Kidney International, Vol. 29 (1986), pp. 41–45

Metabolic fuels along the nephron: Pathways and intracellular mechanisms of interaction

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Substrates in large numbers are removed from the blood by the kidney in linear relationship to their arterial concentrations [1, 2]. At normal arterial blood levels, the kidney utilizes significant amounts of free fatty acids, lactate, glutamine, 3-hydroxybutyrate, and citrate. Furthermore, the kidney removes substrates like pyruvate, α-ketoglutarate, glycerol, proline, and some other amino acids of low arterial concentrations. However, when blood levels of these substances increase, their renal uptake rates likewise increase [1, 2]. Metabolic fates of these substrates in the kidney are related intimately to major functions of the kidney including excretion of waste materials, reabsorption of life conserving substances and water, and other important endocrine and metabolic functions.

When studied in vitro, the capacity of renal tissue to take up substrates was shown to be far in excess of the rates occurring under in vivo conditions [2]. This indicates that saturation is not reached in vivo due to suboptimal substrate concentrations. For lactate, pyruvate, glutamine, proline, fatty acids, and ketone bodies, normal arterial levels are below or around the half-maximal concentration kinetically determined in in vitro uptake studies [3–8]. However, even under this nonsaturating condition, the rates of substrate uptake in vivo exceed the quantities of fuel needed to meet the energy demands of the kidney as calculated from oxygen uptake [1, 9]. Table 1 summarizes the calculated oxygen uptake and ATP formation rates for some substrates. From the theoretical and the experimental data on substrate uptake rates and measurements of oxygen consumption [1, 2], it becomes clear that the kidney takes up more substrates than could be accounted for by oxidation.

The term "incomplete oxidation" was introduced by Cohen [1] to explain this phenomenon. For example, 3-hydroxybutyrate taken up by the kidney is partially released as acetoacetate [8]. On the other hand, no net product release was found for other substrates taken up in excess. From recent in vitro studies, it was concluded that the kidney can utilize substrates by metabolic pathways that do not lead to their oxidation [2–7]. Thus, lactate, glycerol, glutamine, and other substrates are in part converted to glucose through the gluconeogenic pathway, whereas fatty acids which cannot be converted to glucose are recovered mainly as triacylglycerol [5, 10].

Received for publication June 12, 1985

Two major questions may be raised at this point: *(1)* What nephron cells are responsible for the substrate uptake rates observed? *(2)* What are the mechanisms regulating intracellular interactions of various substrates?

This review will briefly summarize some recent findings on intercellular heterogeneity and intracellular regulatory mechanisms that may help explain the metabolic balances observed in vivo.

Intrarenal heterogeneity of metabolic pathways

Since the studies of Gyoergy, Keller, and Brehme, [11] it has been known that renal cortex and medulla exhibit marked differences in metabolism. This compartmentation of metabolic pathways in different parts of the kidney was further substantiated and confirmed by measuring rate-limiting enzymes in different segments of the nephron. Thus, hexokinase, pyruvate kinase, and phosphofructokinase—enzymes of the glycolytic pathway—were found to be abundant throughout the distal structures of the nephron and were low in the proximal convoluted and straight tubule [12]. On the other hand, enzymes of the gluconeogenic pathway and the capacity to form glucose seem to be restricted to the proximal tubule [12, 13]. This explains why the kidney in vivo does not show any arteriovenous difference for glucose. Only when using radioactive tracers can the rate of glucose uptake and release be calculated in whole kidney studies [14, 15]. Recent metabolic studies with isolated nephron segments confirm that the proximal tubule does not produce appreciable amounts of $^{14}CO_2$ from ^{14}C-glucose whereas the thick ascending limb of Henle's loop oxidizes ^{14}C-glucose to a considerable extent [16, 17]. Lactate, on the other hand, is oxidized by both segments [16, 17]. More recently, Wittner et al [18] demonstrated in the isolated perfused thick ascending limbs of rabbit kidney that glucose, lactate, and pyruvate were equally effective to sustain short-circuit current [18].

