

Fuel selection by the kidney: adaptation to starvation

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Sélection de substrats énergétiques par le rein

RÉSUMÉ

Pour couvrir ses besoins énergétiques *in vivo*, le rein, qui a un apport sanguin et une consommation d'O₂ importants, est capable de métaboliser une grande variété de substrats. Le néphron, qui est l'unité fonctionnelle du rein, est fait de segments successifs morphologiquement, fonctionnellement et métaboliquement différents. Les déterminants de la sélection des substrats énergétiques par un segment spécifique de néphron qui agissent ensemble sont (1) l'existence et les propriétés cinétiques des transporteurs de substrats à travers les membranes apicale ou basolatérale des cellules rénales, (2) l'existence et l'activité de voies métaboliques pour l'apport et la consommation d'énergie, (3) l'environnement local et (4) l'état métabolique et hormonal de l'organisme qui détermine non seulement la disponibilité et la compétition des substrats au niveau du rein, mais aussi les modifications adaptatives des transporteurs, des voies métaboliques et de leurs régulations dans le rein. Les principales sources potentielles d'énergie pour le rein sont le glucose, le lactate, les acides gras, les corps cétoniques, le citrate, le glycérol et la glutamine. La manière dont ces substrats sont sélectionnés dans le rein intact et dans différents segments de néphron est illustrée dans des conditions métaboliques différentes (à l'état nourri, pendant le jeûne et pendant l'acidose métabolique). Ces exemples montrent que (1) l'apport de substrats au rein *in vivo* dépasse largement ses besoins énergétiques, (2) chaque segment de néphron a ses caractéristiques propres pour la sélection de substrats et (3) on sait souvent imparfaitement ou pas du tout où est le site précis de l'utilisation des substrats *in vivo*, dans quelle mesure chacun des substrats captés est utilisé comme source d'énergie, et quelle est la base moléculaire des modifications adaptatives de l'utilisation des substrats par le rein.

Although the kidney tissue represents less than 0.5% of the body weight, it receives 20–25% of the cardiac output and accounts for about 10% of whole-body O₂ consumption, representing intense synthetic activity supplying the ATP needed to reclaim most of the solutes filtered through glomerular membranes (Cohen & Barac-Nieto, 1973). Since energy reserves, in the form of glycogen and phosphocreatine (Needleman *et al.* 1968; Bastin *et al.* 1987) or neutral lipids (Hohenegger & Schuh, 1980; Tannenbaum *et al.* 1993), are very low the kidney must extract and degrade circulating substrates to supply its energetic needs. The aim of the present review is to provide a general picture of the

nature and amounts of substrates taken up by the rat kidney and of the cellular and molecular mechanisms whereby the kidney selects its fuels in the fed and fasted states.

SUBSTRATE UTILIZATION AT THE WHOLE-ORGAN LEVEL

Identification and quantification of the major substrates taken up by the kidney can be achieved by determining renal clearances, blood flow and arterio-venous concentration differences. Using microanalytical methods based on enzymic cycling, Elhamri *et al.* (1993) measured simultaneously the renal uptake or release of twelve different circulating metabolites in both fed and 72 h-starved rats (Table 1). In the fed state, lactate and possibly glucose were the main substrates taken up by the rat kidney; other substrates taken up to a smaller but significant extent included fatty acids, ketone bodies, citrate, glutamine and glycerol. Glutamate, α -ketoglutarate and pyruvate, taken up in only small amounts, could not make a significant contribution to the renal energy provision (Elhamri *et al.* 1993). Other studies have shown that lactate (Squires *et al.* 1976; Goldstein, 1987), ketone bodies and citrate (Goldstein, 1987), but not glucose or glutamine (Squires *et al.* 1976; Hohenegger & Schuh, 1980; Goldstein, 1987), are utilized by the kidney of fed rats. In contrast with the results of Elhamri *et al.* (1993), a net release of glucose (Churchill *et al.* 1973; Kida *et al.* 1978), total free fatty acids, free glycerol and triacylglycerols (Hohenegger & Schuh, 1980) was observed in the renal vein of fed rats.

Table 1. Concentrations of renal substrates in arterial blood, their uptake by the kidney and the oxygen equivalents of uptakes in fed and 72 h-starved rats (From data of Elhamri *et al.* 1993)

(The values are means. The sum of O₂ equivalents of the substrates taken up was calculated without taking into account the uptake of glucose)

Substrate	Fed rats			Starved rats		
	Blood concentration (mM)	Uptake†	O ₂ equivalent†	Blood concentration (mM)	Uptake†	O ₂ equivalent†
Citrate	0.17	1.5	6.8	0.14*	1.3	5.9
Lactate	1.23	6.1	18.3	0.57*	2.2*	6.6
Glycerol	0.10	1.1	3.9	0.11	1.8*	6.3
AcAc	0.36	1.9	7.6	1.05*	5.5*	22.0
β -OH-B	0.18	0.6	2.7	1.81*	4.8*	21.6
Glutamate	0.13	0.2	0.9	0.14	0.2	0.9
Glutamine	0.45	0.9	4.1	0.44	2.3*	10.4
FFA	0.91	0.5	11.5	1.57*	2.8*	64.4
Glucose	7.34	6.6	39.6	4.39*	4.0	24.0
Sum	—	—	55.8	—	—	138.1

FFA, plasma free fatty acids; AcAc and β -OH-B, acetoacetate and β -hydroxybutyrate respectively.

