Fate of Glutamate Carbon and Nitrogen in Isolated Guinea-Pig Kidney-Cortex Tubules

EVIDENCE FOR INVOLVEMENT OF GLUTAMATE DEHYDROGENASE IN GLUTAMINE SYNTHESIS FROM GLUTAMATE

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1. The pathways and the fate of glutamate carbon and nitrogen were investigated in isolated guinea-pig kidney-cortex tubules. 2. At low glutamate concentration (1 mM), the glutamate carbon skeleton was either completely oxidized or converted into glutamine. At high glutamate concentration (5 mM), glucose, lactate and alanine were additional products of glutamate metabolism. 3. At neither concentration of glutamate was there accumulation of ammonia. 4. Nitrogen-balance calculations and the release of $^{14}\text{CO}_2$ from L-[1- ^{14}C]glutamate (which gives an estimation of the flux of glutamate carbon skeleton through α -oxoglutarate dehydrogenase) clearly indicated that, despite the absence of ammonia accumulation, glutamate metabolism was initiated by the action of glutamate dehydrogenase and not by transamination reactions as suggested by Klahr, Schoolwerth & Bourgoignie [(1972) *Am. J. Physiol.* **222**, 813–820] and Preuss [(1972) *Am. J. Physiol.* **222**, 1395–1397]. Additional evidence for this was obtained by the use of (i) amino-oxyacetate, an inhibitor of transaminases, which did not decrease glutamate removal, or (ii) L-methionine DL-sulphoximine, an inhibitor of glutamine synthetase, which caused an accumulation of ammonia from glutamate. 5. Addition of NH_4Cl plus glutamate caused an increase in both glutamate removal and glutamine synthesis, demonstrating that the supply of ammonia via glutamate dehydrogenase is the rate-limiting step in glutamine formation from glutamate. NH_4Cl also inhibited the flux of glutamate through glutamate dehydrogenase and the formation of glucose, alanine and lactate. 6. The activities of enzymes possibly involved in the glutamate conversion into pyruvate were measured in guinea-pig renal cortex. 7. Renal arteriovenous-difference measurements revealed that *in vivo* the guinea-pig kidney adds glutamine and alanine to the circulating blood.

Since the initial work of Krebs (1935), it is known that, in contrast with kidney-cortex slices of dog, cat, pig, rat, sheep and pigeon, slices of guinea pig and rabbit do not release ammonia when incubated with glutamate. Krebs concluded that this was so in both the latter species because the ammonia formed by the deamination of glutamate via glutamate dehydrogenase was used for glutamine synthesis (see Krebs, 1935). However, the role of glutamate dehydrogenase in the metabolism of glutamate by guinea-pig and rabbit kidney slices has been questioned by Klahr *et al.* (1972) and Preuss (1972), because these authors observed that glucose was formed from glutamate without concomitant accumulation of

ammonia; they concluded that the initial step in glutamate metabolism was transamination rather than oxidative deamination. So the pathways of renal metabolism of glutamate in these species remain uncertain.

In an attempt to clarify this subject, we studied glutamate metabolism in isolated guinea-pig kidney tubules. The data obtained support the view of Krebs (1935) that glutamate dehydrogenase plays a central role in the renal metabolism of glutamate in this species. They also provide precise information on the metabolic fate of both the nitrogen and the carbon fragment of glutamate in guinea-pig kidney-cortex.

Experimental

Guinea pigs

Kidneys were from female guinea pigs (400–600 g) of the Dunkin–Hartley albino strain, obtained from Iffa-Credo, St. Germain-sur-l'Arbresle, France. The animals were fed on a standard diet (U.A.R., Villemoisson-sur-Orge, France).

Preparation of kidney-cortex tubules

Kidney tubules were prepared as described by Guder *et al.* (1971), as modified by Baverel *et al.* (1978).

Incubation procedures

Incubations were carried out at 37°C in a shaking water bath, in 25 ml stoppered Erlenmeyer flasks in an atmosphere of O₂/CO₂ (19:1). In all experiments the tubular fragments obtained were incubated with or without L-glutamate (1 or 5 mM) in 4 ml of Krebs–Henseleit medium (Krebs & Henseleit, 1932). In all experiments each experimental condition was carried out in duplicate. Incubations were terminated by adding HClO₄ (2%, v/v, final concn.) to each flask. In all experiments, zero-time flasks, with and without substrates, were prepared by adding HClO₄ before the tubules. After removal of the denatured protein by centrifugation (4000 g for 10 min), the supernatant was neutralized with 20% (w/v) KOH before metabolites were assayed.

