Characteristics of glutamine metabolism in human precision-cut kidney slices: a ^{13}C -NMR study

Anne VITTORELLI, Catherine GAUTHIER, Christian MICHOUDET, Guy MARTIN and Gabriel BAVEREL¹

Laboratoire de Physiopathologie Métabolique et Rénale, Institut National de la Santé et de la Recherche Médicale, U 499, Faculté de Médecine R.T.H. Laennec, 69372 Lyon Cedex 08, France

The metabolism of glutamine, a physiological substrate of the human kidney, plays a major role in systemic acid–base homeostasis. Not only because of the limited availability of human renal tissue but also in part due to the lack of adequate cellular models, the mechanisms regulating the renal metabolism of this amino acid in humans have been poorly characterized. Therefore given the renewed interest in their use, human precision-cut renal cortical slices were incubated in Krebs–Henseleit medium (118 mM NaCl, 4.7 mM KCl, 1.18 mM KH_2PO_4 , 1.18 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 24.9 mM NaHCO_3 and 2.5 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) with 2 mM unlabelled or ^{13}C -labelled glutamine residues. After incubation, substrate utilization and product formation were measured by enzymatic and NMR spectroscopic methods. Glutamate accumulation tended to plateau but glutamine removal and ammonia, alanine and lactate production as well as flux through GLDH (glutamate dehydrogenase) increased to various extents with time for up to 4 h of incubation indicating the metabolic viability of the slices.

Valproate, a stimulator of renal glutamine metabolism, markedly and in a dose-dependent fashion increased ammonia production. With [^{13}C]glutamine as a substrate, and in the absence and presence of valproate, [^{13}C]glutamate, [^{13}C]alanine and [^{13}C]lactate accounted for 81 and 96%, 34 and 63%, 30 and 46% of the glutamate, alanine and lactate accumulations measured enzymatically respectively. The slices also metabolized glutamine and retained their reactivity to valproate during incubations lasting for up to 48 h. These results demonstrate that, although endogenous metabolism substantially operates in the presence of glutamine, human precision-cut renal cortical slices are metabolically viable and strongly respond to the ammoniagenic effect of valproate. Thus, this experimental model is suitable for metabolic and pharmacotoxicological studies.

Key words: ammoniogenesis, ^{13}C NMR, gluconeogenesis, glutamine metabolism, human kidney, valproate.

INTRODUCTION

It is well established that the human kidney takes up glutamine from the circulating blood under various physiological and pathophysiological situations [1–6]. The renal metabolism of glutamine, the major precursor of the ammonium ions excreted in the urine, is of central importance for the regulation of systemic acid–base homeostasis and for energy provision to renal cells (see [7–9] for reviews). Despite this, the mechanisms regulating or disturbing the human renal metabolism of glutamine, which cannot be studied *in vivo*, remain poorly understood; indeed, only a few studies have been devoted to glutamine metabolism in human renal cells *in vitro* [10–14]. For these studies, either cultured cells or thick cortical slices prepared by free-hand dissection with a razor blade or suspensions of proximal tubules or microdissected proximal nephron segments have been used.

Each of these experimental models has advantages and drawbacks. Kidney tubules and microdissected nephron segments, which are isolated with collagenase, remain fully differentiated but are only viable for a maximum of a few hours [14,15]; therefore, they are not suitable for the study of long-term regulatory effects. Human primary renal cells [11], like rabbit primary renal cells [15], can be cultured for several days but they partially dedifferentiate [11,15].

Recently, there has been a renewed interest in the use of precision-cut slices particularly for hepatotoxicity and, to a lesser extent, for nephrotoxicity studies (see [16–18] for reviews);

indeed, such slices, which maintain cell heterogeneity and tissue architecture and whose preparation does not require the use of proteolytic enzymes, retain their viability for at least 24–48 h. Surprisingly, despite the potential interest of human precision-cut renal cortical slices for a variety of biological studies, their metabolism has not been investigated specifically. Therefore, to characterize the metabolic viability of this experimental model better, human precision-cut renal cortical slices were incubated in the presence of glutamine as substrate and their glutamine metabolism, schematically represented in Figure 1, has been characterized. Their pharmacotoxicological reactivity to valproate, a widely used anti-epileptic drug and a hyperammonemic agent [19,20] that stimulates human renal glutamine metabolism *in vivo* [21], has also been studied.

METHODS

Reagents

Enzymes, coenzymes and L-lactate were supplied by Roche Molecular Biochemicals (Meylan, France). L-Glutamine, D-glucose and valproate were obtained from Sigma Chemicals (St. Louis, MO, U.S.A.). L-[1- ^{13}C]Glutamine, L-[3- ^{13}C]glutamine and D-[1- ^{13}C]glucose were obtained from Euriso-Top (St. Aubain, France). These ^{13}C -labelled compounds had a 99% isotopic abundance. The other chemicals used were of analytical grade.

Abbreviations used: AlaAT, alanine aminotransferase; GLDH, glutamate dehydrogenase; GLNase, glutaminase; LDH, lactate dehydrogenase; 2 OG, 2-oxoglutarate; OGDH, 2-oxoglutarate dehydrogenase; PEPCK, phosphoenolpyruvate carboxykinase; PSerAT, phosphoserine aminotransferase.

¹ To whom correspondence should be addressed (email baverel@sante.univ-lyon1.fr).

Figure 1 Schematic representation of glutamine metabolism in human precision-cut renal cortical slices

This scheme shows that (i) the C-1 of [1-¹³C]glutamine is released as ¹³CO₂ by the OGDH reaction and (ii) [1-¹³C]2 OG is formed by the GLDH and the alanine, aspartate and PSerAT reactions. AspAT, aspartate aminotransferase; PK, pyruvate kinase; PDH, pyruvate dehydrogenase; CS, citrate synthase.

Preparation of precision-cut renal cortical slices

Fresh human normal kidney cortex was obtained from the uninvolved pole of kidneys removed from 18 h fasted patients. The kidneys were decapsulated and cylindrical cores of 5 mm diameter were prepared from cortical sections with a tissue coring tool (Alabama R&D, Alabama, U.S.A.). The cores were maintained in oxygenated (95 % O₂ and 5 % CO₂) ice-cold Krebs–Henseleit buffer and transferred to a Krumdieck tissue slicer (Alabama R&D) filled with oxygenated ice-cold Krebs–Henseleit buffer. Preliminary experiments were used to determine settings that produced slices of approx. 250 µm thickness.

Incubation procedure

Short-term incubations were performed for 1, 2 or 4 h at 37 °C in Krebs–Henseleit medium in a shaking water bath in 25 ml stoppered Erlenmeyer flasks with an atmosphere of 95 % O₂–5 % CO₂. Four slices were incubated in 2 ml of Krebs–Henseleit buffer (pH 7.4) with either 2 mM unlabelled or ¹³C-labelled glutamine in the absence or presence of various concentrations (0.01–1 mM) of valproate, or 2 mM lactate, or 0.2 mM glucose or 5 mM [1-¹³C]-glucose as substrate. When long-term incubations were performed, the slices were floated on to Teflon®/Vitron rollers, which were carefully blotted and loaded into scintillation vials containing 2 ml of William's medium E No. 22551 supplemented with

2 mM glutamine. Vials were closed with a cap that had a central hole, and placed horizontally on a vial rotator in a humidified incubator set at 37 °C and gassed with 40 % O₂ and 5 % CO₂. Then the slices were incubated either between 0 and 24 h or between 24 and 48 h after a prior 24 h incubation period.

