Effects of Metabolic Acidosis and Starvation on the Content of Intermediary Metabolites in Rat Kidney

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1. Metabolite contents were determined in freeze-clamped kidney from acidotic and starved rats in order to elucidate the rate-controlling steps which are responsible for the acceleration of gluconeogenesis in these situations. 2. In the kidney of rats which were made mildly acidotic by replacing drinking water with 1.5% ammonium chloride for 7 to 10 days (when the plasma bicarbonate concentration was 20 mm) the content of phosphoenolpyruvate was increased from the control value of 35 to 63 nmol/g and that of 3-phosphoglycerate from 85 to 154 nmol/g. 3. Similar but smaller changes in these metabolites occurred in the kidney of starved rats but there were no such changes in the kidney of rats 12 h after an infusion of 0.275 M hydrochloric acid, although plasma bicarbonate concentration fell to about 10 mm on this treatment. 4. The renal concentration of glucose 6-phosphate was not raised in rats that received ammonium chloride, but was increased in starved and acutely acidotic rats. 5. The concentrations of \( \alpha \)-oxoglutarate, malate and citrate were less than half the normal value in the kidney of both groups of acidotic rats. These changes can be accounted for on the basis of equilibrium relationships among reversible reactions, particularly as a result of the increase in intracellular ammonia content. A less marked decrease in \( \alpha \)-oxoglutarate and malate was found in the kidney of starved rats. 6. The renal cortical cytoplasmic oxaloacetate concentration was calculated to be decreased in acidotic and starved rats. 7. These results are discussed in the light of the known enhancement by acidosis and starvation of renal gluconeogenesis. In particular they support the suggestion that the phosphoenolpyruvate carboxykinase reaction is a site of control of gluconeogenesis in kidney in these conditions.

As the kidney plays a key role in acid–base balance, acidosis is associated with major alterations of renal metabolism. These include an increased release of ammonia to neutralize fixed acid in the urine and to conserve Na+ (for review see Pitts, 1964). The main precursor of the urinary ammonia is known to be plasma glutamine (Van Slyke et al. 1943; Davies & Yudkin, 1952; see also Pitts, 1964; Goldstein & Schooler, 1967; Lotspeich, 1967; Addae & Lotspeich, 1968a), which is formed at least partly in the liver (Addae & Lotspeich, 1968a,b). Glutamine is broken down in the kidney by the glutaminase reaction, and the subsequent deamination of glutamate, to release further ammonia (see Pitts, 1964), leaves for disposal the carbon skeleton of the original glutamine, namely \( \alpha \)-oxoglutarate. Goodman, Fuisz & Cahill (1966) suggested that the increased rate of gluconeogenesis in cortex slices from acidotic rats (which has been confirmed by Goorno, Rector & Seldin, 1967; Alleyne, 1970; Churchill & Malvin, 1970) may be interpreted as a major mechanism for disposing of the carbon skeleton of glutamine (see also Pagliara & Goodman, 1970).

Thus the question arises of which enzymic mechanisms regulate the rate of gluconeogenesis in relation to acid–base balance. Alleyne (1968, 1970) investigated this problem by measuring the content of several intermediates of gluconeogenesis and of the tricarboxylic acid cycle in the freeze-clamped kidney. He found a rise in the content of phosphoenolpyruvate in acidotic rat kidneys and suggested that the site at which the rate of gluconeogenesis is regulated is in acidosis is the phosphoenolpyruvate carboxykinase reaction. The present work extends
the range of intermediates determined in the freeze-clamped acidotic kidney. In addition, the enhancement of renal gluconeogenesis by starvation (Krebs, Bennett, de Gasquet, Gascoyne & Yoshiida, 1963) has been investigated by measurement of the content of intermediates in tissue.

EXPERIMENTAL

Animals. Male albino Wistar rats (250–350g) were fed on a standard diet (see Krebs et al. 1963). Starved animals were deprived of food for 48 h.

