Metabolism of Glutamine and Glutamic Acid by Isolated Perfused Kidneys of Normal and Acidotic Rats

By D. A. HEMS*

*Metabolic Research Laboratory, Nuffield Department of Clinical Medicine,
Radcliffe Infirmary, Oxford OX2 6HE, U.K.*

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1. When isolated kidneys from fed rats were perfused with glutamine the rate of ammonia release at pH 7.4 ($110\text{--}360\ \mu\text{mol/h per g dry wt.}$) was one to two times that of glutamine removal. Glucose formation from 5 mM-glutamine was $16\ \mu\text{mol/h per g}$. If kidneys were perfused with glutamine at pH 7.1 (10–13 mM-sodium bicarbonate) there was no increase in glutamine removal or in the formation of ammonia or glucose. 2. When isolated kidneys from fed rats were perfused with glutamate at pH 7.4, glucose formation was $59\ \mu\text{mol/h per g}$, glutamine formation was $182\ \mu\text{mol/h per g}$ and ammonia release was negligible. At pH 7.1 glutamine synthesis was inhibited and formation of ammonia and glucose were increased. 3. In perfused kidneys from acidotic rats, which had received 1.5% (w/v) NH_4Cl to drink for 7–10 days, gluconeogenesis from glutamine was enhanced ($101\ \mu\text{mol/h per g}$). Glutamine removal and ammonia formation were also increased, compared with the rates in perfused kidney from normal rats. The extra glutamine consumed was equivalent to the extra glucose formed. 4. When the kidney from the 7–10-day-acidotic rat was perfused with glutamate gluconeogenesis was increased ($113\ \mu\text{mol/h per g}$). Synthesis of glutamine was decreased, and ammonia release was approximately equal to the rate of glutamate removal. 5. The time-course of these metabolic alterations was investigated after the rapid induction of acidosis by infusion of 0.25M-HCl into the right side of the heart. The increase in gluconeogenesis from glutamine developed gradually over several hours. When kidneys from 6h-acidotic rats were perfused with glutamate, formation of glucose and glutamine were both rapid. 6. In acidotic rat kidneys perfused with glutamine, tissue concentrations of glutamate and glucose 6-phosphate were increased compared with those in control perfused kidneys from non-acidotic rats. 7. The results are discussed in terms of control of the renal metabolism of glutamine. In particular, it is suggested that in acidotic rats glucose formation is the major fate of the carbon of the extra glutamine utilized by the kidney, and that inhibition of glutamine synthetase could contribute to the increase in intracellular ammonia concentration in the kidney.

The kidney plays an important role in acid–base balance, and adapts during acidosis to bring about increased excretion of ammonia in urine, to neutralize fixed acid and to conserve Na^+ (for review see Pitts, 1964). The main circulating precursor of urinary ammonia is glutamine (Van Slyke *et al.*, 1943; see also Davies & Yudkin, 1952; and for reviews see Pitts, 1964; Goldstein & Schooler, 1967; Lotspeich, 1967; Orloff & Burg, 1971).

The accelerated excretion of ammonia in acidosis mainly derives from an increased tissue ammonia concentration (Denis *et al.*, 1964; Goldstein, 1967; Hems & Brosnan, 1971) and the increased H^+ concentration gradient between the tissue and the tubular lumen, through which a substantial quantity

of the ammonia produced in the kidney is ‘trapped’ in the urine (Pitts, 1964). The required metabolic function of the kidney in acidosis is to generate ammonia at a sufficient rate to maintain the increased tissue concentration. Since ammonia diffuses rapidly through the whole kidney (Stone *et al.*, 1967) this process may occur at any site within the kidney. Hence an isolated, perfused rat kidney is a preparation suitable for the study of metabolic aspects of ammonia production, despite the fact that tubular function is not fully retained after isolation. In the present paper an investigation of glutamine metabolism in perfused rat kidneys is reported, with particular regard to the relationship between the formation of ammonia and glucose during acidosis. Various aspects of such an association have been investigated previously by Goodman *et al.* (1966), Goorno *et al.* (1967), Kamm & Cahill (1969), Alleyne (1970), Kamm & Asher

* Present address: Department of Biochemistry, Imperial College of Science and Technology, London SW7, U.K.

(1970), Churchill & Malvin (1970*a,b*), Pagliara & Goodman (1970) and Klahr (1971). Also, results are presented on the inhibitory effect of acidosis on the renal capacity for synthesis of glutamine from glutamate (Lyon & Pitts, 1969; Damian & Pitts, 1970).

Materials and Methods

Animals

Male albino Wistar rats (270–370 g) were used for all experiments. They were fed on a standard diet of rat cubes (see Krebs *et al.*, 1963) and had free access to water. Starvation was for 48 h.