Incubations were terminated by adding HClO₄ [2 % (v/v), final concentration]. In the flasks in which LDH (lactate dehydrogenase) activity was measured, slices were used for intracellular LDH activity measurement and the medium was collected for extracellular LDH activity measurement before HClO₄ addition. In each experiment, zero-time flasks were prepared with slices by adding HClO₄ before the slices. In all experiments, each experimental condition was performed at least in duplicate.

Analytical methods

ATP and protein content

After the incubation, two slices were homogenized in 0.2 ml of cold 7 % HClO₄ (v/v) using an Ultraturrax homogenizer at 9500 rev./min, and after centrifugation of the homogenate for 5 min at 3000 g, the supernatant was neutralized with 20 % KOH (w/v)/1 % H₃PO₄ (v/v) before ATP measurement. The slice ATP concentration was quantified by using the method of Lamprecht and Trautschold [22]. Pellets were solubilized in 0.5 M NaOH for protein determination. Total protein was determined

Figure 2 Time course of glutamine metabolism in human precision-cut renal cortical slices

The values are means of four experiments performed in duplicate.

according to the method of Lowry et al. [23] using BSA as a standard.

LDH release

At the end of the incubation period, slices were removed, frozen in liquid nitrogen and thawed three times to release intracellular LDH. Enzyme leakage was determined in the incubation medium and compared with total LDH activity (extracellular + intracellular LDH). LDH activity was determined using the method described by Bergmeyer and Bernt [24].

Metabolite assays

After removing the denaturated protein by centrifugation (3000 *g* for 5 min), the supernatant was neutralized with 20% KOH/1% H_3PO_4 . Glutamine, glutamate, ammonia, alanine, glucose, lactate, pyruvate and aspartate residues were determined as described previously [25,26].

^{13}C -NMR techniques

Data were recorded as indicated previously [27,28] at 125.75 MHz on a Bruker AM-500 WB spectrometer using a 5 mm broadband probe thermostated at $8 \pm 0.5^\circ\text{C}$. In brief, magnet homogeneity was adjusted using the deuterium lock signal. Supernatants, obtained from three flasks for each experimental condition with one among L-[1- ^{13}C]glutamine, L-[3- ^{13}C]glutamine or [1- ^{13}C]glucose as substrates, were pooled and lyophilized; then, the lyophilized material was redissolved in $^2\text{H}_2\text{O}$ containing [2- ^{13}C]glycine as an internal standard, and centrifuged (5000 *g* at 4°C for 5 min). To obtain absolute quantitative results, special care was taken for data acquisition.

Acquisition parameters were as follows: spectral width, 25000 Hz; tilt angle, 90° ; data size, 32 k; repetition time, 50 s; number of scans, 600. We used a standard (WALTZ 16) pulse sequence for inverse-gated proton decoupling [29]. We did not use the NOESY during proton decoupling to avoid the use of the corresponding correction factors. A 1 Hz line broadening was applied prior to Fourier transformation. Peak areas were determined by measuring the integral heights. Chemical shifts were expressed as p.p.m. with respect to tetramethylsilane. Assignments were made by comparing the chemical shifts obtained with those given in the literature [30,31].

Calculations and statistical analysis

Net substrate utilization and product formation were calculated as the difference between the total flask contents at the start (zero-time flasks) and after the period of incubation.

When [^{13}C]glutamine species or [1- ^{13}C]glucose was the substrate, the transfer of the C-1 of glutamine or glucose, or of the C-3 of glutamine to a given position in a given metabolite was calculated using the formula: $(L_m - l_m)/(E_s - e_s)$, L_m is the amount of ^{13}C measured in the corresponding NMR peak (or resonance), l_m the natural ^{13}C abundance (1.1%) multiplied by the amount of metabolite assayed enzymatically, E_s the ^{13}C abundance of the C-1 or C-3 of glutamine or of the C-1 of glucose and e_s is the natural ^{13}C abundance. Note that $(E_s - e_s)$ is the specific enrichment of the corresponding species.

Results, presented as means $\pm$ S.E.M., are expressed in μmol of metabolite produced or removed $\cdot (\text{g of protein})^{-1} \cdot (\text{incubation time})^{-1}$ (1, 2 or 4 h). Comparisons of two groups were made by Student's *t* test for paired data. Multiple group comparisons were made by ANOVA on the StatView software. $P < 0.05$ was considered to be statistically significant.

RESULTS

Time course of glutamine metabolism in human precision-cut renal cortical slices

In each experiment, human renal cortical slices were incubated for 1, 2 or 4 h in Krebs–Henseleit medium. The cellular ATP level was measured as an index of cellular energy metabolism whereas LDH release into the incubation medium was taken as a marker of cell lysis. After 4 h of incubation, the ATP level was $6.9 \pm 0.8 \mu\text{mol/g}$ of protein and the LDH release was $< 0.5\%$ of the intracellular LDH ($n = 4$); the intracellular LDH activity was $680 \pm 88 \mu\text{mol} \cdot (\text{g of protein})^{-1} \cdot \text{min}^{-1}$ and the extracellular LDH activity was $2 \pm 1 \mu\text{mol} \cdot (\text{g of protein})^{-1} \cdot \text{min}^{-1}$. Moreover, the capacity of slices to maintain their metabolic activity after 4 h of incubation was studied by measuring glutamine removal and metabolism as a function of time. Figure 2 shows that glutamine removal and ammonia, alanine and lactate accumulation increased with time. By contrast, the accumulation of glutamate, which is an intermediate of glutamine metabolism, tended to plateau after 1 h of incubation. In these experiments, flux through GLDH

Table 1 Effect of 1 mM valproate on the short-term metabolism of 2 mM [^{13}C]glutamine in human precision-cut renal cortical slices

Slices (2.8 ± 0.3 mg of protein/flask) were incubated for 4 h in Krebs–Henseleit medium with 2 mM [^{13}C]glutamine in the absence or in the presence of 1 mM valproate. Net substrate utilization and product formation were calculated as the difference between the incubated flasks and the zero-time flasks. Results are expressed as means $\pm$ S.E.M. for six experiments performed in triplicate. Statistical difference was analysed by the paired Student's *t* test comparing values obtained in the absence with those in the presence of 1 mM valproate (**P* < 0.05). The ^{13}C -NMR results corresponding to these experiments are reported in Tables 2 and 3.