In experiments where L-[1-¹⁴C]glutamate was used as substrate, incubation, deproteinization, collection and measurement of the ¹⁴CO₂ formed were carried out as described by Baverel & Lund (1979); the medium was then treated as described above.

Analytical methods

Lactate, pyruvate, glucose, alanine, aspartate, citrate, 2-oxoglutarate, fumarate, malate and glutamate, and also the dry weight of the amount of tubules added to the flasks, were determined as previously described (Baverel *et al.*, 1978). Glutamine was measured by the method of Lund (1974), and ammonia by the method of Kun & Kearney (1974). Glycogen was measured by the method of Keppler & Decker (1974).

Measurement of enzyme activities

Pieces of guinea-pig renal cortex were rapidly excised, weighed, and homogenized in 10 vol. of ice-cold double-distilled water in a Potter–Elvehjem glass homogenizer with a motor-driven Teflon pestle. The homogenates were frozen in liquid nitrogen, thawed and re-frozen twice, and then centrifuged at 30000 g for 30 min at 4°C. The supernatant was immediately used for measurement of the activities of glutamate dehydrogenase (EC 1.4.1.2),

alanine aminotransferase (EC 2.6.1.2), phosphoenolpyruvate carboxykinase (EC 4.1.1.32), pyruvate kinase (EC 2.7.1.40), 'malic' enzyme (EC 1.1.1.40) and oxaloacetate decarboxylase (EC 4.1.1.3). Glutamate dehydrogenase was assayed as described by Schmidt (1974), alanine aminotransferase as described by Pollak *et al.* (1965), phosphoenolpyruvate carboxykinase in the direction of oxaloacetate formation by the method of Holten & Nordlie (1965), pyruvate kinase by the method of Feliu *et al.* (1976), 'malic' enzyme by the method of Hsu & Lardy (1969), and oxaloacetate decarboxylase as described for 'supernatant decarboxylase' by Dean & Bartley (1973).

Measurement of the activity of oxaloacetate decarboxylase was carried out at 37°C; all other measurements of enzyme activity were carried out at room temperature (24–26°C). The unit of enzyme activity is defined as the amount of enzyme that transforms 1 μmol of substrate/min under the conditions described.

Reagents

L-Glutamate, glutaminase (grade V), amino-oxyacetic acid, quinolinic acid and L-methionine DL-sulphoximine were supplied by Sigma Chemical Co. (St Louis, MO., U.S.A.). Other enzymes and co-enzymes came from Boehringer Mannheim G.m.b.H. (Mannheim, Germany). L-[1-¹⁴C]glutamate (50 mCi/mmol) was obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). The other chemicals used were of analytical grade.

Calculations

Net substrate utilization and product formation were calculated as the difference between the total contents of the flask (tissue + medium) at the start (zero-time flasks) and after the period of incubation. The metabolic rates are expressed in μmol of substance removed, or produced, per g dry wt. of tubule fragments per unit time. They are reported as means ± S.E.M.

The metabolic fate of the glutamate added as substrate was established by calculating carbon and nitrogen balance. Since the label of the L-[1-¹⁴C]glutamate is released as ¹⁴CO₂ by α-oxoglutarate dehydrogenase, the flux of the glutamate carbon skeleton through this step was calculated by dividing the radioactivity in the ¹⁴CO₂ released by the specific radioactivity of L-[1-¹⁴C]glutamate measured in the zero-time samples (it was assumed that the specific radioactivity of [1-¹⁴C]glutamate remained constant during the incubation period). The difference between the glutamate removed and the flux through α-oxoglutarate dehydrogenase was taken as the amount of [1-¹⁴C]glutamate carbon skeleton converted into glutamine. Complete oxidation of glutamate was calculated as the difference

between the release of $^{14}\text{CO}_2$ minus the sum of glucose (expressed in C_3 units, because 2 molecules of glutamate are needed for the synthesis of each glucose molecule), alanine, aspartate and lactate formed. This method of calculation was used by Baverel & Lund (1979).