* Mean values for starved rats were significantly different from those for fed rats ($P < 0.05$).

† Values of uptake and O₂ equivalents are expressed in $\mu\text{mol/kg}$ body weight per min for one kidney. O₂ equivalents: 3 for lactate; 3.5 for glycerol, 4.0 for acetoacetate, 4.5 for glutamine, glutamate, β -hydroxybutyrate and citrate, 6 for glucose and 23 for FFA.

Elhamri *et al.* (1993) recognized that, given the small percentage of circulating glucose extracted by the kidney, their data on renal handling of this substance, like those of other authors, are not reliable. Thus, depending on whether the glucose uptake is included in the calculations (Table 1), the sum of the oxygen equivalents required for complete oxidation of the substrates taken up by the kidney of fed rats exceeds, or is in agreement with, the published values (Weinstein & Szyjewick, 1976) of renal O₂ consumption in the rat *in vivo*. Neglecting the contribution of glucose and assuming complete oxidation of all the other substrates taken up, oxidation of lactate, fatty acids, ketone bodies and citrate would account for 33, 21, 19 and 12% of total calculated renal O₂ consumption (see Table 1). Since the synthesis of fatty acids in the kidney is very low (Hohenegger & Schuh, 1980), the only possible fate of the ketone bodies metabolized by the kidney is complete oxidation. Therefore, even in the fed state, ketone bodies and particularly acetoacetate, appear to contribute substantially to the renal energy provision.

As shown in Table 1, Elhamri *et al.* (1993) found that starvation for 72 h led to a large increase in blood ketone body and fatty acid concentrations and to a large fall in blood lactate and glucose concentrations; the circulating concentrations of other potential renal substrates, except citrate which fell slightly, did not significantly change. Starvation caused a large stimulation of the renal uptake of ketone bodies and fatty acids and, to a lesser extent, of glutamine and glycerol and, by contrast, lactate uptake was diminished whereas that of glucose did not change significantly. The changes in the renal uptake of ketone bodies, fatty acids and lactate were proportional to the changes in the arterial supply of these compounds. The renal blood flow, glomerular filtration rate and urine volume were not altered by starvation. Again, neglecting the contribution of glucose, the complete oxidation of all other substrates taken up by the kidney would lead to a considerable increase in renal O₂ consumption (Table 1). Unfortunately, the concomitant O₂ consumption was not measured in these experiments. Unless there was some uncoupling effect of oxidative phosphorylation caused for example by the increased supply of fatty acids, such an increase in renal O₂ consumption is unlikely because the renal reabsorption of Na, the main process responsible for renal O₂ consumption (Cohen & Barac-Nieto, 1973), remained unchanged in the 72 h-starved rats when compared with that in fed rats (505 *v.* 486 $\mu\text{mol/kg}$ per min for one kidney respectively). This means that a large proportion of the substrates taken up has probably been converted into compounds not measured in this study that were either stored *in situ* or exported via the renal vein. A substantial uptake of free fatty acids and glycerol and their probable incorporation into triacylglycerols is suggested by a significant increase in the concentration of triacylglycerols in the renal cortex of the rat after a 24 h fast (Hohenegger & Schuh, 1980; Wirthensohn *et al.* 1980); in agreement with this view also is the demonstration by the same authors that the net renal release of glycerol and total fatty acids found in fed rats was converted into a net uptake in 24 h-starved rats. Thus, the latter observations together with the results obtained by Elhamri *et al.* (1993; see also Table 1) lead to the unavoidable conclusion that ketone bodies are the preferred fuels of renal respiration during starvation. However, this conclusion does not rule out the possibility that the other substrates taken up (free fatty acids, citrate, lactate, glycerol, glutamine and perhaps glucose) also make some contribution to the renal energy supply.

