GLUCONEOGENESIS AND AMMONIA PRODUCTION IN THE ISOLATED PERFUSED RAT KIDNEY: THE EFFECT OF STARVATION, ACIDOSIS AND DIABETIC KETOSIS

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ABSTRACT

Isolated rat kidneys were perfused with a cell-free perfusate, and their creatinine clearance was found to be 106 $\mu l/min./g$ wet weight. Gluconeogenesis from pyruvate, lactate, fructose, α-oxoglutarate, oxaloacetate, glutamate and glutamine as the substrates at a concentration of 10 mM and ammonia production from glutamine were studied in the kidneys from fasted rats, rats with NH_4Cl-induced acidosis, and streptozotocin diabetic rats, using cell-free perfusates. 1. Fasting caused the gluconeogenesis from all substrates except pyruvate and the ammonia production from glutamine to increase. 2. In the kidneys from the rats with chronic acidosis, the gluconeogenesis from the substrates other than pyruvate, lactate and fructose was increased, and the gluconeogenesis and ammonia production from glutamine were both greater than those in the kidneys from the 7-day fasted rats. 3. In the kidneys from the streptozotocin diabetic rats, the gluconeogenesis from all substrates other than fructose was increased and was greater than that in the kidneys from the fasted rats and the rats with chronic acidosis. Also, despite the markedly increased gluconeogenesis from glutamine, ammonia production did not increase. From these findings, we believe that gluconeogenesis and ammonia production are not always coupled with each other, especially under some conditions in the isolated perfused rat kidneys.

Key words: Gluconeogenesis, Ammonia production, Isolated perfused rat kidney

INTRODUCTION

The most important of the roles of the kidney as one of the life-sustaining organs of the organism may be its so-called renal functions, such as the elimination of waste matters and maintenance of water, electrolyte and acid-base balances. However, since the renal cortex is the only site other than the liver where gluconeogenesis is carried out, the kidney is also placed in an important position even in regard to glucose homeostasis. It has been reported that renal gluconeogenic flux may rise to as much as 50% of the total glucose production in prolonged starvation (1). Krebs *et al.* (2) have also described that the gluconeogenic capacity per unit weight of the renal cortex is comparable to that of the liver. The amount of glycogen reserved in the kidneys, on the other hand, is as low as 0.1%, compared with 10% per unit wet weight in the liver; and, in view of the resemblance of key enzymes in carbohydrate metabolism in the renal cortex to those in the liver, the kidney may be an organ suitable for studying

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gluconeogenesis.

Almost all reports on gluconeogenesis in the kidney have been obtained from experiments using slice technique. However, there have been no reports on the effects of ketone bodies on gluconeogenesis and ammonia production in the perfused kidney.

For this reason, we perfused the kidneys isolated from fasted rats, from rats with experimentally induced chronic metabolic acidosis and from those with experimentally induced diabetes, in the renal cortex of which gluconeogenesis could be persumed to be elevated. We then compared the gluconeogenic capacities of the kidneys using various substrates. We also studied the longstanding problem as to whether ammonia productivity might in any way have a bearing on gluconeogenesis in the kidney.

MATERIALS AND METHODS

Male Wistar rats, weighing 250 to 400 g, were used as the experimental animals. Prior to their use in the study, the rats were divided into 5 groups: 1) well-fed rats; 2) rats fasted for 24 hours preceding the study; 3) rats fasted for 7 days preceding the study; 4) rats with experimentally induced chronic acidosis (prepared by giving the rats free access to 280 mM NH_4Cl solution as the sole drinking water for 7 days, fasted during the 24 hours preceding the experiment, and given 1 mM of NH_4Cl per 100 g of body weight 4 hours before the experiment); and 5) streptozotocin diabetic rats (stz-DM rats) (prepared by injecting the rats intravenously with 70 mg/kg of streptozotocin, controlling them by subcutaneous injection of 3 units/day of lente insulin for 4 days, and then withdrawing the insulin for 3 days to induce ketosis, and fasting the animals during the 24 hours preceding the experiment).

