The rabbit kidney does not readily metabolize but synthesizes glutamine at high rates by pathways that remain poorly defined. Therefore, the metabolism of variously labeled \([1^3C]\)- and \([1^{14}C]\)glutamates has been studied in isolated rabbit kidney tubules with and without acetate. \(CO_2\), glutamine, and alanine were the main carbon and nitrogenous end products of glutamate metabolism but no ammonia accumulated. Absolute fluxes through enzymes involved in glutamate metabolism, including enzymes of four different cycles operating simultaneously, were assessed by combining mainly the \(13C\) NMR data with a new model of glutamate metabolism. In contrast to a previous conclusion of Klahr et al. (Klahr, S., Schoolwerth, A. C., and Bourgoignie, J. J. (1972) \textit{Am. J. Physiol.} 222, 813–820), glutamate metabolism was found to be initiated by glutamate dehydrogenase at high rates. Glutamate dehydrogenase also operated at high rates in the reverse direction; this, together with the operation of the glutamine synthetase reaction, masked the release of ammonia. Addition of acetate stimulated the operation of the “glutamate \(\rightarrow\) \(\alpha\)-keto-glutarate \(\rightarrow\) glutamate” cycle and the accumulation of glucose but reduced both the net oxidative deamination of glutamate and glutamine synthesis. Acetate considerably increased flux through \(\alpha\)-keto-glutarate dehydrogenase and citrate synthase at the expense of flux through phosphoenolpyruvate carboxykinase; acetate also caused a large decrease in flux through alanine aminotransferase, pyruvate dehydrogenase, and the “substrate cycle” involving oxaloacetate, phosphoenolpyruvate, and pyruvate. Phosphate-activated glutaminase (hereafter referred to as glutaminase), the enzyme which initiates glutamine degradation and releases both glutamate and ammonia, is present in the rabbit kidney (1–3). However, its activity is low when compared with that in the kidney of other species (2, 4). This is not surprising because the rabbit, like other herbivorous species, excretes an alkaline urine (2) that does not need to contain large amounts of ammonium ions. This also explains why rabbit kidney tubules do not readily use glutamine as substrate (5, 6). By contrast, the rabbit kidney has a high capacity to trap ammonia and synthesize glutamine (1, 2, 4–8) by pathways that are far from being fully defined. In his initial work on glutamine synthesis in mammalian tissues (1), Krebs demonstrated that addition of glutamate to the incubation medium significantly increased glutamine synthesis by rabbit kidney cortex slices; since no ammonia accumulated under this condition, he concluded that the ammonia released by the glutamate dehydrogenase reaction was immediately utilized for the synthesis of glutamine (1). Later, Klahr (9) and Klahr \textit{et al.} (4) have also shown that glucose synthesis by rabbit kidney cortex slices is increased in the presence of glutamate. Since the latter authors observed glucose synthesis without concomitant accumulation of ammonium ions, they concluded, unlike Krebs (1), that glutamate metabolism in rabbit kidney slices is initiated by transamination rather than by oxidative deamination by glutamate dehydrogenase (4). More recently, Watford \textit{et al.} (10) stated that rabbit kidney tubules do not readily use glutamate or glutamine as gluconeogenic substrate, possibly because of inhibition of glutamate dehydrogenase by ammonia (4). However, none of the above authors measured glutamate uptake in their study. Recent studies in our laboratory (5, 6) have also shown that endogenous glutamate is converted at significant rates into glutamine in isolated rabbit kidney-cortex tubules which contain a high activity of glutamine synthetase (1, 7–9), the enzyme responsible for glutamine synthesis. Thus, all the above observations suggest that both rabbit renal cortical slices and tubules have the capacity to remove glutamate, but the extent to which the rabbit renal cortex can utilize glutamate as substrate and the pathways involved in glutamate metabolism by this tissue remain uncertain. In an attempt to clarify this subject, we decided to study glutamate metabolism in rabbit kidney tubules; for this, we conducted a study involving metabolic balance of substrate, incorporation of label into metabolites of glutamate, and modeling of glutamate metabolism (44). Our results clearly establish that rabbit kidney tubules readily utilize glutamate as substrate and convert it mainly into glutamine, alanine, and \(CO_2\) and to a lesser extent into glucose and serine. Modeling of the data obtained by \(13C\) NMR spectroscopy indicates that, during glutamate utilization via mainly glutamate dehydrogenase, glutamine synthetase, and alanine aminotransferase, glutamate synthesis also occurs at high rates. In addition, our study demonstrates that addition of acetate reduces net removal of glutamate by inhibiting not only net flux through glutamate dehydrogenase in the oxidative deamination direction but also by inhibiting glutamine, alanine, and serine synthesis. Finally, the rate of the cycle involving pyruvate, oxaloacetate, and phosphoenolpyruvate that was high with glutamate as sole substrate is drastically reduced.
in the presence of acetate, whereas glucose synthesis is stimulated.

EXPERIMENTAL PROCEDURES

Reagents

Glutaminase (grade V) was from Sigma. Other enzymes and coenzymes were purchased from Boehringer Mannheim (Meylan, France). \( ^{13} \)-[\( 1-^{13} \)C]Glutamate (2.04 GBq/mmole) and \( ^{13} \)-[\( 1,3-^{13} \)C]glutamate (2.15 GBq/mmole) were obtained from Amersham Corp. (Les Ulis, France). \( ^{13} \)-[\( 1-^{13} \)C]Glutamate (9.25 GBq/mmole) and \( ^{13} \)Na\( ^{13} \)CO\( _3 \) (2.07 GBq/mmole) were supplied by Dositek (Orsay, France). \( ^{13} \)-[\( 1,2-^{13} \)C]Glutamate, \( ^{13} \)-[\( 3-^{13} \)C]glutamate, and \( ^{13} \)-[\( 5-^{13} \)C]glutamate (isotopic enrichment of 99% for the three labeled compounds) were obtained from Merck, Sharp and Dohme (Montreal, Canada).

Rabbits

Female rabbits (1.8–2 kg; New Zealand albino strain) were obtained from the Elevage de Dombes (Châtillon-sur-Chalaronne, France) and were fed a standard diet (U.A.R., Villemaison-sur-Orge, France). All experiments were performed with kidney tubules from fed rabbits. Access to water was not limited.