It should be emphasized that the conclusions drawn from renal substrate balance experiments, which yield useful information about the relative importance of potential fuels of the kidney *in vivo*, suffer from a number of important limitations. Because of the

high renal blood flow and small arterio-venous concentration differences, large errors are inherent in this experimental approach, particularly for substances such as glucose circulating at high concentrations. The rates of substrate uptake or metabolite release measured are only net rates and do not take into account the possibility that synthesis and degradation of a given substrate occur simultaneously. Indeed, it is well established that whole rat kidney has the capacity to utilize glucose via glycolysis (Hems & Gaja, 1972; Cohen *et al.* 1977; Anaizi & Cohen, 1991) and synthesize glucose via gluconeogenesis (Nishiitsuji *et al.* 1967; Bowman, 1970; Hems & Gaja, 1972). Similarly, whole rat kidney can form lactate by glycolysis under aerobic or anaerobic conditions (Hems & Gaja, 1972; Bartlett *et al.* 1984) and utilize lactate as a substrate (Nishiitsuji *et al.* 1967; Bowman, 1970; Cohen *et al.* 1977; Bartlett *et al.* 1984). Also, rat kidney (Damian & Pitts, 1970; Hems, 1972) contains significant activities of both glutamine synthetase (Richterich & Goldstein, 1958; Lemieux *et al.* 1976; Burch *et al.* 1978a), the enzyme responsible for glutamine synthesis, and phosphate-activated glutaminase (Richterich & Goldstein, 1958; Curthoys & Lowry, 1973), which initiates the degradation of glutamine. Since the renal activity of β -hydroxybutyrate dehydrogenase is high (Robinson & Williamson, 1980), acetoacetate and β -hydroxybutyrate are readily interconverted in whole rat kidney (Goldstein *et al.* 1982). Similarly, glycerol and free fatty acids can be incorporated into and released from triacylglycerols by rat kidney *in vivo* (Hohenegger & Schuh, 1980). Citrate may also be degraded and synthesized by rat kidney (Simpson, 1983). Therefore, if all or some of these opposing metabolic processes occur concurrently *in vivo*, the real contribution of each potential fuel to the provision of energy to the kidney is difficult to establish on the basis of renal balance studies performed *in vivo*. Furthermore, these experiments do not allow identification of the precise nephron segment where substrate metabolism occurs, nor the extent of substrate oxidation and interaction or the cellular mechanisms of adaptation to changing situations. Therefore, complementary information is needed at the cellular and subcellular levels.

SUBSTRATE UTILIZATION AND OXIDATION AT THE NEPHRON SEGMENT LEVEL

Metabolic heterogeneity of the rat kidney

Excellent reviews are available on the metabolic heterogeneity of the kidney (Guder & Schmidt, 1976; Ross & Guder, 1982; Guder & Ross, 1984; Kurokawa *et al.* 1985; Guder *et al.* 1986; Wirthensohn & Guder, 1986; Guder & Morel, 1992). In brief, the kidney comprises the cortex, which represents about 70% of the organ weight, and the medulla which is divided into the outer and inner medulla (Pfaller & Rittinger, 1980). About 90% of the renal blood flow is distributed to the cortex, allowing a large O₂ supply and a high pO₂ in this renal zone (Leichtweiss *et al.* 1969). The renal blood flow and the O₂ supply to the outer medulla may become rapidly limiting and, therefore, the metabolism of this renal zone may shift from aerobic to anaerobic. The paucity of mitochondria (Guder & Morel, 1992) and the low pO₂ (Leichtweiss *et al.* 1969) of the inner medulla suggest that the nephron segments contained in this part of the kidney rely on anaerobic glycolysis to supply their energetic needs. The osmolality, progressively increasing in the medulla along the cortico-medullary axis, is an additional factor liable to influence the renal medullary energy metabolism as demonstrated in microdissected rat medullary collecting tubules and thick ascending limbs of Henle (Hus-Citharel & Morel, 1986). Fig. 1 shows

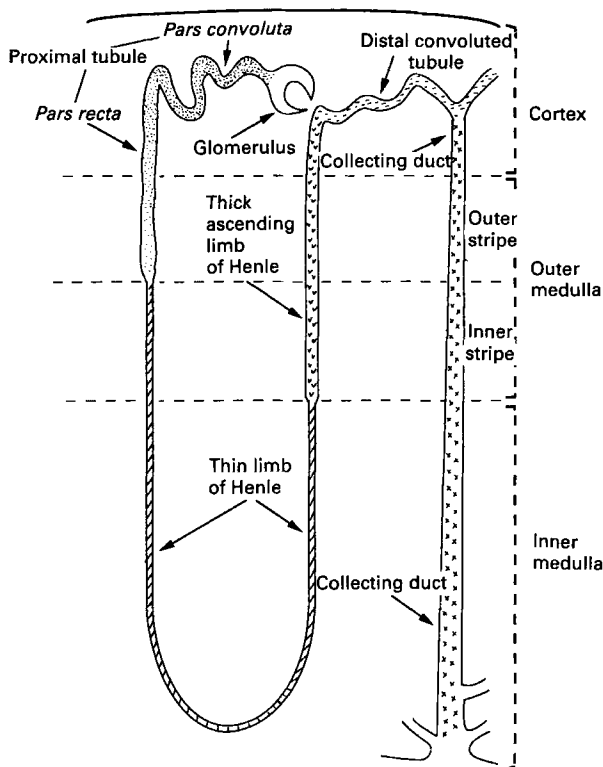


Fig. 1. Interrelationships between the various segments of the nephron and the different renal zones.

schematically the interrelationships between the various segments of the nephron, the functional unit of the kidney, and the different renal zones (for details, see Kriz & Bankir, 1988).