Procedure for perfusion of isolated kidneys

Each rat was anesthetized with thiamylal sodium (80 mg/kg, i.p.); an abdominal crucial incision was then performed, and a pp-10 polyethylene catheter was inserted into the right ureter to a level close to the pelvis of the kidney, and the ureter was tied in place with a suture. (In the experiments other than those for renal function, the ureter was not cannulated but the urine was returned directly into the perfusate so as to prevent metabolites from being lost.) The left renal vein, superior mesenteric artery, left renal artery and right adrenal artery were ligated and severed. One silk suture was then applied to a part of the abdominal aorta proximal to the site where the right renal artery arises, and 2 silk sutures to a part distal to that site. After ligation with the distal of the 2 sutures, a silastic tube (2 mm in external diameter and 1.0 mm in caliber) was inserted into the aorta. In order to fix the tube in place, the aorta was ligated with the remaining silk suture distal to the site from which the renal artery arises, and the aorta was ligated with the proximal silk suture. At the same time an injection with a syringe of 25 ml of a perfusate, oxygenated with a mixture of 95% O_2 and 5% CO_2 beforehand and containing 300 units of heparin, was started in order to prevent ischemia in the kidney. The right kidney was then isolated, stripped of the adhering adipose tissue, and connected to a perfusing apparatus. It took about 15 minutes to complete these procedures. Fig. 1 illustrates the apparatus. The perfusion was performed on a recirculation system, and the internal temperature of the apparatus was maintained at 38 to 40°C. The flow rate of the perfusate was adjusted with a microtube pump (Tokyo Rikakikai Co. LTD.) so that the perfusate pressure would be 90 to 120 mm Hg, at which the flow rate was 15 to 20 ml/min., and the pulse pressure was 10 mm Hg. For oxygenation of the perfusate, the aforementioned O_2-CO_2 mixture was used, and the oxygen tension in the recirculation system was maintained at 450 to 500 mm Hg. During the experiments, the pH value of the perfusate was maintained constant with a
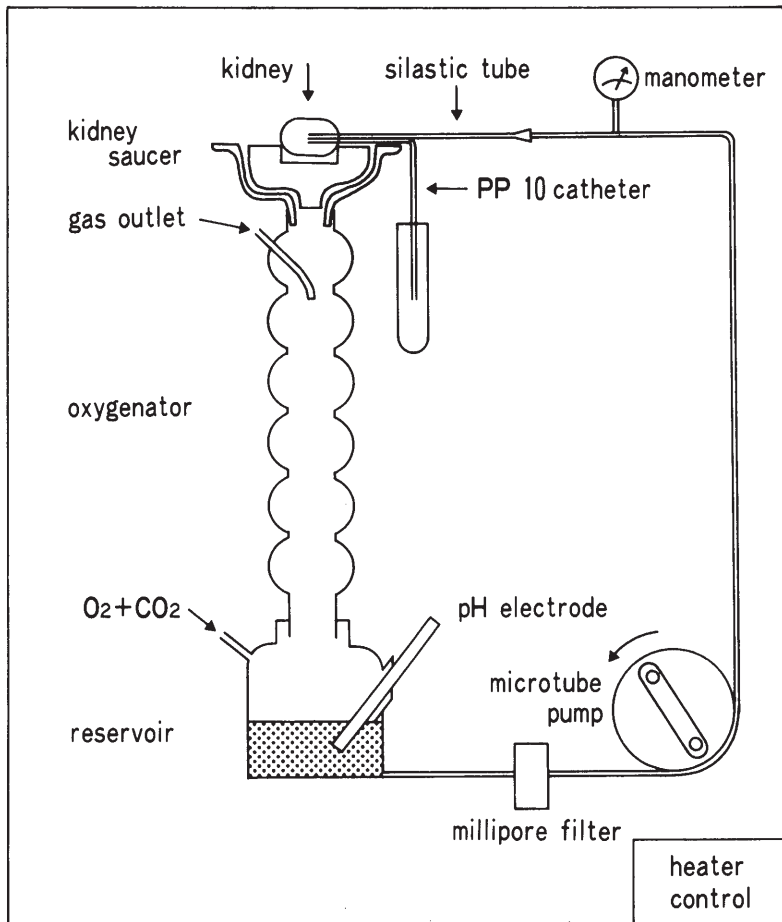
Fig. 1   Apparatus

pH-stat (TOA Electronics LTD.). The perfusate was made aseptic by first sterilizing it in an autoclave and then filtering it through a 0.45-micron millipore filter. Table 1 shows the composition of the perfusate, which in order to avoid the effects of glucose consumption by red blood cells, was made cell-free. Bovine albumin (Cohn fraction V; Daiichi Pure

Table 1   Modified Krebs-Henseleit solution

NaCl	118 mM
KCl	4.7 mM
CaCl$_2$	2.5 mM
MgSO$_4$	1.2 mM
KH$_2$PO$_4$	1.2 mM
NaHCO$_3$	25 mM
Bovine Albumin	4.5 g/dl