Induction of acidosis. Two procedures were employed:

(i) NH₄Cl (1.5%, w/v) was given as drinking water for 7–10 days (see Haldane, 1921). The daily intake was 5.0–6.5 mmol/day per 300 g rat, and ammonia excretion was 4.0–5.5 mmol/day per 300 g rat, compared with 0.6 in control rats. Average plasma bicarbonate concentrations were 25 mmol in control rats, and 20 mmol in acidotic rats; thus the acidosis was well compensated. Rats receiving NH₄Cl ate about 5 g of food pellets/day per 300 g rat, which was less than the usual intake. Control experiments showed that non-acidotic rats fed with only 5 g/day did not develop a significant alteration of renal gluconeogenesis. Hence the effects of the NH₄Cl regime were not due to food deprivation. The rate of gluconeogenesis from 5 mm-glutamine by the isolated perfused kidney from acidotic rats was 101 μmol/h per g dry wt., compared with 16 for normal kidney (D. A. Hems & H. A. Krebs, unpublished work).

(ii) HCl (12 ml of 0.25 M/300 g rat) was infused into the right atrium. The rat was anaesthetized with ether, and, after making a small skin incision, a fine polythene cannula (Portex PP25) was inserted through an Intracath needle into the right jugular vein. The cannula was pushed in to approximately the position of the right atrium (judged by a mark on the cannula) and tied into the vein. The needle was pulled off the cannula, and the skin was then sutured. Most animals recovered consciousness within 15 min, despite tying the jugular vein. The rate of infusion of acid was 3–4 ml/h, by means of a small roller or 'delta' pump (Watson–Marlow Ltd., Falmouth, Cornwall, U.K.). During an infusion, rate were kept in a restraining cage, and showed no signs of stress or discomfort. Acidification of the urine (pH 5.5–5.7) occurred within 1 h of the start of infusion. During the first 2 h, urine flow rate increased to about 0.1 ml/min (about ten times the normal rate), and urine NH₄ concentration rose to 30–40 mm. Occasionally there was mild haemoglobinuria.

The above procedure was chosen after trial infusions in which it was observed that larger doses or concentrations of acid were not well tolerated. Intragastric infusion of acid was also not satisfactory, in sedated or conscious rats.

After the 3–4 h of an infusion, the tube was tied and cut, close to the skin. The kidney was freeze-clamped or perfused 12 h after the start of infusion, and at that time the cannula was checked as being in the right atrium or inferior vena cava. The initial rate of gluconeogenesis from 5 mm-glutamine by isolated perfused kidney of rats which were acidotic for 12 h was 70 μmol/h per g dry wt. (D. A. Hems & H. A. Krebs, unpublished work). Their plasma bicarbonate concentration was 8–10 mm.

Preparation of extracts and analytical methods. Freeze-clamping of whole kidneys, preparation of extracts of whole kidney and 'cortex', and calculation of results, were as described in Hems & Brosnan (1970). The analytical methods, which consisted of NADH-linked enzymic techniques (except for the determination of P₇, which was by the method of Martin & Doty, 1949), were as described in Hems & Brosnan (1970). Bicarbonate was determined manometrically.

RESULTS

Renal content of intermediates of gluconeogenesis. In the kidney from 7–10-day-acidotic rats the content of phosphoenolpyruvate was increased (Table 1) as found by Alleyne (1968, 1970). In addition, the concentrations of 3-phosphoglycerate, triose phosphate, fructose diphosphate and α-glycerophosphate were increased. The content of hexose 6-phosphates did not show any increase (Table 1). There was no alteration of P₇ concentration in the kidney from 7–10-day-acidotic rats (Table 1), which confirms the finding of Goldstein (1967). The pattern of intermediates in 'cortex' extracts from 7–10-day-acidotic rats was the same as that in whole kidney (Table 1). Thus, no major intra-renal gradients of metabolites developed during acidosis, and the total renal metabolite concentration reflected that in cortex (as in kidney from fed rats; Hems & Brosnan, 1970). In kidney from rats which were acidotic for 12 h there was no change in the content of phosphorylated intermediates whereas that of hexose 6-phosphates was increased (Table 1).

