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QUARTERLY

Ketone bodies activate gluconeogenesis in isolated rabbit renal cortical tubules incubated in the presence of amino acids and glycerol*

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In isolated rabbit renal kidney-cortex tubules 2 mM glycerol, which is a poor gluconeogenic substrate, does not induce glucose formation in the presence of alanine, while it activates gluconeogenesis on substitution of alanine by aspartate, glutamate or proline. The addition of either 5 mM 3-hydroxybutyrate or 5 mM acetoacetate to renal tubules incubated with alanine+glycerol causes a marked induction of glucose production associated with inhibition of glutamine synthesis. In contrast, the rate of the latter process is not altered by ketones in the presence of glycerol and either aspartate, glutamine or proline despite the stimulation of glucose formation. Acceleration of gluconeogenesis by ketone bodies in the presence of amino acids and glycerol is probably due to (i) stimulation of pyruvate carboxylase activity, (ii) activation of malate-aspartate shuttle as concluded from elevated intracellular levels of malate, aspartate and glutamate, as well as (iii) diminished supply of ammonium for glutamine synthesis from alanine resulting from a decrease in glutamate dehydrogenase activity.

High activities of 3-hydroxybutyrate dehydrogenase, 3-oxoacid-CoA transferase and acetoacetyl-CoA thiolase in the kidney make this organ capable of utilizing 3-hydroxybutyrate (3-HB) and acetoacetate (AcAc) as oxidative fuels *via* their conversion into acetyl-CoA [1–3]. It has been reported that AcAc is readily taken up by rat kidney and provides, depending on its concentration, up to 80% of the respiratory fuel whereas endogenous respiration is suppressed [4]. Both 3-HB and AcAc have been reported to exert a stimulatory effect on renal gluconeogenesis from lactate, pyruvate and glycerol, probably by increasing intracellular acetyl-CoA content resulting in stimulation of pyruvate carboxylase activity [5]. Moreover, 3-HB was shown to inhibit ammoniagenesis from glutamine in isolated kidneys and mitochondria [6], indicating that ketone bodies may act not only as respiratory fuels but also as regulators of renal metabolism.

Despite the existence of amino acid aminotranferases and glutamate dehydrogenase

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Abbreviations: 3-HB, 3-hydroxybutyrate; AcAc, acetoacetate; MSO, L-methionine sulfoximine; G-3P, glycerol-3-phosphate; DHAP, dihydroxyacetone phosphate; DABS-Cl, 4-dimethylaminoazobenzene-4′-sulfonyl chloride.

activities in guinea pig and rabbit renal cortical tubules, amino acids such as alanine, glutamate and aspartate are not utilized for gluconeogenesis when used as the sole substrates [7–11], while glutamine synthesis is very efficient [8, 10, 11]. Glycerol, which is also a very poor gluconeogenic substrate in rabbit kidney-cortex tubules [9], has been shown to induce gluconeogenesis from glutamate [9] and aspartate [10] by a mechanism involving inhibition of glutamine synthesis and acceleration of glyceraldehyde-3-phosphate dehydrogenase activity due to elevation of the cytosolic redox state [10]. The aim of the present investigation was to study the effect of ketone bodies on glucose formation from amino acids in rabbit renal cortical tubules incubated with glycerol.

MATERIAL AND METHODS

Preparation and incubation of kidney-cortex tubules. Fed rabbits (male, California strain, 2–3 kg body weight) were anesthetized with pentobarbital injected into ear marginal vein (30 mg per kg body weight). In some experiments rabbits starved for 48 h were used. Renal cortex tubules were isolated as described previously [12] and incubated (about 10 mg dry weight) at 37°C in 2 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) in 25 ml plastic Erlenmeyer flasks sealed with rubber stoppers under the atmosphere of 95% O_2 + 5% CO_2. Substrates were added at concentrations given in legends to Tables and Figure. For the measurement of total production of metabolites in renal tubule suspension the reaction was stopped after 60 min of incubation by addition of 35% perchloric acid (0.1 vol. of tubule suspension).