Experimental condition	Metabolite removal (–) or production ($\mu\text{mol} \cdot 4 \text{ h}^{-1} \cdot \text{g of protein}^{-1}$)					
	Glutamine	Glutamate	Ammonia	Alanine	Lactate	Aspartate
Glutamine (2 mM)	-528 ± 54	241 ± 12	765 ± 67	89 ± 14	100 ± 32	7 ± 7
+ Valproate (1 mM)	$-754 \pm 86^*$	261 ± 24	$1093 \pm 104^*$	$178 \pm 11^*$	$198 \pm 78^*$	16 ± 8
No added substrate	–	30 ± 3	80 ± 17	30 ± 3	97 ± 42	9 ± 4
+ Valproate (1 mM)	–	$21 \pm 4^*$	110 ± 16	$25 \pm 2^*$	92 ± 23	$13 \pm 6^*$

calculated as the difference between the ammonia produced and the glutamine removed was 38 ± 11 , 90 ± 21 and $195 \pm 11 \mu\text{mol/g}$ of protein after 1, 2 and 4 h of incubation respectively. Considering that a glutamine molecule contains two nitrogen atoms and that an ammonia, glutamate and alanine molecule contains one nitrogen atom, the nitrogen balance was calculated as the difference between twice the glutamine removed and the sum of the glutamate, ammonia and alanine accumulated; these calculations reveal that the nitrogen found exceeded by 9 ± 13 , 24 ± 42 and $97 \pm 25 \mu\text{mol/g}$ of protein when compared with the nitrogen removed as glutamine (332 ± 42 , 490 ± 44 and $664 \pm 60 \mu\text{mol/g}$ of protein) after 1, 2 and 4 h of incubation respectively. This suggests that part of the nitrogen found arose from endogenous substrates. This also suggests that part of the carbon products formed in the presence of glutamine might also have been formed from endogenous substrates presumably represented mainly by amino acids arising from cellular proteolysis.

Effect of 1 mM valproate on the metabolism of [^{13}C]glutamine in human precision-cut renal cortical slices

In a series of four preliminary experiments, the effect of increasing concentrations of valproate, a compound known to stimulate ammonia production from glutamine in isolated human kidney tubules [12], was studied in slices incubated for 4 h with 2 mM glutamine. In the presence of 0.01, 0.1 and 1 mM valproate, ammonia production was stimulated in a statistically significant manner by 34, 51 and 75 % respectively. In the absence of valproate, negligible amounts of glucose [$12 \pm 2 \mu\text{mol} \cdot (\text{g of protein})^{-1} \cdot (4 \text{ h})^{-1}$] were formed. In the presence of 0.01, 0.1 and 1 mM valproate, glucose synthesis was 13 ± 3 , 7 ± 1 and $2 \pm 0.3 \mu\text{mol} \cdot (\text{g of protein})^{-1} \cdot (4 \text{ h})^{-1}$ respectively.

Then, a series of six experiments was performed to determine precisely the extent to which the carbon products that accumulated in precision-cut human renal cortical slices metabolizing glutamine were formed from glutamine and/or from endogenous substrates. For this, slices were incubated in each experiment for 4 h in the presence of 2 mM [^{13}C]glutamine, or 2 mM [^{1-13}C]glutamine in the absence and presence of valproate at the concentration of 1 mM, which produced the largest effects in the preceding series of experiments; then, substrate utilization and product accumulation were determined by combining enzymatic and ^{13}C -NMR spectroscopic measurements.

Table 1 shows glutamine utilization and product accumulation measured enzymatically. As expected, valproate stimulated mean glutamine utilization without altering glutamate accumulation; it also enhanced mean ammonia, alanine and lactate accumulations but did not alter the small amount of aspartate accumulated. In

the absence of added substrate, substantial amounts of ammonia, lactate, glutamate and alanine were also formed from endogenous substrates; under this condition, valproate diminished glutamate and alanine accumulations and stimulated aspartate accumulation in a statistically significant manner (see Table 1).

Figures 3(A) and 3(B) show the ^{13}C -NMR spectra obtained after 4 h of incubation of the slices in the presence of 2 mM [^{13}C]glutamine, and in the absence and presence of 1 mM valproate, respectively. The corresponding values of the different resonances are presented in Table 2. Both in the absence and presence of valproate, the [^{13}C]glutamine utilized was in good agreement with the glutamine utilization measured enzymatically (see Table 1). Comparisons of the results presented in Tables 1 and 2 show that, 81 (195/241) and 96 % (251/261) of the glutamate accumulated was labelled on its C-3 in the absence and presence of valproate respectively. It can also be seen that the C-2 of glutamate, which was not labelled at the start of incubation, became labelled during incubation with [^{13}C]glutamine (Table 2). This demonstrates the existence of a concomitant degradation and synthesis of glutamate both in the absence and in the presence of valproate; the latter labelling (which also indicates a re-synthesis of the C-3 of glutamate) resulted from the conversion of the [^{13}C]oxaloacetate synthesized from [^{13}C]glutamine during the first turn of the tricarboxylic acid cycle into [^{13}C]2 OG (where 2 OG stands for 2-oxoglutarate) and then into [^{13}C]glutamate, thanks to GLDH operating in the reductive amination direction.

Table 2 shows that aspartate, alanine, lactate and serine were approximately equally labelled on their C-2 and C-3; this clearly demonstrates the passage of the glutamine carbon skeleton through the symmetrical molecules, succinate and fumarate, in the tricarboxylic acid cycle to give rise to oxaloacetate molecules labelled either on their C-2 or on their C-3. The sum of the labelled C-2 and C-3 of alanine, lactate and aspartate (Table 2) divided by the alanine, lactate and aspartate accumulation measured enzymatically (Table 1) gives the proportion of the alanine, lactate and aspartate carbon skeletons arising from glutamine; in the absence and in the presence of valproate, the corresponding values were 34 and 63 %, 30 and 46 %, 57 and 69 % respectively. Note that valproate greatly stimulated the labelling of alanine and lactate but not that of aspartate and serine. Note also that the production of $^{13}\text{CO}_2$ from [^{13}C]glutamine, calculated as the [^{13}C]glutamine removed and not accounted for by the non-volatile ^{13}C -products accumulated, was not altered by valproate (Table 2). It is also important to emphasize that, both in the absence and in the presence of valproate and in agreement with the very low glucose production measured enzymatically and reported above, none of the glucose carbons was found to be labelled from [^{13}C]glutamine in the NMR spectra shown in Figure 3.

Figure 3 ^{13}C -NMR spectra of neutralized HClO_4 extracts obtained from human kidney slices incubated with $[3\text{-}^{13}\text{C}]\text{glutamine}$ in the absence (A) and presence (B) of valproate

1, alanine C-3 (17.4); 2, lactate C-3 (21.4); 3, glutamine C-3 (27.4); 4, glutamate C-3 (28.1); 5, aspartate C-3 (37.7); 6, glycine C-2 (42.7); 7, alanine C-2 (51.8); 8, aspartate C-2 (53.4); 9, glutamate C-2 (55.8); 10, serine C-2 (57.6); 11, serine C-3 (61.4); 12, lactate C-2 (69.7). The chemical shifts in parentheses, expressed in p.p.m., are measured with respect to tetramethylsilane.