Chemicals Co., Tokyo) was washed 3 times with 10 volumes of methyl alcohol, dissolved in the perfusate, dialyzed with a modified Krebs-Henseleit solution for 1 week, and adjusted to a pH of 7.2; and 50 ml of the resulting solution were used. For the experiments by perfusion, the substrates were added to the perfusate; the mixtures were well stirred, and the isolated kidneys were connected with the perfusing apparatus and perfused for 1 hour, while being sampled at 15-minute intervals, with the time of connection of the appratus with the kidney as 0 hours of perfusion. The kidneys under perfusion were investigated for renal function by perfusing each of them with 100 ml of the pH-7.4 perfusate, adding 20 mg of creatinine to the perfusate at 30 minutes of perfusion and at 30-minute intervals thereafter for a total of 90 minutes. The perfusate was assayed for metabolites first by deproteinizing it with $HClO_4$, then neutralizing it with KOH, and finally determining the metabolites enzymatically. Glucose was determined by the glucose oxidase method; pyruvate, by Bücher's method; lactate, by Hohorst's method; fructose, α-oxoglutarate and glutamate, by Bergmeyer's method; oxaloacetate, by Wahlefeld's method; creatinine, by Seeling's method, and ammonia, by Fujii-Okuda's method (using the ammonia test kit commercially available from Wako Pure Chemical Industries, Osaka).

RESULTS

1. *Renal function of the isolated perfused rat kidneys*

In the experiments for renal function, the kidneys from the well-fed rats were perfused with a perfusate containing 5 mM glucose and 5 mM pyruvate as energy sources. The creatinine clearance reached a peak of 106 ± 26 $\mu l/min./g$ wet weight at 30 to 60 minutes of perfusion, then tended to steadily decrease with the passage of time, reaching 80 ± 21 $\mu l/min./g$ wet weight at 60 to 90 minutes. Our values were slightly lower than the values of 159 to 106 $\mu l/min./g$ wet weight, respectively, reported by Nishiitsutsuji-Uwo (3).

Fig. 2 illustrates changes in glucose, pyruvate and lactate concentrations when the kidneys from the well-fed rats were perfused with a perfusate containing glucose as the substrate at a physiological concentration of 5 mM. The glucose concentration decreased by 0.30 $\mu mole/ml$ during the first 15 minutes of perfusion, increased slightly during the following 15 minutes, and then decreased and remained unchanged during the last 15 minutes. As a result, 11.2 ± 2.9 $\mu moles/g$ wet weight/hour of glucose were consumed. This value agreed with 54 ± 15 $\mu moles/g$ dry weight/hour reported by Nishiitsutsuji-Uwo (3) and 65 ± 8 $\mu moles/g$ dry weight/hour reported by Hems et al. (4) Both the pyruvate and the lactate concentrations, on the other hand, increased during the first 30 minutes and remained practically unchanged during the following 30 minutes. The output of lactate amounted to 14.8 ± 7.5 $\mu moles/g$ wet weight/hour, which was almost equivalent to the consumed amount of glucose. In other words, about half of the consumed amount of glucose seemed to have been utilized for the production of lactate.

Histologic examination of the kidneys perfused for 90 minutes revealed no striking changes except for slight enlargment of the glomerulus, slight flattening of the renal tubule epithelium, slight dilatation of the renal tubule lumen, and only slight changes in the nucleus. From these findings, the renal function seemed histologically normal. These findings also indicated that the isolated perfused kidneys used in this study were under relatively physiological conditions.

