SOME EFFECTS OF METABOLIC ACIDOSIS ON CARBOHYDRATE METABOLISM IN THE RAT

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SUMMARY

1. Metabolic acidosis was induced by feeding ammonium chloride to rats which were maintained on a carbohydrate diet for 48 h.

2. Fasting blood glucose was the same in acidotic and control animals, but there was an increase in liver glycogen in the former. Muscle glycogen was unchanged.

3. In vitro glycogenolysis was the same in liver slices from normal rats when incubated at a range of pH from 6-90 to 7-40.

4. The peak blood glucose in response to intraperitoneal injections of glucagon was the same in control and acidotic rats. The rate of disappearance of glucose was slower in acidotic rats both after the glucagon induced hyperglycaemia and after intravenously injected glucose.

5. Liver phosphorylase, total glycogen synthetase and the I form of this enzyme were unchanged in acidosis.

6. The data are compatible with the hypothesis that in the acidotic rat there is a block in glycolysis—possibly at the phosphofructokinase step.

In vivo studies in dogs and in vitro experiments with blood have shown that metabolic acidosis impairs glucose metabolism (Mackler, Lichtenstein & Guest, 1951, 1952; Mackler & Guest, 1953) and it was originally postulated that a block in phosphorylation of glucose was responsible for the defect. More recently it has been shown that phosphofructokinase (PFK)—one of the irreversible enzymes of the glycolytic pathway—is sensitive to changes in pH (Ui, 1966). Gevers & Dowdle (1963) have shown that alkalosis causes increased glycolysis in muscle and Halperin, Connors, Relman & Karnovsky (1969) have also shown that in guinea-pig leucocytes, phosphofructokinase is inhibited by a decrease in pH.

In view also of recent interest in carbohydrate metabolism in uraemic subjects who may be chronically acidotic (Hampers, Soeldner, Doak & Merrill, 1966; Harton, Johnson & Lebovitz, 1968) experiments were designed to investigate the effects of metabolic acidosis on carbo-
hydrate metabolism in rats. Since there were already data on glucose utilization by acidotic dogs (Mackler et al., 1951, 1952), in the present studies more attention was paid to glycogen metabolism in rats under conditions of metabolic acidosis.

METHODS

Virgin female Sprague-Dawley rats (150–200 g) were used in all experiments.

Induction of acidosis. Rats were removed from solid food and for 48 h were tube fed 12-hourly with 20% (w/v) glucose (controls) or 20% (w/v) glucose with 200 mm NH₄Cl (acidotic). A volume of 5 ml/100 g body wt of these fluids was given. The rats were killed 12 h after the last feed. Tap water was allowed freely to both groups.

Anaesthetic. Sodium pentobarbitone, 45 mg/kg, was given intraperitoneally before death. For experiments in which serial blood samples were taken, an initial injection of 20 mg/kg was used and subsequent small doses given as required.

Handling of samples. After anaesthesia, the abdomen was opened, and where necessary, blood taken from the abdominal aorta into heparinized syringes. The livers were removed rapidly, weighed and aliquots of 100 to 200 mg taken for glycogen estimations. Muscle samples were taken from the quadriceps femoris.

Response to glucagon. Rats were anaesthetized and 1·0 mg heparin given intraperitoneally to facilitate blood sampling. Glucagon 0·05 mg/100 g body wt was injected intraperitoneally and blood samples were taken from the clipped tail at 5, 15, 30, 45, 60, 75 and 90 min for estimation of glucose.

Glucose tolerance. Rats were anaesthetized and heparinized as above and glucose 50 mg/100 g body wt given into a tail vein. Blood samples were taken from the end of the tail at 5, 10, 15, 30, 45 and 60 min.