The values derived from the ^{13}C -NMR spectra (results not shown) obtained with $[1\text{-}^{13}\text{C}]\text{glutamine}$ as substrate without and with valproate are given in Table 3. Again, the $[1\text{-}^{13}\text{C}]\text{glutamine}$ removed was in good agreement with the glutamine removal measured enzymatically (Table 1) or with $[3\text{-}^{13}\text{C}]\text{glutamine}$ as

Table 3 Effect of 1 mM valproate on the short-term metabolism of 2 mM $[1\text{-}^{13}\text{C}]\text{glutamine}$ in human precision-cut renal cortical slices

Slices (2.8 ± 0.3 mg of protein/flask) were incubated for 4 h in Krebs–Henseleit medium with $[1\text{-}^{13}\text{C}]\text{glutamine}$ in the absence or in the presence of 1 mM valproate. $[1\text{-}^{13}\text{C}]\text{glutamine}$ removal was calculated as the difference between the values found in zero-time flasks and those in incubated flasks. Results are reported as means $\pm$ S.E.M. for six experiments performed in triplicate. The paired Student's *t* test was used to measure the statistical difference against the control without valproate: **P* < 0.05. Substrate utilization and product formation, measured enzymatically, are reported in Table 1.

Experimental condition	Amount of labelled metabolites removed (–) or accumulated ($\mu\text{mol} \cdot 4 \text{ h}^{-1} \cdot \text{g}$ of protein $^{-1}$)	
	$[1\text{-}^{13}\text{C}]\text{Glutamine}$	$[1\text{-}^{13}\text{C}]\text{Glutamate}$
$[1\text{-}^{13}\text{C}]\text{Glutamine}$ (2 mM)	-536 ± 78	173 ± 9
+ Valproate (1 mM)	$-756 \pm 120^*$	222 ± 24

substrate (Table 2). The fact that, both in the absence and in the presence of valproate, the $[1\text{-}^{13}\text{C}]\text{glutamate}$ accumulated (Table 3) tended to be slightly lower than the $[3\text{-}^{13}\text{C}]\text{glutamate}$ accumulated (Table 2) was due to the fact that part of the labelled C-3 of glutamate, equal to the labelled C-2 of glutamate, resulted from the re-synthesis of glutamate after having passed through the tricarboxylic acid cycle. Table 3 also shows that the release of the C-1 of glutamine as CO_2 at the level of the OGDH (2-oxoglutarate dehydrogenase) reaction was stimulated by valproate.

Table 4 shows the absolute values of fluxes through the enzymes involved in glutamine metabolism. These values are derived from the combination of the enzymatic and ^{13}C -NMR spectroscopy results shown in Tables 1–3 with our mathematical model developed for studying renal glutamate and glutamine metabolism [28]. As expected, valproate stimulated mainly flux through GLNase (glutaminase) and, to a much lesser extent, through GLDH, resulting in the increased ammonia production observed in Table 1. Note that the net synthesis of 2 OG by GLDH and the alanine, aspartate and PserAT (phosphoserine aminotransferase) reactions shown in Table 4 are in relatively good agreement with the release of $^{13}\text{CO}_2$ from $[1\text{-}^{13}\text{C}]\text{glutamine}$ that can be calculated from the results shown in Table 3, both with and without valproate. As expected from the results shown in Tables 1 and 2, valproate stimulated fluxes through AlaAT (alanine aminotransferase) and LDH. Note that the increase in the synthesis of 2 OG caused by valproate during the first turn of the tricarboxylic acid cycle was approximately balanced by the increases in fluxes through PEPCK (phosphoenolpyruvate carboxykinase) and pyruvate kinase. In agreement with the recycling of oxaloacetate, mentioned above to explain the re-synthesis of glutamate, and with the large proportion of the C-3 of glutamine utilized that was not accounted for by the non-volatile ^{13}C -products found to accumulate

Table 2 Effect of 1 mM valproate on the short-term metabolism of 2 mM $[3\text{-}^{13}\text{C}]\text{glutamine}$ in human precision-cut renal cortical slices

Slices (2.8 ± 0.3 mg of protein/flask) were incubated for 4 h in Krebs–Henseleit medium with $[3\text{-}^{13}\text{C}]\text{glutamine}$ in the absence or in the presence of 1 mM valproate. $[3\text{-}^{13}\text{C}]\text{glutamine}$ removal was calculated as the difference between the values found in zero-time flasks and those in incubated flasks. Results are means $\pm$ S.E.M. for six experiments performed in triplicate. The paired Student's *t* test was used to measure the statistical difference against the control without valproate: **P* < 0.05. Substrate utilization and product formation, measured enzymatically, are reported in Table 1.

Experimental condition	Amount of labelled metabolites removed (–) or accumulated ($\mu\text{mol} \cdot 4 \text{ h}^{-1} \cdot \text{g}$ of protein $^{-1}$)									
	Glutamine		Glutamate		Alanine		Lactate		Aspartate	
	C-3	C-2	C-3	C-2	C-3	C-2	C-3	C-2	C-3	C-2
$[3\text{-}^{13}\text{C}]\text{Glutamine}$ (2 mM)	-521 ± 58	18 ± 2	195 ± 17	12 ± 1	18 ± 3	16 ± 3	14 ± 2	1 ± 1	3 ± 2	16 ± 2
+ Valproate (1 mM)	$-745 \pm 93^*$	17 ± 2	251 ± 25	$57 \pm 6^*$	$56 \pm 6^*$	$50 \pm 9^*$	$42 \pm 8^*$	4 ± 2	7 ± 3	23 ± 5

Table 4 Effects of 1 mM valproate on fluxes through pathways of ^{13}C -glutamine metabolism in human precision-cut renal cortical slices

Values, reported as means $\pm$ S.E.M. for six experiments performed in triplicate, were calculated from Tables 1–3. The paired Student's *t* test was used to measure the statistical differences between the results obtained in the absence and those in the presence of 1 mM valproate; **P* < 0.05. Abbreviations: AspAT, aspartate aminotransferase; CS, citrate synthase; PK, pyruvate kinase.

Experimental condition	Flux (μmol of C_3 units $\cdot 4 \text{ h}^{-1} \cdot \text{g}$ of protein $^{-1}$)									
	Unidirectional fluxes									
	GLNase	Glu $\rightarrow$ 2 OG	2 OG $\rightarrow$ Glu	Net Glu $\rightarrow$ 2 OG	AlaAT	AspAT	PSerAT	GLDH Net Glu $\rightarrow$ 2 OG	OGDH	PEPCK
Glutamine (2 mM)	528 $\pm$ 58	401 $\pm$ 55	93 $\pm$ 14	308 $\pm$ 43	80 $\pm$ 12	11 $\pm$ 9	86 $\pm$ 18	130 $\pm$ 16	1565 $\pm$ 409	154 $\pm$ 16
+ Valproate (1 mM)	745 $\pm$ 93*	533 $\pm$ 73*	57 $\pm$ 8*	476 $\pm$ 69*	192 $\pm$ 24*	19 $\pm$ 6	90 $\pm$ 22	177 $\pm$ 30*	1292 $\pm$ 351	346 $\pm$ 53*
										LDH
										CS
										74 $\pm$ 8
										154 $\pm$ 29*
										1257 $\pm$ 364
										815 $\pm$ 287

Table 5 Time course of the metabolism of 0.2 mM D-glucose in human precision-cut renal cortical slices

Slices (4.1 ± 0.3 and 4.3 ± 0.3 mg of protein/flask incubated for 2 and 4 h respectively) were incubated in Krebs–Henseleit medium with and without 0.2 mM glucose. Net substrate utilization and product formation were calculated as the difference between the values in the incubated flasks and those in the zero-time flasks. Results are expressed as means $\pm$ S.E.M. for four experiments performed in duplicate.