On the other hand, fatty acids can be oxidized in all nephron segments, and the rates of their oxidation can parallel the mitochondrial mass of the nephron segments [12, 19, 20]. However, as shown for other substrates, only part of the fatty acids taken up are oxidized whereas the major part is incorporated into triacylglycerol [6, 10]. When esterification of fatty acids was studied along the rabbit nephron, the highest rate of triacylglycerol formation was found in the proximal convoluted tubule [21]. Moreover, gluconeogenic substrates increase the

Table 1. Calculated values for oxidative metabolism of some renal substrates[a]

Substrate	O_2 uptake	CO_2 formation	ATP formation	RQ
Glucose	6	6	38	1.00
Palmitate	23	16	130	0.70
3-Hydroxybutyrate	4.5	4	25	0.89
Acetoacetate	4	4	22	1.00
Glutamine	4.5	5	27	1.11
Lactate	3	3	18	1.00

[a] Values represent mole/mole substrate.

esterification in proximal tubules whereas in distal structures only glucose stimulates it [21]. These data are consistent with the notion that the gluconeogenic pathway provides glycerol in the proximal tubules.

The enzymes of glutamine metabolism as well as ammonia formation from glutamine have also been mapped along the nephron [12, 13, 22]. In the rat, distal convoluted tubules exert highest activities under control conditions whereas in metabolic acidosis the proximal tubule increases its capacity several-fold.

Weidemann and Krebs demonstrated that ketone bodies [23] compete with fatty acids as energy fuels for the kidney depending on their concentrations. Recently, we measured two enzymes of ketone body metabolism along the rabbit, rat, and mouse nephron [24–26]. The activity of 3-oxoacid-CoA-transferase was similar in mouse and rat kidney and paralleled the distribution pattern of the amounts of mitochondria. In rabbit kidney, however, this enzyme activity was very low and present almost exclusively in the cortical ascending limb of Henle's loop [25]. On the other hand, the 3-hydroxybutyrate dehydrogenase exhibited similar activities in the three species, although there was some difference between the relative distibution pattern along the proximal tubule [24]. These findings are consistent with the observation of Wittner et al. [18] in isolated perfused rabbit cortical ascending limbs of Henle that both acetoacetate and 3-hydroxybutyrate are effective in sustaining short-circuit current.

Studies on enzyme distribution along the nephron can provide information on certain metabolic abilities (Fig. 1). However, these studies do not attempt to draw any conclusions about the relative metabolic rates of the respective substrates. Likewise, they do not explain how substrates interact with each other when offered simultaneously a condition which prevails in vivo.