Preparations of Kidney Tubules and Incubations

Kidney cortex tubules were prepared by collagenase treatment of renal cortex slices as described by Baverel et al. (11). Incubations other than those involving radioactive substances were performed for 30 or 60 min at 37 °C in a shaking water bath, in 25-ml stopped Erlenmeyer flasks in an atmosphere of \( \text{O}_2 \text{CO}_2 \) (19/1). The flasks contained 1 ml of the tubule suspension plus 3 ml of Krebs-Henseleit medium (12) either unsupplemented or supplemented with substrates, i.e. 5 mm (final concentration) \( ^{13} \)-[\( 1-^{13} \)C]glutamate (10 Bq/flask), \( ^{13} \)-[\( 1,5-^{13} \)C]glutamate (2.10\(^2\) Bq/flask), \( ^{13} \)-[\( 1,3-^{13} \)C]glutamate (5.10\(^2\) Bq/flask), \( ^{13} \)-[\( 1,2-^{13} \)C]glutamate, \( ^{13} \)-[\( 3-^{13} \)C]glutamate, \( ^{13} \)-[\( 5-^{13} \)C]glutamate, in the absence and the presence of 10 mm (final concentration) acetate or 25 mm \( \text{NaH}^{14} \)CO\( _3 \) (10\(^6\) Bq/flask). These differently labeled glutamate were used in an attempt to define the fate of all the glutamate carbons which depends on the metabolic pathways involved (see Fig. 1). It should be mentioned that the C-1 of glutamate could be released as \( ^{14} \)CO\( _2 \) by the \( \alpha \)-ketoglutarate dehydrogenase reaction. The C-2 and C-5 on one hand, and the C-3 and C-4 on the other hand, were expected to behave symmetrically by the stage of succinyl-CoA. Therefore, the \( ^{13} \)C glutamates used allowed us to measure and/or calculate easily the contribution of each glutamate carbon to the release of \( \text{CO}_2 \). Finally, \( ^{13} \)C-labeled bicarbonate was used to measure the pyruvate carboxylase-mediated incorporation of the bicarbonate carbon into the C-1 of alanine and of glutamate + glutamine. In all experiments, each experimental condition was performed in quadruplicate. Incubation was stopped by adding perchloric acid (final concentration 2%, v/v) to each flask. Metabolite assays were conducted on the neutralized supernatant. In all experiments, zero time flasks, with and without substrates, were prepared by adding perchloric acid before the tubules. When radioactive glutamate or bicarbonate was present in the medium, incubation, deproteinization, collection, and measurement of the \( ^{14} \)CO\( _2 \) formed were performed as described by Baverel and Lund (13). After removal of the denaturated protein by centrifugation, the supernatant was neutralized with a mixture of 20% (v/v) KOH and 1% (v/v) \( \text{H}_2\text{PO}_4 \) (8 m) for metabolite determination, measurement of bicarbonate fixation, and NMR spectroscopy measurements.

Analytical Methods

Metabolite Assays—Glucose, glycogen, lactate, pyruvate, glutamate, glutamine, alanine, aspartate, citrate, \( \alpha \)-ketoglutarate, fumarate, malate, glycerol, and glycerol 3-phosphate were determined by usual enzymatic methods, and the dry weight of tubules added to the flasks was determined as described previously (11, 13).

\(^{13} \)C NMR Techniques—These were recorded at 100.6 MHz on a Bruker AM-400 WB spectrometer using a 10-mm broadband probe thermostatted at 8 ± 0.5 °C. Magnet homogeneity was adjusted using the deuterium lock signal. Supernatants, obtained from four flasks for each experimental condition with either \( ^{13} \)-[\( 1,2-^{13} \)C]glutamate, or \( ^{13} \)-[\( 3-^{13} \)C]glutamate, with or without acetate as substrates, were pooled and lyophilized. Then, the freeze-dried material was re-dissolved in 3 ml of \( \text{H}_2\text{O} \) and centrifuged (5000 \( \times \) g, 4 °C, 15 min). In order to obtain absolute quantitative results, special care was taken for data acquisition. Relaxation times, saturation, and nuclear Overhauser effects were minimized, and resolution was optimized. To reduce long relaxation times, 20 \( \mu \)l of a \( \text{H}_2\text{O} \) solution containing 13 mm sodium EDTA and 11 mm gadolinium nitrate and \( ^{2} \)-[\( 13 \)C]glucine as internal standard were added to each milliliter of sample. Under this condition, all the T1 relaxation times determined for the carbons of interest in our experiments and measured using the inversion-recovery method were less than 10 s. Acquisition parameters were as follows: spectral width, 25000 Hz; tilt angle, 90°; data size, 32K; repetition time, 30 s; number of scans, 2700; Proton decoupling was carried out during the data acquisition (0.65 s) using a standard (WALTZ 16) pulse sequence for inverse-gated proton decoupling (14). We did not use the the freeze-dried material was re-dissolved in 3 ml of \( \text{H}_2\text{O} \) and centrifuged (5000 \( \times \) g, 4 °C, 15 min). In order to obtain absolute quantitative results, special care was taken for data acquisition. Relaxation times, saturation, and nuclear Overhauser effects were minimized, and resolution was optimized. To reduce long relaxation times, 20 \( \mu \)l of a \( \text{H}_2\text{O} \) solution containing 13 mm sodium EDTA and 11 mm gadolinium nitrate and \( ^{2} \)-[\( 13 \)C]glucine as internal standard were added to each milliliter of sample. Under this condition, all the T1 relaxation times determined for the carbons of interest in our experiments and measured using the inversion-recovery method were less than 10 s. Acquisition parameters were as follows: spectral width, 25000 Hz; tilt angle, 90°; data size, 32K; repetition time, 30 s; number of scans, 2700; Proton decoupling was carried out during the data acquisition (0.65 s) using a standard (WALTZ 16) pulse sequence for inverse-gated proton decoupling (14). We did not use the

### Table I

| Added substrate | Incubation | Glutamate | NH\(_4^+\) | Glutamine | Alanine | Aspartate | Glucose | Lactate |
|----------------|-----------|-----------|----------|-----------|---------|-----------|---------|---------|
| 5 mm glutamate | 30 min    | 64.4 ± 3.8| 44.2 ± 4.3| 11.9 ± 1.6| 3.8 ± 0.6| 3.6 ± 0.4 |
| None           | 30 min    | 18.5 ± 1.7| 1.0 ± 0.4 | 0.1 ± 0.4 | 0.0 ± 0.1| 0.7 ± 0.3 |
| 5 mm glutamate | 60 min    | 126.5 ± 8.8| 40.6 ± 12.7| 4.2 ± 0.7 | 8.4 ± 1.8| 3.6 ± 1.1 |
| None           | 60 min    | 30.5 ± 2.3| 1.2 ± 0.7 | 1.2 ± 0.3 | 0.1 ± 0.1| 1.0 ± 0.1 |

RESULTS

As shown in Table 1, rabbit kidney tubules metabolized glutamate at high rates; glutamate utilization was linear with time over 60 min. Glutamine, which was the main nitrogenous product found, accumulated linearly with time; since glutamate

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**4706**

**13C NMR and Glutamate Metabolism in Rabbit Kidney**

**TABLE I**

Time course of the metabolism of 5 mm L-glutamate in rabbit kidney tubules

Kidney tubules (55.5 ± 2.7 mg dry wt per flask) were incubated as described under “Experimental Procedures.” Results (\( \mu \)mol/g dry wt/h) for metabolite removal (−) or production are reported as means ± S.E. for four experiments performed in duplicate.
mine synthesis is an ATP-consuming reaction, this means that our tubules were metabolically viable (their ATP content was 6.5 ± 0.4, 7.4 ± 0.6, and 7.5 ± 0.5 μmol/g dry wt after 0, 30, and 60 min of incubation, respectively; n = 4; these mean values take into account the values found both in the absence and the presence of acetate because they were not significantly different). Significant amounts of alanine also accumulated at both incubation times, but alanine accumulation was not linear with time; nitrogen balance calculations reveal that alanine nitrogen accounted for 31.5 and 15.3% of the nitrogen removed as glutamate at 30 and 60 min, respectively. Small amounts of aspartate, glucose, and lactate were also found to accumulate but with no significant accumulation of pyruvate, glycogen, glyceraldehyde-3-phosphate, glyceraldehyde, acetate, ketone bodies, or tricarboxylic acid cycle intermediates occurred. As found by Krebs (1) and Klahr (9) and Klahr et al. (4) in rabbit kidney slices, no ammonia accumulated; on the contrary, a small amount of the ammonia brought by the tubules at zero time was utilized as substrate during the incubation.

Nitrogen balance calculations also indicate that the nitrogen found as glutamine, alanine, and aspartate exceeded the nitrogen removed as glutamate and ammonia; this means that the glutamine synthesis observed from endogenous sources in the absence of exogenous substrate (see Table I) still occurred, at least partially, in the presence of glutamate.