Rats were rendered acidotic by one of three techniques: (i) drinking water was replaced by 1.5% (w/v) (0.28 M) NH_4Cl for 7–10 days; (ii) 12 ml of 0.25 M-HCl was infused for 4 h into the right side of the heart (both these techniques are described by Hems & Brosnan, 1971); (iii) acidosis of 24 h duration was induced by giving two intragastric doses of 5 ml of 0.75 M- NH_4Cl /300 g rat under light ether anaesthesia with a 5 h interval (see Lotspeich, 1967) [the plasma bicarbonate concentration of these animals was $19 \pm 1(4)$ mm].

Chemicals

Bovine serum albumin (fraction V) was obtained from Armour Pharmaceutical Co. (Eastbourne, Sussex, U.K.). L-Glutamine and L-glutamic acid were from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). *Escherichia coli* glutaminase was from Worthington Chemical Corp. (Freehold, N.J., U.S.A.) or Sigma Chemical Co.

Perfusion of isolated kidney

This was carried out as described by Nishiitsutsuji-Uwo *et al.* (1967). The standard perfusion medium consisted of 60–100 ml of bicarbonate-buffered saline (Krebs & Henseleit, 1932) containing 4.5% (w/v) of dialysed bovine serum albumin. The gas phase was usually $\text{CO}_2 + \text{O}_2$ (5:95) so that the pH was about 7.4 when the medium contained 25 mM-sodium bicarbonate (Krebs & Henseleit, 1932). Acidification of the medium was achieved by adding HCl until the bicarbonate concentration was 10–13 mM; when the medium was gassed with $\text{CO}_2 + \text{O}_2$ (5:95) the pH was about 7.1. Substrates were usually added before the start of perfusion. Glutamine was added as a 0.15 M solution in bicarbonate-buffered saline, and glutamate as a 0.2 M solution of the sodium salt. Sodium oleate was prepared as described by Ross *et al.* (1967). Ammonia was added as a solution of NH_4Cl .

Neutralized perchloric acid extracts of freeze-clamped kidney, perfusion medium, or urine, were prepared as described by Weidemann *et al.* (1969).

Metabolic rates were measured by analysing at least four samples of perfusion medium between 20 and 100 min of perfusion, when the time-courses of the major processes were approximately linear. Urine was collected from the ureter in portions of at least 0.3 ml. When the concentrations of ammonia or glucose were measured in urine, the rate of their formation was added to that in the medium. Metabolic rates were not dependent on whether urine was collected. The dry weight of kidneys was measured at the end of perfusions.

Slice experiments

Incubations of kidney-cortex slices were carried out to compare the rates of glutamine metabolism in this system with those of the perfused kidney. Slices were cut freehand from moist kidney, and incubated for 1 h at 37°C in 4 ml of bicarbonate-buffered saline (Krebs *et al.*, 1963). The incubation was terminated with perchloric acid, after slices were removed from the medium with forceps, and ammonia and glucose were measured in the incubation medium.

Analytical methods

Metabolic substrates and products (including glutamine, ammonia and glutamate) and tissue intermediates, were in general measured by established NADH- or NADPH-linked enzymic methods, as described by Hems & Brosnan (1970). Determination of P_i was by the method of Martin & Doty (1949), bicarbonate was measured manometrically, and glucose by a glucose oxidase method. The pH of perfusion medium or urine was determined with a glass electrode (Jena dual electrode, from Schott and Gen., Mainz, Germany).

Glutamine was determined as ammonia or glutamate after acid hydrolysis (1.6 M- H_2SO_4 at 100°C for 5 min). To follow decreases in the concentration of glutamine in the perfusion medium, glutamate was determined (Bernt & Bergmeyer, 1965) after acid hydrolysis and alkaline treatment (45 min at 100°C in 1 M-KOH) to convert any pyroglutamate into glutamate. Glutamine synthesis from glutamate in perfusions was followed by measuring the ammonia produced by acid hydrolysis of glutamine. After incubation in H_2SO_4 , samples were made 0.1 M with respect to sodium phosphate and adjusted to pH 7.4 with NaOH. Ammonia was determined enzymically (Kirsten *et al.*, 1963). No asparagine (measured as acid-labile aspartate) was formed from glutamine during perfusion, so that asparagine did not interfere with this procedure by contributing acid-labile ammonia. In a few experiments, glutamine hydrolysis was achieved with *E. coli* glutaminase (Lund, 1970), and then glutamate or ammonia were measured as described above.

Measurement of the production of ammonia from glutamine by kidneys was a major aim of the present work. In control experiments, the characteristics of this process were investigated in the perfusion apparatus in the absence of a kidney. Formation of ammonia from glutamine was not more than 1%/h; the rates of ammonia release by kidneys have not been corrected for this factor. Ammonia loss from the perfusion apparatus during circulation of the medium at 37°C, measured with 2–8 mM-NH₄Cl, was negligible at pH 7.4.