Experimental condition	Incubation time (h)	Metabolite removed (–) or produced ($\mu\text{mol} \cdot \text{time of incubation}^{-1} \cdot \text{g}$ of protein $^{-1}$)			
		Glucose	Pyruvate	Lactate	Alanine
Glucose (0.2 mM)	2	–21 $\pm$ 5	7 $\pm$ 1	41 $\pm$ 5	13 $\pm$ 2
No added substrate	2	3 $\pm$ 1	2 $\pm$ 1	12 $\pm$ 3	12 $\pm$ 2
Glucose (0.2 mM)	4	–37 $\pm$ 5	9 $\pm$ 1	50 $\pm$ 7	23 $\pm$ 4
No added substrate	4	6 $\pm$ 2	4 $\pm$ 1	11 $\pm$ 5	23 $\pm$ 8

(see Table 2), total flux through OGDH was several times greater than the flux through the latter enzyme during the first turn of the tricarboxylic acid cycle (that can be calculated from the results shown in Table 3), both in the absence and in the presence of valproate which did not alter this total flux.

Lactate and glucose metabolism in human precision-cut renal cortical slices

To test if the absence of glucose synthesis from glutamine in human kidney slices was due to a poor gluconeogenic capacity of these slices, a series of five experiments was performed in duplicate in which the slices were incubated for 4 h in 2 ml of Krebs–Henseleit medium containing 2 mM lactate, a physiological substrate of the human kidney *in vivo* [32,33] and a gluconeogenic precursor in isolated human renal proximal tubules [34–36]. The utilization of lactate and the accumulation of glucose, pyruvate and alanine were 415 ± 49 , 81 ± 12 , 42 ± 1 and $69 \pm 3 \mu\text{mol/g}$ of protein/4 h respectively (3.1 ± 0.1 mg of protein/flask; $n = 4$). This clearly demonstrates that our slices retained an important capacity for glucose synthesis. This also means that the four key-gluconeogenic enzymes, pyruvate carboxylase, PEPCK, fructose-1-6-bisphosphatase and glucose-6-phosphatase, were well functional and that the ATP needed for glucose synthesis from lactate was supplied in adequate amounts.

Then, we tested if the glucose probably synthesized from glutamine might have been reutilized by our slices. For this, we performed a new series of four experiments in duplicate in which slices were incubated for 2 and 4 h in the presence of a low concentration (0.2 mM) of glucose simulating the maximal glucose concentration that might have been generated by slices incubated with 2 mM glutamine in the preceding series of experiments. The maximal possible formation of glucose from 2 mM glutamine was taken as the mean glutamine unaccounted for by the accumulation of labelled glutamate, alanine, serine, aspartate and lactate over a 4 h incubation period (see Table 2).

Table 5 shows the results obtained in these experiments. They clearly demonstrate that glucose was used as substrate in a virtually linear manner with time; this linearity was verified in two additional experiments in which the slices were incubated for 1, 2, 3 and 4 h (results not shown). Table 5 also shows that the glucose utilization was accompanied by the accumulation of lactate and alanine. Note that, in the absence of glucose as substrate, very small amounts of glucose were synthesized from endogenous substrates; by contrast, the alanine synthesized from endogenous substrates was in the same range as that synthesized in the presence of 0.2 mM glucose.

Figure 4 ^{13}C -NMR spectrum of a neutralized HClO_4 extract obtained from human kidney slices incubated with 5 mM D-[1- ^{13}C]glucose

1, alanine C-3 (17.4); 2, lactate C-3 (21.4); 3, glycine C-2 (42.7); 4, unidentified, also present at zero time (50.1); 5, glucose C-6 $\alpha + \beta$ (61.7 + 61.8); 6, lactate C-2 (69.7); 7, glucose C-4 $\alpha + \beta$ (70.7); 8, glucose C-2 α (72.5); 9, glucose C-5 α (72.6); 10, glucose C-3 α (73.8); 11, glucose C-2 β (75.2); 12, glucose C-5 β (76.7); 13, glucose C-3 β (77.0); 14, unidentified (90.0); 15, glucose C-1 α (93.1); 16, glucose C-1 β (96.9); 17, glycine C-1 (173.8); 18, lactate C-1 (183.8). The chemical shifts in parentheses, expressed in p.p.m., are measured with respect to tetramethylsilane.

Table 6 Metabolism of 5 mM D-[1- ^{13}C]glucose in human precision-cut renal cortical slices

Slices (3.3 ± 0.5 mg of protein/flask) were incubated for 4 h in Krebs–Henseleit medium with 5 mM [1- ^{13}C]glucose. [1- ^{13}C]Glucose removal was calculated as the difference between the values found in zero-time flasks and those in incubated flasks. Results are expressed as means $\pm$ S.E.M. for three experiments performed in duplicate. The $^{13}\text{CO}_2$ released was calculated as the difference between the [1- ^{13}C]glucose removed and the sum of the non-volatile ^{13}C -products found (in these calculations, it was assumed that the pyruvate found was labelled as the lactate found; indeed, the ^{13}C -pyruvate could not be measured because it was not stable during the lyophilization procedure). Enz. means 'lactate, alanine and pyruvate accumulation measured enzymatically'.

Experimental condition	Amount of labelled metabolite utilized (–) or accumulated ($\mu\text{mol} \cdot 4 \text{ h}^{-1} \cdot \text{g of protein}^{-1}$)									
	Glucose		Lactate				Alanine		Pyruvate	$^{13}\text{CO}_2$
	C-1	C-6	Enz.	C-1	C-2	C-3	Enz.	C-3	Enz.	C-1
[1- ^{13}C]Glucose (5 mM)	-901 ± 156	28 ± 2	711 ± 65	3 ± 1	9 ± 1	286 ± 27	60 ± 2	19 ± 2	76 ± 6	524 ± 126

Human precision-cut renal cortical slices were incubated with a physiological concentration (5 mM) of [1- ^{13}C]glucose to characterize the glucose metabolism better. Substrate utilization and product formation, measured after 2 and 4 h of incubation, were approximately linear with time ($n=2$; results not shown). A representative ^{13}C -NMR spectrum obtained after 4 h of incubation is shown in Figure 4 and the values obtained after 4 h in three experiments are presented in Table 6. Glucose was utilized at high rates, and the only non-volatile products found to accumulate were mainly lactate and to a lesser extent pyruvate and alanine. The large labelling of the C-3 of lactate, and to a lesser extent of alanine, indicates that these compounds were formed from the pyruvate derived from glucose through the glycolytic pathway. The fact that the sum of the labellings of lactate was almost half the lactate enzymatically measured reveals that virtually all the lactate accumulated arose from glucose. The small but significant labelling of the C-6 of glucose means that a small resynthesis of glucose occurred. Finally, the results presented in Table 6 show that a large fraction of the glucose removed was oxidized (see the $^{13}\text{CO}_2$ formed).