Table 2 shows the arterial blood pH values and blood glucose levels in the well-fed rats, the 24-hour fasted rats, the 7-day fasted rats, the rats with chronic acidosis and the diabetic rats,
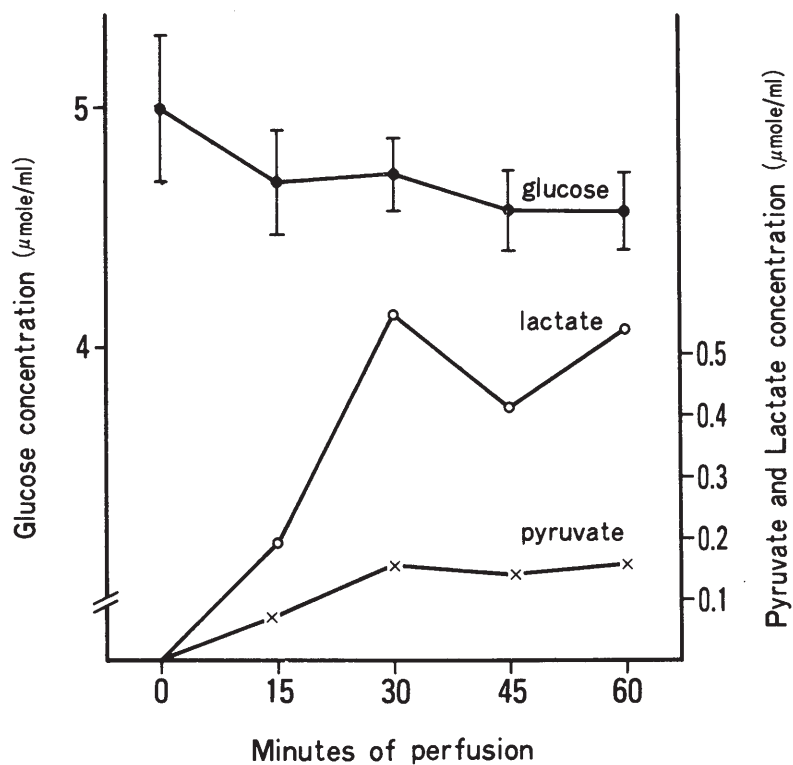
Fig. 2 Time-course of glucose consumption and pyruvate and lactate formation from glucose in isolated perfused rat kidney

The rats were well fed. Glucose (5 μmole/ml) was added at the start of the perfusion. The initial perfusion volume was 50 ml. The perfusate was adjusted to a pH of 7.4. Each curve is the mean of six perfusions.

Table 2 The arterial blood pH values and blood glucose levels in the well-fed rats, 24h fasted rats 7day fasted rats, chronic acidosis rats and diabetic rats

	No. of rat	Blood pH	Plasma HCO_3^- m mole/l	Blood glucose mg/dl
well-fed	4	7.51±0.02	27.5±3.4	181± 4
24h fasted	5	7.43±0.03	21.6±1.3	94±20
7day fasted	3	7.31±0.04	24.2±2.0	92±11
NH₄ Cl treated + 24h fasted	7	7.12±0.12	12.0±2.4	114±12
stz-DM + 24h fasted	4	7.45±0.05	23.8±4.0	367±35

all of which were used in the experiments by kidney perfusion. The pH value of the arterial blood was 7.51 ± 0.02 in the well-fed rats, 7.43 ± 0.03 in the 24-hour fasted rats, 7.31 ± 0.04 in the 7-day fasted rats, and as low as 7.12 ± 0.12 in the rats with chronic acidosis, while the diabetic rats used were ketotic but non-acidotic, i.e. 7.45 ± 0.05. The blood glucose was 181 ± 4 mg/dl in the well-fed rats, 94 ± 20 mg/dl in the 24-hour fasted rats, 92 ± 11 mg/dl in the 7-day fasted rats, and 114 ± 12 mg/dl in the rats with chronic acidosis, while it was as high as 367 ± 35 mg/dl in the diabetic rats.

2. *Gluconeogenesis and ammonia production from varying substrates in fasting*

Table 3 shows the gluconeogenesis from varying substrates in the kidneys from the 24-hour fasted and the 7-day fasted rats in comparison with that in the kidneys from the well-fed rats. In these experiments, all substrates were added at a concentration of 10 mM, and the perfusate was maintained at a pH of 7.2 during the experiments. Out of the substrates added, fructose gave rise to the highest gluconeogenesis, irrespective of whether or not the rats were fasted. In the kidneys from the well-fed rats, gluconeogenesis was higher with fructose, pyruvate, α-oxoglutarate, oxaloacetate, lactate, glutamate and glutamine in decreasing order. In the kidneys from the 24-hour fasted rats, it was higher with fructose, pyruvate, α-oxoglutarate, glutamate, oxaloacetate, glutamine and lactate in decreasing sequence, and in the kidneys from the 7-day fasted rats, it was higher with fructose, α-oxoglutarate, pyruvate, oxaloacetate, lactate, glutamate and glutamine. The fasting for either period proved inert on the gluconeogenesis from pyruvate. On the other hand, fasting significantly increased the gluconeogenesis from lactate, oxaloacetate, α-oxoglutarate, fructose, glutamate and glutamine with $P < 0.05$ except for that from lactate in the kidneys from the 24-hour fasted rats and for that from oxaloacetate in the kidneys from the 7-day fasted rats. The ammonia production from glutamine increased significantly with $P < 0.01$ in the kidneys from the 7-day fasted rats (Table 4). The consumed amounts of substrates in the two fasted groups were compared only in terms of the consumed amount of glutamate. The consumed amounts of glutamate in the kidneys from both the 24-hour and the 7-day fasted rats were significantly greater, with $P < 0.05$, than the consumed amount of the same substrate in the kidneys from the well-fed rats (Data not shown).