Results and Discussion

Time-course of glutamate metabolism: role of glutamate dehydrogenase

When 1 mM - $[1-^{14}\text{C}]$ glutamate was the sole substrate, glutamate removal was linear with time over a 60 min incubation period (Table 1). As found by Krebs (1935), Klahr *et al.* (1972) and Preuss (1972), no ammonia accumulated. Glutamine was the only nitrogenous product formed in significant amount. Glutamine has previously been shown to be an important end-product of glutamate metabolism (see Krebs, 1935; Klahr *et al.*, 1972). There were very small increments of glucose, alanine, aspartate and lactate. The release of $^{14}\text{CO}_2$ shows that 63% of the glutamate carbon skeleton removed was metabolized through the stage of α -oxoglutarate dehydrogenase. No accumulation of α -oxoglutarate, fumarate, malate, citrate or pyruvate was observed. The nitrogen-balance calculations reveal that the amount of nitrogen found (mainly as glutamine) greatly exceeds the amount of nitrogen removed as glutamate, indicating that part of the glutamine found came from endogenous sources; this is in agreement with the high formation of glutamine observed without added glutamate (see Table 1). Since part of the glutamine found was formed from endogenous sources, when glutamate was added as a substrate, the carbon balance cannot be used to estimate the true contribution of the added glutamate to glutamine synthesis. In these experiments, $[1-^{14}\text{C}]$ glutamate is therefore very useful, because it allows calculation of the flux through α -oxoglutarate dehydrogenase and glutamate dehydrogenase. Without the $^{14}\text{CO}_2$ data it would be difficult to calculate the fraction of the added glutamate carbon skeleton that is converted into glutamine (calculated as glutamate removal minus the release of $^{14}\text{CO}_2$), because glutamine synthesis from endogenous sources still occurred in the presence of added glutamate. Thanks to the $^{14}\text{CO}_2$ data, complete oxidation of glutamate could also be calculated, as indicated under 'Calculations'.

The addition of glutamate (1 mM) + NH_4Cl (1 mM) caused a moderate increase in glutamate removal after 30 min (Table 1). The high rate of ammonia removal was accompanied by a considerable increase in glutamine formation. The addition of NH_4Cl caused a decrease in the release of $^{14}\text{CO}_2$, indicating that NH_4Cl partly diverted glutamate from utilization through glutamate dehydrogenase

and α -oxoglutarate dehydrogenase to glutamine synthesis. The nitrogen balance shows that glutamine synthesis from endogenous sources also occurred in the presence of glutamate + NH_4Cl .

When 5 mM - $[1-^{14}\text{C}]$ glutamate was the substrate, the rates of glutamate removal and glutamine synthesis, and the release of $^{14}\text{CO}_2$, were higher than those observed with 1 mM substrate concentration (Table 2). Here again, no ammonia accumulated, but linear glucose formation occurred. Significant amounts of alanine, aspartate and lactate were also produced, especially after 30 min incubation.

The addition of glutamate (5 mM) + NH_4Cl (5 mM) caused a very large increase in the yield of glutamine, which can be explained by an increased conversion of glutamate into glutamine carbon skeleton and amino nitrogen, as well as by an increased provision of ammonia (in the form of NH_4Cl) for the synthesis of the glutamine amide group. The addition of NH_4Cl also suppressed the formation of glucose, alanine, lactate and aspartate. Such an effect of NH_4Cl has been observed by Klahr (1971) and Klahr *et al.* (1972) in rabbit and guinea-pig kidney-cortex slices. No formation of tricarboxylic acid-cycle intermediates, pyruvate or glycogen could be detected. This indicates that the glutamate carbon skeletons that have passed through the stage of α -oxoglutarate dehydrogenase, and which cannot be accounted for by the formation of glucose, alanine, aspartate or lactate, were completely oxidized.

In the presence of glutamate as sole substrate, the large amount of glutamine formed, which cannot be entirely explained by endogenous formation, indicates that added glutamate must contribute not only to the carbon skeleton and the amino nitrogen of glutamine, but also to its amide nitrogen. This clearly demonstrates that some of the glutamate removed must be deaminated via glutamate dehydrogenase to provide the ammonia needed for the synthesis of the glutamine amide group. Thus, in the absence of NH_4Cl , glutamate dehydrogenase appears to be the rate-limiting step of glutamine synthesis, by providing ammonia for the formation of the glutamine amide group. In these experiments, the nitrogen-balance calculations and the high rate of $^{14}\text{CO}_2$ release, which cannot be explained by the small rates of alanine and aspartate synthesis, provide conclusive evidence that glutamate dehydrogenase plays an important role in glutamate metabolism in guinea-pig kidney cortex. Our data therefore are in agreement with the view of Krebs (1935) about the role of this enzyme, but they disagree with the conclusion of Klahr *et al.* (1972) and Preuss (1972), who did not notice that the release of ammonia by oxidative deamination of glutamate was masked by the high capacity of guinea-pig kidney cortex for glutamine synthesis.