In kidney from 48 h-starved rats the pattern of gluconeogenic intermediates resembled that in kidney from 7–10-day-acidotic rat (Table 1), except that the concentration of glucose 6-phosphate was raised. The increases of phosphorylated intermediates was less marked than in 7–10-day-acidotic rat kidney (Table 1).

Renal content of adenine nucleotides. Interpretation of tissue concentrations of metabolites is most valid when they resemble those in vivo. If there is delay in fixation of tissue, the concentrations of adenine nucleotides alter rapidly in liver and kidney (see Hems & Brosnan, 1970). For this reason, and since these metabolites are important modifiers of carbohydrate metabolism (Newsholme & Gevers, 1967), adenine nucleotides were determined in the present experiments. The contents of adenine nucleotides in acidotic rats (μmol/g of fresh kidney) were: ATP, 1.49±0.17; ADP, 1.07±0.08; AMP, 0.49±0.10 (12 h-acidotic rats anaesthetized with Nembutal, four observations) and ATP, 1.34±0.04; ADP, 1.22±0.08; AMP, 0.46±0.05 (7–10 day-acidotic rats immobilized by cervical dislocation, seven observations). These values are similar to
those in the kidney from well-fed or starved rats (Hems & Brosnan, 1970).

Renal content of amino acids and tricarboxylic acid-cycle metabolites. In kidney from acidotic rats, the contents of glutamine and glutamate were decreased (Table 2), as reported by Goldstein (1967; see also Goldstein & Schooler, 1967). The concentrations of malate, α-oxoglutarate and citrate were decreased to less than half their normal values in kidney from both groups of acidotic rats (Table 2), confirming the observations of Alleyne (1968, 1970).

The intracellular ammonia concentration, calculated from the renal venous ammonia concentration (Denis, Preuss & Pitts, 1964; see Hems & Brosnan, 1970) was increased in 7–10-day-acidotic rat kidney (Table 2; in agreement with Goldstein, 1967). Renal venous blood samples were not obtained from 12h-acidotic rats, so that intracellular ammonia content cannot be calculated in this condition. However, total (i.e. intracellular, extracellular and tubular luminal) ammonia content was increased in 12h-acidotic rat kidney (compared with normal kidney) to about the same extent as in 7–10-day-acidotic rat kidney, suggesting that intracellular content was increased after 12h of acidosis.

In kidney from 48h-starved rats, concentrations of the measured amino acids were decreased, although not to a major extent and α-oxoglutarate and malate also decreased. The calculated intracellular ammonia content was not increased in the kidney of starved rats (Table 2).

Calculation of the renal cytoplasmic oxaloacetate content. A key intermediate of gluconeogenesis is oxaloacetate, especially in connexion with acidosis, since phosphoenolpyruvate carboxykinase appears to be regulatory. This metabolite is unstable and present in low amounts in tissues. However, two independent methods of indirectly estimating the renal cytoplasmic oxaloacetate content are available from the present data. These calculations (Table 3) are based on the assumption that the following reactions are at or near equilibrium in the cytoplasm.

(i) The lactate dehydrogenase and malate dehydrogenase reactions. Thus the cytoplasmic oxaloacetate content has been calculated from the renal content of lactate, pyruvate and malate, as described by Williamson, Lund & Krebs (1967). A change in [H+] during acidosis would not affect this calculation, since the term \([\text{free NAD}^+]/[\text{free NADH}[\text{H}^+]]\) cancels out.

