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 0.25 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 α -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 rise in intracellular ammonia content. A less marked decrease in α -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, 1968*a*), which is formed at least partly in the liver (Addae & Lotspeich, 1968*a,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 α -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 in acidosis is the phosphoenolpyruvate carboxykinase reaction. The present work extends

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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 & Yoshida, 1963) has been investigated by measurement of the content of intermediates in tissue.

EXPERIMENTAL

Animals. Male albino Wistar rats (250–350 g) 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) NH_4Cl (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 mM in control rats, and 20 mM in acidotic rats; thus the acidosis was well compensated. Rats receiving NH_4Cl 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 NH_4Cl 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 $\mu\text{mol/h}$ 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 right 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, rats 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 NH_3 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 $\mu\text{mol/h}$ 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 P_i , 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 P_i 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 ($\mu\text{mol/g}$ 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

Table 1. Renal content of intermediates of gluconeogenesis

Kidneys were obtained from rats that were killed by cervical dislocation except for two groups in which rats were anaesthetized with Nembutal (as indicated). 'Cortex' was obtained as described by Hems & Brosnan (1970). The values for well-fed rats have been reported previously (Hems & Brosnan, 1970). Further details are in the text. Results are means $\pm$ S.E.M. (numbers of observations in parentheses). Abbreviations: Triose-P, sum of triose phosphate; Fru-1,6-P, fructose 1,6-diphosphate; Fru-6-P, fructose 6-phosphate; Glc-6-P, glucose 6-phosphate; P_i, inorganic phosphate.

State of rats	Content of metabolites (nmol/g of fresh kidney)							
	Phospho-enol		2-Phospho-glycerate		3-Phospho-glycerate		α -Glycero-phosphate	
	Lactate	Pyruvate	Pyruvate	Triose-P	Triose-P	Fru-1,6-P	Fru-6-P	Glc-6-P
Well-fed	480 $\pm$ 62 (13)	27 $\pm$ 3 (13)	35 $\pm$ 3 (13)	14 $\pm$ 2 (12)	85 $\pm$ 9 (12)	16 $\pm$ 2 (12)	120 $\pm$ 8 (13)	39 $\pm$ 4 (12)
Well-fed (Nembutal)	850 $\pm$ 113 (6)	48 $\pm$ 6 (6)	47 $\pm$ 6 (4)	<10	100 $\pm$ 8 (4)	14 $\pm$ 5 (4)	130 $\pm$ 11 (6)	69 $\pm$ 14 (5)
Acidotic, 12h (Nembutal)	1330 $\pm$ 41 (4)	58 $\pm$ 17 (4)	44 $\pm$ 6 (4)	<10	88 $\pm$ 10 (4)	14 $\pm$ 7 (3)	180 $\pm$ 40 (4)	89 $\pm$ 12 (3)
Acidotic, 7-10 days	480 $\pm$ 3 (13)	29 $\pm$ 2 (13)	63 $\pm$ 6 (13)	21 $\pm$ 3 (13)	154 $\pm$ 12 (13)	23 $\pm$ 3 (8)	180 $\pm$ 6 (13)	34 $\pm$ 4 (12)
Starved, 48h	420 $\pm$ 50 (14)	28 $\pm$ 3 (14)	50 $\pm$ 5 (11)	16 $\pm$ 4 (9)	114 $\pm$ 10 (12)	17 $\pm$ 2 (8)	180 $\pm$ 12 (14)	59 $\pm$ 2 (9)
Acidotic, 7-10 days ('cortex')	650 $\pm$ 57 (3)	33 $\pm$ 6 (3)	65 $\pm$ 8 (3)	16 $\pm$ 7 (3)	148 $\pm$ 23 (3)	26 $\pm$ 7 (3)	80 $\pm$ 10 (3)	34 $\pm$ 3 (3)
								4100 $\pm$ 65 (3)

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 $[free\ NAD^+]/[free\ NADH][H^+]$ cancels out.

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

The results of both procedures suggest that the

Table 2. Renal content of non-gluconeogenic intermediates

Kidneys were obtained from rats anaesthetized with Nembutal, except that in one group of starved animals, and for all measurements of citrate and of P_i in 7-10-day-acidotic rats, kidneys were obtained after cervical dislocation. 'Cortex' was obtained as described in Hems & Brosnan (1970). Further details are in the text. The values for well-fed and one group of starved rats have been reported previously (Hems & Brosnan, 1970). Results are means $\pm$ S.E.M. (numbers of observations in parentheses).