Table 1. *Metabolism of low concentration (1 mM) of L-[1-¹⁴C]glutamate in guinea-pig kidney tubules*

Kidney tubules (11.2 ± 0.7 mg dry wt. per flask) were incubated as described in the Experimental section. Results ($\mu\text{mol/g}$ dry wt.) for metabolite removal (–) or production (+) are reported as means $\pm$ s.e.m. for five experiments, each performed in duplicate. Statistical difference was measured by the paired Student's *t* test against the control without NH_4Cl : * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$. Carbon and nitrogen balances were calculated as the difference between the sum of the carbon or nitrogenous substances removed minus the sum of those produced (in the balance calculations, a – sign indicates a deficit and a + sign an excess of carbon skeletons or nitrogen found as compared with those removed).

Substrate added	Incubation (min)	Metabolite removal or production								Carbon balance (C ₃ units)	Nitrogen balance
		Glutamate	Glutamine	Ammonia	Glucose	Alanine	Aspartate	Lactate	¹⁴ CO ₂		
[1- ¹⁴ C]Glutamate (1 mM)	30	–151.2 ± 9.1	+94.0 ± 6.0	–3.9 ± 2.7	+7.1 ± 2.5	+6.3 ± 2.3	+3.1 ± 1.1	+6.4 ± 2.6	+95.0 ± 2.9	–27.2	+42.3
[1- ¹⁴ C]Glutamate (1 mM) + NH_4Cl (1 mM)	30	–193.5 $\pm 12.2^{**}$	+191.5 $\pm 11.0^{***}$	–146.9 ± 15.2	+1.7 ± 1.8	–4.2 $\pm 1.9^*$	+0.1 ± 0.4	–2.5 $\pm 2.2^*$	+62.8 $\pm 1.7^{***}$	–5.2	+38.5
None	30	–5.3 ± 0.6	+40.4 ± 3.9	–5.8 ± 6.4	–0.5 ± 0.3	–5.5 ± 1.5	–1.3 ± 0.7	–1.1 ± 0.9	—	+26.2	+62.9
[1- ¹⁴ C]Glutamate (1 mM)	60	–273.0 ± 13.8	+182.1 ± 8.8	+2.7 ± 3.5	+6.9 ± 2.1	+4.1 ± 2.4	+3.6 ± 2.2	+8.9 ± 2.0	+171.2 ± 7.4	–60.5	+101.6
[1- ¹⁴ C]Glutamate (1 mM) + NH_4Cl (1 mM)	60	–283.2 ± 15.0	+282.5 $\pm 16.1^{***}$	–191.0 ± 24.8	–0.7 $\pm 0.4^*$	–4.9 $\pm 1.1^*$	+0.3 ± 0.9	–2.2 $\pm 1.1^{**}$	+112.4 $\pm 4.2^{***}$	–8.9	+86.2
None	60	–5.5 ± 0.7	+57.3 ± 4.5	+11.4 ± 6.6	–0.5 ± 0.4	–5.7 ± 1.8	–2.5 ± 1.0	–1.6 ± 1.4	—	+43.5	+112.3

Table 2. *Metabolism of high concentration (5 mM) of L-[1-¹⁴C]glutamate in guinea-pig kidney tubules*

Kidney tubules (25.8 ± 0.9 mg dry wt. per flask) were incubated as described in the Experimental section. Results ($\mu\text{mol/g}$ dry wt.) for metabolite removal (–) or production (+) are reported as means $\pm$ s.e.m. for three experiments, each performed in duplicate. Statistical difference was measured by the paired Student's *t* test against the control without NH_4Cl : * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$. Carbon and nitrogen balances were calculated as the sum of the carbon or nitrogenous substances removed minus the sum of those produced (in the balance calculations, a – sign indicates a deficit and a + sign an excess of carbon skeletons or nitrogen found as compared with those removed).