Long-term metabolism of glutamine and effect of valproate in human precision-cut renal cortical slices

Table 7 shows that, in William's medium E which contains various amino acids and 11 mM glucose, the slices retained the capacity to metabolize glutamine for up to 48 h of incubation although at lower rates than those observed with freshly prepared slices incubated in Krebs–Henseleit medium for 4 h (see Table 1 for comparison). The fact that, both in the absence and in the presence of valproate, only a part of the glutamine removed was accounted for by the glutamate accumulated clearly indicates that a large fraction of the glutamate synthesized from glutamine by GLNase was further metabolized by AlaAT and GLDH. The fact that the ammonia found was larger when compared with the glutamine removed is in favour of the operation of GLDH under all experimental conditions. However, the nitrogen balance calculations reveal that the nitrogen found as glutamate, ammonia and alanine greatly exceeded the nitrogen removed as glutamine (two nitrogen atoms per glutamine molecule); this means that nitrogenous substrates other than glutamine (other amino acids present

Table 7 Effect of 1 mM valproate on the long-term metabolism of 2 mM glutamine in human precision-cut renal cortical slices

Slices (1.2 ± 0.3 and 1.6 ± 0.2 mg of protein/roller in the 0–24 and 24–48 h incubation experiments respectively) were incubated between 0 and 24 h and between 24 and 48 h in William's medium E with 2 mM glutamine in the absence and in the presence of 1 mM valproate. Net substrate utilization and product formation were calculated as the difference between the incubated flasks and the zero-time flasks. Results are expressed as means $\pm$ S.E.M. for five experiments in each experimental series. Statistical difference was analysed by the paired Student's *t* test comparing values obtained in the absence with those in the presence of 1 mM valproate (**P* < 0.05).

Experimental condition	Incubation period (h)	Metabolite removal (–) or production ($\mu\text{mol} \cdot \text{incubation period}^{-1} \cdot \text{g of protein}^{-1}$)				
		Glutamine	Glutamate	Ammonia	Alanine	Lactate
Glutamine (2 mM)	0–24	-716 ± 37	262 ± 67	1688 ± 90	423 ± 29	1977 ± 190
+ Valproate (1 mM)	0–24	$-1493 \pm 122^*$	150 ± 25	$2450 \pm 115^*$	$1003 \pm 86^*$	2538 ± 203
Glutamine (2 mM)	24–48	-620 ± 86	179 ± 46	1501 ± 149	612 ± 165	2223 ± 601
+ Valproate (1 mM)	24–48	$-1209 \pm 28^*$	$102 \pm 29^*$	$2131 \pm 212^*$	$1093 \pm 175^*$	2292 ± 459

in the incubation medium and endogenous amino acids formed by proteolysis of slice proteins) were also metabolized. An important observation is that valproate addition to the incubation medium approximately doubled glutamine utilization. The increase in glutamine utilization was associated with an approximately equivalent increase in ammonia production and alanine accumulation, indicating an equivalent stimulation of fluxes through GLNase and AlaAT, but no change through GLDH. Note that the sum of the glutamate, alanine and lactate found exceeded by far the glutamine removed. This strongly suggests that a part of the glucose present in William's medium E was metabolized and provided a substantial fraction of the lactate and alanine found. The metabolic viability of the slices at the end of the long-term incubation periods is indicated by their ATP content and their low release of LDH which were 6.9 ± 1.2 and $7.4 \pm 0.7 \mu\text{mol/g}$ of protein and 1.5 ± 0.5 and $0.9 \pm 0.6\%$ at the end of the 0–24 and 24–48 h incubation periods respectively.

DISCUSSION

At first sight, it might appear surprising to use again renal cortical slices, an experimental model that has been virtually abandoned since the 1970s when collagenase became available to isolate kidney tubules or nephron segments or renal cells. In fact, it should be emphasized that the latter experimental models, that are very useful for many studies, suffer from a lack of sufficiently long viability that does not exceed some hours [14,15]. Similarly, cultured primary renal cells tend to dedifferentiate [11,15]; for example, they become highly glycolytic and have a decreased mitochondrial oxidative metabolism [15]. Thus, there is a need for a model that retains its metabolic viability and differentiation over a long period of incubation. As part of a broad study aimed at evaluating the metabolic performances of fresh and cryopreserved slices, we have tested in the present work if precision-cut cortical slices freshly prepared from human kidney could be used for short-term and long-term metabolic and pharmacotoxicological studies related to glutamine metabolism.

Metabolic viability of human precision-cut renal cortical slices used for short-term and long-term incubations

The fact that the removal of glutamine and the production of ammonia, lactate and alanine increased with time indicates that our freshly prepared human renal cortical slices were metabolically viable for at least 4 h of incubation. Further evidence for the viability of our slices is their ATP level, a classical marker of cellular energy metabolism, which was in the same range as that found recently in isolated human renal proximal tubules [35,36].

Given that the mean wet weight/dry weight ratio of the human renal cortex is equal to 5.2 [34] and that proteins represented $90 \pm 2\%$ of the dry weight of our slices ($n=5$ experiments in which the slices were desiccated to constant weight), it can be calculated that the mean ATP concentration of our slices was approx. $1.2 \mu\text{mol/g}$ fresh weight, a value representing 75% of that measured *in vivo* by NMR spectroscopy in the intact human kidney [37]. Moreover, unlike isolated human renal proximal tubules whose preparation requires exposition to collagenase and associated proteases [35,36], the slices used in the present study, released virtually no LDH. This clearly indicates that these slices maintained their plasma membrane integrity well over 48 h of incubation.