The distribution of the activities of oxidative enzymes parallels the content of mitochondria and the activity of Na^+, K^+ -ATPase (EC 3.6.1.37), considered to be the main enzyme responsible for the reabsorptive transport of Na^+ across the basolateral membrane of renal cells (Guder & Morel, 1992). For example, the highest activities of enzymes of the citric acid cycle, of 3-oxoacid-CoA transferase (EC 2.8.3.5) and of β -hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35; Le Hir & Dubach, 1982*a,b*; Burch *et al.* 1984) are found in the thick ascending limb of Henle, the distal convoluted tubule and the proximal tubule whereas lower activities of these enzymes are found in the glomerulus, collecting duct and deep medullary segments of the nephron. Key enzymes of glycolysis (hexokinase (EC 2.7.9.1), phosphofructokinase 1 (EC 2.7.1.11) and pyruvate kinase (EC 2.7.1.40)) are most active in all distal nephron segments (Burch *et al.* 1974; Schmidt *et al.* 1975; Schmidt & Dubach, 1976) and much less active in the proximal tubule (*pars convoluta* and *pars recta*) which is the only nephron segment containing significant activities of glycerol kinase (EC 2.7.1.30; Burch *et al.* 1982) and of the key enzymes (pyruvate carboxylase (EC 6.4.1.1), phosphoenolpyruvate carboxykinase (EC 4.1.1.49), fructose biphosphatase (EC 3.1.3.11) and glucose-6-

phosphatase (*EC* 3.1.3.9) of gluconeogenesis (Burch *et al.* 1978*b*; Salto *et al.* 1991). Significant activity of lactate dehydrogenase (*EC* 1.1.1.27), an enzyme common to glycolysis and gluconeogenesis, is present along the entire length of the nephron (Burch *et al.* 1974). The activity of phosphate-activated glutaminase (*EC* 3.5.1.2; Curthoys & Lowry, 1973; Wright & Knepper, 1990*b*) is present in all nephron segments of the rat, whereas that of glutamine synthetase (*EC* 6.3.1.2) is restricted to the straight portion (the *pars recta*) of the proximal tubule (Burch *et al.* 1978*a*).

Incubation of microdissected rat nephron segments with equimolar concentrations of ¹⁴C-labelled substrates has given interesting information about the relative capacity of the proximal convoluted tubule and the medullary and cortical thick ascending limb of the Henle loop to produce ¹⁴CO₂. Among the physiological substrates shown to be taken up *in vivo* by the rat kidney, glucose and lactate do not appear to be oxidized to a significant extent in the proximal convoluted tubule whereas glutamine and citrate and, to a much lesser extent, palmitate are readily oxidized by this nephron segment (Klein *et al.* 1981); in the thick ascending limb of Henle, glucose, lactate, glutamine and palmitate are oxidized at high rates and citrate at the lowest rate (Klein *et al.* 1981). Le Bouffant *et al.* (1984) also found that glucose is poorly oxidized in the proximal convoluted tubule, whereas it is oxidized at high rates, as is lactate, in the thick ascending limb of Henle, the distal convoluted tubule, and the cortical and the medullary collecting ducts. Le Bouffant *et al.* (1984) also observed that when glucose and lactate were available together lactate was the preferred fuel in the thick ascending limb of Henle, whereas the cortical collecting tubule oxidized glucose preferentially to lactate. An important role for long-chain free fatty acids as a source of energy has been demonstrated in rat renal cortical slices (Weidemann & Krebs, 1969; Hohenegger *et al.* 1973) and outer medullary slices (Hohenegger *et al.* 1973). In contrast with the findings of Klein *et al.* (1981), a large proportion of the lactate taken up by proximal tubule suspensions from fed and starved rats has been shown to undergo complete oxidation (Guder & Wieland, 1972; Janssens *et al.* 1980).

In microdissected proximal tubules, the best substrate (at a concentration of 2 mM) to maintain the cellular ATP content during incubation is glutamine followed by lactate and β-hydroxybutyrate but glucose is inefficient, whereas in microdissected cortical and medullary thick ascending limb of Henle, in distal convoluted tubule and in cortical and outer medullary collecting tubule, glucose and lactate are the best substrates followed by β-hydroxybutyrate and glutamine (Jung *et al.* 1989). Consistent with an important role for glucose in the provision of energy to distal, but not proximal, nephron segments is the observation that lactate accumulated from 5 mM-glucose only in microdissected rat thick ascending limb of Henle, distal convoluted tubule and cortical, outer and inner medullary collecting tubules, both in the absence and the presence of antimycin, an inhibitor of oxidative phosphorylation (Bagnasco *et al.* 1985).