(ii) The aspartate aminotransferase reaction. The cytoplasmic content of oxaloacetate has been calculated as \([\text{aspartate}[\alpha-\text{oxoglutarate}]/6.7[\text{glutamate}]) since 6.7 is the equilibrium constant for the aspartate aminotransferase reaction (see Vecch, Eggleston & Krebs, 1969).

The results of both procedures suggest that the
cytoplasmic oxaloacetate content of kidney is decreased in acidotic and starved rats (Table 3).

**Mass-action ratios of aminotransferase reactants.**

The approximate agreement between the above two independent calculations of cytoplasmic oxaloacetate concentration suggests that the aspartate aminotransferase reaction is near equilibrium in kidney. The mass-action ratio for its reactants has been calculated (Table 4), taking the oxaloacetate concentration calculated from the presumed malate dehydrogenase equilibrium (see Table 3). The mass-action ratio for the pyruvate aminotransferase reactants has also been calculated (Table 4). The equilibrium constants for these reactions (quotients as in Table 4) are 1.5 and 6.7 respectively (see Veech et al. 1969). The calculations suggest that both these aminotransferase reactions are not far from equilibrium in kidney in the conditions tested (Table 4).

**Redox state of NAD couple in kidney from acidotic rats.** The redox state of the mitochondrial NAD couple may be calculated from the [glutamate]/[α-oxoglutarate]/[NH₄⁺] and [β-hydroxybutyrate]/[acetocacetate] ratios, and that of the cytoplasmic NAD couple from the [lactate]/[pyruvate] ratios, as described by Williamson et al. (1967). This approach has been applied to the kidney from well-fed or 48h-starved rats (Hems & Brosnan, 1970), there being no major differences between these states.

In acidotic rat kidney such calculations may be questioned on account of the doubtful validity of any assumption about the constancy of intracellular pH, especially in the highly acidotic rats. In the rats that were mildly acidotic for 7–10 days, intracellular pH probably did not alter much, because acidosis was largely compensated (the bicarbonate concentration in the plasma being 20mM). Thus the lack of a major alteration in the [lactate]/[pyruvate] ratio in the kidney from 7–10-day-acidotic rats (Tables 1 and 3) may be taken as evidence that the redox state of the cytoplasmic NAD couple did not alter significantly. The lack of a major alteration of the renal mitochondrial [free NAD⁺]/[free NADH] ratio in 7–10-day-acidotic rats is suggested by the following two observations.

(i) In 7–10-day-acidotic rats that were starved for 48h, the [β-hydroxybutyrate]/[acetocacetate] ratio in kidney was 5.6, which was similar to 7.0 in control starved rats (animals anaesthetized with Nembutal; see Hems & Brosnan, 1970).

(ii) Considering the glutamate dehydrogenase reactants, the rise in calculated intracellular ammonia concentration in 7–10-day-acidotic rats was associated with a decrease in α-oxoglutarate and no major alteration of glutamate (Table 2).
Table 3. Calculated cytoplasmic content of oxaloacetate in kidney cortex

The results of Table 2 (Nembutal-treated rats) have been used to calculate cortical cytoplasmic oxaloacetate content, by employing the presumed equilibria of malate dehydrogenase and aspartate aminotransferase, as described in the text, i.e. the calculated ratio [free NAD⁺]/[free NADH] does not take account of any changes in cytoplasmic [H⁺].

<table>
<thead>
<tr>
<th>State of rats</th>
<th>[Lactate]</th>
<th>[Pyruvate]</th>
<th>[free NAD⁺] (calculated from [lactate]/[pyruvate])</th>
<th>Calculated oxaloacetate content (nmol/g of fresh kidney)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well-fed</td>
<td>13.1</td>
<td>695</td>
<td>7.1</td>
<td>16.4</td>
</tr>
<tr>
<td>Acidotic, 12h</td>
<td>22.9</td>
<td>400</td>
<td>1.9</td>
<td>3.9</td>
</tr>
<tr>
<td>Acidotic, 7–10 days</td>
<td>15.2</td>
<td>600</td>
<td>2.3</td>
<td>6.2</td>
</tr>
<tr>
<td>Starved, 48h</td>
<td>14.8</td>
<td>615</td>
<td>4.6</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Table 4. Mass-action ratios of the aminotransferase reactions in kidney