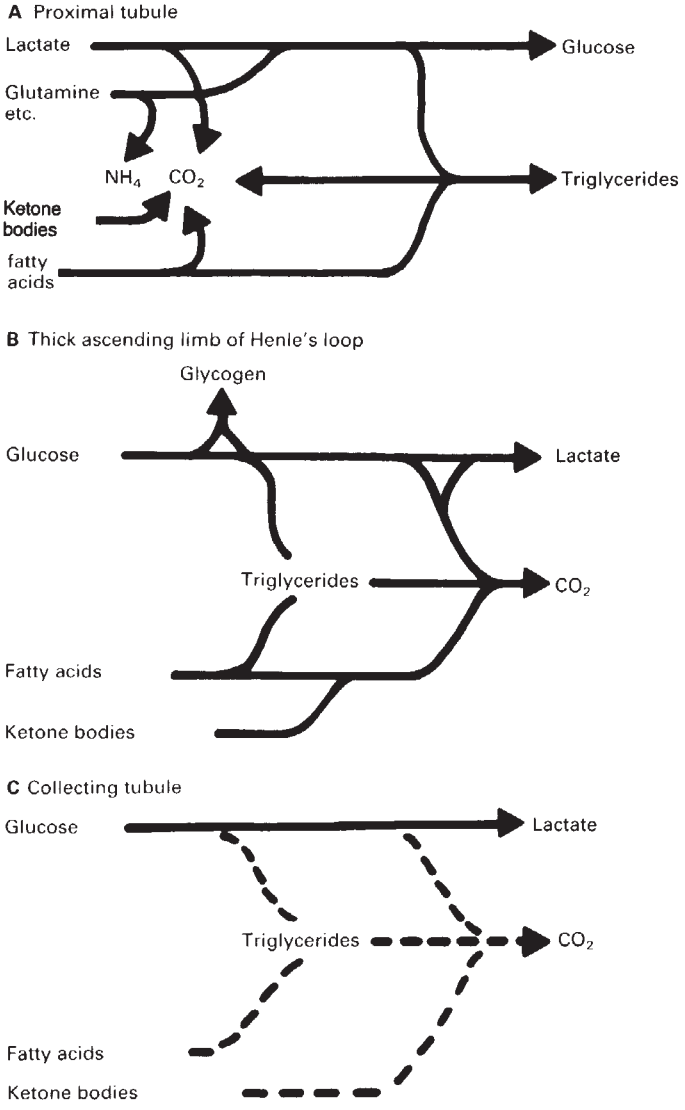

Fig. 1. *Biochemical pathways of different nephron segments of the kidney.*

Metabolic interactions

Studies on metabolic interactions of substrates in the kidney performed with isolated tubule suspensions have established a hypothetic hierarchy of energy providing fuels. Because more than 90% of cortical tubules consist of proximal convoluted tubule cells, the results obtained using cortical tubule suspension can be attributed most probably to this nephron segment. As Weidemann and Krebs [23] and Krebs, Speake, and Hems [27] have shown, fatty acids and ketone bodies inhibit the oxidative metabolism of lactate in isolated tubules from kidney cortex (Fig. 2). In the presence of fatty acid, lactate uptake is reduced, and a relatively greater proportion of lactate carbon taken up by the kidney is converted to glucose [28]. The mechanism of this substrate interaction was shown to be due to inhibition of pyruvate dehydrogenase by allosteric inhibition and by enzymatic conversion of the enzyme to the inactive phosphorylated form [29]. A similar mechanism seems to regulate lactate and glucose oxidation in distal nephron segments of the rat, because outer medullary pyruvate dehydrogenase is found to be inactivated under conditions of increased fatty acid supply in vivo [30]. $^{14}CO_2$ formation from ^{14}C-lactate was almost completely suppressed by oleate in isolated cortical ascending limbs of rat kidney (Guder WG, Morel F, unpublished observations). Likewise, ketone bodies inhibited conversion of glucose to CO_2 and led to an increase in lactate formation from glucose [31].

Lactate and oleate inhibit tubular glutamine metabolism, leading to a decrease in ammonia and glucose formation from glutamine [5]. This interaction which was first observed in in