Adaptative changes in fuel selection by the rat kidney during starvation

In the light of information available from arterio-venous difference measurements and on the metabolic segmentation of the nephron, one may attempt to provide a general picture of the selection of fuels by the various nephron segments of both fed and 72 h-starved rats.

It is very likely that glucose, via anaerobic glycolysis, provides energy to all inner

medullary structures irrespective of the nutritional state. Since glucose reabsorption is restricted to the proximal tubule (Frohnert *et al.* 1970), the glucose used is probably transported across the basolateral membrane of the cells of these inner medullary nephron segments by the facilitated diffusion glucose transporters GLUT 1 and GLUT 2 (Thorens *et al.* 1990; Chin *et al.* 1993). The very limited reserves of glycogen contained in these segments may represent a source of energy but only for very short periods, as shown during ischaemia (Needleman *et al.* 1968; Bastin *et al.* 1987). Although glucose and lactate, when present as the only substrates, seem to be the best energy providers to the thick ascending limb of Henle, the distal convoluted tubule and the cortical and outer medullary collecting tubule in the rat, it is conceivable, but not proven, that ketone bodies and perhaps also free fatty acids are the predominant fuels of these nephron segments, especially during starvation, because these substrates are known to reduce both glucose and lactate removal and oxidation by inhibiting pyruvate dehydrogenase (EC 1.2.4.1; Guder & Wieland, 1972; Guder & Schmidt, 1976). It should be mentioned that the basolateral membrane of the thick ascending limb of Henle is the only nephron segment containing the insulin-stimulated glucose transporter GLUT 4 (Chin *et al.* 1993). To our knowledge, the existence and the characteristics of apical and basolateral lactate transporters in these distal nephron segments in the rat remain to be established.

Although the transport of long-chain free fatty acids by the isolated perfused rat kidney is saturable (Trimble, 1982) and, therefore, possibly carrier-mediated (Trimble, 1989), the existence of such a carrier has not been demonstrated so far. It should be mentioned here that the kinetic characteristics of this transport do not change in perfused kidneys isolated from starved rats (Trimble, 1980). It is also noteworthy that no alteration in the activity of fatty acid-binding protein, present in the kidney and considered to play a role in the control of intracellular fatty acid metabolism, has not been reported during starvation (Paulussen *et al.* 1986).

Using rat renal cortical slices, Weidemann & Krebs (1969) have demonstrated that ketone bodies inhibit the oxidation of free fatty acids; therefore, it is conceivable that during starvation ketone bodies inhibit fatty acid oxidation in the nephron segments present in the cortex, i.e. the proximal tubule, the distal convoluted tubule, the cortical thick ascending limb of Henle and the cortical collecting duct, which contain β -oxidation and citric acid cycle enzymes. The increased availability and uptake of fatty acids, leading to their increased conversion into triacylglycerols in the rat proximal tubule (Wirthensohn & Guder, 1980), probably explains, at least in part, the augmented accumulation of triacylglycerols observed in the cortex of starved rats (Hohenegger & Schuh, 1980; Wirthensohn *et al.* 1980). Whether the latter process contributes to reduced utilization of free fatty acids as fuels of respiration in the proximal tubule of starved rats is unknown.

The available evidence suggests that the proximal tubule reabsorbs all the glucose filtered through the glomerulus without any significant metabolism in the cells of the proximal tubule. Reabsorption of glucose in the proximal tubule occurs via the coupling of Na^+ -glucose cotransporters across the apical membrane (Dominguez *et al.* 1992) and of two facilitated diffusive glucose transporters, GLUT 1 and GLUT 2, which are localized in the basolateral membrane of proximal convoluted and straight tubules respectively (Thorens *et al.* 1990; Dominguez *et al.* 1992; Chin *et al.* 1993). It is probable that, *in vivo*, the proximal tubule also contributes to the basolateral efflux of the glucose synthesized by gluconeogenesis which has been shown to occur in various experimental