The results of Table 2 have been used to calculate the [alanine][α-oxoglutarate]/[glutamate][pyruvate] and [aspartate] [α-oxoglutarate]/[glutamate] [oxaloacetate] ratios. [Oxaloacetate] has been calculated from the presumed malate dehydrogenase equilibrium (see Table 3). The equilibrium constants (written as defined above) are 1.5 and 6.7 respectively (see the text).

<table>
<thead>
<tr>
<th>State of rats</th>
<th>Alanine aminotransferase</th>
<th>Aspartate aminotransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well-fed</td>
<td>0.96</td>
<td>15.5</td>
</tr>
<tr>
<td>Acidotic, 12h</td>
<td>—</td>
<td>13.9</td>
</tr>
<tr>
<td>Acidotic, 7–10 days</td>
<td>0.29</td>
<td>17.9</td>
</tr>
<tr>
<td>Starved, 48h</td>
<td>0.82</td>
<td>14.5</td>
</tr>
</tbody>
</table>

DISCUSSION

Fall in renal content of α-oxoglutarate, citrate and malate in acidosis. The decrease in concentration of glutamate in acidic rat kidney (Goldstein, 1968) has been attributed to accelerated gluconeogenesis, with consequent withdrawal of intermediates from the tricarboxylic acid cycle (Goodman et al. 1966). The decreases during acidosis of renal content of α-oxoglutarate (Goldstein, 1967; Freuss, 1969) and of malate and citrate (Alleyne, 1968) have been similarly explained, especially by acceleration at phosphoenolpyruvate carboxykinase (Alleyne, 1968, 1970).

An alternative explanation for the decline in content of these metabolites in acidosis invokes the assumption that they reflect alterations in cellular equilibria. This could occur primarily as a result of the rise in intracellular ammonia concentration, as follows.

(i) The glutamate dehydrogenase reaction is probably at equilibrium in kidney (Hems & Brosnan, 1970). There appears to be no major change in the mitochondrial [free NAD⁺]/[free NADH] ratio in 7–10-day-acidotic rat kidney (see the Results section). Hence an increase in ammonia concentration would cause a decrease in mitochondrial α-oxoglutarate, in the absence of an increase in glutamate.

(ii) A decrease in α-oxoglutarate could cause a decrease in isocitrate and citrate concentrations, if equilibrium holds at the aconitase reaction and at the isocitrate dehydrogenase reaction, and if there is no major change in the redox state of the relevant nicotinamide nucleotide couple.

(iii) A decrease in concentration of α-oxoglutarate would also cause a decrease in oxaloacetate concentration, if the aspartate aminotransferase reactants are at or near equilibrium in normal and acidic kidney (as seems likely from the present results), since the [glutamate]/[aspartate] ratio did not change in acidosis.

(iv) A decrease in oxaloacetate concentration would explain that of malate, since the cortical cytoplasmic [free NAD⁺]/[free NADH] ratio as determined from the [lactate]/[pyruvate] ratio does not alter in acidosis, and the cytoplasmic malate dehydrogenase may reasonably be presumed to be at equilibrium.

Alleyne (1968) suggested that accelerated removal of oxaloacetate (to form glucose) contributes to the decline in concentrations of α-oxoglutarate, citrate and malate in the kidney in acidosis. This could occur within the above network of cellular equilibria, by the reverse process to that described, although the direction of changes in metabolite concentrations would be similar.