Results

Formation of ammonia and glucose from glutamine in perfused kidneys from fed rats

During perfusion of kidneys with glutamine, ammonia was released into the medium and urine. The total ammonia released was 1–1.7 mol/mol of glutamine removed (Table 1). The rates of ammonia formation and glutamine utilization were dependent on the concentration of glutamine over the range 1–10 mM (Table 1). The rate of glucose formation from glutamine (Table 1) was about one-third the rate from glutamate (Table 2) and was not significantly stimulated by 0.5 mM-sodium oleate (Table 1). During perfusion with glutamine (5 mM) release of glutamate into the medium was negligible (0–30 µmol/h per g of dry kidney). When kidneys were perfused with 5 mM-glutamine in the presence of 3 mM-ammonia (three perfusions) the average rates of addition of further ammonia to the medium (225 µmol/h per g) and of glutamine removal (160 µmol/h per g) were less than in the absence of ammonia (see Table 1).

The hydrolysis of glutamine (5 mM) by perfused kidneys was not greatly affected by anaerobiosis [gas phase CO₂+N₂ (5:95)]; ammonia release was 331 µmol/h per g dry wt. of kidney (average rate from two experiments). However, glutamate release was 215 µmol/h per g (two experiments). Thus anaerobic conditions inhibited the further metabolism of glutamate derived from glutamine, which shows that the release of ammonia from the amide group of glutamine is not dependent on the disposal of the resulting glutamate as glucose. In the anaerobic situation lactate formation from glutamine was 47 µmol/h per g (two perfusions) and no glucose, aspartate or citrate were released into the medium.

During aerobic perfusions with glutamine, the lactate concentration in the perfusion medium was low and variable; the rate of release of lactate was usually less than 15 µmol/h per g dry wt. Alanine was measured in the medium during three aerobic perfusions with 5 mM-glutamine and 0.5 mM-oleate; its rate of formation was 5 µmol/h per g in the absence of added glucose (average of two perfusions) and 17 µmol/h per g in its presence (one perfusion). No aspartate, asparagine, oxoglutarate or malate could be detected in the medium during perfusion with 5 mM- or 10 mM-glutamine; thus the concentration of these products, if they were present in the perfusion medium, was less than 10 nM.

Formation of ammonia and glucose from glutamate in the perfused kidney from fed rats

A major reaction of glutamate is its conversion into glutamine (Krebs, 1935; Nishiitsutsuji-Uwo *et al.*, 1967), which accounted in the present experiments

Table 1. *Metabolism of glutamine by perfused kidneys from fed and from starved rats*

Kidneys were perfused with bicarbonate-buffered saline. The initial bicarbonate concentration was either 25 mM (pH 7.4) or 10–13 mM (pH 7.1). Starvation of the rats was for 48 h. Other details are given in the text. Results are given as means ± S.E.M. for the numbers of observations indicated in parentheses.

State of rat	Concn. in medium (mM)		Initial pH of medium	Metabolic changes (µmol/h per g dry wt.)		
	Glutamine	Other additions		Glutamine	Glucose	Ammonia
Fed	—	—	7.4	—	<2 (5)	47 ± 4 (5)
Fed	—	Oleate (0.5) + glucose (5)	7.4	—	—	11 ± 7 (3)
Fed	1	—	7.4	−45 ± 5 (3)	—	119 ± 9 (3)
Fed	5	—	7.4	−215 ± 27 (5)	16 ± 4 (6)	358 ± 24 (5)
Fed	5	Oleate (0.5)	7.4	−185 ± 12 (3)	22 ± 5 (3)	245 ± 19 (3)
Fed	5	Oleate (0.5) + glucose (5)	7.4	−195 ± 14 (5)	—	186 ± 14 (5)
Fed	5	—	7.1	−182 ± 29 (6)	16 ± 2 (6)	326 ± 27 (6)
Fed	10	—	7.4	—	26 ± 7 (3)	540 ± 70 (3)
Starved	5	—	7.4	−267 ± 53 (4)	46 ± 7 (5)	354 ± 18 (5)
Starved	5	—	7.1	−187 ± 13 (3)	33 ± 3 (3)	300 ± 64 (3)

Table 2. *Metabolism of glutamate by perfused kidneys from fed and from starved rats*

Kidneys were perfused as described in Table 1. Other details are given in the text. Results are given as means $\pm$ S.E.M. for the numbers of observations indicated in parentheses.

State of rat	Concn. of glutamate in medium (mM)	Initial pH of medium	Metabolic changes (μ mol/h per g dry wt.)			
			Glutamate	Glutamine	Glucose	Ammonia
Fed	5	7.4	-445 ± 39 (6)	182 ± 16 (3)	59 ± 5 (7) [†]	15 ± 4 (7)
Fed	5	7.1	-430 ± 30 (8)	137 ± 14 (6)	68 ± 4 (10) [†]	135 ± 11 (5)
Fed	10	7.4	—	220 ± 19 (3)	79 ± 7 (3)	52 ± 21 (3)
Starved	5	7.4	-518 ± 49 (8)*	196 ± 14 (3)*	108 ± 3 (3)	10 ± 5 (3)
Starved	5	7.1	—	—	89 ± 6 (4)	—

* From Nishiitsutsuji-Uwo *et al.* (1967).