preparations of the rat kidney cortex from lactate (Krebs *et al.* 1963; Weidemann & Krebs, 1969; Guder & Wieland, 1972; Janssens *et al.* 1980), glutamine (Watford *et al.* 1980), citrate (Kurokawa & Rasmussen, 1973) and glycerol (Krebs *et al.* 1963; Guder *et al.* 1971). This glucose production probably masks, at the whole-organ level *in vivo*, the concurrent utilization of glucose by the distal segments of the nephron. Several authors have demonstrated that glucose can be metabolized by various preparations of the rat kidney (Underwood & Newsholme, 1967; Weidemann & Krebs, 1969; Hems & Gaja, 1972; Ross *et al.* 1973; Anaizi & Cohen, 1991). Although Elhamri *et al.* (1993) could not demonstrate a statistically significant change in renal glucose handling during starvation, there was a tendency for glucose removal to decrease in their study. This may be due to (1) a decrease in glucose utilization in distal segments of the nephron resulting from an intrinsic decrease in the ability to metabolize and oxidize glucose (Underwood & Newsholme, 1967; Weidemann & Krebs, 1969; Hems & Gaja, 1972) and/or the increased availability and metabolism of ketone bodies and fatty acids (Underwood & Newsholme, 1967; Weidemann & Krebs, 1969) and (2) a starvation-induced stimulation of gluconeogenesis from lactate (Krebs *et al.* 1963, 1965, 1966; Janssens *et al.* 1980), glutamine (Iynedjian & Peters, 1974; Bennett *et al.* 1975), and glycerol (Krebs *et al.* 1963; Underwood & Newsholme, 1967). In the proximal tubule, the increased conversion of lactate into glucose at the expense of lactate oxidation during starvation may result from the sparing effect of fatty acids and ketone bodies (Krebs *et al.* 1965, 1966) and from the stimulation of pyruvate carboxylase by an elevated cellular acetyl-CoA level (Krebs *et al.* 1965). Increased transcription of the phosphoenolpyruvate carboxykinase gene known to occur in the kidney of starved rats (Meisner *et al.* 1985) and leading to the increased synthesis (Iynedjian *et al.* 1975) and activity of renal phosphoenolpyruvate carboxykinase (Iynedjian & Peters, 1974; Bennett *et al.* 1975; Iynedjian *et al.* 1975), and to the increased levels of the corresponding mRNA (Pollock, 1989) also shown in acidosis (Iynedjian & Hanson, 1977), represents another important mechanism whereby glucose synthesis not only from lactate but also from glutamine and perhaps from citrate is increased in the proximal tubule of starved rats. Burch *et al.* (1978*b*) have also demonstrated increased activity of phosphoenolpyruvate carboxykinase, fructose-1,6-bisphosphatase and glucose-6-phosphatase in the rat proximal tubule during starvation.

Given that glycerokinase, the enzyme initiating glycerol metabolism and forming 3-glycerophosphate, has a significant activity exclusively in the rat proximal tubule (Burch *et al.* 1982), the glycerol taken up across both the basolateral and apical membranes of the proximal tubular cells (Elhamri *et al.* 1993) can be either incorporated into triacylglycerols whose synthesis occurs in these cells (Wirthensohn & Guder, 1980) or converted into glucose or CO₂ (Krebs *et al.* 1963; Underwood & Newsholme, 1967). Although the supply of glycerol to the kidney did not change during starvation, the significant increase in glycerol utilization observed across the basolateral membrane of the proximal tubule (Elhamri *et al.* 1993) does not necessarily mean that glycerol provided more energy (if any) to the proximal tubule because the renal cortex of starved rats stores more triacylglycerols (Hohenegger & Schuh, 1980; Wirthensohn *et al.* 1980) and probably forms more glucose from glycerol as a result of the increased activity of fructose bisphosphatase and glucose-6-phosphatase (Burch *et al.* 1978*b*). The increase in glycerol-3-phosphate dehydrogenase (*EC* 1.1.1.8) activity observed by Robinson & Newsholme (1969) in the kidney of starved rats may also be part of the mechanism

responsible for the increased renal utilization of glycerol observed *in vivo* (Elhamri *et al.* 1993).

The observation that citrate was taken up across both the apical and basolateral membranes of renal cells (Elhamri *et al.* 1993) is consistent with the existence of tricarboxylate carriers at both poles of the renal cells (Windus *et al.* 1986; Burckhardt & Ullrich, 1989). Since virtually all the filtered citrate is reabsorbed in the proximal tubule (Simpson, 1983), and since reabsorption provided half the citrate metabolized by the kidney (Elhamri *et al.* 1993), one may conclude that, in both the fed and starved state, at least half the citrate utilization occurs in the proximal tubule. It is likely that most of the basolateral uptake of citrate also occurs in the proximal tubule, but, since citrate has been shown to undergo some oxidation in microdissected thick ascending limb of Henle (Klein *et al.* 1981), one cannot rule out the possibility that some citrate was transported across the basolateral membrane of this nephron segment and then oxidized. In rat renal cortical slices, citrate is converted at least in part into CO₂ (Adler & Preuss, 1972); thus, citrate represents a source of energy for the proximal tubule even if it is substantially converted into glucose, because the latter process yields energy. It is unknown whether, as has been demonstrated in isolated rat cortical tubules incubated in acidic pH (Kurokawa & Rasmussen, 1973), the synthesis of glucose from citrate is augmented in the proximal tubule of starved rats at the expense of complete oxidation of citrate as a result of stimulation of the key enzymes of gluconeogenesis located in the pathway between oxaloacetate and glucose. Whether the increase in citrate oxidation observed in rat renal cortical slices of chronically acidotic rats (Adler & Preuss, 1972) also holds true in the renal cortex of starved rats and whether this increase in citrate oxidation is due to the partial oxidation associated with glucose synthesis or to an increase in complete oxidation of citrate remains to be studied.