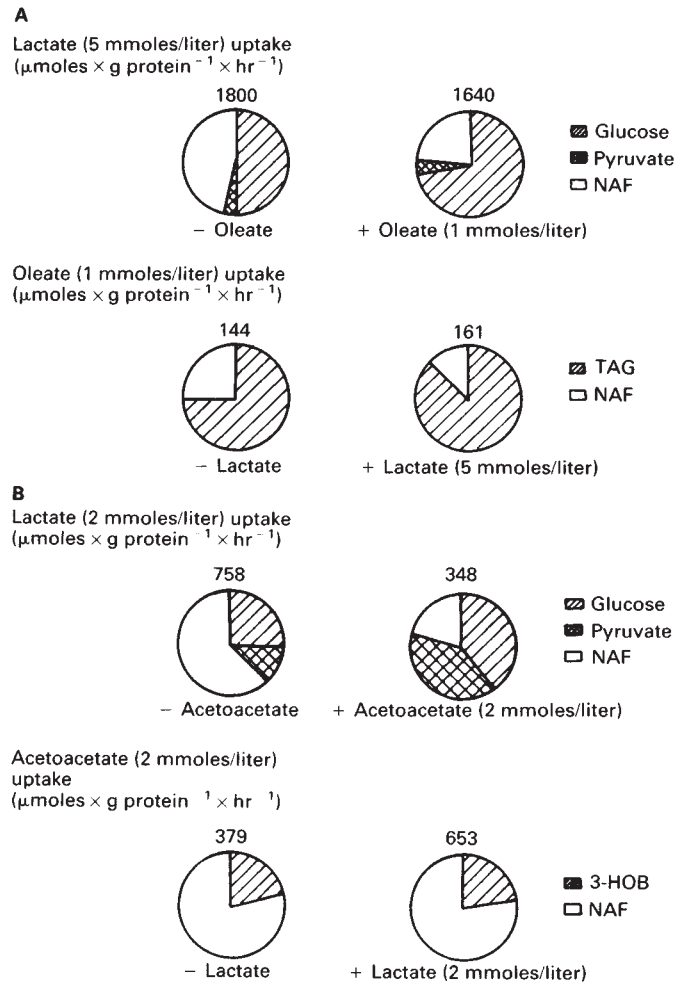

Fig. 2. *Interactions of lactate with oleate and acetoacetate in isolated cortical tubule suspension.* Rat kidney cortical tubule suspensions were prepared and incubated as described in [28]. Substrates were assayed enzymatically. The *open areas* in the *circles* represent the substrate uptake not accounted for (NAF) most probably due to oxidation to CO_2. (Results taken from [7, 28].)

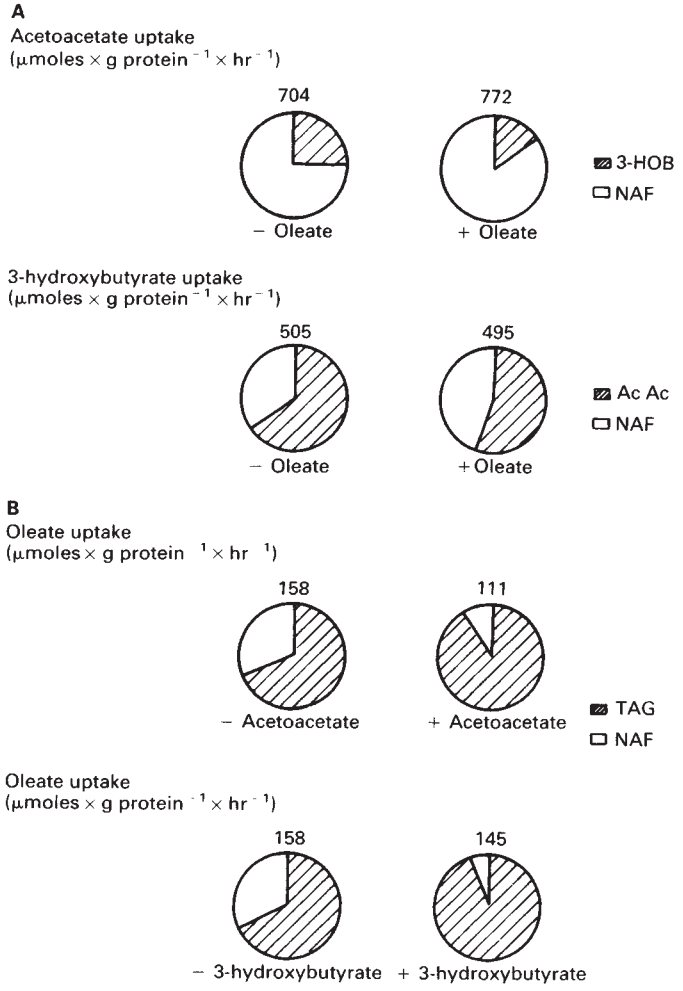

Fig. 3. *Interactions between oleate and ketone bodies in isolated cortical tubule suspensions of fed rats in the presence of 2 mM glutamine.* Rat cortical tubule suspensions were prepared and incubated as described in [28]. The *circles* represent substrate uptake rates. The *open areas* in the *circles* are not accounted for (NAF) by the products and probably represent CO_2-formation. Incorporation of oleate into phospholipids represented less than 5% of oleate taken up [6]. (Data obtained from [7, 35].)

