EFFECT OF INSULIN ON PYRUVIC ACID FORMATION IN DEPANCREATEIZED DOGS*

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In normal human subjects the administration of glucose produces an increase in blood pyruvic acid (1–3). In patients with diabetes mellitus the rise in blood pyruvate under the same conditions is either delayed and smaller or does not occur at all (2, 4). Insulin, administered to diabetic patients either simultaneously with glucose or several hours after glucose ingestion, produces a marked increase in blood pyruvate (2, 4). The present studies on the relation of insulin to the formation and disappearance of pyruvate were conducted on completely depancreatized dogs. With such a preparation the effects of any residual functioning pancreas are eliminated.

EXPERIMENTAL

Depancreatized dogs were maintained in good condition on bovex, pancreatin, and insulin for several weeks before experimentation. Insulin was withheld for 72 hours and food for 24 hours before each experiment. In order to avoid the increase in blood pyruvate caused by muscular contraction the animals were given pentobarbital, 25 mg. per kilo, or less, 1 to 2 hours before the observations were begun. Blood samples were taken from the femoral artery. Blood glucose and pyruvate were determined by methods used in previous publications (1, 5).

Pyruvic acid (Eastman Kodak, c.p.) used in these experiments was redistilled three times immediately before injection, appropriately diluted with ice-cold water, and then neutralized at 2–4° with NaOH to pH 6.4.

Results

In normal dogs (fasted for 24 hours) the intravenous injection of 2 gm. per kilo of glucose was followed by a significant rise in blood pyruvate (Fig. 1). The maximum rise usually occurred 30 to 45 minutes after the injection and the increase averaged about 100 per cent over the fasting values.

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Blood pyruvate failed to increase after a similar injection of glucose into the depancreatized dogs in observations extended over a period of 5 hours (Fig. 2). If insulin (crystalline insulin, Eli Lilly) was administered to depancreatized dogs 90 to 120 minutes after the glucose injection, a marked elevation in blood pyruvate occurred which coincided with a drop in blood sugar (Fig. 3).

When insulin was given together with glucose to the same depancreatized dogs, a rise in blood pyruvate was observed. This rise reached its maximum from 1 to 3 hours after the first glucose injection. A second injection
of glucose alone 4 hours after the beginning of the experiment produced a second rise in blood pyruvate (Fig. 4). When the same experiment was performed with the omission of insulin, no rise in blood pyruvate occurred. Since with the hydrazone method \(\alpha\)-ketoglutaric acid is determined as well as pyruvic acid, an attempt was made to differentiate these two keto acids. It was found that in contrast to pyruvic acid (5) \(\alpha\)-ketoglutaric acid does not disappear when added to oxalated blood. Blood samples of four depancreatized dogs that had been injected with glucose were allowed to stand in 0.2 per cent oxalate for 30 minutes at room temperature before and after insulin administration. The blood pyruvate before insulin injection varied between 0.74 and 1.10 mg. per cent, and 1 hour after insulin between 1.72 and 2.91 mg. per cent respectively. When the blood had stood in oxalate for 30 minutes at room temperature, the pyruvic acid content fell to values between 0.21 and 0.44 mg. per cent both in the samples before and
after insulin administration, indicating that insulin did not produce a rise in α-ketoglutaric acid.

FIG. 4. Blood pyruvic acid after the simultaneous intravenous injection of 2 gm. of glucose per kilo and insulin (25 to 40 units) into depancreatized dogs followed by the intravenous injection of another 2 gm. per kilo of glucose. The dotted line represents the blood pyruvic acid values after the second glucose injection. The corresponding blood sugar values (in mg. per cent) were as follows: Curve 16 (without the second glucose injection), 337, 725, 445, 279, 243, 121, 117, 128; Curve 17 (without the second glucose injection), 413, 815, 627, 462, 230, 208, 175; Curve 18, 267, 568, 428, 327, 242, 214, 502, 386, 334; Curve 19, 340, 348, 348, 161, 165, 394, 311, 252; Curve 20, 310, 452, 289, 235, 242, 580, 384, 330; Curve 21, 372, 640, 565, 368, 215, 143, 125, 395, 310, 262, 232.

When the blood sugar of a depancreatized dog was raised to between 700 and 1000 mg. per cent by a continuous infusion (300 ml. of a 5 per cent solution per hour after a preliminary injection of 3 gm. of glucose per kilo), an
FIG. 5. Blood pyruvic acid (left-hand scale) and glucose (right-hand scale) in mg. per cent, after the intravenous injection (2 gm. per kilo) followed by continuous infusion (300 ml. of a 5 per cent solution per hour) of glucose into depancreatized dogs.