3. *Gluconeogenesis and ammonia production from varying substrates in the kidneys from the rats with NH₄Cl-induced chronic acidosis*

Table 5 shows the gluconeogenesis from varying substrates in the kidneys from the rats with NH_4Cl-induced chronic acidosis compared with that in the kidneys from the 24-hour fasted rats as the controls. The gluconeogenesis from fructose, pyruvate and lactate remained the same as in the kidneys from the control rats; the gluconeogenesis from oxaloacetate increased by 39%; the gluconeogenesis from α-oxoglutarate increased by 55%; the gluconeogenesis from glutamate, by 54% and that from glutamine, by 78%. The ammonia production from glutamine increased by 113% (Table 6). There was no significant difference in the consumed amount of any substrate between the kidneys from the 2 groups of rats (Data not shown).

4. *Gluconeogenesis and ammonia production from varying substrates in the kidneys from stz-DM rats*

Table 7 shows the gluconeogenesis from the varying substrates in the kidneys from the stz-DM rats in comparison with that from the substrates in the kidneys from the 24-hour fasted rats. The gluconeogenesis from all substrates in the kidneys from the stz-DM rats was increased significantly with $P < 0.01$ over that in the kidneys from the 24-hour fasted rats with the exception of gluconeogenesis from fructose. The increase rate was 226% with glutamine, 192% with lactate, 109% with glutamate, 92% with oxaloacetate, 64% with α-oxoglutarate

and 46% with pyruvate. The ammonia production from glutamine did not increase, despite the markedly increased gluconeogenesis in the kidneys from the stz-DM rats (Table 8). On the other hand, there was no difference in the consumption of any substrate between the 2 groups (Data not shown).

Table 3 Effect of starvation on gluconeogenesis from various substrates in isolated perfused rat kidney

substrate	well-fed μmoles/g wet wt./h	24h fasted μmoles/g wet wt./h	7day fasted μmoles/g wet wt./h
pyruvate	28.4±5.8(4)	28.0±3.3 (3)	26.2±4.7 (5)
lactate	7.6±0.4(3)	7.4±0.5 (4)	11.1±0.4***(3)
fructose	36.6±2.2(3)	52.6±4.6** (3)	42.0±1.4* (3)
oxaloacetate	9.7±0.6(3)	12.8±1.3* (3)	14.3±3.1 (3)
α-oxoglutarate	14.8±1.3(3)	22.2±3.8* (3)	27.6±1.1***(4)
glutamate	5.1±0.7(3)	12.9±1.0***(3)	10.6±0.7***(3)
glutamine	3.8±0.4(5)	8.9±1.1***(5)	10.3±2.6** (5)

The values are means ±SD. Numbers in parentheses are numbers of perfusions. The rats were well fed, fasted for 24 hours, and fasted for 7 days. Substrates were initially present in the media in 10 mM concentrations. Significant differences are indicated: * P<0.05; ** P<0.01; *** P<0.001 well-fed rats V.S. 24h or 7day fasted rats. For other details see the text.

Table 4 Effect of starvation on ammonia production from glutamine in isolated perfused rat kidney

substrate	well-fed μmoles/g wet wt./h	24h fasted μmoles/g wet wt./h	7day fasted μmoles/g wet wt./h
glutamine	39.0±4.7(5)	53.6±9.9(5)	81.7±12.0**(5)

The values are means ±SD. Numbers in parentheses are numbers of perfusions. The rats were well fed, fasted for 24 hours, and fasted for 7 days. Glutamine was initially present in the media in 10 mM concentrations. Significant differences are indicated: ** P < 0.01 well-fed rats V.S. 24h or 7day fasted rats. For other details see the text.