vivo studies by Lemieux *et al* in dogs [32] is probably due to a change in mitochondrial NADH/NAD ratio leading to accumulation of glutamate in the mitochondrial matrix, which in turn inhibits glutamine deamination [22]. A similar inhibitory effect is found when ketone bodies are offered together with glutamine [7, 31]. These observations indicate that oleate and ketone bodies seem to be utilized preferentially over lactate and glutamine by the kidney.

More recently, we studied the interactions of ketone bodies with other renal subtrates in more detail [34]. Interestingly, acetoacetate and 3-hydroxybutyrate inhibited the uptake and oxidation of oleate (Fig. 3). In the presence of substrates providing glycerol moieties such as glutamine and lactate, ketone bodies led to an increase in fatty acid esterification. Oleate, on the other hand, did not significantly alter ketone body metabolism, whereas gluconeogenic substrates increased the uptake and contribution of ketone bodies to oxidative metabolism [7, 34, 35]. The mechanism of this interaction is not clear. The tight coupling of acetoacetate and succinate metabolism through 3-oxoacid-CoA-transferase makes it likely that ketone bodies lead to preferential oxidation of succinate, which is linked to FAD as electron transfer mechanism. This, in turn, may inhibit the oxidation of NADH and thereby the suppression of NAD-dependent pathways. Direct measurements of the redox state of components of the respiratory chain may confirm this effect of ketone bodies [9].

Our data as well as others seem to suggest that acetoacetate is the best candidate for the preferred fuel of respiration of the whole kidney. At least in proximal tubules of the rat, the hierarchy of the oxidative substrate hierarchy seems to include fatty acids, lactate, and probably glutamine (Fig. 4). The relative contribution of these substrates to tubular energy balance depends on their relative concentration and the metabolic state of the animal. In addition, hormonal influences may modify the relative contribution of these substrates by direct tubular or indirect effects on their blood levels.

A

Substrates acting on	The uptake of			
	Acetoacetate	Fatty acid	Lactate	Glutamine
Acetoacetate		—	—	—
Fatty acid	0		—	—
Lactate	+	+		—
Glutamine	+	+	+	

B

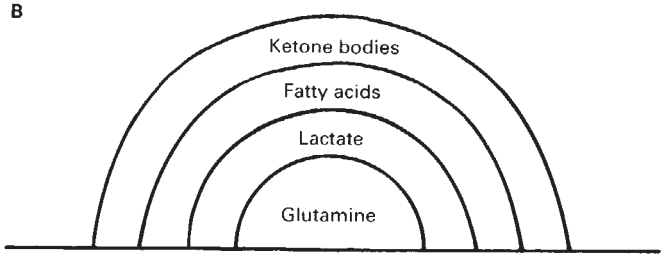

Fig. 4. **A** *Substrate interactions in isolated kidney cortex tubules.* Symbols are: 0, no effect; +, stimulatory; −, inhibitory. **B** *Proposed substrate hierarchy as oxidative fuels.*

Acknowledgments

Our work cited herein was supported by the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg (Sonderforschungsbereich 51, Munchen and project GU 82/2-1) and the Fonds der Chemischen Industrie, Frankfurt/Main. We thank S. Lefrank, S. Pürschel, and E. Vollrath for assistance in our studies and preparation of the manuscript.