State of rats	Content of metabolites (μ mol/g of fresh kidney)										Renal venous NH_4^+ (μ mol/ml of blood)	Calculated intracellular NH_4^+ (μ mol/g of fresh kidney)
	Glutamine	Glutamate	α -Oxo-glutarate	Total tissue NH_4^+	Alanine	Aspartate	Citrate	Malate	Lactate	Pyruvate	P _i	
Well-fed	1.72 $\pm$ 0.17 (6)	3.01 $\pm$ 0.23 (9)	0.30 $\pm$ 0.06 (6)	0.88 $\pm$ 0.10 (6)	0.86 $\pm$ 0.07 (6)	1.11 $\pm$ 0.06 (6)	0.39 $\pm$ 0.08 (6)	0.37 $\pm$ 0.05 (6)	1.17 $\pm$ 0.15 (6)	0.089 $\pm$ 0.01 (6)	2.81 $\pm$ 0.15 (3)	0.12 $\pm$ 0.02 (6)
Acidotic, 12h	0.87 $\pm$ 0.13 (3)	2.33 $\pm$ 0.27 (4)	0.08 $\pm$ 0.02 (4)	4.60 $\pm$ 0.55 (3)	—	0.77 $\pm$ 0.03 (3)	—	0.17 $\pm$ 0.04 (5)	1.33 $\pm$ 0.05 (5)	0.058 $\pm$ 0.017 (5)	4.86 $\pm$ 0.23 (4)	—
Acidotic, 7-10 days	1.17 $\pm$ 0.11 (5)	2.48 $\pm$ 0.27 (6)	0.11 $\pm$ 0.02 (5)	3.89 $\pm$ 0.38 (5)	0.67 $\pm$ 0.05 (4)	0.93 $\pm$ 0.08 (5)	0.18 $\pm$ 0.01 (8)	0.14 $\pm$ 0.02 (5)	1.58 $\pm$ 0.24 (6)	0.104 $\pm$ 0.01 (5)	3.83 $\pm$ 0.16 (11)	0.46 $\pm$ 0.06 (4)
Starved, 48h	1.09 $\pm$ 0.05 (8)	2.70 $\pm$ 0.02 (12)	0.24 $\pm$ 0.02 (11)	1.71 $\pm$ 0.14 (6)	0.52 $\pm$ 0.04 (3)	0.75 $\pm$ 0.03 (6)	—	0.27 $\pm$ 0.02 (8)	0.83 $\pm$ 0.10 (11)	0.056 $\pm$ 0.05 (11)	—	0.09 $\pm$ 0.02 (7)
Starved, 48h (cervical dislocation)	0.84 $\pm$ 0.10 (5)	2.59 $\pm$ 0.15 (5)	0.32 $\pm$ 0.03 (8)	1.01 $\pm$ 0.14 (8)	—	0.74 $\pm$ 0.11 (6)	0.37 $\pm$ 0.02 (10)	0.22 $\pm$ 0.02 (6)	0.53 $\pm$ 0.07 (10)	0.038 $\pm$ 0.01 (10)	4.25 $\pm$ 0.15 (10)	—
Acidotic, 7-10 days ('cortex')	0.82 $\pm$ 0.14 (3)	2.06 $\pm$ 0.09 (3)	0.12 $\pm$ 0.02 (3)	—	—	0.90 $\pm$ 0.06 (3)	0.21 $\pm$ 0.03 (3)	0.15 $\pm$ 0.02 (3)	1.10 $\pm$ 0.20 (3)	0.081 $\pm$ 0.01 (3)	2.88 $\pm$ 0.08 (3)	—

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 near to 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][NH_4^+] and [β -hydroxybutyrate]/[acetoacetate] 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]/[acetoacetate] 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 rat kidney 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 $[\text{free NAD}^+]/[\text{free NADH}]$ does not take account of any changes in cytoplasmic $[\text{H}^+]$.

State of rats	$\frac{[\text{Lactate}]}{[\text{Pyruvate}]}$	Cytoplasmic $[\text{free NAD}^+]$	Calculated oxaloacetate content (nmol/g of fresh kidney)	
		$\frac{[\text{free NADH}]}{[\text{lactate}]/[\text{pyruvate}]}$	From malate dehydrogenase	From aspartate aminotransferase
Well-fed	13.1	695	7.1	16.4
Acidotic, 12h	22.9	400	1.9	3.9
Acidotic, 7-10 days	15.2	600	2.3	6.2
Starved, 48h	14.8	615	4.6	10.0

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

The results of Table 2 have been used to calculate the $[\text{alanine}]/[\alpha\text{-oxoglutarate}]/[\text{glutamate}]/[\text{pyruvate}]$ and $[\text{aspartate}]/[\alpha\text{-oxoglutarate}]/[\text{glutamate}]/[\text{oxaloacetate}]$ ratios. $[\text{Oxaloacetate}]$ 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).

State of rats	Mass-action ratio	
	Alanine aminotransferase	Aspartate aminotransferase
Well-fed	0.96	15.5
Acidotic, 12h	—	13.9
Acidotic, 7-10 days	0.29	17.9
Starved, 48h	0.82	14.5

DISCUSSION

Fall in renal content of α -oxoglutarate, citrate and malate in acidosis. The decrease in concentration of glutamate in acidotic rat kidney (Goldstein, 1966) 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; Preuss, 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 $[\text{free NAD}^+]/[\text{free NADH}]$ 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 acidotic kidney (as seems likely from the present results), since the $[\text{glutamate}]/[\text{aspartate}]$ ratio did not change in acidosis.

(iv) A decrease in oxaloacetate concentration would explain that of malate, since the cortical cytoplasmic $[\text{free NAD}^+]/[\text{free NADH}]$ ratio as determined from the $[\text{lactate}]/[\text{pyruvate}]$ 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 concentration of phosphoenolpyruvate in the kidney of 2-day-acidotic rats that activation of phosphoenolpyruvate 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 carboxykinase 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 by Alleyne & Scullard (1969) and Alleyne (1970). It has been found that, assayed under optimum conditions, the renal capacity of phosphoenolpyruvate 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 concentrations of all intermediates from phosphoenolpyruvate to 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 oxaloacetate in the kidneys of acutely acidotic rats suggests that acceleration at the phosphoenolpyruvate 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 12h of the onset of acidosis (Goodman *et al.*

1966; Alleyne 1970; see Kamm, Fuisz, Goodman & Cahill, 1967). However, the lack of an increase in C_3 intermediates in the kidney from such rats suggests that additional factors were controlling the concentrations of C_3 intermediates, or that acceleration at phosphoenolpyruvate carboxykinase was not maximal by 12h *in vivo*.

Activation of renal gluconeogenesis by starvation. The rise in C_3 phosphorylated intermediates, combined with the calculated decrease in oxaloacetate, suggests that acceleration of the phosphoenolpyruvate 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 concentration 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 $[H^+]$:

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

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 α -oxoglutarate.

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