FIG. 6. Blood pyruvate after the intravenous injection of sodium pyruvate (1 gm. of pyruvic acid per kilo) into normal and into depancreatized dogs. Each curve represents the average of four experiments.
The rise in blood pyruvate produced by insulin after glucose injection in depancreatized dogs may be attributed to either an increased formation or a decreased removal of pyruvate. Flock, Bollman, and Mann (6) have previously reported that the utilization of pyruvate is the same in normal and depancreatized animals. In agreement with these observations it was found that after the injection of 1 gm. of pyruvic acid (as Na pyruvate) per

<table>
<thead>
<tr>
<th>Time after glucose injection (min.)</th>
<th>Pyruvic acid (mg. per cent)</th>
<th>Glucose (mg. per cent)</th>
<th>Pyruvic acid (mg. per cent)</th>
<th>Glucose (mg. per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>1.38</td>
<td>101</td>
<td>0.86</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>1.92 ± 0.54</td>
<td>673</td>
<td>1.11 ± 0.25</td>
<td>575</td>
</tr>
<tr>
<td>20</td>
<td>2.32 ± 0.94</td>
<td>428</td>
<td>1.82 ± 0.96</td>
<td>450</td>
</tr>
<tr>
<td>30</td>
<td>2.49 ± 1.11</td>
<td>313</td>
<td>1.05 ± 1.00</td>
<td>294</td>
</tr>
<tr>
<td>45</td>
<td>2.66 ± 1.28</td>
<td>181</td>
<td>2.03 ± 1.17</td>
<td>199</td>
</tr>
<tr>
<td>60</td>
<td>2.41 ± 1.03</td>
<td>103</td>
<td>1.95 ± 1.09</td>
<td>152</td>
</tr>
<tr>
<td>90</td>
<td>1.92 ± 0.54</td>
<td>87</td>
<td>1.73 ± 0.87</td>
<td>120</td>
</tr>
<tr>
<td>120</td>
<td>1.92 ± 0.54</td>
<td>87</td>
<td>1.73 ± 0.87</td>
<td>120</td>
</tr>
</tbody>
</table>

A dog trained to remain under basal conditions during the whole experimental period was used in order to determine whether pentobarbital anesthesia has an effect on the blood pyruvate level after glucose injection. The results obtained before and after pancreatectomy are similar to those observed on the animals anesthetized with pentobarbital (Table I). In addition after glucose injection barbiturate anesthesia does not influence the rise in blood lactate in dogs (7) nor the increase of blood pyruvate in human
It is, therefore, probable that nembutal anesthesia did not have any effect on the blood pyruvate levels reported in this paper.

**DISCUSSION**

An increase in blood pyruvate in vivo can be produced (1) by supplying large amounts of glucose and (2) by insulin. If glucose is supplied in amounts sufficient to maintain the blood sugar of depancreatized dogs at levels between 750 and 1000 mg. per cent, a moderate increase in pyruvate takes place even in the absence of insulin. In addition the injection of glucose into depancreatized dogs that had previously (4 hours before) received insulin and glucose produces a rise in pyruvate even without further insulin injection. On the other hand insulin, without the administration of glucose, produces no increase even in large doses in normal (8, 3) or diabetic subjects (2). Insulin, therefore, increases the blood pyruvate in the depancreatized animal or diabetic or normal (3) subjects only if large amounts of glucose are supplied.

Since the rate of disappearance of injected pyruvate from the blood is the same whether insulin is absent or present in normal or excessive amounts, the increase in blood pyruvate produced by insulin cannot be due to a decreased pyruvate removal. It is, therefore, concluded that after the administration of glucose to depancreatized dogs insulin increases the formation of pyruvic acid. This effect is also indicated by the observation that under aerobic conditions insulin increases the phosphorylation of glucose in vitro (9); i.e., insulin acts on a stage of carbohydrate metabolism preceding the formation of pyruvic acid.

**SUMMARY**

1. In contrast to normal animals the injection of glucose (2 gm. per kilo) into depancreatized dogs does not produce a rise in blood pyruvate.

2. If insulin is administered to depancreatized dogs together with or shortly after the injection of glucose, a marked rise in blood pyruvate takes place.

3. A moderate increase in blood pyruvate occurs in depancreatized dogs despite the absence of insulin if excessively large amounts of glucose are injected. Under these conditions insulin produces a further rise in blood pyruvate.

4. Since insulin does not decrease the removal of pyruvic acid in depancreatized dogs, it is concluded that insulin increases the formation of pyruvic acid in vivo.

\[1\] Unpublished observations.
BIBLIOGRAPHY

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