In vitro glycogenolysis. Slices were cut by hand from the livers of rats which had not been fasted and aliquots of 100–150 mg were placed in flasks containing 10 ml Krebs-Ringer bicarbonate buffer which had been equilibrated with either a 5% CO₂, 95% O₂ or a 20% CO₂, 80% O₂ gas mixture. Flasks were shaken at 100 oscillations per min in a metabolic shaker at 37° and continuously flushed with the appropriate gas mixture. Flasks were taken from the bath at appropriate intervals, liver slices were removed, blotted, weighed rapidly on a torsion balance and the glycogen estimated. The pH of the buffer equilibrated with the 5% CO₂, 95% O₂ gas mixture was 7·36–7·40 as opposed to 6·90–7·10 for that equilibrated with the 20% CO₂, 80% O₂ gas mixture.

Analyses. Glycogen was measured by the method of Good, Kramer & Somogyi (1933). The glucose resulting from acid hydrolysis was estimated with glucose oxidase. Blood hydrogen ion concentration was measured at 37° with the Radiometer micro Astrup blood pH assembly, and total CO₂ was measured manometrically in a Natelson microgasometer (Scientific Industries Inc., Springfield, Mass., U.S.A.). For estimation of liver composition, samples of fresh liver were placed in tared vials and dried at 90° for 48 h. The dried tissue was extracted with several changes of petroleum ether and fat estimated by weight differences. The dry, fat-free tissue was ground to a fine powder in a mortar and aliquots dissolved in 2·0 N NaOH for estimation of protein by the method of Lowry, Roseborough, Farr & Randall (1951) with bovine albumin as a standard. Total phosphorylase (E.C. 2.4.1.1.) was measured in liver homogenates by Hers' method (1959). Glycogen synthetase (UDP glucose: α-1, 4, glucan α-4
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glucosyltransferase E.C. 2.4.1.11) was assayed by measuring the incorporation of $[^{14}C]$uridine diphosphate glucose into glycogen (Steiner, Randa & Williams, 1961). The incubation was carried out in 0·1 M glycyl glycine buffer (pH 7·5) for 15 min at 30°. The $[^{14}C]$glycogen was counted in a Dioxan scintillation mixture which contained 10 g/100 ml naphthalene, 0·4 g/100 ml 2,5-diphenyloxazole and thixcin 0·5 g/100 ml (Nuclear Enterprises Ltd, Sighthill, Edinburgh, Scotland). Radioactivity was measured in a Beckman L.S. 150 liquid scintillation counter.

Glucose oxidase, peroxidase, adenosine monophosphate (AMP), glucose-6-phosphate and uridine diphosphate glucose (UDP glucose) were obtained from the Sigma Chemical Company. $[^{14}C]$UDP glucose was a product of the Radiochemical Centre, Amersham, England.

RESULTS

Table 1 shows the blood glucose, blood hydrogen ion concentration and plasma total CO$_2$ from control and acidotic rats. There was no difference in blood glucose, but the changes in hydrogen ion concentration and CO$_2$ indicated a significant degree of metabolic acidosis.

<table>
<thead>
<tr>
<th></th>
<th>Blood glucose (mg/100 ml)</th>
<th>Blood H$^+$ (nEq/l)</th>
<th>Plasma total CO$_2$ (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>73.9±8.6 (14)</td>
<td>42.2±1.4 (7)</td>
<td>25.2±1.3 (6)</td>
</tr>
<tr>
<td>Acidotic</td>
<td>76.2±5.9 (12)</td>
<td>54.9±1.7 (8)</td>
<td>18.3±1.0 (6)</td>
</tr>
<tr>
<td>$P$</td>
<td>NS</td>
<td>&lt;0·001</td>
<td>&lt;0·001</td>
</tr>
</tbody>
</table>

NS = not significant

Table 2. Liver composition in control and acidotic rats. Values are means±SEM. Figures in parentheses refer to the number of animals

<table>
<thead>
<tr>
<th></th>
<th>Water (% of fresh tissue)</th>
<th>Fat (% of fresh tissue)</th>
<th>Protein (% fat-free dry tissue)</th>
<th>Glycogen (% of fresh tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>69.9±0.8 (8)</td>
<td>2.31±0.35 (5)</td>
<td>78.0±2.3 (8)</td>
<td>1.61±0.22 (24)</td>
</tr>
<tr>
<td>Acidotic</td>
<td>70.0±0.7 (8)</td>
<td>1.88±0.29 (6)</td>
<td>73.8±2.2 (8)</td>
<td>2.81±0.26 (23)</td>
</tr>
<tr>
<td>$P$</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = not significant

In Table 2 is shown the composition of the livers of the control and acidotic rats. There is a significant increase in liver glycogen in the acidotic animal, but water, fat and protein content are unchanged.