To test whether acetate, a substrate that circulates at high levels in the rabbit blood (18) and is readily metabolized by rabbit kidney tissue (19), can alter the synthesis of glutamine in rabbit kidney tubules by providing additional carbons, increasing concentrations of acetate (0.5–10 mM) were added to the incubation medium containing 5 mM glutamate. Fig. 2 shows that acetate addition induced a dose-dependent inhibition of both glutamate removal and glutamine accumulation; alanine accumulation was reduced only at the highest concentration of acetate used, whereas an increase in glucose accumulation was observed at all acetate concentrations employed.

In order to determine the precise pathways of glutamate metabolism (see Fig. 1) and the effect of 10 mM acetate (that had the greatest effect of the concentrations used) on these pathways, we performed a series of experiments in which we combined enzymatic, radioactive, and 13C NMR spectroscopy measurements using specifically or uniformly 14C- and/or 13C-labeled glutamate in the absence and the presence of unlabelled acetate. Table II shows that 10 mM acetate, which was avidly removed by rabbit kidney tubules, caused effects that have already been partially commented above (see Fig. 2). This table provides the values (obtained by enzymatic methods) necessary to the 13C (see the “Calculation” section) and flux calculations in which the glutamate removal is a key parameter. In addition, this table allows us to compare the formation of end products with calculated fluxes through the corresponding enzymes. Note also that the presence of glutamate stimulates the utilization of acetate.

Table III presents the release of 14CO2 from various 14C-labeled glutamates (5 mM) as well as the formation of 1-[14C]glutamate + 1-[14C]glutamine (referred to as [1-14C]Glx) and of [1-13C]alanine in the presence of glutamate (5 mM) + NaH14CO3 (25 mM). With glutamate as sole substrate, it can be seen that a large proportion of the C-1 of this amino acid was released as CO2 by the α-ketoglutarate dehydrogenase reaction; since the alanine and aspartate formed by the alanine and aspartate aminotransferase reactions can account for only 34% (64.6: 190.6) of the α-ketoglutarate that has been decarboxylated, our data clearly indicate that most (66%) of the α-keto-glutamate synthesis from glutamate occurred thanks to the glutamate dehydrogenase reaction. The difference between the releases of 14CO2 from [1,5-14C]glutamate and [1-14C]glutamate gives the release of 14CO2 from [5-14C]glutamate which is equal to a mean value of 153 μmol/g dry wt. Since the releases as CO2 of the C-2 and of the C-5 of glutamate, which occur beyond the stage of succinate and fumarate (two symmetrical molecules), are assumed to be equal, one can deduce that the release of 14CO2 from [2-14C]glutamate is also equal to 153 μmol/g dry wt. Thus, the C-1, C-2, and C-5 of glutamate accounted for about 80% (497:2.635:2) of the total release of CO2 from glutamate measured as the 14CO2 release from [U-14C]glutamate (see Table III). Therefore, it appears that only a small proportion (about 10%) of the C-3 and of the C-4 of glutamate, which are assumed to behave symmetrically beyond the stage of succinyl-CoA, was released as CO2 in rabbit kidney

**Table II**

| Experimental condition | Glutamate | Acetate | NH₄⁺ | Glutamine | Alanine | Aspartate | Lactate | Glucose |
|------------------------|-----------|---------|------|-----------|---------|-----------|---------|---------|
| 5 mM glutamate         | -218.4 ± 5.1 | 1.9 ± 0.5 | -1.8 ± 0.7 | 107.1 ± 1.4 | 57.4 ± 10.1 | 7.2 ± 0.8 | 2.5 ± 1.1 | 5.5 ± 1.9 |
| 5 mM glutamate +       | -90.2 ± 5.5*** | -406.1 ± 10.6*** | -2.8 ± 1.4 | 78.5 ± 7.2a | 11.2 ± 0.9a | 8.2 ± 0.7 | 2.1 ± 0.2 | 14.5 ± 1.5** |
| 10 mM acetate          | 3.1 ± 4.0 | -0.3 ± 0.1 | -0.5 ± 0.9 | 41.1 ± 1.4 | -1.1 ± 0.2 | 1.4 ± 0.2 | -2.5 ± 0.4 | 0.0 ± 0.1 |
| No added substrate     | 19.0 ± 2.2* | -302.5 ± 1.5*** | -1.9 ± 3.1 | 35.2 ± 1.0 | -2.6 ± 0.7 | -0.2 ± 0.4* | -2.7 ± 0.4* | 2.0 ± 1.0 |

**Fig. 1. Pathways of glutamate metabolism in rabbit kidney tubules.** Numbers refer to enzymes involved in the different reactions: 1, aspartate aminotransferase; 2, phosphoserine aminotransferase; 3, alanine aminotransferase; 4, glutamate dehydrogenase; 5, α-ketoglutarate dehydrogenase; 6, citrate synthase; 7, isocitrate dehydrogenase; 8, phosphoenolpyruvate carboxykinase; 9, phosphoglyceromutase; 10, glucose-6-phosphatase; 11, 3-phosphoglycerate dehydrogenase; 12, pyruvate kinase; 13, pyruvate carboxylase; 14, pyruvate dehydrogenase; 15, lactate dehydrogenase; 16, glutamine synthetase; 17, glutaminase; 18, acetyl-CoA synthetase.
enzymatically, are reported in Table II. Statistical difference was measured by the paired Student's t test against the control without acetate. Results (μmol/g dry wt/h) for metabolite removal (−) or production are reported as means ± S.E. for four experiments performed in duplicate. Statistical difference was measured by the paired Student's t test against the control without acetate. *p < 0.05.

Table III

Effect of 10 mM acetate on the release of 14CO2 from [1-14C]-, [1,5-14C]-, and [U-14C]glutamate and on the accumulation of [1-14C]glutamate plus glutamine and [1-14C]alanine during the incorporation of 14CO2 into glutamate metabolism in rabbit kidney tubules

Kidney tubules (37.9 ± 2.5 mg dry wt per flask) were incubated for 60 min as described under "Experimental Procedures." Results (μmol/g dry wt/h) for substrate utilization and product formation, measured enzymatically, are reported in Table II. Statistical difference was measured by the paired Student's t test against the control without acetate. *p < 0.05, **p < 0.001. Glu = glutamate; Glx = glutamate plus glutamine; Ala = alanine.

| Experimental condition | 14CO2 from [1-14C]Glu | 14CO2 from [1,5-14C]Glu | 14CO2 from [U-14C]Glu | [1-14C]Glu from Glu + H14CO3 | [1-14C]Glu from Ala + H14CO3 |
|------------------------|-----------------------|------------------------|------------------------|-----------------------------|-----------------------------|
| 5 mM [14C]glutamate    | 190.6 ± 4.5           | 343.9 ± 13.3           | 635.2 ± 43.0           | 53.4 ± 1.5                  | 25.0 ± 5.4                  |
| + 10 mM acetate        | 191.2 ± 12.2          | 316.3 ± 12.3**         | 579.4 ± 21.6           | 86.7 ± 4.2*                 | 5.2 ± 0.2*                  |
shown), acetate stimulated the synthesis and accumulation of labeled glutamate carbons but reduced the accumulation of labeled glutamine. In all spectra, small amounts of labeled serine carbons could also be identified.