[†] $P < 0.05$.

for most of the nitrogen and nearly half of the carbon of the glutamate which was consumed (Table 2). Glucose formation accounted for further carbon (Table 2). Addition of glucose (5mM) plus oleate (0.5mM) did not affect the rate of glutamine synthesis from 5mM-glutamate. Synthesis of glutamine was linear with time until the glutamine concentration in the medium reached at least 1.3mM. During perfusion with glutamate at pH 7.4 the formation of ammonia was negligible (Table 2). Aspartate formation occurred at 30–40 μ mol/h per g dry wt. initially, and then slowed.

During perfusion of kidneys from normal rats with 5mM-glutamine and 5mM-glutamate together (in the presence or in the absence of sodium oleate and glucose) ammonia was released into the medium at approximately the rate of glutamate removal. Net formation or breakdown of glutamine was not detected. In the absence of glucose gluconeogenesis (74 μ mol/h per g) occurred at the rate normally observed from 5mM-glutamate alone, and glutamate removal and ammonia formation were about 300 μ mol/h per g (two perfusions). When glucose (5mM) and oleate (1mM) were present in addition to glutamate and glutamine (each 5mM), glutamate removal and ammonia formation rates were approx. 100 μ mol/h per g (two perfusions). These results confirm in general the observations made by Preuss (1971) with isolated kidney tubules.

Formation of glucose and ammonia from glutamine or glutamate by the perfused kidney of the 7–10-day acidotic rat

Gluconeogenesis from glutamine was enhanced in perfused kidneys from rats that had been given 1.5% (w/v) (0.28M) NH_4Cl to drink for 7–10 days (Table 3). The increase in utilization of glutamine was approximately the carbon equivalent of the extra glucose formed.

The ratio of glutamine removed to ammonia released closely approached 1:2 in the acidotic rat kidney. The effect of sodium oleate (0.5mM) and glucose (5mM) in producing a decrease in ammonia production from glutamine (see Table 1) also occurred in acidotic rat kidneys (Table 3).

When kidneys from rats given NH_4Cl to drink were perfused with 5mM-glutamate, glutamine synthesis was much slower than in normal kidney, whereas ammonia release was almost equivalent to the removal of glutamate from the medium (Table 3). Gluconeogenesis was enhanced (Table 3).

To check whether the effects of administered NH_4Cl were due to ammonia, NH_4HCO_3 (also 0.28M; checked after administration to the rat) was given as drinking fluid to rats for 5 days. Perfused kidneys from rats treated in this way exhibited the glutamine or glutamate metabolism characteristic of the normal fed state (each a single perfusion).

Metabolism of glutamine and glutamate by perfused kidneys from starved rats

The rate of gluconeogenesis from glutamate or glutamine was increased by previous starvation of the animals for 48h (Tables 1 and 2). However, formation of ammonia by the kidney was not increased in perfused kidneys from 48h-starved rats (Table 1). This result shows that the stimulation of gluconeogenesis by starvation is not solely due to acidosis.

Characteristics and metabolism of the kidney perfused with acidified medium

To assess the effects of acute acidosis, kidneys from fed or starved rats were perfused with medium acidified with HCl so that the bicarbonate concentration was 10–13mM. Since the gas phase was

Table 3. Metabolism of glutamine and of glutamate by perfused kidneys from acidotic rats

Kidneys were perfused with bicarbonate-buffered saline at pH7.4. The methods for induction of acidosis and other experimental details are given in the text. Results are given as the averages of two perfusions or as means $\pm$ s.e.m. for the numbers of observations indicated.

Duration of acidosis (days)	Concn. of substrate in medium (mM)	No. of observations	Metabolic changes (μ mol/h per g dry wt.)			
			Glutamate	Glutamine	Glucose	Ammonia
7-10	—	5	—	—	6 $\pm$ 1	50 $\pm$ 3
0.25	Glutamine (5)	2	10	-180	59	465
0.25	Glutamate (5)	3	-505 $\pm$ 35	190 $\pm$ 8	96 $\pm$ 11	140 $\pm$ 3
0.5	Glutamine (5)	2	30	-240	74	550
1	Glutamine (5)	2	10	-300	89	565
1	Glutamate (5)	3	-410 $\pm$ 134	21 $\pm$ 11	94 $\pm$ 13	300 $\pm$ 65
7-10	Glutamine (1)+oleate (0.5)+glucose (5)	3	—	-212 $\pm$ 23	—	331 $\pm$ 70
7-10	Glutamine (5)	5	25 $\pm$ 6	-387 $\pm$ 37	101 $\pm$ 11	721 $\pm$ 57
7-10	Glutamine (5)+oleate (0.5)+glucose (5)	3	20 $\pm$ 6	-325 $\pm$ 49	—	538 $\pm$ 76
7-10	Glutamate (5)	6	-345 $\pm$ 40	30 $\pm$ 6	113 $\pm$ 14	321 $\pm$ 37

Fig. 1. Dependence on pH of ammonia concentration in urine from the perfused rat kidney

Kidneys from fed rats were perfused with bicarbonate-buffered saline at pH7.1 or 7.4; the urine pH was 6.3-6.9 or 6.8-7.1 respectively. The concentration of glutamine in the medium was 1 mM (●) or 5 mM (o). Other details are given in the text. Each point represents a single perfusion.