Analytical methods. Both utilization of alanine and glycerol and production of amino acids, lactate, ammonium and glucose were estimated from measurements of metabolites in samples withdrawn from the reaction medium following 60 min of incubation. The intracellular content of metabolites in isolated kidney tubules was measured in samples following centrifugation of tubule suspension through silicone oil into a perchloric acid solution as described previously [12]. The isotopic studies used for investigation of the contribution of different substrates to glucose formation were performed according to Exton & Park [13] and Pilkis *et al.* [14]. Fixation of $^{14}CO_2$ by renal tubules was determined in the incubation mixture containing a trace amount of sodium [^{14}C]bicarbonate [8]. Glucose, ammonium, lactate, malate, pyruvate, 3-HB, AcAc, glycerol, G-3P, DHAP and 2-oxoglutarate were estimated either spectrophotometrically or fluorimetrically by standard enzymatic techniques [15]. Serum levels of ketone bodies were measured enzymatically in deproteinized blood samples. Amino acids were determined by HPLC after derivatization of samples with DABS-Cl [16].

Enzymes and chemicals. Collagenase (EC 3.4.24.3), type IV, 4-dimethylaminoazobenzene-4′-sulfonyl chloride (DABS-Cl), L-alanine, 3-hydroxybutyrate, acetoacetate and L-methionine sulfoximine (MSO) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Enzymes for metabolite determination were from Boehringer (Mannheim, Germany), Reanal (Budapest, Hungary) and P.O.Ch. (Gliwice, Poland). [U-^{14}C]Glycerol and [U-^{14}C]L-alanine were from Amersham Searle Corporation (Amersham, U.K.), while [^{14}C]sodium bicarbonate was from Świerk (Poland). Other reagents were from P.O.Ch. (Gliwice, Poland).

RESULTS AND DISCUSSION

Glucose synthesis

In agreement with the data obtained for guinea pig [8], rat and dog [17], the rate of gluconeogenesis in rabbit renal cortical tubules with 1 mM alanine added as the sole substrate was negligible (Table 1). Although the addition of various concentrations of either ketone bodies or glycerol to renal tubules incubated with 1 mM alanine did not affect glucose formation (not shown), increasing concentrations of either AcAc or 3-HB in the presence of both 1 mM alanine and 2 mM glycerol resulted in a marked induction of gluconeogenesis (Fig. 1). The maximal rate of glucose synthesis was exhibited in the pres-

Table 1. The effect of 3-hydroxybutyrate on the rate of gluconeogenesis in renal tubules incubated with amino acids and glycerol

Substrates	Glucose synthesis (μmol/h per g dry wt.)	
	−3-HB	+3-HB
Glycerol	4.8±0.2	14.8±1.7[a]
Alanine+glycerol	3.9±0.5	53.8±4.9[a]
Aspartate+glycerol	33.5±3.3	85.5±3.0[a]
Proline+glycerol	40.5±4.5	60.0±5.0[a]
Glutamate+glycerol	38.5±3.3	53.5±5.2[a]

Renal tubules were incubated for 60 min. Amino acids and glycerol were added at 1 and 2 mM concentration, while 3-HB an AcAc were 5 mM. Values are means ±S.D. for 5–10 experiments. [a] $P < 0.05$ *vs* corresponding control value with no ketone body. The rates of gluconeogenesis in the presence of alanine, aspartate, proline and glutamate applied as the sole substrates were 3.5±0.2, 3.5±0.6, 4.3±2.2 and 4.1±2.8 μmol/h per g dry weight, respectively.