The liver weight in relation to the body weight is shown in Table 3. When expressed as a fraction of the body weight at death, liver weight is higher in the acidotic animals, but the
Acidotic rats lost more body weight during the 48 h of acidosis. Hence when the liver weights are related to the initial weights of the rats there is no significant difference between the two groups.

**Table 3.** Liver weight as a fraction of body weight and weight loss in control and acidotic rats. Values are means ± SEM. Figures in parentheses refer to the number of animals.

<table>
<thead>
<tr>
<th></th>
<th>Liver weight ( % final body wt)</th>
<th>Liver weight ( % initial body wt)</th>
<th>Weight loss ( % initial body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.78 ± 0.04 (19)</td>
<td>2.61 ± 0.07 (8)</td>
<td>9.58 ± 0.69 (11)</td>
</tr>
<tr>
<td>Acidotic</td>
<td>3.02 ± 0.08 (19)</td>
<td>2.66 ± 0.04 (8)</td>
<td>13.17 ± 0.66 (10)</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.02</td>
<td>NS</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

NS = not significant

**Fig. 1.** The fall in liver glycogen in liver slices incubated at pH from 6.90 to 7.10 (acid) and from 7.36 to 7.40 (control).

There was no difference in rates of glycogenolysis at the different levels of pH (Fig. 1). Glycogen levels were higher here than in any other experiment because the rats were not starved before death.

The blood glucose response to intraperitoneal injections of glucagon is shown in Fig. 2. There was a slight insignificant fall in glucose at 5 min after glucagon in both groups of animals, but thereafter blood glucose rose in control and acidotic animals. The peak value was the same in both groups but was reached at 15 min in the controls and at 60 min in the acidotic
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379 rats. At 90 min when blood sugar had returned to normal in the control animals, it was still elevated in the acidotic ones.

The pertinent data from the glucose tolerance tests are shown in Table 4. The initial blood glucose as well as the maximum rise is the same in both groups, but the rate of disappearance of glucose is slower in the acidotic animals as shown by the longer glucose half-life.

![Blood glucose response of control and acidotic rats to intraperitoneal injections of glucagon.](image)

**TABLE 4. Results of intravenous glucose tolerance tests in control and acidotic rats**

<table>
<thead>
<tr>
<th></th>
<th>Initial blood glucose (mg/100 ml)</th>
<th>Peak blood glucose after intravenous glucose injected (mg/100 ml)</th>
<th>Half-life of injected glucose (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (8)</td>
<td>65.6 ± 1.1</td>
<td>284.1 ± 15.9</td>
<td>29.9 ± 2.5</td>
</tr>
<tr>
<td>Acidotic (10)</td>
<td>64.0 ± 3.0</td>
<td>324.5 ± 22.3</td>
<td>45.5 ± 3.3</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

NS = not significant

The results of measurement of phosphorylase and glycogen synthetase activities are shown in Table 5. There is no difference in phosphorylase activity between the two groups. Neither the total glycogen synthetase nor the I form of the enzyme changes with acidosis.