In the fed state, the rat kidney takes up glutamine only from the lumen fluid and an efflux of glutamine is observed at the basolateral pole of renal cells (Elhamri *et al.* 1993). Since the filtered glutamine is almost quantitatively reabsorbed in the proximal tubule (Silbernagl, 1980), this means that most, if not all, the glutamine utilization occurs in the cells of this nephron segment which contains not only significant activities of glutaminase and glutamate dehydrogenase (*EC* 1.4.1.2; Curthoys & Lowry, 1973; Wright & Knepper, 1990*a,b*) but also the enzymes of the tricarboxylic acid cycle and of gluconeogenesis (see pp. 201–202). Several authors have demonstrated that glutamine may be converted into glucose and CO₂ by rat proximal tubules or slices *in vitro* (Kamm & Strobe, 1972; Baverel & Lund, 1979; Watford *et al.* 1980; Baverel *et al.* 1984). The net provision of energy during glutamine metabolism results from both complete oxidation of its carbon skeleton and, to a lesser extent, gluconeogenesis (Halperin *et al.* 1982). Therefore, irrespective of the relative importance of complete oxidation and glucose synthesis from glutamine, this amino acid appears to be an energy supplier to the proximal tubule of fed rats.

Starvation was found to increase the uptake of glutamine by the rat kidney *in vivo* by conversion of the net basolateral efflux of glutamine into a net influx (Elhamri *et al.* 1993); this effect, which cannot result from a change in the kinetic properties of the basolateral glutamine transporter (Foreman *et al.* 1983), was accompanied by a stimulation of the production, excretion and venous release of NH₄⁺ without any concomitant increase in glutamine release; this indicates that flux through glutaminase and glutamate dehydrogenase was augmented (Elhamri *et al.* 1993). Although, in the

latter study, no systemic metabolic acidosis was observed after 72 h of starvation, such a stimulation of renal ammoniogenesis from glutamine is probably indicative of an adaptative response of the kidney to the initial metabolic acidosis (fully compensated after 72 h of starvation) due to the increased production of acetoacetic and β -hydroxybutyric acids by the liver. The metabolic acidosis-induced stimulation of glutamine metabolism, which is due to (1) a stimulation of glutaminase activity (Curthoys & Lowry, 1973; Wright & Knepper, 1990b) resulting from increased synthesis of this enzyme via the stabilization of glutaminase mRNA (Hwang & Curthoys, 1991) and (2) the stimulation of the activity and of the flux through glutamate dehydrogenase (Schoolwerth *et al.* 1978; Wright & Knepper, 1990a), together with the associated enhancement of NH_4^+ production from glutamine, is restricted to the S1 and S2 segments of the proximal tubule (Good & Burg, 1984; Nonoguchi *et al.* 1986). Other factors capable of stimulating glutamine metabolism are a fall in intramitochondrial pH (Schoolwerth & Gesek, 1990), which leads to a stimulation of oxoglutarate dehydrogenase (*EC* 1.2.4.2) activity (Lowry & Ross, 1980), and the stimulation of phosphoenolpyruvate carboxykinase already mentioned.

Under acidotic conditions, flux through glutamine synthetase has been found to be decreased in the rat kidney *in vivo* (Damian & Pitts, 1970) and glutamine synthesis from glutamate is also decreased in the isolated perfused rat kidney (Hems, 1972). Together with the stimulation of unidirectional glutamine utilization, this may contribute to the increased net glutamine utilization observed in the kidney of starved rats (Elhamri *et al.* 1993). Inhibition of glutamine utilization and, therefore, of glutamine oxidation by β -hydroxybutyrate in the fed state observed by Goldstein *et al.* (1980, 1982) is probably reduced in the starved state because, under ketotic conditions, the rat kidney does not appear to be sensitive to the inhibitory effects of ketone bodies. Since experimental evidence obtained in the isolated perfused rat kidney suggests that the extra glutamine taken up in acidosis is accounted for by the extra glucose formed (Hems, 1972), it is likely that the 3-fold stimulation of glutamine utilization observed *in vivo* in the kidney of starved rats (Elhamri *et al.* 1993) was accompanied by an enhanced contribution of glutamine to the provision of energy in the proximal tubule. Despite the presence of the enzymic machinery for glutamine metabolism and the evidence for some glutamine oxidation (Klein *et al.* 1981; Jung *et al.* 1989), it remains unknown whether or not significant amounts of glutamine are taken up across the basolateral membrane and oxidized in other nephron segments *in vivo*.