Table 5 Effect of chronic metabolic acidosis on gluconeogenesis from various substrates in isolated perfused rat kidney

substrate	24h fasted μmoles/g wet wt./h	NH$_4$Cl-induced + 24h fasted μmoles/g wet wt./h
pyruvate	28.0±3.3(3)	28.2±2.9 (4)
lactate	7.4±0.5(4)	7.5±0.5 (3)
fructose	52.6±4.6(3)	55.0±3.0 (3)
oxaloacetate	12.8±1.3(3)	17.8±2.3* (4)
α-oxoglutarate	22.2±3.8(3)	34.5±1.8**(3)
glutamate	12.9±1.0(3)	19.9±2.2**(4)
glutamine	8.9±1.1(5)	15.8±2.8**(4)

The values are means ±SD. Numbers in parentheses are numbers of perfusions. The rats were fasted for 24 hours and NH$_4$Cl-induced plus fasted for 24 hours. Substrates were initially present in the media in 10 mM concentrations. Significant differences are indicated: * P < 0.05; ** P < 0.01 24h fasted rats V.S. NH$_4$Cl induced plus 24h fasted rats. For other details see the text.

Table 6   Effect of chronic metabolic acidosis on ammonia production from glutamine in isolated perfused
rat kidney

substrate	24h fasted μmoles/g wet wt./h	NH$_4$Cl-induced + 24h fasted μmoles/g wet wt./h
glutamine	53.6±9.9(5)	113.9±27.5**(4)

The values are means ±SD. Numbers in parentheses are numbers of perfusions. The rats were fasted for 24
hours and NH$_4$Cl-induced plus fasted for 24 hours. Glutamine was initially present in the media in 10 mM
concentrations. Significant differences are indicated: ** P < 0.01 24h fasted rats V.S. NH$_4$Cl induced plus
fasted rats. For other details see the text.

Table 7   Effect of diabetic ketosis on gluconeogenesis from various substrates in isolated perfused rat
kidney

substrate	24h fasted μmoles/g wet wt./h	stz-DM + 24h fasted μmoles/g wet wt./h
pyruvate	28.0±3.3(3)	41.0± 5.3** (5)
lactate	7.4±0.5(4)	21.6± 3.2***(4)
fructose	52.6±4.6(3)	59.8± 6.2 (4)
oxaloacetate	12.8±1.3(3)	24.6± 2.2** (3)
α-oxoglutarate	22.2±3.8(3)	36.5± 4.5** (4)
glutamate	12.9±1.0(3)	27.0± 4.4** (5)
glutamine	8.9±1.1(5)	29.0±11.9** (5)

The values are means ±SD. Numbers in parentheses are numbers of perfusions. The rats were fasted for 24
hours and stz DM plus fasted for 24 hours. Substrates were initially present in the media in 10 mM
concentrations. Significant differences are indicated: ** P < 0.01; *** P < 0.001 24h fasted rats V.S. stz DM
plus 24h fasted rats. For other details see the text.

Table 8   Effect of diabetic ketosis on ammonia production from glutamine in isolated perfused rat kidney

substrate	24h fasted μmoles/g wet wt./h	stz-DM + 24h fasted μmoles/g wet wt./h
glutamine	53.6±9.9(5)	55.2±14.1(5)

The values are means ±SD. Numbers in parentheses are numbers of perfusions. The rats were fasted for 24
hours and stz-DM plus fasted for 24 hours. Glutamine was initially present in the media in 10mM
concentrations. There is no significant difference between the 2 groups of rats. For other details see the next.

DISCUSSION

There are many papers on gluconeogenesis and ammonia production in the kidney. Most
of these papers, however, described experiments using slices of the kidney, but few papers
have reported the findings from experiments by perfusion of the kidney under more
physiological conditions (3—6).

The gluconeogenesis per unit weight of the renal cortex is greater than that of the liver, and
because the blood flow into the two organs is almost equal, these two organs are thought to be
supplied with the same amounts of substrates (2). For these reasons, gluconeogenesis in the

renal cortex is of significance to the organism. Especially, since renal gluconeogenesis increases during fasting or with low-carbohydrate diet (7), it is accepted that when man is starved for a long time, the renal cortex undertakes 50% of the glucose production (1).

In our present experiments for gluconeogenesis from varying substrates of the same concentration in the kidneys from the well-fed rats, the gluconeogenesis from fructose was the highest, followed by that from pyruvate, α-oxoglutarate, oxaloacetate, lactate, glutamate and glutamine. In the liver, the gluconeogenesis from pyruvate is comparable to that from lactate (8), but in the kidney, the former is 3 to 4 times as high as the latter (3, 6).