The observations (i) that, both in the absence and in the presence of valproate, the $[1-^{13}\text{C}]$ glutamine removed was only partially accounted for by the $[1-^{13}\text{C}]$ glutamate accumulated and (ii) that no $[1-^{13}\text{C}]2$ OG accumulated indicates that OGDH also operated at high rates. Interestingly, like the human kidney *in vivo* [1,3], human precision-cut renal cortical slices produced alanine and serine as shown by the enzymatic and/or the ^{13}C -NMR measurements. This implies that both pyruvate and 3-phosphohydroxypyruvate were synthesized from glutamine and metabolized by the alanine and P-SerAT reactions respectively. In addition, the synthesis of $[2-^{13}\text{C}]$ glutamate from $[3-^{13}\text{C}]$ glutamine clearly indicates that the entire tricarboxylic acid cycle functioned in our slices. Moreover, the production of $^{13}\text{CO}_2$ from $[3-^{13}\text{C}]$ glutamine, which provides a measure of the complete oxidation of glutamine [38], reveals that a substantial fraction of the glutamine removed in the absence and in the presence of valproate was completely oxidized (Table 2). Thus, the above findings reveal that precision-cut renal cortical slices, which are much thinner compared with the slices prepared free-hand or with a Stadie–Riggs microtome, were well-oxygenated. Further evidence for this was obtained in two experiments in which four thin slices (1.24 mg of protein/flask) and four thick slices (5.72 mg of protein/flask) were prepared from the same tissue cores with the Krumdieck slicer and free-hand respectively and incubated in 4 ml of Krebs–Henseleit medium for 4 h with 2 mM glutamine as substrate. In thin slices, the removal of glutamine, the accumulation of glutamate, ammonia, alanine, glucose, lactate, pyruvate and glutamine complete oxidation were 646, 339, 865, 104, 16, 62, 8 and $101 \mu\text{mol/g}$ of protein/4 h respectively; the corresponding values in thick slices were 340, 193, 487, 108, 18, 51, 7 and $0 \mu\text{mol/g}$ of protein/4 h respectively (mean values of two experiments). These additional results clearly show that, on the same protein weight basis, thin slices removed much more glutamine and produced much more ammonia than thick slices. A substantial fraction of the glutamine used was completely oxidized in thin slices but not in thick slices.

Maintenance of gluconeogenesis in human precision-cut renal cortical slices

In view of the observation that the human kidney *in vivo* produces small amounts of labelled glucose from infused-labelled glutamine [5], the absence of ^{13}C -glucose synthesis and the very low glucose found in the presence of 2 mM $[3-^{13}\text{C}]$ glutamine might raise some concerns about the metabolic viability of our slices. In fact, the presence of glucose synthesis observed with 2 mM lactate as substrate rules out the possibility that the gluconeogenic capacity of these slices was lost.

A possible explanation for the virtual absence of glucose synthesis from glutamine in human precision-cut slices is that glutamine is in fact a poor gluconeogenic substrate in the human kidney, at least *in vitro*; in this regard, it should be pointed out that the amount of glucose synthesized from physiological or near-physiological concentrations of glutamine by suspensions of isolated human proximal tubules [12] and by microdissected human proximal nephron segments [14] did not exceed by far the amount of glucose either present at zero time or synthesized from endogenous substrates in the absence of glutamine. In agreement with this observation is the low rate of glucose synthesis from 5 mM glutamine observed by Watford et al. [10] in isolated human proximal tubules and from 10 mM glutamine reported by Nakada et al. [13] in human renal cortical slices. Consistent with our view that glutamine is a poor gluconeogenic substrate when compared with lactate is the recent observation by Meyer et al. [33] that lactate gluconeogenesis in the human kidney *in vivo* is approx. 10 times greater than that from glutamine.

An alternative explanation, which does not rule out the preceding one, for the virtual absence of glucose synthesis from glutamine in our slices is that the glucose synthesized was degraded by the glycolytic segments of the human nephrons present in our slices. The results presented in Tables 5 and 6 and Figure 4 show for the first time that, both at very low and at a physiological concentration of glucose, human renal cortical slices have the capacity to remove this substrate, making this explanation likely.

Reactivity to the pharmaco-toxicological effect of valproate

It is of interest to underline that our slices used in short-term incubations were at least as sensitive as isolated tubules [12] to the ammoniagenic effect of valproate. Interestingly, the slices used in long-term incubations were even more sensitive to the ammoniagenic effect of valproate; this may be due to the presence of glucose (as an incubation medium component) that provided pyruvate which in turn favoured alanine synthesis and therefore glutamate metabolism through the AlaAT reaction, and glutamine metabolism through the GLNase reaction, the main target of the ammoniagenic effect of valproate.

Another interesting new observation made from the results presented in Table 2 is that the labelled C-3 of glutamine that was removed and unaccounted for by the ^{13}C -labelled products accumulated did not change in the presence of valproate. Thus, the valproate-induced stimulation of glutamine removal was accompanied by an increase in partial oxidation but not in complete oxidation of the glutamine carbon skeleton metabolized. Therefore this indicates that flux through pyruvate dehydrogenase, which was not detected by the labelling of the C-4 and C-5 of glutamate in the presence of $[3-^{13}\text{C}]$ glutamine (see [38] for further explanation), was not increased despite an increased synthesis of pyruvate. Finally, another new piece of information provided by this study is that, at least *in vitro*, serine carbon may originate from glutamine carbon.

In conclusion, our study clearly demonstrates that human precision-cut renal cortical slices incubated not only for short periods

(up to 4 h) but also for much longer periods (up to 48 h) are metabolically viable at least when glutamine metabolism is concerned. In addition, like suspensions of human proximal tubules [12] and the human kidney *in vivo* [21], they retain their full capacity to respond to the ammoniagenic effect of valproate, a well-established stimulator of human renal glutamine metabolism. Thus, compared with other experimental models used *in vitro*, these slices appear to be suitable for metabolic and pharmacotoxicological studies.

We thank Professor J. M. Dubernard and his co-workers and Dr R. Bouvier and co-workers for their collaboration. This work was supported by a grant from the European Commission Biotech program (project number: BIO4-CT97-2145).