ence of alanine, glycerol and 5 mM 3-HB. A lower glucose production observed in the presence of 5 mM AcAc and alanine+glycerol was not caused by a slower AcAc metabolism since the rates of 3-HB and AcAc consumption in the presence of alanine and glycerol were similar (56.5±6.1 and 55.0±7.3 μmol/h per g dry wt., respectively). As gluconeogenic activity with alanine, glycerol and 5 mM 3-HB was by about 45% higher than that measured in the presence of 5 mM AcAc, we have studied the effect of 5 mM 3-HB on glucose formation in renal tubules incubated with glycerol and several other amino acids. As shown in Table 1, 2 mM glycerol induced gluconeogenesis in the presence of aspartate, glutamate or proline, confirming our previous observations concerning glutamate and aspartate [9, 10]. Interestingly, the addition of 3-HB to renal tubules incubated with glycerol and either aspartate, proline or glutamate resulted in a significant acceleration of gluconeogenesis.

Since the most effective action of ketone bodies on gluconeogenesis was observed in the presence of glycerol and either alanine or aspartate, we have studied the effect of ketones on the incorporation of [U-^{14}C]substrates into glucose in tubules incubated with these amino acids and glycerol. As presented in Table 2, both 3-HB and AcAc augmented the incorporation of [U-^{14}C]alanine into glucose in the presence of glycerol 8.5- and 5.5-fold, respectively, while [U-^{14}C]glycerol utilization for gluconeogenesis was increased 11- and 7-fold, respectively. Moroever, the addition of 3-HB to tubules incubated with aspartate+glycerol doubled both [U-^{14}C]aspartate and [U-^{14}C]glycerol incorporation into glucose.

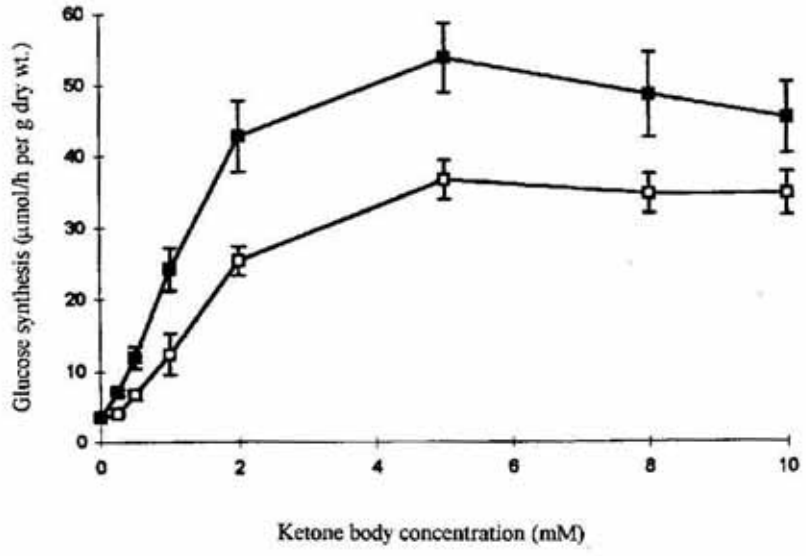

Figure 1. The effect of increasing concentrations of either 3-HB or AcAc on glucose formation in renal tubules incubated with alanine+glycerol.

Alanine and glycerol were applied at 1 and 2 mM concentration, respectively. Values are expressed as means ±S.D. for 5–10 experiments. (■) +3-HB; (□) +AcAc.

Table 2. The effect of 3-hydroxybutyrate and acetoacetate on the incorporation of [U-^{14}C]alanine, [U-^{14}C]aspartate and [U-^{14}C]glycerol into glucose

^{14}C-Labeled substrate	Unlabeled substrate	Ketone bodies	[^{14}C]Glucose synthesis (μmol $^{14}C_3$ units/h per g dry wt.)
[U-^{14}C]Alanine	glycerol	None	2.5±0.4
[U-^{14}C]Alanine	glycerol	3-HB	21.6±2.2[a, b]
[U-^{14}C]Alanine	glycerol	AcAc	14.4±1.5[a]
[U-^{14}C]Aspartate	glycerol	None	12.2±1.1
[U-^{14}C]Aspartate	glycerol	3-HB	23.2±2.2[a]
[U-^{14}C]Glycerol	alanine	None	7.8±1.7
[U-^{14}C]Glycerol	alanine	3-HB	89.6±2.4[a, b]
[U-^{14}C]Glycerol	alanine	AcAc	56.9±6.5[a]
[U-^{14}C]Glycerol	aspartate	None	45.3±2.3
[U-^{14}C]Glycerol	aspartate	3-HB	90.5±5.5[a]