CO₂ + O₂ (5:95) the pH was 7.1-7.15. In this situation the urine produced by the kidney was more acidic (pH6.2-6.9) than during perfusion at pH7.4 (urine pH6.8-7.2). The concentration of ammonia in the urine was dependent on that in the medium (Fig. 1), and was higher when the urine became more acid (Fig. 1). This increase occurred within a few minutes of acidification of the medium during perfusion. These characteristics occur in the intact animal during acute acidosis, and they suggest that ammonia is as rapidly diffusible in the isolated kidney as it is *in vivo* (Stone *et al.*, 1967).

Reabsorption from the urine of glutamine or glutamate, present in the perfusion medium at a concentration of 5mM, was negligible during perfusion at normal pH; however, if the perfusion medium and the urine were acidified (as described above), tubular reabsorption of glutamine or glutamate occurred such that their concentration in the urine became about one-half that in the perfusion medium.

During perfusion with glutamine in acidified medium there was no stimulation of gluconeogenesis, substrate removal or total ammonia release, relative to rates at pH7.4, in kidneys from fed or starved rats (Table 1). This lack of an effect of acidification was confirmed in other experiments with 1 mM-glutamine. In contrast with the results with glutamine, acidification of the medium perfusing the kidney from fed rats (but not starved rats) stimulated formation of glucose and ammonia from glutamate (Table 2). There was no increase of glutamate uptake; thus increased glucose formation was at the expense of glutamine synthesis (Table 2).

Time-course of the alterations in rates of glutamine and glutamate metabolism during acidosis

The alteration in glutamine metabolism caused by chronic acidosis could be due to changes in either amount or activity of the relevant enzymes. Elucidation of the time-course of the alteration provides one possible way of distinguishing between these alternatives. The results of the experiments at pH 7.1 (Table 1) demonstrate that no alteration in the rate of glutamine metabolism is detectable in perfused kidney within 1–2 h of acidification of the medium.

To elucidate the further time-course of metabolic alterations, induction of rapid controlled acidosis was required. The technique of infusion of HCl (0.25 M) into the right side of the heart was employed (Hems & Brosnan, 1971). After a time-interval kidneys were perfused with 5 mM-glutamine at normal pH or with acidified medium. The enhancement of gluconeogenesis and ammonia production from glutamine was well developed after 6 or 12 h acidosis

(Table 3). During perfusions of 6 h-acidotic rat kidneys at normal pH an eventual fall-off in the rate of ammonia formation and of gluconeogenesis was observed after about 90 min; however, at pH 7.1 ammonia release from glutamine (about 500 $\mu\text{mol/h}$ per g) was rapid, and did not fall off during perfusion.

In the perfused kidney from acutely (6 h-) acidotic rats the enhancement of gluconeogenesis from glutamate was already as marked as that in acidosis of longer duration (Table 3). Ammonia release was increased, relative to that of the normal kidney perfused at pH 7.4. No decrease of glutamine synthesis was detected when kidneys from 6 h-acidotic rats were perfused at pH 7.4, although inhibition at pH 7.1 was present (results not tabulated) as in kidney from rats in normal acid-base balance (Table 2).

To assess the metabolic alteration present in perfused kidney 24 h after induction of acidosis intragastric administration of 0.75 M- NH_4Cl was used.

Table 4. *Metabolism of glutamine and of glutamate by kidney-cortex slices from fed rats*

Kidney-cortex slices were incubated in bicarbonate-buffered saline at 38°C; the initial bicarbonate concentration was 25 mM or 10 mM. Other details are given in the text. Results are given as means $\pm$ S.E.M. for the numbers of observations indicated.

Concn. of substrate in medium (mM)	Initial pH of medium	No. of observations	Metabolic changes ($\mu\text{mol/h}$ per g final dry wt.)	
			Glucose	Ammonia
—	7.4	4	7 $\pm$ 2	61 $\pm$ 15
Glutamine (5)	7.4	8	34 $\pm$ 4	550 $\pm$ 47
Glutamine (5)	7.1	7	44 $\pm$ 6	548 $\pm$ 57
Glutamate (5)	7.4	8	42 $\pm$ 3	100 $\pm$ 12
Glutamate (5)	7.1	8	56 $\pm$ 4	115 $\pm$ 5

Table 5. *Metabolism of glutamine and of glutamate by kidney-cortex slices from acidotic rats*

Kidney-cortex slices from 7–10-day-acidotic rats were incubated at pH 7.4, as described in Table 4. Other experimental details are given in the text. Results are given as means $\pm$ S.E.M. for the numbers of observations indicated.