REFERENCES

- Owen, O. E. and Robinson, R. R. (1963) Amino acid extraction and ammonia metabolism by the human kidney during the prolonged administration of ammonium chloride. *J. Clin. Invest.* **42**, 263–276
- Wahren, I. and Felig, P. (1975) Renal substrate exchange in human diabetes mellitus. *Diabetes* **24**, 730–734
- Tizianello, A., De Ferrari, G., Garibotto, G., Gurreri, G. and Robaldo, C. (1980) Renal metabolism of amino acids and ammonia in subjects with normal renal function and in patients with chronic renal insufficiency. *J. Clin. Invest.* **65**, 1162–1173
- Brundin, T. and Wahren, J. (1994) Renal oxygen consumption, thermogenesis, and amino acid utilization during an infusion of amino acids in man. *Am. J. Physiol.* **267**, E648–E655
- Meyer, C., Dostou, J., Nadkarni, V. and Gerich, J. (1998) Effects of physiological hyperinsulinemia on systemic, renal, and hepatic substrate metabolism. *Am. J. Physiol.* **275**, F915–F921
- Cersosimo, E., Garlick, P. and Ferretti, J. (2000) Renal substrate metabolism and gluconeogenesis during hypoglycemia in humans. *Diabetes* **49**, 1186–1193
- Curthoys, N. P. and Watford, M. (1995) Regulation of glutaminase activity and glutamine metabolism. *Annu. Rev. Nutr.* **15**, 133–159
- Nissim, I. (1999) Newer aspects of glutamine/glutamate metabolism: the role of acute pH changes. *Am. J. Physiol.* **277**, F493–F497
- Welbourne, T., Routh, R., Yudkoff, M. and Nissim, I. (2001) The glutamine/glutamate couplet and cellular function. *News Physiol. Sci.* **16**, 157–160
- Watford, M., Vinay, P., Lemieux, G. and Gougoux, A. (1980) Inhibition of renal gluconeogenesis and phosphoenolpyruvate carboxykinase activity by 3-mercaptopicolinic acid: studies in rat, guinea pig, dog, rabbit, and man. *Can. J. Biochem.* **58**, 440–445
- Nissim, I. and States, B. (1989) Ammoniogenesis by cultured human renal cortical epithelial cells: study with ^{15}N . *Am. J. Physiol.* **256**, F187–F196
- Martin, G., Durozard, D., Besson, J. and Baverel, G. (1990) Effect of the antiepileptic drug sodium valproate on glutamine and glutamate metabolism in isolated human kidney tubules. *Biochim. Biophys. Acta* **1033**, 261–266
- Nakada, J., Machida, T., Masuda, F., Kondou, I., Furuta, N., Suzuki, H. and Endou, H. (1994) Renal gluconeogenesis in man: comparison with rats and rabbits. *Jpn. J. Nephrol.* **36**, 39–42
- Conjard, A., Martin, M., Guitton, J., Baverel, G. and Ferrier, B. (2001) Gluconeogenesis from glutamine and lactate in the isolated human renal proximal tubule: longitudinal heterogeneity and lack of response to adrenaline. *Biochem. J.* **360**, 371–377
- Chauvin, M. F., Bolon, C., Conjard, A., Martin, G., Ferrier, B., Martin, M., Michoudet, C., Durozard, D., Lareal, M. C., Gauthier, C. et al. (1996) Advantages and limitations of the use of isolated kidney tubules in pharmacotoxicology. *Cell Biol. Toxicol.* **12**, 283–287
- Parrish, A. R., Gandolfi, A. J. and Brendel, K. (1995) Precision-cut tissue slices: applications in pharmacology and toxicology. *Life Sci.* **57**, 1887–1901
- Bach, P. H., Vickers, A. E. M., Fisher, R., Baumann, A., Brittebo, E., Carlile, D. J., Koster, H. J., Lake, B. G., Salmon, F., Sawyer, T. W. et al. (1996) The use of tissue slices for pharmaco-toxicological studies. *ATLA-Altern. Lab. Anim.* **24**, 893–923
- Lerche-Langrand, C. and Toutain, H. J. (2000) Precision-cut liver slices: characteristics and use for *in vitro* pharmaco-toxicology. *Toxicology* **153**, 221–253
- Simon, D. and Penry, J. K. (1975) Sodium di-n-propylacetate (DPA) in the treatment of epilepsy. A review. *Epilepsia* **16**, 549–573
- Coulter, D. L. and Allen, R. J. (1980) Secondary hyperammonemia: a possible mechanism for valproate encephalopathy. *Lancet* **1**, 1310–1311
- Warter, J. M., Marescaux, C., Chabrier, G., Rumbach, L., Micheletti, B. and Imler, M. (1984) Renal glutamine metabolism in man during treatment with sodium valproate. *Rev. Neurol.* **140**, 370–371

- 22 Lamprecht, W. and Trautchohl, I. (1974) Adenosine-5'-triphosphate. Determination with hexokinase and glucose-6-phosphate dehydrogenase. In *Methods of Enzymatic Analysis*, vol. 4 (Bergmeyer, H. U., ed.), pp. 2101–2110, Academic Press, New York
- 23 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275
- 24 Bergmeyer, H. U. and Bernt, E. (1974) Lactate dehydrogenase: UV assay with pyruvate and NADH. In *Methods of Enzymatic Analysis*, vol. 2 (Bergmeyer, H. U., ed.), pp. 574–579, Academic Press, New York
- 25 Baverel, G., Bonnard, M., D'Armagnac de Castanet, E. and Pellet, M. (1978) Lactate and pyruvate metabolism in isolated renal tubules of normal dogs. *Kidney Int.* **14**, 567–575
- 26 Baverel, G. and Lund, P. (1979) A role for bicarbonate in the regulation of mammalian glutamine metabolism. *Biochem. J.* **184**, 599–606
- 27 Chauvin, M. F., Mégnin-Chanet, F., Martin, G., Lhoste, J. M. and Baverel, G. (1994) The rabbit kidney tubule utilizes glucose for glutamine synthesis. A ^{13}C NMR study. *J. Biol. Chem.* **269**, 26025–26033
- 28 Chauvin, M. F., Mégnin-Chanet, F., Martin, G., Mispelter, J. and Baverel, G. (1997) The rabbit kidney tubule simultaneously degrades and synthesizes glutamate. A ^{13}C NMR study. *J. Biol. Chem.* **272**, 4705–4716
- 29 Shaka, A. J., Keeler, J., Frenkiel, T. and Freeman, R. (1983) An improved sequence for broadband decoupling: Waltz 16. *J. Magn. Reson.* **52**, 335–338
- 30 Howarth, O. W. and Lilley, D. M. J. (1978) Carbon-13-NMR of peptides and proteins. *Prog. NMR Spectrosc.* **12**, 1–40
- 31 Canioni, P., Alger, S. R. and Shulman, R. G. (1983) Natural abundance carbon-13 nuclear magnetic resonance spectroscopy of liver and adipose tissue of the living rat. *Biochemistry* **22**, 4974–4980
- 32 Nieth, H. and Schollmeyer, P. (1966) Substrate utilization of the human kidney. *Nature (London)* **209**, 1244–1245
- 33 Meyer, C., Stumvoll, M., Welle, S., Woerle, H. J., Haymond, M. and Gerich, J. (2003) Relative importance of liver, kidney, and substrates in epinephrine-induced increased gluconeogenesis in humans. *Am. J. Physiol.* **285**, E819–E826
- 34 Baverel, G., Bonnard, M. and Pellet, M. (1979) Lactate and pyruvate metabolism in isolated human kidney tubules. *FEBS Lett.* **101**, 282–286
- 35 Dubourg, L., Michoudet, C., Cochat, P. and Baverel, G. (2001) Human kidney tubules detoxify chloroacetaldehyde, a presumed nephrotoxic metabolite of ifosfamide. *J. Am. Soc. Nephrol.* **12**, 1615–1623
- 36 Dubourg, L., Tanière, P., Cochat, P., Baverel, G. and Michoudet, C. (2002) Toxicity of chloroacetaldehyde is similar in adult and pediatric kidney tubules. *Pediatr. Nephrol.* **17**, 97–103
- 37 Kugel, H., Wittsack, H.-J., Wenzel, F., Stippel, D., Heindel, W. and Lackner, K. (2000) Non-invasive determination of metabolite concentrations in human transplanted kidney *in vivo* by ^{31}P MR spectroscopy. *Acta Radiol.* **41**, 634–641
- 38 Vercoutère, B., Durozard, D., Baverel, G. and Martin, G. (2004) Complexity of glutamine metabolism in kidney tubules from fed and fasted rats. *Biochem. J.* **378**, 485–495

Received 2 August 2004/17 November 2004; accepted 3 December 2004

Published as BJ Immediate Publication 3 December 2004, DOI 10.1042/BJ20041309