Experimental conditions were as described in the legend to Table 1 and under Material and Methods. Values for [^{14}C]glucose synthesis are means ± S.D. for 3 experiments. [a] $P < 0.05$ *vs* corresponding control value with no ketone body; [b] $P < 0.05$ *vs* corresponding control value with alanine+glycerol+AcAc.

Utilization of amino acids as well as glutamine and glutamate synthesis

The utilization of 1 mM alanine and aspartate by rabbit renal tubules was similar (180.0±5.0 and 175.0±6.5 μmol/h per g dry wt., ±S.D., respectively, for 5 experiments) and changed neither on addition of glycerol nor of ketone bodies (not shown). As presented in Table 3, addition of glycerol to tubule suspension incubated with alanine did not affect glutamine and glutamate formation. In contrast, the presence of glycerol resulted in a marked inhibition of glutamine synthesis from aspartate, confirming our previous observations [10]. A significant suppression of glutamine production accompanied by a marked glutamate accumulation was observed upon the addition of either AcAc or 3-HB to tubules metabolizing alanine and glycerol, while 3-HB did not affect glutamine synthesis when alanine was substituted by either aspartate (Table 3), proline or glutamate (not shown). Thus, it is likely that ketone body-induced inhibition of glutamine synthesis in the presence of alanine+glycerol might contribute to increased utilization of amino-acid carbon skeleton for gluconeogenesis (cf. Table 2).

The production of ammonium by renal tubules incubated with alanine and glycerol was negligible (not shown) due to a high activity of glutamine synthetase which effectively consumes ammonium ions for glutamine production and therefore masks the activity of glutamate dehydrogenase, as shown previously for rabbit kidney-cortex tubules incubated with aspartate [10] and for guinea pig renal cortical tubules metabolizing glutamate [18], alanine [17] or aspartate [11]. In agreement with these data, the addition of 1 mM MSO, a potent inhibitor of glutamine synthetase [19], to renal tubules incubated with alanine+glycerol resulted in a massive release of ammonium to the reaction mixture. Ammonium formation was markedly lowered in the presence of either 3-HB or AcAc (from 69.0±2.5 to 39.5±4.5 and 46.5±5.3 μmol/h per g dry wt., ±S.D., with alanine+glycerol, alanine+glycerol+3-HB and alanine+glycerol+AcAc, respectively, $P < 0.05$ for 3 experiments), indicating that inhibition of glutamate dehydrogenase activity in the presence of ketone bodies might be responsible for the suppression of glutamine formation (cf. Table 3). The possible mechanisms involved in ketone body-induced inhi-

Table 3. The effect of ketone bodies on glutamine and glutamate synthesis in renal tubules incubated with alanine, aspartate and glycerol

Substrates	Ketone bodies	Glutamine	Glutamate
		(μmol/h per g dry wt.)	
–	None	55.0±4.0	24.5±2.2
Alanine	None	106.0±5.5	39.4±3.3[a]
Alanine+glycerol	None	110.5±3.7	37.5±2.5
Alanine+glycerol	AcAc	60.5±2.0[b]	68.5±5.7[b]
Alanine+glycerol	3-HB	57.5±9.3[b]	73.5±7.3[b]
Aspartate	None	100.5±10.4	50.4±5.6
Aspartate+glycerol	None	50.5±5.6[c]	105.2±10.6[c]
Aspartate+glycerol	3-HB	55.4±6.7[c]	124.4±12.6[d]
Glycerol	None	57.9±2.5	25.0±1.6
Glycerol	AcAc	54.2±5.5	30.6±2.0[a]
Glycerol	3-HB	50.5±8.4	29.5±0.3[a]

