accord with the hypothesis that its primary effect is a stimulation of the hexokinase system and that it has an additional, lesser effect on the reactions involved in the condensation of pyruvate with oxalacetate to enter the citric acid cycle. It had been shown previously (5) that insulin, in addition to increasing glucose utilization and glycogen synthesis, increased the rate of metabolism of glucose to CO₂. In these experiments it was possible to demonstrate that insulin increases the amount of pyruvate made from glucose and that in diabetic muscle less pyruvate is made from glucose than in normal muscle. These experiments show that insulin does not increase the total amount of pyruvate produced but only the amount made from glucose, and hence less pyruvate is made from other sources. This is correlated with the fact that under these conditions the addition of
insulin does not increase the amount of lactate produced. However, when glucose alone is the substrate, the addition of insulin does increase the amount of lactate accumulated during the incubation.

The theory that insulin has some effect on one or more of the reactions involved in the condensation of pyruvate with oxalacetate to give citrate was suggested by the previous observations (9) that the utilization of pyruvate and the metabolism of pyruvate carbons to CO₂ were decreased in diabetic muscle and were increased by the addition of insulin. Similar results were found in the present experiments, in which glucose as well as pyruvate was present in the incubation medium. Again, no effect of insulin on pyruvate utilization or on its metabolism to CO₂ was observed in normal or adrenalectomized muscle. Perhaps this system reaches maximum activity at a relatively low level of insulin, one that is normally present in normal or adrenalectomized muscle.

A comparison of the amount of glycogen synthesized by the diaphragm when incubated with glucose plus pyruvate with the amounts synthesized when it is incubated on each separately shows that it is generally greater than the sum of the two. It would appear that the rates of the enzymes involved in converting glucose-6-phosphate to glycogen are high enough to handle both the glucose-6-phosphate made from glucose by the hexokinase reaction and the glucose-6-phosphate made from pyruvate by a reversal of glycolysis. Results obtained previously (5) suggested also that the limit of the rate at which glucose-6-phosphate can be converted to glucose-1-phosphate and glycogen was considerably higher than the rate at which glucose-6-phosphate can be passed through the Embden-Meyerhof cycle to pyruvate. The calculations presented in Table II show that, although the addition of insulin almost triples the percentage of glycogen produced from glucose and decreases the percentage made from pyruvate to about one-third, it causes such a great increase in the total amount of glycogen synthesized that the amount synthesized from pyruvate is the same or slightly increased when insulin is added.

In both these experiments and the previous ones (5), it is clear that muscle from adrenalectomized rats synthesizes less glycogen than normal muscle. However, the percentage of glycogen derived from glucose is greater in adrenalectomized muscle than in normal muscle (Table II), as would be expected on the Cori hypothesis of the action of adrenal hormone in inhibiting the hexokinase reaction. The smaller amount of glycogen synthesized in adrenalectomized muscle would appear to be due to some effect of the absence of the adrenals on the conversion of glucose-6-phosphate to glycogen.

Adrenalectomy also decreases the amount of lactic acid produced by diaphragm muscle and increases the amount of pyruvate metabolized to
CO₂. This effect of adrenalectomy was found both in the present experiments and in the earlier ones with pyruvate alone as the substrate. Thus it appears that in normal muscle some adrenal hormone favors the accumulation of lactic acid. It is tempting to suppose that this action is on the same step in the condensation of pyruvate with oxalacetate previously shown to be accelerated by insulin and that this reaction is a second example of a metabolic step whose rate is controlled by antagonistic actions of insulin and adrenal hormones.

SUMMARY

1. The glucose and pyruvate utilization, glycogen synthesis, lactate accumulation, and metabolism of glucose and pyruvate to carbon dioxide by isolated rat diaphragm have been measured in muscle from normal, diabetic, and adrenalectomized rats incubated in a phosphate-saline buffer containing glucose and pyruvate. In all three types of animals, the addition of insulin increased glucose utilization, glycogen synthesis, and the amount of glucose metabolized to CO₂.

2. The amount of pyruvate produced from all sources and from glucose was calculated. The addition of insulin did not increase the total amount of pyruvate produced but did increase the amount of pyruvate made from glucose. The production of pyruvate from glucose is less in diabetic muscle than in normal or adrenalectomized muscle.

3. The utilization of pyruvate was less in diabetic muscle than in normal or adrenalectomized muscle and was increased to normal by the addition of insulin. Insulin had no effect on pyruvate utilization in normal or adrenalectomized muscle.

4. Although muscle from adrenalectomized rats synthesized less glycogen than normal muscle, a greater percentage of the glycogen that was formed came from glucose.

5. Carbon balances, including glucose and pyruvate utilized and lactate, glycogen, and CO₂ produced, were calculated; it was possible to account for 85 per cent of the carbons disappearing in normal muscle but for only 50 per cent in adrenalectomized muscle plus insulin.

6. When pyruvate is added to the incubation medium, less CO₂ is derived from glucose, but when glucose is added, the amount of CO₂ derived from pyruvate remains essentially constant.

7. The percentage of glycogen made from glucose is increased and the percentage made from pyruvate is decreased when insulin is added, but the actual amount of glycogen synthesized from pyruvate is unchanged by the addition of insulin.

8. Confirmatory evidence, obtained by experiments in vitro, is presented that muscle has no glucose-6-phosphatase activity. No evidence for a glycerol-phosphorylating enzyme was found in muscle.
9. The experimental evidence accords well with the theory that insulin produces a stimulation of the hexokinase system and that it has an additional effect on one or more of the reactions involved in the condensation of pyruvate with oxalacetate to enter the citric acid cycle.

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