From the spectra obtained with [3-13C]-, [5-13C]-, and [1,2-13C]glutamate as substrate (in the absence and in the presence of acetate), in which virtually all the significant resonances could be identified, we calculated the amounts of labeled products after correction for the 13C natural abundance as described under "Experimental Procedures." With [3-13C]glutamate as substrate in the absence and the presence of acetate (Table IV), the labeled C-2 of alanine was of the same order of magnitude as the labeled C-3 of alanine, and the labeled C-4 of glutamate and glutamine was virtually equal to the labeled C-5 of glutamate and glutamine; this is in agreement with the view that the C-3 and C-4 of the glutamate converted into α-ketoglutarate by either glutamate dehydrogenase or by alanine or aspartate or phosphoserine aminotransferase (see Fig. 1), and further metabolized in the tricarboxylic acid cycle, passed through the stage of succinate and fumarate, two symmetrical molecules. The passage through these symmetrical molecules is also consistent with the fate of the C-2 and the C-5 of the glutamate converted into the α-ketoglutarate that was further metabolized in the tricarboxylic acid cycle because the labeling of the C-1 (singlet) of glutamate, glutamine, and alanine in the presence of [5-13C]glutamate as substrate were approximately the same as those obtained when [1,2-13C]glutamate was the substrate (see Tables V and VI).

Since the C-2 and the C-3 of the oxaloacetate derived from the added [3-13C]glutamate were converted at the same rates to the C-3 and C-2 (respectively) of the glutamate synthesized and therefore to the C-3 and C-2 of the glutamine derived from the latter glutamate, one may calculate that with glutamate as sole substrate, a mean value of 67 μmol/g dry wt of glutamine arose directly from the added [3-13C]glutamate, whereas 10.8 μmol/g dry wt of [3-13C]glutamine derived from synthesized [3-13C]glutamate (see Table IV); in the presence of [3-13C]glutamate plus acetate, the corresponding values are 34.6 and 11.3 μmol/g dry wt, respectively. The latter values obtained for estimating the direct accumulation of glutamine from added [3-13C]glutamate in the absence and the presence of acetate are in relatively good agreement with those obtained by measuring the accumulation of [5-13C]glutamine from [5-13C]glutamate (Table V) as well as by measuring the multiplets (spin-spin 13C coupling of C-1 and C-2) resulting from the synthesis of [1,3-13C]glutamine from [1,2-13C]glutamate (Table VI).

Table IV also shows that the addition of acetate stimulated the accumulation of the [2-13C]glutamate and [3-13C]glutamate
synthesized from [3-13C]glutamate but not that of the [2-13C]glutamine and [3-13C]glutamate synthesized from the corresponding synthesized glutamates. Another interesting observation drawn from Table IV is that, in the presence of acetate, the indirect accumulation of [2-13C]glutamine and [3-13C]glutamine (from synthesized [2-13C]glutamate and [3-13C]glutamate, respectively) did not significantly fall as did the direct accumulation of [3-13C]glutamine from added [3-13C]glutamate (Table IV). Note that the direct accumulation of [3-13C]glutamine from added [3-13C]glutamate was virtually identical to the accumulation of [5-13C]glutamine from added [5-13C]glutamate (Table V) or of [1,2-13C]glutamine from added [1,2-13C]glutamate (Table VI).

Table IV also shows that, with [3-13C]glutamate as sole substrate, the C-2 and the C-3 of aspartate, lactate, alanine, and serine became labeled to the same extent. This reveals that the C-3 of glutamate, after having passed through the stage of succinate and fumarate, two symmetrical molecules, and then through the stage of malate and oxaloacetate, was metabolized by the action of phosphoenolpyruvate carboxykinase, pyruvate kinase, lactate dehydrogenase, aspartate, alanine, and phosphoserine aminotransferases. The metabolism of [3-13C]gluta-

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**Table IV**

| Experimental condition | Amount of labeled products | Glutamate | Lactate | Aspartate | Serine |
|------------------------|----------------------------|----------|---------|-----------|--------|
| [3-13C]Glu             |                           | C1       | C2      | C3        | C4     | C5       | C6       | C7       | C8       | C9       |
| [3-13C]Glu + acetate   |                           |          |         |           |        |          |          |          |          |          |
|                        |                           | 12 ± 0.2 | 16.1 ± 2.0 | 134.4 ± 15.5 | 4.1 ± 0.3 | 3.5 ± 0.8 | -       | 2.0 ± 0.6 | 1.8 ± 0.5 | 3.0 ± 0.6 | 2.9 ± 0.4 |

**Table V**

| Experimental condition | Amount of labeled products | Glutamate | Glutamine | Alanine | Aspartate | Serine |
|------------------------|----------------------------|----------|-----------|---------|-----------|--------|
| [5-13C]Glu             |                           | C1       | C5       | C1      | C5       | C1     |
| [5-13C]Glu + acetate   |                           |          |          |         |          |        |
|                        |                           | 7.2 ± 1.4 | 131.7 ± 18.8 | 4.6 ± 0.1 | 73.3 ± 4.0 | 11.2 ± 2.2 | 1.1 ± 0.1 | 6.6 ± 0.4 |

**Table VI**

| Experimental condition | Amount of labeled products | Glutamate | Glutamine | Alanine | Serine |
|------------------------|----------------------------|----------|-----------|---------|--------|
| [1,2-13C]Glu           |                           | C2-1     | C1       | C1       | C1     |
| [1,2-13C]Glu + acetate |                           |          |          |         |        |
|                        |                           | 131.5 ± 18.0 | 133.7 ± 19.3 | 7.7 ± 1.5 | 72.0 ± 3.1 | 72.9 ± 3.7 | 5.1 ± 0.1 | 12.7 ± 3.4 | 6.9 ± 0.2 |

*Note: *Glutamate also resulted in the labeling of the C-4 and C-5 of glutamate and glutamine (Table IV). This indicates that the labeled C-5 of glutamate led to the labeling of the C-2 and C-1 of acetyl-CoA that were converted to the C-4 and C-5 of citrate and then to the C-4 and C-5 of glutamate and glutamine. Thus, the pyruvate derived from glutamate underwent oxidative decarboxylation by the pyruvate dehydrogenase reaction. Addition of acetate did not alter the labeling of the C-2 and C-3 of aspartate but reduced the labeling of the C-2 and C-3 of lactate, alanine, and serine as well as the labeling of the C-4 and C-5 of glutamate and glutamine (Table IV). Since the sum of the labeled C-2 and C-3 of alanine (Table IV) was close to the labeling of the C-4 and C-5 of glutamate and glutamine (Table IV). Therefore, the labeling of alanine found in Table II, virtually all the carbons of the alanine found were derived from glutamate.
Table VII

Various proportions through pathways of glutamate metabolism in the absence or in the presence of 10 mM acetate in rabbit kidney tables.

Values, given as means ± S.E. for four experiments, were calculated from those of Tables III–VI. The various proportions are shown in Fig. 2 of the accompanying paper. The paired Student’s t test was used to measure the statistical difference against the control with glutamate as sole substrate: *, p < 0.05; **, p < 0.01; ***, p < 0.001. TCA, tricarboxylic acid cycle; Lac, lactate.