Concn. of substrate in medium (mM)	No. of observations	Metabolic changes ($\mu\text{mol/h}$ per g final dry wt.)	
		Glucose	Ammonia
—	4	15 $\pm$ 3	112 $\pm$ 11
Glutamine (5)	6	130 $\pm$ 13	880 $\pm$ 56
Glutamine (10)	6	133 $\pm$ 17	1100 $\pm$ 45
Glutamate (5)	9	128 $\pm$ 13	369 $\pm$ 23
Glutamate (10)	6	135 $\pm$ 18	385 $\pm$ 25
2-Oxoglutarate (1)	4	80 $\pm$ 5	57 $\pm$ 16
2-Oxoglutarate (2)	4	136 $\pm$ 10	89 $\pm$ 24
2-Oxoglutarate (5)	5	205 $\pm$ 19	57 $\pm$ 8

The rate of gluconeogenesis from glutamine was similar to that in rats which were acidotic for 7–10 days (Table 3). In a control experiment rats received NH_4HCO_3 in the same dose; 24 h later the metabolism of glutamine was the same as in kidney from normal rat (two perfusions).

The rate of glutamine synthesis from glutamate in perfused kidney was decreased 20–30 h after a 4 h infusion of HCl (to about $100\text{ }\mu\text{mol/h}$ per g at pH 7.4; single perfusions), or in perfused kidney from animals to which NH_4Cl was administered 24 h before (Table 3). Thus a decline in the capacity for glutamine synthesis in perfused kidney developed gradually between 6 and 30 h after an acute acid load. Part of the effect of NH_4Cl may have been due to the ammonia load, since administration of NH_4HCO_3 to rats (in similar amount to the NH_4Cl) caused a partial loss of capacity for glutamine synthesis (measured 24 h later at pH 7.4; single perfusion).

Metabolism of glutamine and glutamate by kidney-cortex slices

The observation by Kamm *et al.* (1967) that gluconeogenesis from glutamine in kidney-cortex slices is stimulated at pH 7.1 was confirmed (Table 4). In other experiments the rate of glucose formation from glutamine (5 or 10 mM) or glutamate was tested at bicarbonate concentrations of 5–25 mM. The gas phase was CO_2+O_2 (5:95) so that pH at 37°C was 6.9–7.4. Glucose formation was fastest at pH 7.1–7.2. Although glucose formation from glutamine was faster at pH 7.1 than at pH 7.4 no increase in ammonia release was detected (Table 4).

The enhancement of gluconeogenesis from glutamine and glutamate by acidosis, reported for kidney-cortex slices (Goodman *et al.*, 1966; Goorno *et al.*, 1967; Alleyne, 1970; Churchill & Malvin, 1970*a,b*), was confirmed (compare Tables 4 and 5).

Concentrations of metabolites in the kidney perfused with 5 mM-glutamine

To elucidate the sites at which the acceleration of gluconeogenesis was achieved by acidosis, the concentrations of relevant pathway intermediates were measured during perfusion with glutamine. In particular the contents of glutamate, glucose 6-phosphate and ATP were increased in perfused kidneys from rats which were acidotic for 12 h or 7–10 days (Table 6). The increase in the concentrations of C_3 intermediates of glycolysis and the decrease in those of certain intermediates of the citrate cycle, which occur in the kidneys of acidotic rats *in vivo* (Hems & Brosnan, 1971), were not discernible in perfused kidneys (Table 6).

Table 6. Concentrations of metabolic intermediates in rat kidneys perfused with 5 mM-glutamine

Kidneys from 12 h- or 7–10-day-acidotic rats were perfused with bicarbonate-buffered saline at pH 7.4 and freeze-clamped after 40 min. Other details are given in the text. Results were calculated as described by Weidemann *et al.* (1969), and are given as means $\pm$ S.E.M.; the number of observations is in parentheses if different from the number of perfusions.

Duration of acidosis	No. of perfusions	Concn. of metabolite (nmol/g fresh wt. of kidney)									
		Lactate	Pyruvate	Phosphoenolpyruvate	2-Phosphoglycerate	3-Phosphoglycerate	Triose phosphates	1-Glycerophosphate	Fructose 1,6-diphosphate	Fructose 6-phosphate	
Control	4	380 ± 60	27 ± 7	94 ± 11	23 ± 3	171 ± 16	10 ± 4	157 ± 41	<10	<10	
12h	3	290 ± 125	26 ± 6	100 ± 20	18 ± 3	179 ± 38	13 ± 4	158 ± 58	<10	<10	
7–10 days	4	280 ± 50	39 ± 6	83 ± 35	20 ± 4	194 ± 17	10 ± 4	196 ± 13	<10	11 ± 5	
Duration of acidosis	No. of perfusions	Glucose 6-phosphate	2-Oxoglutarate	Glutamate	Aspartate	Malate	ATP	ADP	AMP	P _i	
Control	4	12 ± 3	277 ± 56	2930 ± 300	920 ± 60	81 ± 17	1190 ± 79 (3)	595 ± 120 (3)	210 ± 23 (3)	4030 ± 390 (3)	
12h	3	34 ± 3	243 ± 36	4080 ± 400	1150 ± 50	76 ± 9	1310 ± 58	635 ± 75	175 ± 35	3830 ± 725	
7–10 days	4	52 ± 4	244 ± 10	6230 ± 260	1120 ± 50	66 ± 20	1920 ± 145	700 ± 90	188 ± 23	3565 ± 270	