Since at low (physiological) concentrations, filtered ketone bodies are reabsorbed mainly in the proximal tubule (Ferrier *et al.* 1992), and since ketone bodies are also taken up across the basolateral membrane of proximal tubular cells by a diffusion mechanism (Ullrich & Papavassiliou, 1986), it is very likely that a large proportion of the metabolism of the ketone bodies taken up by the rat kidney *in vivo* (Elhamri *et al.* 1993) occurs in the proximal tubule both in fed and starved rats. The preferential utilization of acetoacetate in the fed state may be due to its higher blood concentration (Elhamri *et al.* 1993). It should be noted that the capacity or affinity of the apical transporter of ketone bodies did not change in brush-border-membrane vesicles prepared from starved rats (Barac-Nieto, 1986). By contrast, although the circulating concentration of β -hydroxybutyrate was much greater than that of acetoacetate, the rates of utilization of these two compounds were similar during starvation and the basolateral efflux of β -hydroxybutyrate observed in fed rats persisted and even increased in starved animals (Elhamri *et al.* 1993). This may

reflect a limitation of the metabolism of β -hydroxybutyrate by β -hydroxybutyrate dehydrogenase (*EC* 1.1.1.30) secondary to a reduction of the activity of this enzyme (Kante *et al.* 1990) and to the shift to a more reduced state of the NAD–NADH couple during starvation as reflected by the blood β -hydroxybutyrate:acetoacetate value which increased in starved animals when compared with fed rats (Elhamri *et al.* 1993, see also Table 1).

Micropuncture experiments have shown that ketone bodies are not reabsorbed along the distal convoluted tubule or the collecting duct but a reabsorption of ketone bodies in the thick ascending limb of Henle when blood ketone body concentration is elevated cannot be excluded (Ferrier *et al.* 1992). Whether monocarboxylate carriers, whose existence has been demonstrated in the apical membrane of the proximal tubule (Garcia *et al.* 1980; Jørgensen & Sheikh, 1985), exist and transport ketone bodies across the apical membrane of the thick ascending limb of Henle in the rat remains unknown. Similarly, the possible existence of basolateral transporters of ketone bodies in certain distal nephron segments in the rat has not been demonstrated so far. However, because of the high rate of ketone-body utilization during starvation, the fate of these compounds, which seems to be only oxidation, and the fact that their oxidation would account for most of the O_2 consumed by the rat kidney *in vivo* (see Table 1), it is likely that these compounds represent the main fuels of all the nephron segments equipped with the enzymes of ketone-body degradation.

CONCLUSION

At the whole-organ level, lactate and perhaps glucose seem to be the main fuels of the kidney of fed rats, but all the other substrates taken up in significant amounts (ketone bodies, free fatty acids, citrate, glutamine and glycerol) probably also contribute to the provision of energy; during starvation, ketone bodies and perhaps free fatty acids and, to a lesser extent, glucose seem to be the preferred fuels of the kidney, whereas the stimulation of gluconeogenesis known to occur in this nutritional situation probably limits the contribution of lactate and glycerol and augments the contribution of glutamine and perhaps citrate to the synthesis of ATP.

At the nephron segment level, glucose is not a fuel in the proximal tubule but probably represents the only significant source of energy of all the nephron segments located in the inner medulla. The proximal tubule has the capacity to oxidize all the other substrates taken up. It is conceivable, therefore, that they all supply energy to the proximal tubule in fed rats but, given the quantitative importance of renal lactate uptake *in vivo*, it is likely that lactate represents the main fuel of this segment. In starved rats, the proximal tubule probably relies mainly on ketone bodies for its energetic needs; the free fatty acids taken up by this segment seem to undergo, at least in part, esterification and storage and the extent to which they are oxidized is uncertain. It is also possible that the glycerol and perhaps the citrate taken up by the proximal tubule of starved rats are diverted away from oxidation. The other nephron segments (cortical and outer medullary thick ascending limb of Henle, the distal convoluted tubule and the cortical and outer medullary collecting duct) have the capacity to oxidize significant amounts of glucose, lactate, ketone bodies and free fatty acids; it is likely that in fed rats they use, to variable extents, a mixture of these substrates as energy sources. In starved rats there is little doubt that these nephron segments shift their preference to ketone bodies and fatty acids as respiratory fuels.

Thus, the complexity of the structural, functional and biochemical organization of the kidney makes the conclusions uncertain and much remains to be learned about the relative quantitative importance of (1) the fuels utilized by the whole kidney under different nutritional situations and (2) the successive nephron segments involved in energy production. Only some of the mechanisms by which the kidney selects its fuels in a given nutritional state and under changing situations are known; in particular, as recognized by Guder & Morel (1992), relatively little is known about the regulation of renal intermediary metabolism by hormones.

Finally, it should be emphasized that the conclusions drawn from results obtained in the rat kidney do not necessarily apply to the kidney of other species. For example, the proximal tubules of the rabbit and guinea-pig kidney substantially oxidize glucose (Gullans *et al.* 1984; Chauvin *et al.* 1994; Michoudet *et al.* 1994), and the human kidney *in vivo*, like the rat kidney, takes up large amounts of β -hydroxybutyrate during starvation but, in contrast to the rat kidney, releases large amounts of acetoacetate (Owen *et al.* 1969).

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