Substrate added	Incubation (min)	Metabolite removal or production								Carbon balance (C ₃ units)	Nitrogen balance
		Glutamate	Glutamine	Ammonia	Glucose	Alanine	Aspartate	Lactate	¹⁴ CO ₂		
[1- ¹⁴ C]Glutamate (5 mM)	30	–326.2 ± 14.5	+154.8 ± 4.2	–2.1 ± 3.0	+17.1 ± 3.5	+23.3 ± 5.5	+12.5 ± 0.8	+15.4 ± 3.0	+215.7 ± 3.8	–86.0	+17.1
[1- ¹⁴ C]Glutamate (5 mM) + NH_4Cl (5 mM)	30	–531.1 $\pm 10.5^{***}$	+448.6 $\pm 20.0^{***}$	–362.8 ± 14.4	–0.1 $\pm 0.2^*$	–0.6 $\pm 1.9^*$	+4.1 ± 4.1	–2.3 $\pm 1.6^*$	+156.5 $\pm 5.2^{***}$	–81.5	+6.8
None	30	–8.6 ± 3.0	+48.3 ± 4.1	+3.3 ± 1.3	–0.7 ± 0.5	–2.9 ± 1.5	–2.8 ± 1.0	–2.1 ± 3.4	—	+30.5	+85.6
[1- ¹⁴ C]Glutamate (5 mM)	60	–646.0 ± 7.1	+354.1 ± 11.2	+5.0 ± 4.2	+34.9 ± 3.5	+19.7 ± 5.2	+0.8 ± 1.3	+6.5 ± 2.3	+387.8 ± 6.5	–195.7	+87.7
[1- ¹⁴ C]Glutamate (5 mM) + NH_4Cl (5 mM)	60	–747.3 $\pm 12.4^*$	+622.2 $\pm 27.7^{***}$	–465.3 ± 18.7	–0.7 $\pm 0.2^{**}$	–3.4 $\pm 2.6^*$	+1.0 ± 0.5	–5.9 ± 1.6	+236.7 $\pm 25.6^*$	–134.8	+29.4
None	60	–10.1 ± 3.9	+68.2 ± 9.2	+15.1 ± 5.0	–0.7 ± 0.3	–4.8 ± 2.7	–4.3 ± 0.1	–3.2 ± 2.9	—	+44.6	+132.3

Table 3. *Effect of amino-oxyacetate on the metabolism of 5 mM-[1-¹⁴C]glutamate in guinea-pig kidney tubules*

Kidney tubules (18.5 ± 1.1 mg dry wt. per flask) were incubated for 60 min as described in the Experimental section. Results ($\mu\text{mol/g}$ dry wt.) for metabolite removal (–) or production (+) are reported as means $\pm$ S.E.M. for three experiments performed in duplicate. Statistical difference was measured by the paired Student's *t* test against the control without amino-oxyacetate: **P* < 0.05; ***P* < 0.02; ****P* < 0.01.

Experimental condition	Metabolite removal or production							
	Glutamate	Glutamine	Ammonia	Glucose	Alanine	Lactate	Aspartate	¹⁴ CO ₂
[1- ¹⁴ C]Glutamate (5 mM)	–666.5 ± 57.8	+317.5 ± 43.2	+9.7 ± 2.7	+35.3 ± 4.1	+38.6 ± 6.9	+16.4 ± 3.2	+8.1 ± 1.2	+440.8 ± 44.7
[1- ¹⁴ C]Glutamate (5 mM) + amino-oxyacetate (0.1 mM)	–681.6 ± 61.3	+336.9 ± 51.2	+13.5 ± 4.6	+22.2 $\pm 5.8^{**}$	+12.8 $\pm 1.9^{*}$	+50.0 $\pm 8.5^{**}$	—	+344.0 $\pm 34.3^{**}$
[1- ¹⁴ C]Glutamate (5 mM) + amino-oxyacetate (0.8 mM)	–661.2 ± 47.2	+356.9 ± 16.3	+13.6 ± 8.0	+15.2 $\pm 5.0^{***}$	+11.8 $\pm 2.3^{*}$	+52.3 $\pm 4.8^{***}$	—	+299.7 $\pm 31.6^{**}$

Table 4. *Effect of L-methionine DL-sulphoximine (MSO) on the metabolism of 5 mM-glutamate in guinea-pig kidney tubules*

Kidney tubules (18.4 ± 1.4 mg dry wt. per flask) were incubated for 60 min as described in the Experimental section. Results ($\mu\text{mol/g}$ dry wt.) for metabolite removal (–) or production (+) are reported as means $\pm$ S.E.M. for three experiments performed in duplicate. Statistical difference was measured by the paired Student's *t* test against the control without MSO: **P* < 0.05; ***P* < 0.02; ****P* < 0.01.