There was no significant difference in muscle glycogen between the control (0.498 ± 0.034 mg/100 wet wt) (mean ± SEM, n = 9) and the acidotic animals (0.532 ± 0.057 mg/100 mg wet wt, n = 10) (P>0.05).
TABLE 5. Liver phosphorylase and glycogen synthetase activities in control and acidotic rats. Phosphorylase activity expressed in μmol inorganic P min⁻¹ g protein⁻¹. Total glycogen synthetase and glycogen synthetase I expressed as counts min⁻¹ 100 mg protein⁻¹

<table>
<thead>
<tr>
<th></th>
<th>Phosphorylase activity</th>
<th>Glycogen synthetase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>Control</td>
<td>120.0±5.2</td>
<td>73.3±6.5</td>
</tr>
<tr>
<td>Acidotic</td>
<td>113.3±8.2</td>
<td>93.9±12.6</td>
</tr>
</tbody>
</table>

P

NS = not significant

DISCUSSION

These experiments have shown that in acidotic rats there is glucose intolerance similar to that described in acidotic dogs (Mackler et al., 1951, 1952). These workers had never investigated glycogen metabolism in their acidotic dogs. The increased liver glycogen in our rats must have been caused either by increased synthesis of glycogen or impaired glycogenolysis. Fasting blood sugar was the same in the acidotic and control animals. In the face of impaired peripheral glycolysis this may indeed indicate some impairment of hepatic glycogenolysis in vivo or a decreased rate of gluconeogenesis. Previous work has shown that acidosis causes increased renal gluconeogenesis but there was no suggestion of increased hepatic gluconeogenesis under these conditions (Goodman, Fuisz & Cahill, 1966; Alleyne & Scullard, 1969). The results of the glycogenolysis experiments indicate that under the conditions which obtained in vitro, there was no impairment of glycogen breakdown in livers from normal rats at an acid pH. Injection of glucagon produced a rise in blood glucose in the normal rats but a delayed hyperglycaemic response in the acidotic rats. This would indicate that there is no gross impairment of glycogenolysis in these acidotic animals and the enzymes involved in glycogen breakdown must be functionally competent. Glucagon acts by stimulating the adenyl cyclase system and activating the conversion of the enzyme phosphorylase b to phosphorylase a. It has previously been shown that hepatic glucose-6-phosphatase activity is unchanged in acidosis (Alleyne & Scullard, 1969). The small fall in blood glucose after glucagon reflects the glucagon induced stimulation of insulin release. The normal values of phosphorylase in the livers of acidotic rats is in keeping with the other data which show that there is no impairment of glycogenolysis.

There must therefore be an increase in glycogen synthesis. Glycogen synthetase is the important enzyme concerned with glycogen synthesis, and exists in two forms—I (independent of glucose-6-phosphate for maximal activity) and D (dependent on glucose-6-phosphate) (Leloir & Cardini, 1957; Danforth, 1965). Alterations in glycogen synthesis are reflected most clearly in the activity of the I form which also responds most markedly to hormonal influences (Smith, Taylor & Whelan, 1968). In the acidotic rats there is however no significant change in the total synthetase activity.

The increased hepatic glycogen shows that in this tissue there is not decreased phosphorylation of glucose as was proposed for blood cells (Mackler & Guest, 1953). A normal muscle glycogen in the presence of increased hepatic glycogen is of interest. There are two different
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enzymes responsible for glucose phosphorylation in mammalian tissues—glucokinase and hexokinase. The former is mainly responsible for glucose phosphorylation in liver, is not allosterically inhibited by glucose-6-phosphate and has a Michaelis constant for glucose of 10 mmol. The latter—hexokinase—on the contrary is the important enzyme in muscle, is allosterically inhibited by glucose-6-phosphate and has a Michaelis constant for glucose of 0.01 mmol (Sols, 1968). If acidosis inhibits glycolysis at the phosphofructokinase step, as others have proposed, there would be accumulation of hexose monophosphates in the tissues. In muscle this would serve to reduce glucose phosphorylation, but this clearly would not occur in liver. Thus in liver with continued glucose phosphorylation and impaired glycolysis there must be increased synthesis of glycogen.

The effect of acidosis on blocking glycolysis, with consequent decreased glucose phosphorylation in all tissues which do not possess a glucokinase with a high Michaelis constant for glucose, is the most likely explanation for the impaired glucose tolerance of acidotic animals.

Since these experiments were completed it has been reported in abstract form that metabolic acidosis in rats produced increases in hepatic and muscle glycogen (Kamm, 1969).

ACKNOWLEDGMENTS

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REFERENCES