Discussion

Regulation of renal glutamine breakdown in the rat

The rate of ammonia release from glutamine in the perfused kidney from 7–10-day-acidotic rats corresponds to a rate of about 8 mmol/24 h per 300 g rat. This is of the same order as the rate of ammonia excretion in these rats (see Hems & Brosnan, 1971), in accord with the likelihood that circulating glutamine provides most of the excreted ammonia in metabolic acidosis (see Pitts, 1964). The present measurements confirm that both nitrogen atoms of glutamine, and one from glutamic acid, can be released as ammonia in kidney during acidosis (see Pitts *et al.*, 1965; Kamm & Asher, 1970; Preuss, 1971).

The low rates of glutamine utilization and gluconeogenesis from glutamine by perfused kidneys from rats in normal acid–base balance, compared with those when glutamate was the substrate, suggest that the glutaminase reaction controls the uptake of glutamine in kidney of such rats. In contrast, perfused kidneys from 7–10-day-acidotic rats exhibited rates of substrate utilization (and gluconeogenesis) from glutamine and glutamate that were approximately equal. This de-restriction of renal glutamine metabolism in acidotic rats can be explained by an increase in the renal activity of glutaminase (Davies & Yudkin, 1952; Leonard & Orloff, 1955; Rector *et al.*, 1955; Goldstein, 1966; Alleyne & Scullard, 1969).

Control of renal gluconeogenesis by acidosis

The present experiments confirm that renal gluconeogenesis is enhanced by acidosis (Goodman *et al.*, 1966; Goorno *et al.*, 1967; Kamm & Asher, 1970; Alleyne, 1970; Churchill & Malvin, 1970a,b; Pagliara & Goodman, 1970) and show that this phenomenon is demonstrable in the whole kidney. This overall increase in net gluconeogenesis in the whole kidney of acidotic rats is partly due to a diminished rate of medullary aerobic glycolysis (Hems & Gaja, 1972).

In perfused kidneys from 7–10-day-acidotic rats the extra glutamine uptake exactly accounted for the extra glucose formed. Thus it is probable that glucose is the major product of the extra glutamine consumed by the kidney during acidosis, at least in the rat (for discussion see Orloff & Burg, 1971).

Alterations in the renal contents of metabolic intermediates in acidotic rats *in vivo* suggest that a key regulatory step in gluconeogenesis is that catalysed by phosphoenolpyruvate carboxylase (previously known as phosphoenolpyruvate carboxykinase) (Alleyne, 1968, 1970; Flores & Alleyne, 1971; Hems & Brosnan, 1971). In particular, the estimated renal cortical concentration of oxaloacetate is decreased in acidosis, and that of phosphoenolpyruvate is increased. In the perfused kidney, although accelera-

tion of gluconeogenesis by acidosis was about sixfold from glutamine and twofold from glutamate, this pattern of alteration was not detected. The most marked change in the kidney perfused with glutamine was an increase in glucose 6-phosphate in acidosis. This suggests that acceleration at fructose diphosphatase due to acidosis was regulatory in the conditions of perfusion, which differ from those *in vivo*, for example in the near-saturating concentrations of the single substrate (glutamine) and in the recycling of products such as ammonia.

In acidotic rats there is a diminished renal concentration of certain citrate-cycle intermediates, e.g. malate and 2-oxoglutarate (Alleyne, 1968; Goldstein, 1967; Hems & Brosnan, 1971). These decreases were not detected in the perfused kidney, confirming that they are not necessarily connected with other renal metabolic alterations in acidosis (see Hems & Brosnan, 1971). Thus it has been shown that 2-oxoglutarate added to the perfusion medium can diminish ammonia release from amino acids by a perfused rat kidney (Pitts, 1971). The present results show that such control (by cellular 2-oxoglutarate) is not a necessary feature of the events of acidosis, since 2-oxoglutarate was not diminished in the perfused kidney of acidotic rats, although ammonia formation from glutamine was enhanced.

A relationship between gluconeogenesis and renal ammonia production in metabolic acidosis that has been proposed is that a decrease in tissue glutamate, caused by accelerated gluconeogenesis, could de-inhibit the glutaminase enzyme (Goldstein, 1966; Goodman *et al.*, 1966; for review see Orloff & Burg, 1971). However, in the perfused kidney from acidotic rats there was an increase in gluconeogenesis and ammonia production from glutamine, despite an increase in renal glutamate concentration (compared with perfused kidney from normal rats). Hence de-inhibition of glutaminase by glutamate is not obligatory for the occurrence of accelerated glutamine hydrolysis in kidney during acidosis.