Experimental condition	Metabolite removal or production						
	Glutamate	Glutamine	Ammonia	Glucose	Alanine	Lactate	Aspartate
Glutamate (5 mM)	–656.8 ± 46.7	+350.4 ± 42.7	+5.8 ± 7.3	+36.8 ± 7.5	+29.8 ± 6.7	+13.6 ± 1.6	+11.8 ± 3.0
Glutamate (5 mM) + MSO (5 mM)	–333.8 $\pm 23.3^{**}$	+7.8 $\pm 17.9^{***}$	+289.2 $\pm 24.1^{***}$	+6.5 $\pm 1.5^{*}$	+48.7 $\pm 3.8^{*}$	+17.9 ± 1.9	+35.8 $\pm 5.5^{**}$

Taking a fresh-weight/dry-weight ratio of 4.95 ± 0.06 (mean $\pm$ S.E.M. of 26 observations) for the guinea-pig renal cortex and correcting for temperature, we find that the activity of glutamate dehydrogenase (30.1 ± 2.7 $\mu\text{mol/min}$ per g fresh wt. at 25°C; mean $\pm$ S.E.M.; *n* = 6) is sufficient to account for the flux through glutamate dehydrogenase calculated in these experiments (see Table 5). It is noteworthy that this activity is of the same order of magnitude as that in dog renal cortex (Baverel *et al.*, 1980), where glutamate is a good precursor of ammonia (Klahr *et al.*, 1972). Our data contrast with those of Preuss (1972), who did not detect any activity of glutamate dehydrogenase in guinea-pig kidney cortex.

Effect of amino-oxyacetate

Amino-oxyacetate (0.1–0.8 mM), an inhibitor of transaminases (Braunstein, 1964), had no effect on glutamate removal (Table 3). This provides further evidence that glutamate metabolism in guinea-pig renal cortex is not initiated to a great extent by

transamination reactions, as postulated by Klahr *et al.* (1972) and Preuss (1972). The stimulation of lactate formation by amino-oxyacetate probably resulted from increased availability of pyruvate secondary to inhibition of alanine synthesis; the small decrease in glucose synthesis may have resulted from diversion of the reducing equivalents available in the cytosol from glucose to lactate formation. At the present time, we have no explanation for the inhibition by amino-oxyacetate of the release of ¹⁴CO₂, which cannot be entirely explained by the inhibition of alanine and aspartate formation.

Effect of L-methionine DL-sulphoximine

Additional evidence of the role of glutamate dehydrogenase was obtained by the addition of L-methionine DL-sulphoximine, an inhibitor of glutamine synthetase (Meister, 1968), which completely suppressed glutamine synthesis both from endogenous substrates and from the added glutamate (Table 4). Inhibition of glutamine synthesis was accompanied by the appearance of a high rate of

accumulation of ammonia (released by the glutamate dehydrogenase reaction) and by a large decrease in glutamate removal. A similar observation was made by Krebs (1935), who inhibited glutamine synthesis by either As_2O_3 or HCN. The fact that, with L-methionine DL-sulphoximine, the formation of glucose was decreased, and that of alanine, aspartate and lactate increased, indicates that some unidentified mechanism prevented the utilization of the oxaloacetate (formed from glutamate) for glucose synthesis and directed it to the formation of aspartate and pyruvate (used for lactate and alanine synthesis). Since, in these experiments, the formation of alanine and aspartate still occurred in the presence of L-methionine DL-sulphoximine, the role of glutamine-pyruvate transaminase and glutamine-oxaloacetate transaminase can be excluded in the formation of these amino acids, because no glutamine was available. The activity of alanine aminotransferase, which was found to be $6.51 \pm 0.87 \mu\text{mol/min per g fresh wt.}$ (mean $\pm$ S.E.M. of six observations), is sufficient to account for the alanine formation observed.

Pathways of glutamate metabolism and fate of glutamate carbon skeleton and nitrogen

Table 5 shows the calculations of the flux of the carbon skeleton and nitrogen of the added [$1-^{14}\text{C}$]glutamate through various pathways, under the experimental conditions reported in Tables 1 and 2, as well as the percentage distribution of the products of glutamate metabolism after 60 min. In the absence of NH_4Cl , the main fraction of the glutamate removed was metabolized via glutamate dehydrogenase, and only a small fraction was transaminated to alanine and aspartate; the remainder of the glutamate used was converted into glutamine by the action of glutamine synthetase. In the presence of NH_4Cl , the reverse was true: the flux through glutamine synthetase was higher than that through glutamate dehydrogenase. Table 5 also shows that beyond the stage of glutamate dehydrogenase most of the glutamate carbon skeleton was completely oxidized; complete oxidation represents up to 50% of the glutamate carbon removed.