Proportion of the direct conversion

| Substance Converted to | Parameter notation | Parameter value |
|------------------------|--------------------|-----------------|
|                        |                    | Without acetate | With acetate |
| OAA                    | Cit                | 0.22 ± 0.01     | 0.80 ± 0.01*** |
| OAA                    | PEP                | 0.75 ± 0.01     | 0.18 ± 0.01*** |
| OAA                    | Asp                | 0.01 ± 0.01     | 0.02 ± 0.01*** |
| PEP                    | Pyr                | 0.86 ± 0.01     | 0.69 ± 0.05*** |
| PEP                    | 3P-glycerate       | 0.04 ± 0.01     | 0.31 ± 0.05*** |
| Pyr                    | OAA                | 0.81 ± 0.02     | 0.66 ± 0.06*  |
| Pyr                    | Ala                | 0.11 ± 0.03     | 0.15 ± 0.03*  |
| Pyr                    | AcCoA              | 0.07 ± 0.01     | 0.14 ± 0.03*** |
| Pyr                    | Lac                | 0.01 ± 0.01     | 0.05 ± 0.01*** |
| 3P-glycerate           | Glc                | 0.35 ± 0.08     | 0.71 ± 0.01*  |
| 3P-glycerate           | Ser                | 0.65 ± 0.08     | 0.29 ± 0.01*  |
| αKG                    | OAA                | 0.73 ± 0.06     | 0.52 ± 0.02*  |

Proportions taking into account the recycling through “Glu → αKG → Glu” and “Glu → Glu → αKG” cycles

Added Glu (αKG) 0.74 ± 0.09 0.95 ± 0.12*
Added Glu Accumulated Glu (’Glu → ’Glu) 0.27 ± 0.04 0.41 ± 0.04***
Added Glu Accumulated Glu (’Glu → ’Gln) 0.20 ± 0.02 0.10 ± 0.01*
Cit-derived αKG Glu (αKG → Glu) 0.33 ± 0.09 0.70 ± 0.06***
Cit-derived αKG OAA (αKG → OAA) 0.88 ± 0.02 0.76 ± 0.02

Other parameters

| Substance | Parameter notation | Parameter value |
|-----------|--------------------|-----------------|
|           |                    | Without acetate | With acetate |
| OAA inversion | (OAA)             | 0.32 ± 0.04     | 0.46 ± 0.02  |
| Citric acid cycle | (TCA [n]) | (OAA → Cit/[1-13C]αKG → OAA) | 0.19 ± 0.01 | 0.61 ± 0.02*** |
| “OAA → PEP → Pyr → PEP” cycle | (Pyr [n] OAA) | (OAA → PEP)(Pyr → Pyr)(Pyr → OAA) | 0.60 ± 0.03 | 0.08 ± 0.01*** |
| “OAA → PEP → Pyr → AcCoA → Cit → OAA” cycle | (AcCoA [n] OAA) | (OAA → PEP)(Pyr → Pyr)(Pyr → AcCoA)([1-13C]αKG → OAA) | 0.05 ± 0.01 | 0.01 ± 0.01 |
| Recycling ratio of αKG through “Glu → αKG → Glu” and “Glu → Glu → αKG” cycles | [αKG [n] Glu + Gln] = [1-(Glu [n] Glu)]/[1-(Glu [n] αKG)-Glu] | 1.21 ± 0.07 | 1.46 ± 0.07*** |
| Glu-derived AcCoA condensed with OAA not derived from Glu | (AcCoA + OAA) | 0.19 ± 0.01 | 0.19 ± 0.03 |
| Glu-derived OAA condensed with AcCoA not derived from Glu | (OAA + AcCoA) | 0.78 ± 0.05 | 0.98 ± 0.01* |

The C-2 and C-5 of glutamate behaved in a symmetrical way when the [1,2-13C]- or [5-13C]-α-ketoglutarate synthesized from [1,2-13C]glutamate or [5-13C]glutamate was further metabolized in the tricarboxylic acid cycle to lead to the accumulation of alanine and to the synthesis of glutamate which accumulated as glutamate and glutamine. Both in the absence and the presence of acetate, the labeling of the C-1 (singlet) of glutamate and alanine and of alanine were almost identical when [5-13C]glutamate or [1,2-13C]glutamate was the substrate. Tables V and VI also show that, with [5-13C]glutamate and [1,2-13C]glutamate as substrate, both in the absence and the presence of acetate, the labeling of the C-1 (singlet) of glutamate, glutamine, and alanine was virtually half that of the C-2 of glutamate, glutamine, and alanine when [3-13C]glutamate was the substrate (see Table IV).

When [3-13C]glutamate was the substrate in the absence and the presence of acetate, the labeling (in mol% dry wt) of the different carbons of glucose (α + β anomer) was as follows: C-1, 2.2 ± 0.5 and 3.4 ± 0.2; C-6, 1.8 ± 0.5 and 2.6 ± 0.1; C-2, 1.7 ± 0.6 and 2.6 ± 0.4; C-5, 2.5 ± 0.9 and 4.5 ± 0.2, respectively; the C-3 and C-4 were not enriched. When [5-13C]- and [1,2-13C]glutamate were the substrates, the C-3 and C-4 of glucose were enriched only in the presence of acetate; the values were 2.0 ± 0.4 and 1.1 ± 0.1 for the C-3, respectively; the corresponding values for the C-4 were 2.4 ± 0.5 and 1.7 ± 0.2, respectively. Thus, in agreement with the data of Table II, it appears that acetate tended to stimulate the conversion of glutamate carbons into glucose carbons, although the 13C resonances of the glucose carbons were small.

Table VII contains the proportions of each metabolite converted into the next one(s). It should be emphasized here that these proportions which constitute the basis of our model do not give direct access to fluxes. Fluxes also take into account the formation of the substrate of the reaction of interest. Table VII shows that with glutamate as sole substrate, the proportion of oxaloacetate synthesized that was converted into phosphoenolpyruvate was much greater than that converted into citrate, whereas the contrary was true in the presence of acetate. Under the control condition without acetate, almost all the phosphoenolpyruvate was converted into pyruvate, a very small fraction being converted into 3-phosphoglycerate; addition of acetate increased the proportion of phosphoenolpyruvate converted into 3-phosphoglycerate at the expense of that converted into pyruvate. Most of the pyruvate synthesized was converted into oxaloacetate both in the absence and the presence of acetate; addition of acetate significantly changed the proportions of pyruvate converted into oxaloacetate, alanine, and lactate. Table VII also shows that the proportion of 3-phosphoglycerate synthesized from glutamate as sole substrate that was converted into 3-phosphohydroxybutyrate and then into serine was much greater than that converted into glucose; the reverse was observed upon addition
of acetate. The proportion ($\alpha$KG$^1$ $\rightarrow$ OAA)$^3$ of $\alpha$-ketoglutarate that was directly converted into oxaloacetate was diminished in the presence of acetate.

Of the added glutamate removed and not recycled in the tricarboxylic acid cycle, ($\text{Glu}^1$ $\rightarrow$ $\alpha$KG), about 74 and 95% was converted into $\alpha$-ketoglutarate in the presence and the absence of acetate, respectively (Table VII); such a proportion, which may appear surprisingly high in view of the significant proportion of glutamate converted into glutamine, takes into account the operation of the "Glu$^1$ $\rightarrow$ $\alpha$KG $\rightarrow$ Glu" and of the "Glu$^1$ $\rightarrow$ Glu" cycles but not the operation of the other cycles (see Fig. 1).

The following proportions presented inside specific braces, [ ], also take into account the recycling through the "Glu$^1$ $\rightarrow$ $\alpha$KG $\rightarrow$ Glu" and the "Glu $\rightarrow$ $\alpha$Kin $\rightarrow$ Glu" cycles but not through the other cycles. The proportion [Glu$^*$ $\rightarrow$ Glu] of the added glutamate removed that accumulated as glutamate was increased in the presence of acetate. On the contrary, the proportion [Glu$^*$ $\rightarrow$ Gln] of added glutamate removed that accumulated as glutamine was significantly reduced by the addition of acetate.