Experimental conditions were as described in the legend to Table 1. Values are means ± S.D. for 5–10 experiments. [a] $P < 0.05$ *vs* control with glycerol; [b] $P < 0.05$ *vs* control with alanine+glycerol; [c] $P < 0.05$ *vs* control with aspartate; [d] $P < 0.05$ *vs* control with aspartate+glycerol.

bition of glutamate dehydrogenase are (i) elevation of the intramitochondrial NADH/NAD^+ ratio and (ii) the increase of intracellular content of 2-oxoglutarate, the end-product of glutamate dehydrogenase, due to stimulation of flux through tricarboxylic acid cycle (cf. Table 4). Similarly, the 3-HB-induced increase in the intramitochondrial redox state was reported to result in a significant suppression of ammoniagenesis in rat kidney mitochondria incubated with glutamine [6], while ketone bodies-induced elevation of intracellular 2-oxoglutarate concentration was associated with a lowered ammonium formation from glutamine in perfused dog kidney [20].

Glycerol utilization and lactate production

It has been reported that transport of reducing equivalents from cytosol into mitochondria might limit gluconeogenesis from glycerol, sorbitol and lactate in both renal cortical tubules and hepatocytes [10, 21–23]. As presented in Table 5, alanine increased both glycerol consumption and lactate formation. Moreover, inclusion of ketone bodies in the incubation media containing alanine plus glycerol produced a significant stimulation of glycerol consumption, while lactate synthesis was slightly diminished in the presence of 3-HB. In contrast, in the absence of alanine neither 3-HB nor AcAc affected lactate production, whereas glycerol consumption was only by about 25% higher than that measured with glycerol as the sole substrate. This suggests that the increased glycerol utilization observed with alanine and ketones might have resulted from the activation of malate-aspartate shuttle.

Intracellular metabolite levels and CO_2 fixation

In the absence of alanine intracellular levels of G-3P were very high and not affected by the addition of ketone bodies (43.0±5.7 and either 46±3.3 or 38.0±2.0 μmol/g dry wt., ± S.D., for glycerol with 3-HB or AcAc, respectively, $P < 0.05$ for 4 experiments). As shown in Table 4, the inclusion of ketones to the incubation mixture containing alanine+glycerol produced a significant decrease in G-3P content accompanied by an elevation of intra-

Table 4. Intracellular levels of metabolites in renal tubules incubated with alanine plus glycerol and either 3-hydroxybutyrate or acetoacetate

	Alanine+glycerol	Alanine +glycerol + 3-HB	Alanine +glycerol + AcAc
Metabolites	Metabolite levels (μmol/g dry weight)		
Glutamate	8.0±0.6	23.2±3.0[a]	14.0 ±1.1[a]
Aspartate	3.7 ±0.1	5.5 ±0.1[a]	4.6 ±0.1[a]
Malate	0.22±0.03	0.50±0.04[a, b]	0.42±0.01[a]
2-Oxoglutarate	0.31±0.01	0.66±0.04[a]	0.66±0.05[a]
G-3P	46.7 ± 4.1	13.3 ±3.7[a, b]	20.7 ±1.7[a]
DHAP	0.06±0.01	0.06±0.01	0.05±0.01
Lactate	3.5 ± 0.4	2.9 ±0.6[a]	3.0 ±0.3[a]
Pyruvate	0.44±0.08	0.46±0.05	0.51±0.01