Nitrogen-balance calculations (Tables 1 and 2) demonstrate that all the glutamate nitrogen was incorporated into glutamine, either as its amino group or as its amide group. Glutamine and complete oxidation were the only important fates of the glutamate carbon skeleton. Significant synthesis of glucose, alanine, lactate and aspartate, which, however, represented only a small fraction of glutamate removal, occurred only with high (5 mM) glutamate concentration; this indicates that, especially at low (1 mM) glutamate concentration, complete oxidation of the carbon skeleton of glutamate competed very efficiently with gluconeogenesis and

the formation of lactate, alanine and aspartate for the removal of oxaloacetate (arising from glutamate). In this respect, it is noteworthy that the energy provided by complete, and to a lesser extent partial, oxidation of glutamate carbon exceeded by far the energy needed for glutamine and glucose synthesis. Thus glutamate functioned as an oxidizable substrate for the generation of ATP needed for glucose and glutamine synthesis, as a precursor of oxaloacetate for glucose and aspartate formation, as a precursor of pyruvate for lactate and alanine formation and, finally, as a carbon and nitrogen donor for glutamine synthesis.

Alanine and lactate accumulation from glutamate, and complete oxidation of glutamate, imply that pyruvate is formed from glutamate. Pyruvate may be formed from malate or oxaloacetate (arising from glutamate) by different enzymic pathways: from malate through 'malic' enzyme, from oxaloacetate through the combined action of phosphoenolpyruvate carboxykinase and pyruvate kinase, or from oxaloacetate through oxaloacetate decarboxylase. The activities of 'malic' enzyme, phosphoenolpyruvate carboxykinase, pyruvate kinase and oxaloacetate decarboxylase were 0.31 ± 0.03 , 2.65 ± 0.40 , 25.8 ± 2.3 , and $2.1 \pm 0.1 \mu\text{mol/min per g fresh wt.}$ respectively (mean $\pm$ S.E.M. of six observations).

When the utilization by phosphoenolpyruvate carboxykinase of the oxaloacetate formed from glutamate was inhibited by quinolinate, aspartate accumulated (Table 6). This indicates that the phosphoenolpyruvate carboxykinase pathway plays an important role in the removal of the oxaloacetate formed; concomitant inhibition of alanine synthesis imply that at least part of the pathway 'glutamate $\rightarrow$ pyruvate' involves phosphoenolpyruvate carboxykinase (see Table 6); this, however, does not prove that phosphoenolpyruvate carboxykinase is involved in complete oxidation of glutamate, and the role of the different pathways possibly involved in complete oxidation of the carbon chain of glutamate deserves further study. A decrease in the flux of [$1-^{14}\text{C}$]glutamate through glutamate dehydrogenase by quinolinate can be attributed to the direct inhibitory action of this compound on glutamate dehydrogenase (Klahr & Schoolwerth, 1972).

Physiological relevance

Since it has a high capacity for glutamine synthesis, and a low glutaminase activity (Richterich & Goldstein, 1958), it might be expected that *in vivo* the guinea-pig kidney would not take up, but rather release, glutamine into the circulating blood. Arteriovenous-difference measurements show that this is the case (Table 7). Finally, the data of Table 7 show that, like the kidney of the dog

Table 5. Pathways and metabolic fate of the carbon skeleton and nitrogen of L-[1-¹⁴C]glutamate

The values, which are derived from those of Tables 1 and 2, are means for five experiments performed in duplicate. These values are given in $\mu\text{mol/g dry wt.}$ of tubules after 60 min of incubation, or as a percentage of glutamate removal given in parentheses. Flux of [1-¹⁴C]glutamate through glutamate dehydrogenase = ¹⁴CO₂ release minus the sum of aspartate and alanine formation; flux of [1-¹⁴C]glutamate through glutamine synthetase = glutamate removal minus ¹⁴CO₂ release; glutamine carbon and amino nitrogen formed from endogenous sources = glutamine found minus flux of [1-¹⁴C]glutamate through glutamine synthetase; complete oxidation = ¹⁴CO₂ release minus the sum of glucose (expressed in C₃ units), alanine, aspartate and lactate formation.

Experimental condition	Flux of [1- ¹⁴ C]-glutamate through glutamate dehydrogenase	Flux of [1- ¹⁴ C]-glutamate through glutamine synthetase	Distribution of glutamate carbon to:				Complete oxidation
			Glucose (in C ₃ units)	Alanine	Aspartate	Lactate	
[1- ¹⁴ C]Glutamate (1 mM)	163.5 (59.9%)	101.8 (37.3%)	13.8 (5.1%)	4.1 (1.5%)	3.6 (1.3%)	8.9 (3.3%)	140.8 (51.6%)
[1- ¹⁴ C]Glutamate (1 mM) + NH ₄ Cl (1 mM)	112.1 (39.6%)	170.8 (60.3%)	—	—	0.3 (0.1%)	—	112.1 (39.6%)
[1- ¹⁴ C]Glutamate (5 mM)	367.3 (56.8%)	258.8 (40.0%)	69.8 (10.8%)	19.7 (3.0%)	0.8 (0.1%)	6.5 (1.0%)	291.0 (45.0%)
[1- ¹⁴ C]Glutamate (5 mM) + NH ₄ Cl (5 mM)	235.7 (31.5%)	510.6 (68.3%)	—	—	1.0 (0.1%)	—	235.7 (31.5%)