The less marked decrease in α-oxoglutarate, citrate or malate in the kidney from starved rats, when gluconeogenesis is enhanced to a similar extent to that in acidosis, is in accord with the suggestion that the rise in intracellular concentration of ammonia is the major cause of the fall in the content of these intermediates in acidosis, since no
such rise occurs in the kidney of starved rats. A
decrease in α-oxoglutarate and malate during
starvation could be the result of withdrawal of
oxaloacetate from the tricarboxylic acid cycle as
described above.

Enhancement of renal gluconeogenesis by acidosis.
Alleyne (1968) inferred from the increased concentra-
tion of phosphoenolpyruvate in the kidney of
2-day-acidotic rats that activation of phosphoenol-
pyruvate carboxykinase could contribute to this
enhancement. This result is confirmed for the
7–10-day-acidotic rat (although not the 12h-
acidotic rat) in the present work. The suggestion of
enhancement of phosphoenolpyruvate carboxy-
kinase in acidosis is supported by the calculated
fall in cytoplasmic oxaloacetate concentration, and
is not incompatible with the different origin for this
fall which was discussed in the previous section.
An enhanced capacity of phosphoenolpyruvate
carboxykinase in acidosis in rats has been reported
It has been found that, assayed under optimum
conditions, the renal capacity of phosphoenol-
pyruvate carboxykinase in 7–10-day-acidotic rats
was 5.6 units/g fresh wt. compared with 3.1 units/
g fresh wt. in normal rats (M. Stubbs & H. A.
Krebs, unpublished work).

If the phosphoenolpyruvate carboxykinase step
were the sole site of enhancement of gluconeogenic
capacity in acidosis, and if formation of glucose
from amino acids by the kidney is presumed to be
faster in vivo during acidosis, then the concentra-
tions of all intermediates from phosphoenolpyruvate
in glucose 6-phosphate should be increased in kidney
from acidotic rats. This was true in the 7–10-day-
acidotic rat kidney for 3-phosphoglycerate and
triose phosphate, but not for hexose 6-phosphates
(see also unpublished observations cited by Alleyne,
1970). Hence a further site of enhancement of
gluconeogenesis may be at the conversion of
glucose 6-phosphate into glucose. If kidney glucose
6-phosphatase resembles that in liver, de-inhibition
by a decrease in bicarbonate concentration (during
acidosis) could contribute to this enhancement
(Dyson, Anderson & Nordlie, 1969).

The decreased cytoplasmic content of oxalo-
acetate in the kidneys of acutely acidotic rats
suggests that acceleration at the phosphoenol-
pyruvate carboxykinase reaction already occurs at
this stage. This is in accord with the increase in
measurable enzyme activity in acutely acidotic
rats (Alleyne & Scullard, 1969; Alleyne, 1970), the
rapid onset of ammonia excretion during acidosis
(see also Alleyne, 1968; Pitts, 1964), and the
enhanced gluconeogenesis from glutamine by
isolated perfused kidney from acutely acidotic rats
(see the Experimental section) and by cortex slices
within 12 h of the onset of acidosis (Goodman et al.
1966; Alleyne 1970; see Kamm, Fuisz, Goodman &
Cahill, 1967). However, the lack of an increase in
C₃ intermediates in the kidney from such rats
suggests that additional factors were controlling
the concentrations of C₃ intermediates, or that
acceleration at phosphoenolpyruvate carboxy-
kinase was not maximal by 12 h in vivo.

Activation of renal gluconeogenesis by starvation.
The rise in C₃ phosphorylated intermediates,
combined with the calculated decrease in oxalo-
acetate, suggests that acceleration of the phospho-
enolpyruvate carboxykinase reaction contributes
to the enhanced gluconeogenesis of starvation.
Henning, Stumpf, Ohly & Seubert (1966) reported
increased activity of this enzyme in the kidney from
starved rats.

The suggestion that the phosphoenolpyruvate
carboxykinase reaction is rate-controlling in both
acidosis and starvation is in accord with the proposal
that the enhancement of renal gluconeogenesis
during starvation is at least partly a consequence of
acidosis (Kamm & Cahill, 1969).