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References

1. COHEN JJ, BARAC-NIETO M: Renal metabolism of substrates in relation to renal function, in *Handbook of Physiology-8: Renal physiology,* edited by ORLOFF J, BERLINER RW, Washington, D.C., American Physiology Society, 1973, pp 909–1002
2. GUDER WG, WIRTHENSOHN G: Renal turnover of substrates, in *Renal Transport of Organic Substances,* edited by GREGER R, LANG F, SILBERNAGL S, Berlin-Heidelberg, Springer-Verlag, 1981, pp 66–77
3. GUDER WG, STUKOWSKI B, WIESNER W, WIELAND OH: Metabolism of isolated kidney tubules. Comparison of the stimulatory action of acetate and adenosine 3′, 5′-monophosphate (cAMP) on glucose formation from l-lactate and pyruvate, in *Biochemical Aspects of Kidney Function,* edited by HOHENEGGER M, MUENCHEN, WILHELM GOLDMANN, 1972, pp 48–63
4. VINAY P, LEMIEUX G, GOUGOUX A, LEMIEUX G: Response of the rat and dog kidney to H^+ concentration in vitro—a comparative study with slices and tubules. *Int J Biochem* 12:89–98, 1980
5. GUDER WG, WIRTHENSOHN G: Metabolism of isolated kidney tubules. Interactions between lactate, glutamine and oleate metabolism. *Eur J Biochem* 99:577–584, 1979
6. GUDER WG, WIRTHENSOHN G: Regulation of fatty acid metabolism in isolated kidney tubules. *Proc 8th Int Congr Nephrol,* Athens, 1981, pp 827–833
7. WAGNER S: Studien zum Ketonkoerperstoffwechsel der Niere. *Dissertation,* Ludwig-Maximilians-Universitaet, Muenchen, 1984
8. OWEN OE, FELIG P, MORGAN AP, WAHREN J, CAHILL GF JR: Liver and kidney metabolism during prolonged starvation. *J Clin Invest* 48:574–583, 1969
9. MANDEL LJ: Primary active sodium transport, oxygen consumption, and ATP; coupling and regulation. *Kidney Int* 29:3–9, 1986
10. WIRTHENSOHN G, GUDER WG: Triacylglycerol metabolism in isolated rat kidney cortex tubules. *Biochem J* 186:317–324, 1980
11. GYOERGY P, KELLER W, BREHME TH: Nierenstoffwechsel und Nierenentwicklung. Biochem Zeitschr 200:356–366, 1928
12. GUDER WG, ROSS BD: Enzyme distribution along the nephron. *Kidney Int* 26:101–111, 1984
13. ENDOU H, NONOGUCHI H, NAKADA J, TAKEHARA Y, YAMADA H: Glutamine metabolism in the kidney: Ammoniagenesis and gluconeogenesis in isolated nephron segments of rats, in *Kidney Metabolism and Function,* edited by DZURIK R, LICHARDUS B, GUDER WG, Boston, Nijhoff Publishers in press, 1985
14. JANSSENS P, HEMS P, ROSS BD: The metabolic fate of lactate in renal cortical tubules. *Biochem J* 190:27–37, 1980
15. KIDA K, NAKAJO S, KAMIYA F, TOYAMA Y, NISHIO T, NAKAGAWA H: Renal net glucose release in vivo and its contribution to blood glucose in rats. *J Clin Invest* 62:721–726, 197
16. LE BOUFFANT F, HUS-CITHAREL A, MOREL F: In vitro $^4CO_2$ production by single pieces of rat cortical thick ascending limbs and its coupling to active salt transport, in *Biochemistry of dney Functions,* edited by MOREL F, Amsterdam, Elsevier Biomedical Press, 1982, pp 363–370
17. KLEIN KL, WANG MS, TORIKAI S, DAVIDSON WD, KUROKAWA K: Substrate oxidation by isolated single nephron segments of the rat. *Kidney Int* 20:29–35, 1981
18. WITTNER M, WEIDTKE C, SCHLATTER E, DI STEFANO A, GREGER R: Substrate utilization in the isolated perfused cortical thick ascending limb of rabbit nephron. *Pflügers Arch* 402:52–62, 1984