Fate of glutamine and glutamate in perfused rat kidney

If the rates of substrate uptake are compared with the formation of detected products in perfused kidney from normal or acidotic rats, about 150 μmol of glutamine or glutamate carbon/h per g dry wt. is not accounted for (assuming accepted metabolic pathways). The most likely fate of most of this extra carbon is oxidation to CO_2 . This would consume 4.5 μmol of $\text{O}_2/\mu\text{mol}$ of glutamine or glutamate, i.e. about 675 $\mu\text{mol/h}$ per g dry wt. This value for extra O_2 consumption is of the same order as the substrate-induced increments in O_2 uptake in kidney-cortex slices (from normal rats) incubated in glutamine or glutamate (D. A. Hems, unpublished work) or in kidney perfused with pyruvate (Nishiitsutsuji-Uwo *et al.*,

1967). Hence CO_2 formation probably explains most of the uptake of glutamine or glutamate unaccounted for in the present experiments. Since the carbon unaccounted for was similar in perfused kidneys from normal and acidotic rats, conversion of glutamine into CO_2 seems unlikely to be much altered by acidosis, at least in the rat (for discussion see Orloff & Burg, 1971).

Regulation of renal glutamine synthesis

The rat, unlike the dog, contains significant renal glutamine synthetase activity (for references see Orloff & Burg, 1971). The role of this enzyme is not well understood, but it is a reasonable assumption that it is implicated in the regulatory events connected with renal ammonia production (see Damian & Pitts, 1970; Lyon & Pitts, 1969). This applies even if the rapid rate of glutamine release by kidney from non-acidotic rats does not reflect events *in vivo*. Indeed, the perfusions with glutamine and glutamate together, in which there was no net glutamine synthesis, suggest that *in vivo*, where glutamine is more abundant than glutamate in blood, net glutamine release by the kidney is not likely to be rapid.

The decreased rate of formation of glutamine from glutamate by perfused kidney from 7–10-day-acidotic rats, which is in accord with observations *in vivo* with ^{14}C -labelled precursors (Damian & Pitts, 1970; Lyon & Pitts, 1969), was not due to lack of availability of ATP, since the tissue ATP concentration in perfused kidneys from 7–10-day-acidotic rats was not decreased. There is no decline in maximal assayable glutamine synthetase activity in acidosis (Janicki & Goldstein, 1969). The inhibitory effect of H^+ on glutamine synthesis in the present experiments suggests that increased tissue H^+ concentration could contribute to the inhibition by acidosis of glutamine synthesis. The increased activity of glutaminase in kidney from acidotic rats (Davies & Yudkin, 1952; Leonard & Orloff, 1955; Rector *et al.*, 1955) could also contribute to the decreased glutamine synthesis in the perfused kidney.

Renal response to acute acidosis in rats

From experiments with kidney-cortex slices incubated at various pH values Kamm *et al.* (1967) (see also Herrera *et al.*, 1966) concluded that acidosis could rapidly and directly initiate activation of renal gluconeogenesis, and thus of glutamine uptake and ammonia excretion. The present results do not support this possibility, in that acidification of the medium perfusing normal kidneys for 2–3 h did not bring about acceleration of glucose or ammonia production from glutamine, or uptake of glutamine. Rather, the perfusions of kidneys from 6–24 h-acidotic rats show that about 6 h *in vivo* is required for

a half-maximal increase in the capacity of renal gluconeogenesis from glutamine. The capacity for utilization of glutamine by the perfused kidney was still increasing 24 h after the induction of acidosis. Such a time-course approximately parallels that of the increase in assayable renal glutaminase activity (Rector *et al.*, 1955; Alleyne & Scullard, 1969).

In contrast with the perfusions with glutamine, acidification of medium containing glutamate did enhance gluconeogenesis and ammonia formation. This confirms the results (obtained with slices) reported by Kamm *et al.* (1967) and Preuss (1969). There was no increase in glutamate utilization, i.e. the increase in ammonia formation was at the expense of glutamine synthesis. Accelerated conversion of renal glutamate (perhaps not derived from blood glutamine) into ammonia and glucose may occur in acute acidosis *in vivo* in the rat. This possibility is supported by the observation that the decrease in renal glutamate concentration is greatest in acute acidosis (Goldstein & Schooler, 1967; Hems & Brosnan, 1971). The nitrogen of such a pool of renal glutamate (i.e. of potential ammonia for excretion) in acute acidosis in the rat could be derived from circulating glutamate, or other amino acids that could transaminate. It has been shown that precursors other than glutamine or glutamate can release ammonia in isolated kidney preparations from acidotic rats (Davies & Yudkin, 1952; Pitts, 1971).

It is noteworthy that inhibition of glutamine synthesis during perfusion at pH 7.1, associated with increased ammonia release from glutamate, was the most clear-cut effect of acidification of the medium that was revealed in the perfusions of normal rat kidney. During acute metabolic acidosis such inhibition of the glutamine synthetase reaction could cause a rapid increase in renal cellular NH_4^+ concentration. This would provide the required increase in the NH_4^+ concentration gradient between the tissue and the tubular lumen, thus accelerating the rate of 'trapping' of ammonia in urine.

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