The gluconeogenesis from fructose and that from pyruvate are high in both the liver and the kidney (3, 6), while it has been shown that the gluconeogenesis from citric acid cycle intermediates other than oxaloacetate and that from glutamate in the liver are greatly lower than those in the kidney. This has been thought to be attributable to the effect of the liver cell membrane in effectively excluding the citric acid cycle intermediates (8). On the other hand, it has been thought that, because the renal cortex permits such intermediates to readily penetrate through, the renal cortex gives rise to higher gluconeogenesis than the liver (9, 10). We achieved similar findings in the experiments for the gluconeogenesis from α-oxoglutarate, oxaloacetate and glutamate. The gluconeogenesis from all substrates except pyruvate increased with both the 24-hour and the 7-day fasting. Especially, the gluconeogenesis from glutamate and glutamine increased markedly, and this finding calls attention to the ammonia production mechanism, the most characteristic metabolism of the kidney. In other words, the production of ammonia from glutamine in the kidney is indispensable for the maintenance of acid-base balance in the organism.

Glutamine is known to be one of the major end products of nitrogen metabolism in the muscle, and the increased gluconeogenesis and ammonia production in the kidney after the 7-day fasting may support this. In an acidotic condition due to prolonged starvation, the blood glutamine is rapidly deamidated by phosphate-dependant glutaminase in the renal cortex, to be further deaminated by glutamate dehydrogenase to α-oxoglutarate. The ammonia produced there buffers protons in order to maintain relatively the same pH value of urine. α-oxoglutarate is then converted to oxaloacetate before it is introduced into the gluconeogenic pathway; in other words, the remaining carbon skeleton of glutamine is converted to glucose. The increased activity of phosphoenolpyruvate carboxykinase (PEPCK) is thought to be responsible for the rate-limiting step in this instance (11, 12). In our experiments by perfusing the kidneys from the 24-hour fasted rats with a perfusate containing glutamine as the substrate, the glucose production increased significantly, and the ammonia production tended to increase. In the experiments by perfusing the kidneys from the 7-day fasted rats with a similar perfusate, both the glucose production and the ammonia production increased markedly.

The metabolism in the kidney varies in many ways with changes in acid-base balance. In the experiments by perfusing the kidneys from the rats with NH_4Cl-induced chronic metabolic acidosis with the perfusates containing pyruvate, lactate and fructose as the substrates, gluconeogenesis remained the same as that in the kidneys from the 24-hour fasted rats, while the gluconeogenesis from oxaloacetate, α-oxoglutarate, glutamate and glutamine increased significantly in the kidneys from the rats with chronic acidosis.

This increase may be thought to have been derived from the increased activity of PEPCK, a key enzyme in the process from oxaloacetate to triose phosphate. This increased activity of PEPCK in the renal cortex of animals with this NH_4Cl-induced acidosis has been reported by Goodman (9), Iynedjian (13), Alleyne (14), Hems (15) and Pogson (12). The gluconeogenesis from glutamine increased and was paralleled by a marked increase in the production of

ammonia. In NH_4Cl-induced metabolic acidosis, a large portion of excess protons are excreted as ammonium ion through the kidney. The activity of PEPCK increases in parallel with the increase in the excretion rate of NH_4^+, leading to increased gluconeogenesis; and the decrease in α-oxoglutarate in the renal tissue accelerates glutamate dehydrogenase, and that in glutamate accelerates phosphate-dependent glutaminase, leading to the increased production of NH_3 (9, 10, 16, 17). Besides the increased activity of PEPCK as a cause for the increased production of ammonia in the presence of chronic metabolic acidosis, Preuss (18-20) held that an increase in the NAD/NADH ratio was responsible; Pitts (21) described the increased activity of glutamate dehydrogenase as being responsible; and Boid (22) and Goldstein (23, 24) reported that the ammonia production increased as a result of the promoted passage of glutamine through the mitochondrial membrane due to decrease in α-oxoglutarate.

In our experiments by perfusing the kidneys from stz-DM rats, only those from the rats which had hyperglycemia and ketosis but no acidosis were used, for purposes of avoiding the effects of acidosis. The gluconeogenesis from all substrates other than fructose was increased, and the results which were obtained were different from those obtained when using the kidneys from the aforementioned fasted rats and the rats with acidosis. Contrary to expectation, the production of ammonia did not increase despite the markedly increased production of glucose. There is a very old report by Teng (25) on experiments for gluconeogenesis in diabetic rats using kidney slice, proving that the gluconeogenesis from pyruvate increased in the kidneys of diabetic rats. Landau (26) later reported that the activity of glucose-6-phosphatase (G6Pase) was increased in the alloxan diabetic rats; Anderson (27) also showed that the activity of PEPCK was increased in such rats; and Joseph (28) described that the activities of G6Pase, fructose-1, 6-diphosphatase, pyruvate carboxylase, PEPCK and glutamate dehydrogenase were increased in similar rats.