Table 6. Effect of quinolinate on the metabolism of 5 mM-[1-¹⁴C]glutamate in guinea-pig kidney tubules

Kidney tubules (20.8 $\pm$ 1.5 mg dry wt. per flask) were incubated for 60 min as described in the Experimental section. Results ($\mu\text{mol/g dry wt.}$) for metabolite removal (—) or production (+) are reported as means $\pm$ S.E.M. for three experiments performed in duplicate. Complete oxidation of glutamate carbon (reported as means) = ¹⁴CO₂ release minus the sum of glucose (expressed in C₃ units) alanine, aspartate, lactate and malate formation. No fumarate accumulated in the presence of quinolinate. Statistical difference was measured by the paired Student's *t* test against the control without quinolinate: **P* < 0.05; ***P* < 0.02; ****P* < 0.01.

Experimental condition	Glutamate	Glutamine	Ammonia	Glucose	Alanine	Aspartate	Lactate	Malate	¹⁴ CO ₂	Complete oxidation
[1- ¹⁴ C]Glutamate (5 mM)	-661.3 ±49.2	+317.4 ±37.7	+3.1 ±4.1	+35.5 ±10.7	+29.1 ±9.0	+8.2 ±3.0	+10.0 ±3.7	-0.6 ±0.1	+423.9 ±15.7	+305.6
[1- ¹⁴ C]Glutamate (5 mM) + quinolinate (5 mM)	-628.3 ±30.0	+265.7 ±31.9*	+5.5 ±5.1	+12.9 ±7.5	+14.1 ±4.8*	+131.3 ±3.0***	+7.0 ±3.2	+8.7 ±0.2	+422.6 ±16.6	+235.7
[1- ¹⁴ C]Glutamate (5 mM) + quinolinate (10 mM)	-592.7 ±21.2	+222.5 ±18.8	+7.9 ±5.9	+12.5 ±7.3*	+10.7 ±3.6*	+135.8 ±4.2***	+6.1 ±1.3	+10.1 ±0.4	+389.4 ±16.0*	+201.7
[1- ¹⁴ C]Glutamate (5 mM) + quinolinate (40 mM)	-467.4 ±7.6*	+187.7 ±12.3*	+1.9 ±4.6	+9.7 ±5.2*	+13.1 ±3.3*	+121.1 ±1.2***	+6.6 ±2.2	+8.6 ±0.3	+334.8 ±7.8**	+166.0

Table 7. *Arteriovenous difference for glutamine, glutamate, ammonia and alanine across the kidney of fed guinea pigs*
 Female guinea pigs were maintained on a normal diet. Blood was taken under nembutal anaesthesia (35 mg/kg body wt., intraperitoneally) by using 5 ml syringes. A catheter was inserted in the carotid artery, and blood from the renal vein was drawn into a syringe with a needle bent for easier insertion. The sample (1–3 ml) was immediately transferred to a weighed tube containing 4 ml of HClO_4 (10%, v/v) and reweighed. Assays were carried out on the deproteinized, neutralized supernatant. Metabolite assays were as described in the Experimental section. Values are given as $\mu\text{mol/ml}$ of whole blood, as means $\pm$ S.E.M. for four guinea pigs. Abbreviation: NS, not significant.

	Arterial blood	Renal-venous blood	Statistical significance (by paired Student's <i>t</i> test)
Glutamine	0.184 ± 0.003	0.252 ± 0.016	$P < 0.02$
Glutamate	0.112 ± 0.012	0.115 ± 0.008	NS
Ammonia	0.102 ± 0.015	0.173 ± 0.009	$P < 0.05$
Alanine	0.120 ± 0.009	0.166 ± 0.014	$P < 0.05$

(Shalhoub *et al.*, 1963) and man (Owen & Robinson, 1963), in which alanine aminotransferase activity has been demonstrated (Pollak *et al.*, 1965; Mattenheimer *et al.*, 1970), and unlike the kidney of the rat (Squires *et al.*, 1976), which does not contain such an enzyme activity (Szepesi *et al.*, 1970), the kidney of the guinea pig adds alanine to the circulating blood.

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