Note that 33 and 70% of the citrate-derived $\alpha$-ketoglutarate ($\text{Cit}^1$ $\rightarrow$ $\alpha$KG $\rightarrow$ Glu) was converted into glutamate in the absence and the presence of acetate, respectively. Note also that a high proportion ($\text{Cit}^1$ $\rightarrow$ OAA) of the citrate-derived $\alpha$-ketoglutarate was converted into oxaloacetate. It can be seen in Table VII that a significant proportion (OAA$^*$) of the oxaloacetate synthesized was inverted as a result of the equilibration with fumarate thanks to the reversible part of the tricarboxylic acid cycle catalyzed by malate dehydrogenase and fumarase. This means that, both in the absence and the presence of acetate, most of the oxaloacetate synthesized by the pyruvate carboxylase reaction underwent equilibration with fumarate. It can also be seen in Table VII that the proportion (TCA$^1$) of oxaloacetate recycled at each turn of the tricarboxylic acid cycle that was relatively small with glutamate as sole substrate, dramatically increased in the presence of acetate. By contrast, the proportion (Pyruvate$^1$ [OAA]) of the oxaloacetate that was metabolized in the "OAA $\rightarrow$ PEPC $\rightarrow$ Pyr $\rightarrow$ OAA" cycle, which was in the absence of acetate, was considerably diminished in the presence of acetate. Both in the absence and the presence of acetate, a very small proportion (AcCoA$^*$) of the oxaloacetate synthesized was metabolized in the "OAA $\rightarrow$ PEPC $\rightarrow$ Pyr $\rightarrow$ AcCoA $\rightarrow$ Cit $\rightarrow$ OAA" cycle.

Table VII shows that the recycling ratio ($\alpha$KG$^*$ $\rightarrow$ Glu$^*$ + Gln)$^1$ of $\alpha$-ketoglutarate through the "Glu$^1$ $\rightarrow$ $\alpha$KG $\rightarrow$ Glu" and "Glu $\rightarrow$ $\alpha$KG$^*$ $\rightarrow$ Glu" cycles, which corresponds to the proportion of the citrate-derived $\alpha$-ketoglutarate that passed through these 2 cycles, was increased in the presence of acetate. A significant proportion (AcCoA + OAA $\rightarrow$ Cit) of glutamate-derived acetyl-CoA was condensed with oxaloacetate that did not originate from glutamate both in the absence and the presence of acetate (Table VII). Finally, a very large proportion (OAA + AcCoA $\rightarrow$ Cit) of glutamate-derived oxaloacetate was condensed with acetyl-CoA that did not originate from glutamate, especially in the presence of acetate.

Table VIII shows the absolute values of fluxes through enzymes involved in glutamate metabolism in the absence and the presence of acetate. It can be seen that high unidirectional fluxes occurred from glutamate to $\alpha$-ketoglutarate and from $\alpha$-ketoglutarate to glutamate at the level of glutamate dehydrogenase under both experimental conditions. Acetate considerably increased these two unidirectional fluxes, resulting in the diminution of the net difference (which was small) in favor of glutamate utilization by the latter enzyme. This effect caused by acetate was accompanied by an inhibition of flux through glutamine synthetase which is in agreement with the reduction of glutamine accumulation shown in Table II. Note that, both in the absence and the presence of acetate, flux through $\alpha$-ketoglutarate dehydrogenase was severalfold greater than the sum of fluxes through glutamate dehydrogenase (net flux in the direction of oxidative deamination of glutamate), alanine, aspartate, and phosphoserine aminotransferases, which lead to $\alpha$-ketoglutarate synthesis; this clearly indicates that most of the $\alpha$-ketoglutarate oxidatively decarboxylated was synthesized by the tricarboxylic acid cycle. This is in agreement with the high flux found through citrate synthase. It is also clear from the data of Table VIII that, upon addition of acetate, the increased flux through $\alpha$-ketoglutarate dehydrogenase can be entirely accounted for by the increase in flux through citrate synthase which occurred at the expense of the very large flux through phosphoenolpyruvate carboxykinase. Table VIII also shows that the acetate-induced diversion of oxaloacetate from phosphoenolpyruvate formation to citrate synthesis was logically accompanied by a large decrease in flux through pyruvate kinase and therefore in a decreased availability of pyruvate leading to a diminution of fluxes through alanine aminotransferase and pyruvate dehydrogenase. The increased glucose synthesis (see flux through glucose-6-phosphate) leading to competition between the gluconeogenic pathway and pyruvate synthesis for utilizing the phosphoenolpyruvate available in decreased amounts in the presence of acetate may also explain in part the decreased availability of pyruvate and of 3-phosphohydroxypyruvate (see flux through 3-phosphoglycerate dehydrogenase in Table VIII).

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1 The abbreviations used are: OAA, oxaloacetate; $\alpha$KG, $\alpha$-ketoglutarate; Ac, acetate; AcCoA, acetyl-coenzyme A; Cit, citrate; Glu, glutamate; 3P, 3-phosphoglycerate; PEPC, phosphoenolpyruvate; Pyr, pyruvate; TCA, tricarboxylic acid.

### Table VIII: Effect of 10 mM acetate on fluxes through pathways of glutamate metabolism in rabbit kidney tubules

| Experimental condition | Glutamate dehydrogenase (Glu $\rightarrow$ $\alpha$KG) | | Glutamine synthetase (Glu $\rightarrow$ $\alpha$KG) | $\alpha$-Ketoglutarate dehydrogenase (AcCoA $\rightarrow$ OAA) | Alanine aminotransferase | Aspartate aminotransferase |
|------------------------|-----------------------------------------------|------------|-------------------------------------|----------------------------|-------------------|-------------------|
| Glutamate              | 221.8 ± 59.3                                 | 155.6 ± 49.0 | 66.2 ± 10.3                         | 86.1 ± 9.3                 | 385.6 ± 20.3    | 69.2 ± 12.0       |
| + acetate              | 593.9 ± 109.2*$^a$                          | 557.1 ± 110.2*$^a$ | 36.8 ± 0.9*$^a$                      | 64.4 ± 1.1*$^a$             | 590.0 ± 84.2*$^a$ | 10.7 ± 0.2*$^a$   |

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$^*$ Values, given as means ± S.E. for four experiments performed in quadruplicate, were calculated from data of Tables II–VII; fluxes are expressed in μmol of C$_3$ units/g dry w/h. The paired Student’s $t$ test was used to measure the statistical difference against the control with glutamate as sole substrate: $^*$, $p < 0.05$; $^{**}$, $p < 0.01$.


DISCUSSION

Removal and Metabolic Fate of Glutamate in Rabbit Kidney Tubules

Our study clearly demonstrates that rabbit kidney tubules readily utilize glutamate as substrate; at equimolar concentration, the rate of glutamate removal observed in the present study is about twice that found for glucose in a recent study (6). This demonstration that glutamate is a substrate of rabbit kidney tubules is in agreement with the findings of Krebs (1) that glutamate is a precursor of glutamine in rabbit kidney slices and of Klahr (9) and Klahr et al. (4) who observed also in rabbit kidney slices that glutamate increases the production of glucose. Our results not only confirm that both glutamine and glutarate are products of glutamate metabolism but also establish the relative importance of these two end products. In addition, they establish the relative importance of alanine and CO₂ as carbon products of glutamate metabolism. From the results obtained, it is clear that glutamate is a potential energy provider to rabbit renal tubular cells.

The main fate of glutamate nitrogen in rabbit kidney tubules is glutamine. Both in the presence of glutamate as sole substrate and in the presence of glutamate + acetate, the significant amount of glutamine formed, which cannot be entirely explained by endogenous formation (see Tables I and II), indicates that added glutamate must have contributed not only to the carbon skeleton but also to its amide nitrogen. This clearly demonstrates that some of the glutamate removed must have been deaminated via glutamate dehydrogenase to provide the ammonia needed for the synthesis of the glutamine amide group. This study also establishes that alanine and, to a much smaller extent, aspartate, and serine are nitrogenous products of glutamate metabolism in rabbit kidney tubules. In agreement with the findings of other authors (1, 4, 9), no ammonia accumulated, an observation explainable by the high rate of glutamate metabolism in rabbit kidney tubules. In agreement with the findings of other authors (1, 4, 9), no ammonia accumulated, an observation explainable by the high rate of glutamate metabolism in rabbit kidney tubules. In addition, the consistency of our analysis and measurements was satisfactory because there were no contradictions either in the qualitative or in the quantitative distributions of the different labeled glutamate carbons in the different products. For example the symmetrical behavior beyond the succinyl-coenzyme A step on the one hand of C-2 and C-5 and on the other hand of the C-3 and C-4 of glutamate was observed from differently labeled glutamates. In addition the number of experimental results was greater than the number of parameters in the model; therefore, several ways of calculations giving virtually identical results were possible. Furthermore, the consistency of our model is also indicated by the fact that calculated and measured amounts of a few labeled products were also almost identical. Finally, virtually identical fluxes were obtained when they were calculated from the data obtained from differently labeled glutamates.