Renal tubules were incubated with substrate concentrations shown in the legend to Table 1 and separated from the reaction medium as described under Material and Methods. The intracellular levels of metabolites are means ±S.D. for 4 separate experiments and were measured following 60 min of incubation. [a] $P < 0.05$ *vs* alanine+glycerol; [b] $P < 0.05$ *vs* alanine+glycerol+AcAc

cellular levels of glutamate, aspartate and malate, indicating activation of the malate-aspartate shuttle, leading to a fall of the cytosolic NADH/NAD^+ ratio and acceleration of G-3P dehydrogenase activity which could result in stimulation of glucose formation from glycerol (cf. Table 2). In agreement with this suggestion, the addition of either 3-HB or AcAc to renal tubules metabolizing alanine and glycerol produced a marked decrease in the [G-3P]/[DHAP] ratio (from 770±78 to 218±11 and 348±25, respectively, $P < 0.05$), while the [lactate]/[pyruvate] ratio was much less affected (8.01±0.45, 6.09±0.35 and 5.88±0.38 without and with AcAc or 3-HB, respectively, $P < 0.05$ for 3 experiments). In view of these observations one might conclude that the redox couples tested are poorly connected, confirming observations for renal tubules [10] and isolated hepatocytes [24].

In view of the markedly increased intracellular levels of aspartate and malate observed on the addition of ketones to renal tubules incubated with alanine+glycerol (cf. Table 4) it is likely that ketone body-induced acceleration of alanine incorporation into glucose (cf. Table 2) and activation of the malate-aspartate shuttle might be due to stimulation of pyruvate carboxylation. The induction of gluconeogenesis from alanine upon the addition of either 5 mM 3-HB or 5 mM AcAc to the reaction medium containing alanine plus glycerol (cf. Table 1), was associated with about 35% and 25% stimulation of $^{14}CO_2$ fixation (from 25.2±0.9 to either 36.4±0.5 or 31.2±0.7 μmol/h per g dry wt., ±S.D., with alanine+glycerol and either 3-HB or AcAc, respectively, $P < 0.05$ for 3 experiments) pointing to acceleration of pyruvate carboxylation. Interestingly, a significant stimulation of $^{14}CO_2$ fixation was also observed on the addition of 3-HB to renal tubules incubated with aspartate+glycerol (from 34.9±3.3 to 46.3±2.2 μmol/h per g dry wt., $P < 0.05$). Pyruvate generation by pyruvate kinase has been postulated to be of great importance for aspartate metabolism, since the inhibition of pyruvate entry into mitochondria by α-cyano-4-hydroxycinnamate in guinea pig kidney-cortex tubules incubated with aspartate suppressed both aspartate removal and glutamine synthesis [11]. Thus, it is likely that the stimulation of pyruvate carboxylation by 3-HB in rabbit renal tubules incubated with asparate+glycerol may also contribute to an increased utilization of aspartate for gluconeogenesis (cf. Table 2). The more pronounced action of 3-HB on gluconeogenesis in the presence of alanine+glycerol compared with that observed with AcAc instead of 3-HB, might have resulted from the ability of 3-HB to increase more effec-

Table 5. The effect of ketone bodies on glycerol removal and lactate formation in renal tubules incubated with glycerol in the absence or presence of alanine

Substrates	Ketone bodies	Glycerol	Lactate
		Metabolite removal (–) or production (+) (μmol/h per g dry wt.)	
Glycerol	None	–106.5±5.2	+50.5±7.8
Glycerol	AcAc	–134.0±4.9[a]	+54.0±5.0
Glycerol	3-HB	–126.5±4.5[a]	+47.0±4.9
Alanine+glycerol	None	–157.5±2.0	+79.5±3.4
Alanine+glycerol	AcAc	–222.0±4.1[a]	+75.7±3.3
Alanine+glycerol	3-HB	–265.5±4.4[a, b]	+56.5±1.4[a, b]

Experimental conditions were as described in the legend to Table 1. Values are means ±S.D. for 5 experiments. [a] $P < 0.05$ *vs* corresponding control value without ketone body; [b] $P < 0.05$ *vs* control with alanine+glycerol+AcAc

tively the mitochondrial redox state [6] since the elevation of the mitochondrial $NADH/NAD^+$ ratio was observed to potentiate the sensitivity of pyruvate carboxylase to its activator, acetyl-CoA [25] as well as to diminish pyruvate dehydrogenase activity [26, 27] resulting in an enhanced supply of pyruvate for carboxylation. The requirement for glycerol in ketone body-induced stimulation of gluconeogenesis from alanine and aspartate results probably from the ability of glycerol to produce a marked increase in the cytosolic redox-state, resulting in acceleration of glyceraldehyde-3-phosphate dehydrogenase activity as shown previously for rabbit renal cortical tubules incubated with glutamate [9] and aspartate [10].