Since there was no detectable rise in fructose
1,6-diphosphate but a rise in glucose 6-phosphate
in the kidney from starved rats (in contrast with
the decrease in the kidney from 7–10-day-acidotic
rats), enhancement of gluconeogenesis at the
fructose diphosphatase reaction also occurred during
starvation.

During starvation, a variety of circulating
precursors may contribute to the enhanced rate of
renal glucose formation, in contrast with acidosis,
when glutamine seems to be the major amino acid
which is taken up from blood at a significantly
faster rate (see Pitts, 1964). Thus pyruvate and its
precursors, such as lactate, alanine and serine, may
be metabolized at a faster rate (see Krebs, 1964).
The pyruvate carboxylase reaction, which is
activated by acetyl-CoA in particular, is thought
to be a control site during starvation, in liver and
kidney (see Newsholme & Gevers, 1967, for review).
The calculated decline in oxaloacetate content in
the kidney from starved rats suggests that any
acceleration of renal pyruvate carboxylase is more
than offset by factors tending to lower the concen-
tration of oxaloacetate, such as acceleration of
phosphoenolpyruvate carboxykinase.

Concentrations of amino acids in kidney in acidosis
and starvation. The decreased concentrations of
glutamine and glutamate in 7–10-day-acidotic rat
kidney confirm the measurements of Goldstein
(1967). Such a decrease of glutamine in acidotic
kidney can be explained by increased glutaminase
capacity in acidosis (see Goldstein & Schooler, 1967,
for discussion). De-inhibition of glutaminase by a
decrease in glutamate concentration may be
especially relevant in acute or severe acidosis, since
glutamate concentrations were lowest in 12h-
acidotic rat kidney, and since the rise in renal glutaminase activity during acidosis is not present at this early stage (Leonard & Orloff, 1955; Rector, Seldin & Copenhaver, 1955; Alleyne & Scullard, 1969).

Any decline in content of glutamine, glutamate, alanine and aspartate in acidotic or starved rat kidney may in general be attributed to their accelerated consumption as gluconeogenic precursors, by the mechanisms discussed above.

*Redox state in acidosis.* For calculations of the redox state of cellular NAD couples from measurements of metabolite concentrations, cellular pH is usually taken to be constant in most physiological conditions (Williamson et al. 1967; Veech et al. 1969). This assumption would not be valid in acidosis, when the consequences of alterations of $[H^+]$ must be considered. The general equation defining the redox state of the NAD couple includes $[H^+]$:

$$\frac{[\text{free NADH}][H^+]}{[\text{free NAD}^+]^2} = K \frac{[\text{reduced metabolite}]}{[\text{oxidized metabolite}]}$$

Thus the [lactate]/[pyruvate] ratio reflects the true redox state of the cytoplasmic NAD-couple at all values of $[H^+]$. During acidosis, the true redox state would not change if the [free NADH]/[free NAD+] ratio altered in inverse relation to $[H^+]$. Conversely, if such an alteration did not occur, the true redox state would become more reduced. Considering all NAD- or NADP-linked dehydrogenases, a large or rapid rise in $[H^+]$ would tend to cause an increase in the [reduced metabolite]/[oxidized metabolite] ratio. In the acutely acidotic rats, such an effect in the cytoplasm could have contributed to (i) the rise in the [lactate]/[pyruvate] ratio, (ii) the increase in glucose 6-phosphate, and (iii) the failure to detect a rise in C3 intermediates, and the increase in P1, as a result of adjustments in the reactants of triose phosphate dehydrogenase (see Veech, Raijman & Krebs, 1970). Similarly, any rise in cytoplasmic or mitochondrial $[H^+]$ would tend to enhance the decline in content of the ‘oxidized’ tricarboxylic acid-cycle intermediates, oxaloacetate and $\alpha$-oxoglutarate.

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