Role of Glutamate Dehydrogenase—In these experiments, the release of 14CO₂ from [14C]glutamate, which cannot be explained by the rates of alanine, aspartate, and serine synthesis (see Tables II and III), provides conclusive evidence that glutamate dehydrogenase plays an important role in initiating glutamate metabolism in rabbit kidney tubules. Our data therefore are in agreement with the view of Krebs (1) about the role of this enzyme, but they disagree with the conclusion of Klahr (9) and Klahr et al. (4) who did not notice that the release of ammonia by oxidative deamination of glutamate was masked by the high capacity of rabbit kidney-cortex slices for glutamine synthesis. Additional evidence for a high flux through glutamate dehydrogenase in the direction of oxidative deamination of glutamate is provided by the results drawn from the combination of the 13C NMR data and the model of glutamate metabolism developed in this study (Table VIII).

Simultaneous Degradation and Synthesis of Glutamate—The fact that, in the presence of glutamate + acetate, the release of 14CO₂ from [1-14C]glutamate, or half the release of 14CO₂ from [1,5-14C]glutamate, or one-fifth of the release of 14CO₂ from [1-13C]glutamate was higher than the removal of glutamate measured enzymatically (Tables II and III) strongly suggests that glutamate removal was accompanied by glutamate synthesis. That this was indeed the case not only in the presence of glutamate + acetate but also in the presence of glutamate as sole substrate was unequivocally demonstrated by the conversion of the C-3 of added [3-13C]glutamate to the C-1, C-2, C-4, and C-5 of glutamate (Table IV). As shown in Table VIII, flux through glutamate dehydrogenase in the direction of the reductive amination of α-ketoglutarate was high both in the absence and the presence of acetate; however, as expected since glutamate was removed in net amounts under both experimental conditions, flux in the oxidative deamination direction exceeded that in the reductive amination direction. The net flux through glutamate dehydrogenase, which was therefore in favor of glutamate deamination (Table VIII), gives the availability of ammonia for glutamine synthesis. It can be seen that, both in the absence and the presence of acetate, the ammonia derived from added glutamate and available for glutamine synthesis was not sufficient to explain all the glutamine found to accumulate (Table II); in agreement with the nitrogen balance calculations mentioned above, this means that the glutamine synthesis from endogenous substrates observed in the absence of exogenous substrates still occurred (at least partially) in the presence of glutamate alone and in the presence of glutamate + acetate (Table II).

The fact that glutamate carbons other than those labeled in the added glutamates became labeled during incubation (Tables IV–VI) implies that the α-ketoglutarate derived from added glutamate as a result of glutamate deamination or transamination was further metabolized in the tricarboxylic
acid cycle to yield newly synthesized glutamate. Thus, at any incubation time point, the glutamate added at the start of incubation was mixed with small amounts of variously labeled glutamates synthesized by rabbit kidney tubules through various pathways. Thanks to the NMR data we were able to identify these pathways and to quantify them by combining the NMR data and the model developed. An interesting observation made from the data of Table IV is that part of the [3-13C]glutamate found at the end of incubation, which is equal to the [2-13C]glutamate found, was synthesized by the tubules; the difference between the [3-13C]glutamate found and the [2-13C]glutamate found gives the amount of added [3-13C]glutamate that was not removed by the tubules or that was removed to give [3-13C]α-ketoglutarate subsequently reconverted into [3-13C]glutamate by the glutamate dehydrogenase reaction. That our way of differentiating the [3-13C]glutamate not removed in net amounts from the [3-13C]glutamate synthesized and accumulated (Table IV) is correct is suggested by the relatively good agreement between the [3-13C]glutamate synthesized and accumulated (Table IV) is correct is suggested by the relatively good agreement between the [3-13C]glutamate synthesized and accumulated (Table IV) and the [3-13C]glutamate found minus the [2-13C]glutamate found (Table IV) on the one hand and the [5-13C]glutamate (Table V) or [1,2,13C]glutamate (Table VI) found on the other hand, both with glutamate alone or with glutamate + acetate.

Note that the two reactions of the glutamate – α-ketoglutarate interconversion could occur within one or different populations of mitochondria or within different cells. In this respect, it should also be emphasized here that no evidence was found for different pools of glutamate as will be discussed in the next section.

**Synthesis of Glutamine**—Although we recently presented some evidence that, in the presence of glucose plus NH4Cl, glutamate accumulation resulting from the action of glutamine synthetase was accompanied by glutamine degradation by glutaminase (6), we obtained in the present study no evidence that glutaminase operated; therefore, we were unable to quantify flux through the latter enzyme which is present along the entire length of the rabbit proximal tubule (3).

In this study, we were able to distinguish between the direct (from added glutamate) and indirect (from synthesized glutamate) synthesis of glutamine (see Tables IV–VI and “Results”). This raises the question of whether or not glutamine was synthesized from a single or from two different glutamate pools. In this respect, the observations made from Tables IV–VI that (i) acetate increased the accumulation of [2-13C]- and [3-13C]glutamate from [3-13C]glutamate but not the indirect accumulation of [2-13C]- and [3-13C]glutamate from the latter glutamates, and (ii) acetate caused a decrease in the direct accumulation of glutamate (Tables IV–VI) but not in the indirect accumulation of this amino acid should be examined with great caution. At first sight, the latter observations might be interpreted as indicative of the existence of two different glutamate pools from which two different glutamine pools would be synthesized. In fact, the absence of a (2-fold) decrease in the indirect accumulation of [2-13C]- and [3-13C]glutamate in the presence of acetate can simply be explained by a large (2-fold) increase in the net synthesis of glutamate as reflected by the accumulation of [2-13C]glutamate + [2-13C]glutamine (see Table IV). Thus, there is no evidence in the present study for the existence of two different glutamate or glutamine pools in rabbit kidney tubules.

Note that it is conceivable that the glutaminase reaction also operated especially in the presence of glutamate as sole substrate when the glutamine concentration reached a level higher (Table II) than that at which the glutaminase reaction has been demonstrated to function in rabbit kidney tubules (5); however, the extent to which this reaction operated, if at all, remains uncertain because of the presence of high concentrations of glutamate, a well established end product inhibitor of renal glutaminase (38). It should also be mentioned here that any operation of the glutaminase reaction in the present study would imply that our value of flux through glutamine synthetase is underestimated.

**Other Pathways of Glutamate Metabolism**—An interesting observation is that the “substrate cycle” involving the operation of phosphoenolpyruvate carboxykinase, pyruvate kinase, and pyruvate carboxylase, which has recently been shown to operate during glucose metabolism in rabbit kidney tubules (6), was also found in the present study to operate at high rates during glutamate metabolism (Tables VII and VIII). Note that the operation of pyruvate carboxylase, which resulted in the labeling of the C-4 of oxaloacetate and then of the C-1 of glutamate and glutamine in the presence of glutamate + [14C]bicarbonate (Table III), cannot explain the labeling of the C-1 of alanine, glutamate, and glutamine as a result of the refixation of the 13CO2 released from the differently 13C-labeled glutamates used as substrates; as a matter of fact, simple calculations taking into account the total “CO2 + bicarbonate” pool of our flasks reveal that the 13CO2 released was considerably diluted by unlabeled CO2 and could account for only negligible labelings of the C-1 of the three latter amino acids.