19. HOHENEGGER M: Lipid metabolism related to kidney function and electrolyte homeostasis, in *Renal Metabolism in Relation to Renal Function,* edited by SCHMIDT U, DUBACH UC, Bern, Huber Publishers, 1976, pp 99–107
20. LEHIR M, DUBACH UC: Distribution of two enzymes of beta-oxidation of fatty acids along the rat nephron, in *Biochemistry of Kidney Functions,* edited by MOREL F, Amsterdam, Elsevier Biomedical Press, 1982, pp 87–94
21. GUDER WG, WIRTHENSOHN G: Triacylglycerol synthesis along the rabbit nephron, in *Biochemistry of Kidney Functions,* edited by MOREL F, Amsterdam, Elsevier Biomedical Press, 1982, pp 95–102
22. VINAY P, LEMIEUX G, GOUGOUX A, HALPERIN M: Regulation of glutamine metabolism in the dog kidney in vivo. *Kidney Int* 29:68–79, 1986
23. WEIDEMANN MJ, KREBS HA: The fuel of respiration of rat kidney cortex. *Bicohem J* 112:149–166, 1969
24. GUDER WG, PUERSCHEL S, WIRTHENSOHN G: Renal ketone body metabolism. Distribution of 3-oxoacid CoA-transferase and 3-hydroxybutyrate dehydrogenase along the mouse nephron. *Hoppe-Seyler's Z Physiol Chem* 364:1727–1737, 1983
25. GUDER WG, PUERSCHEL S, VANDEWALLE A, WIRTHENSOHN G: Bioluminescence procedures for the measurement of NAD(P) dependent enzyme catalytic activities in submicrogram quantities of rabbit and human nephron structures. *J Clin Chem Clin Biochem* 22:129–140, 1984
26. BURCH HB, BROOKS CA, CAMBON N, LOWRY OH: Metabolic systems of nephron segments from hydronephrotic rabbit kidney. *Kidney Metabolism and Function,* edited by DZURIK R, LICHARDUS B, GUDER WG, Boston, Nijhoff Publishers, In press, 1985
27. KREBS HA, SPEAKE RN, HEMS R: Acceleration of renal gluconeogenesis by ketone bodies and fatty acids, *Biochem J* 94:712–720, 1965
28. GUDER WG, WIELAND OH: Metabolism of isolated kidney tubules. Additive effects of parathyroid hormones and free-fatty acids on renal gluconeogenesis. *Eur J Biochem* 31:69–79, 1972
29. GUDER WG, WIELAND OH: Metabolism of isolated kidney tubules. Regulation of pyruvate dehydrogenase by metabolic substrates. *Eur J Biochem* 42:529–538, 1974
30. GUDER WG, SCHMIDT U: Substrate and oxygen dependence of renal metabolism. *Kidney Int* 10:S32–S38, 1976
31. BAVEREL G, FORISSIER M, PELLET M: Lactate and pyruvate metabolism in dog renal outer medulla. Effects of oleate and ketone bodies. *Int J Biochem* 12:163–168, 1980

32. LEMIEUX G, VINAY P, ROBITAILLE P, PLANTE GL, LUSSIER Y, MARTIN P: The effect of ketone bodies on renal ammoniagenesis. *J Clin Invest* 50:1781–1791, 1971
33. VINAY P, LEMIEUX G, CARTIER P, AHMAD M, BAVEREL G: Effect of fatty acids on renal ammoniagenesis in vivo and in vitro studies. *Am J Physiol* 231:880–887, 1976
34. GUDER WG, WIRTHENSOHN G, WAGNER S: Renal ketone body metabolism, in *Kidney Metabolism and Function,* edited by DZURIK R, LICHARDUS B, GUDER WG, Boston, Nijhoff Publishers, In press, 1985
35. BAVEREL G, MARTIN G, FERRIER B, PELLET M: Characteristics of ketone-body metabolism in dog renal cortex and outer medulla, in *Biochemistry of Kidney Function,* edited by MOREL F, Amsterdam, Elsevier Biomedical Press, 1982, pp 177–185