Physiological relevance

Although in the early postabsorptive state the contribution of kidney to gluconeogenesis does not exceed 10%, it increases markedly during prolonged starvation [28]. In normal humans and animals the serum levels of ketone bodies are low but they are dramatically increased in response to alterations in the physiological state. For example, starvation of humans or rats for 48 h results in an elevation of serum ketone bodies from 0.1–0.3 mM up to 2–3 mM concentrations [29]. The serum concentrations of 3-HB and AcAc in fed rabbits were equal to 0.023±0.002 and 0.033±0.010 mM, and after 48 h starvation of animals were elevated about 10- and 5.5-fold, respectively (up to 0.26±0.05 and 0.18±0.03 mM, respectively, $P < 0.001$ for 6 rabbits). Similarly, the serum level of 3-HB in starved rats was reported to increase to a greater extent than that of AcAc [30]. Therefore, it seemed interesting to check the effect of physiological concentrations of 3-HB and AcAc on glucose synthesis in tubules isolated from starved rabbits incubated in the presence of alanine and glycerol. Moreover, we have decided to lower glycerol concentration to 0.4 mM, i.e. to the level found in serum of starved animals [31].

Although there was no significant increase in glucose formation on addition of low concentrations of ketones (i.e. equal to those determined in serum of fed rabbits) to renal tubules isolated from starved animals incubated with 1 mM alanine and 0.4 mM glycerol (10.9±1.8 and 11.7±2.2 μmol/h per g dry wt., ±S.D., in the absence or presence of ketone bodies, respectively, for 4 experiments), the addition of 3-HB plus AcAc at 0.3 and 0.2 mM concentrations, respectively, resulted in an increase in gluconeogenesis up to 22.1±2.8 μmol/h per g dry wt. ($P < 0.05$), indicating the physiological importance of the added compounds.

The data presented in this paper show that ketone bodies play an important regulatory role in renal gluconeogenesis. We have demonstrated for the first time that in the presence of glycerol, ketone bodies induce glucose

formation from alanine and accelerate gluconeogenesis on substitution of alanine by aspartate, proline or glutamate. Moreover, ketone body-induced stimulation of gluconeogenesis in the presence of alanine+glycerol is observed at physiological concentrations of 3-HB and AcAc. In addition, ketone bodies in the presence of amino acids activate glycerol incorporation into glucose, probably due to activation of the malate-aspartate shuttle. This, to our knowledge, might represent a novel mechanism responsible for ketone body-induced stimulation of gluconeogenesis from glycerol, which is known to be efficiently taken up by the kidney [32]. Finally, the data presented in this paper clearly indicate that the contribution of various substrates to glucose synthesis in the kidney established with the use of glucose precursors applied as the sole substrates, may be different from that estimated at substrate concentrations present in the blood in physiological conditions.

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Note added in proof (received in June 12th, 1997)

Recently we have shown (Lietz, T., Rybka, J. & Bryła, J., 1997, *Amino Acids*, accepted for publication) that, similarly to ketone bodies, fatty acids are also of importance for induction of gluconeogenesis in isolated rabbit renal cortical tubules incubated with alanine and either glycerol or lactate. The mechanisms responsible for fatty acid-induced stimulation of glucose synthesis under these conditions include: (i) activation of pyruvate carboxylation, (ii) enhancement of glyceraldehyde-3-phosphate dehydrogenase activity and (iii) inhibition of glutamine production due to elevation of the mitochondrial redox state.