Of the pyruvate synthesized by pyruvate kinase, a significant fraction was metabolized by pyruvate dehydrogenase; this reveals the existence of an additional cycle involving the successive operation of the latter enzyme, the tricarboxylic acid cycle from citrate to oxaloacetate, phosphoenolpyruvate carboxykinase, and pyruvate kinase. Thus, glutamate metabolism in rabbit kidney tubules involves four different cycles; in addition to the latter cycle, the “Glu → αKG → Glu”, the tricarboxylic acid and the “OAA → PEP → Pyr → OAA” cycles are all operative at their own rate which was determined by the enzyme operating at the lowest rate.

The fact that, with glutamate as sole substrate, flux through alanine aminotransferase (Table VIII) was found slightly higher than alanine accumulation (Table II) can be simply explained by the fact that some of the alanine brought by the tubules at the start of incubation was metabolized and replaced by the alanine synthesized from glutamate; this suggests that bidirectional fluxes also occurred at the level of alanine aminotransferase, a fact that is not surprising for a near-equilibrium enzyme.

That some glucose was synthesized from glutamate in our experiments is in agreement with the existence of key gluconeogenic enzymes in the rabbit proximal tubule (39). The small amount of [13C]serine found suggests that, like in the rat kidney (40), 3-phosphoglycerate dehydrogenase, phosphoserine aminotransferase, and phosphoserine phosphatase are also functional in rabbit kidney tubules. It should be mentioned here that, given the very small amounts of glucose synthesized from glutamate in this study, no account was taken of the possible utilization of glucose previously demonstrated in rabbit kidney tubules (6, 41).

**Effect of Acetate on Glutamate Metabolism**

Our data indicate that the rabbit kidney, like the kidney of other species (42, 43), has a high capacity to utilize acetate as substrate (Table II). Furthermore, they show that acetate has a number of important regulatory effects on the metabolism of glutamate in rabbit kidney tubules (Table VIII). The acetate-induced stimulation of flux through glutamate dehydrogenase in the oxidative deamination direction is a priori surprising because one would rather expect an inhibition of this unidirectional flux as a result of the expected shift of the mitochondrial
redox potential toward a more reduced state secondary to acetate oxidation; one partial explanation may be an increased availability of glutamate for its oxidative deamination as a result of the inhibition of glutamate utilization by the glutamine synthetase and glutamate transamination pathways. The stimulation by acetate of the unidirectional flux through glutamate dehydrogenase in the reductive amination direction may have resulted from (i) an increased supply of α-ketoglutarate secondary to the stimulation of flux through citrate synthase caused by acetate and (ii) the more reduced state of the mitochondrial NADH/NAD⁺ couple mentioned above.

The inhibition of flux through glutamine synthetase caused by acetate cannot be attributed to a diminution of glutamate availability which on the contrary increased. Similarly, the provision of ATP (which can be calculated from the data of Table VIII), which is needed for the glutamine synthetase reaction (1), was not decreased but rather was more than doubled in the presence of acetate. This inhibition can be explained by a significant reduction of the release of ammonia, a substrate of the glutamine synthetase reaction, by glutamate dehydrogenase (Table VIII).

It is noteworthy that, despite the increased provision of oxaloacetate via the tricarboxylic acid cycle secondary to the acetate-induced stimulation of flux through α-ketoglutarate dehydrogenase, there was no increase in aspartate accumulation and a large reduction of flux through phosphoenolpyruvate carboxykinase (Table VIII). This can be explained by the increased availability of acetyl-CoA due to acetate utilization which directed oxaloacetate to the citrate synthase reaction at the expense of the aspartate aminotransferase and the phosphoenolpyruvate carboxykinase reactions. The inhibition of phosphoenolpyruvate synthetase caused by acetate led to a reduction of flux through pyruvate kinase and therefore of the synthesis of pyruvate which explains the observed diminution of fluxes through both pyruvate carboxylase and pyruvate dehydrogenase as well as the fall of alanine accumulation. It should be mentioned that, in the presence of acetate, the small fraction of the phosphoenolpyruvate synthesized that was further metabolized in the gluconeogenic pathway was directed to the synthesis of glucose at the expense of the synthesis of 3-phosphoglycerate and glucose-6-phosphate, Table VIII. This effect may result from an increased flux through glyceraldehyde-3-phosphate dehydrogenase due to an increased availability of NADH in the cytosol as a result of acetate oxidation. This is suggested by the unchanged lactate accumulation despite a reduced availability of pyruvate in the presence of acetate. Another possible explanation for the increased accumulation of glucose in the presence of acetate is that the latter compound inhibited the utilization of the glucose synthesized since it is known that glucose is utilized by rabbit kidney tubules (6, 41) and that acetate inhibits glucose utilization in these tubules. Unfortunately, the results obtained in the present study do not allow us to test the latter hypothesis because the glucose synthesis and the 13C resonances of glucose were too small to draw reliable conclusions on the possible existence of glucose utilization.

Physiological Significance

It is clear that in the rabbit kidney, like in the kidney of other herbivorous animals, which excretes an alkaline urine (2) and contains a high glutamine synthetase activity, glutamine synthesis represents an important mechanism whereby the ammonia synthesized by the glutamate dehydrogenase and possibly glutaminase reactions can be trapped. It is therefore of physiological, biochemical, and nutritional interest to characterize not only the metabolism of glutamate, a substrate of glutamine synthetase and a regulator of glutaminase, but also the regulation of glutamate metabolism by important circulating substrates such as acetate, which represents most of the volatile fatty acids in the rabbit blood (18).

It should be emphasized here that the glutamate concentration used in the present study is much higher than the blood glutamate concentration found in the rabbit (0.2 mM). We used such a high glutamate concentration and a large amount of kidney tubules to increase the rate of glutamate removal and the rate of 13C-labeled product formation so that it was possible to measure substrate removal and product formation in a reliable manner to compensate for the poor sensitivity of 13C NMR spectroscopy. This led us also to use a 10 mM acetate concentration so that this substrate, which is avidly metabolized by rabbit kidney tubules (Table II) and is present at millimolar concentrations in the rabbit blood (18), was available in sufficient amounts until the end of the 60-min incubation period.

It should be underlined that the effects of acetate observed in the present study are probably of physiological relevance; indeed, the metabolism of sodium acetate leads to the production of bicarbonate which should be eliminated in the urine, thus leading to urine alkalization and therefore to a decreased need for renal ammonia production and excretion. In the present study, the decreased flux through glutamate dehydrogenase (Table VIII), which releases ammonia, caused by the presence of acetate fits well with a physiological adaptation to the metabolic alkalosis caused by acetate oxidation. In this respect, one would also expect an increased flux through glutamine synthetase and an increased glutamine synthesis which, on the contrary, were found to decrease in this study (Table II and VIII). The absence of this expected increase in glutamine synthesis is probably related to the decreased availability of ammonia mentioned above. Whether acetate, which leads to an increased flux through the tricarboxylic acid and the “Glu → αKG → Glu” cycles and to a decreased flux through the “OAA → PEP → Pyr → OAA” and the “OAA → PEP → Pyr → AcCoA → citrate → OAA” cycles under in vitro conditions, exerts similar actions under in vivo conditions when there is an increased supply of glutamine precursors and acetate to the rabbit kidney during the post-prandial period deserves further study.

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$^{13}$C NMR and Glutamate Metabolism in Rabbit Kidney