From the finding that the increased activity of PEPCK in the kidneys of rats with diabetic acidosis was normalized by insulin treatment, and that the increased activity of PEPCK lowered to the control level with the administration of sodium bicarbonate, Kamm (29) described the increased activity of PEPCK in the renal cortex of diabetic rats as being secondary to the associated acidosis and independent of the defect in carbohydrate metabolism. Furthermore, from the finding that when the alloxan diabetic rats were administered insulin, PEPCK synthesis in the liver was normalized but PEPCK synthesis in the kidney remained unchanged, Iynedjian (13) speculated that the gluconeogenesis in the kidney might be more closely related to the homeostasis of acid-base balance and to the production of ammonia than to the net glucose produciton and glucose homeostasis. Also from the finding in *in vivo* experiments for the production of ammonia in the kidneys of stz diabetic ketoacidosis rats that the production of ammonia was increased in such kidneys and that the addition of β-hydroxybutyrate to the incubation medium for slices of the renal cortex from normal rats inhibited the production of ammonia from glutamine by 60%, Goldstein (30) described that the metabolic acidosis changed the inhibitory effects of *in vivo* β-hydroxybutyrate to the production of ammonia from glutamine. However, no detailed mechanism is yet known. Gluconeogenesis in the kidney has generally been accepted as a phenomenon secondary to the characteristic ammonia-excretory mechanism of the kidney. The findings in our present experiments using the kidneys from 7-day fasted rats and rats with NH_4Cl-induced metabolic acidosis supported this conclusion. However, in the experiments by perfusing the kidneys from acidosis-free stz-DM rats, gluconeogenesis increased markedly without increased production of ammonia; in other words, in the kidneys from the stz-DM rats with ketosis, ammonia production and gluconeogenesis did not always assume a coupled metabolic pattern due to the inhibition of ammonia production by ketone bodies.

CONCLUSION

From the experiments by perfusing isolated rat kidneys with cell-free perfusates, we drew the following conclusions:

1. Rats kidneys perfused with cell-free perfusates by modifications of the perfusing apparatus and procedures as reported by Nishiitsutsuji-Uwo proved capable of maintaining the function of such kidneys sufficiently for over 90 minutes. Histological examination of the kidneys so perfused revealed only slight flattening of the renal tubule epithelium and slight dilatation of the renal tubule lumen. Therefore, the methods used in this study proved satisfactory for studying various metabolisms in the kidney.

2. In the experiments by perfusing the kidneys from well-fed rats, the gluconeogenesis from fructose with the highest compared with that from various other metabolites added in a concentration of 10 mM to the perfusate. Gluconeogenesis from pyruvate, α-oxoglutarate, oxaloacetate, lactate, glutamate and glutamine followed in decreasing order.

3. In the kidneys from the 7-day fasted rats, the gluconeogenesis from all substrates except pyruvate was increased, compared with that in the kidneys from the well-fed rats; especially, the gluconeogenesis and the production of ammonia from glutamine were markedly increased.

4. In the kidneys from the rats with ammonium chloride-induced metabolic acidosis, the gluconeogenesis from the substrates other than pyruvate, lactate and fructose was increased. The gluconeogenesis and ammonia production from glutamine were both greater than those in the 7-day fasted rats.

5. In the kidneys from the streptozotocin diabetic rats, the gluconeogenesis from all substrates other than fructose was increased and was greater than that in the kidneys from the fasted rats and the rats with metabolic acidosis. Furthermore, despite such a markedly increased gluconeogenesis from glutamine, ammonia production was not increased.

From results of experiments by perfusing the isolated rat kidneys, we guessed that ammonia production and gluconeogenesis are not always coupled with each other in non-acidotic, ketotic diabetic rats, and ketone bodies might inhibit the production of ammonia.

REFERENCES

1) Owen, O.E., Felig, P., Morgan, A.P. *et al.* Liver and kidney metabolism during prolonged starvation. *J. Clin. Invest.,* **48**, 574—583, 1969.

2) Krebs, H.A. Gluconeogenesis. *Proc. R. Soc. Lond.,* **159**, 545—564, 1964.

3) Nishiitsutsuji-Uwo, J.M., Ross, B.D. and Krebs, H.A. Metabolic activities of the isolated perfused rat kidney. *Biochem. J.,* **103**, 852—862, 1967.

4) Hems, D.A. and Gaja, G. Carbohydrate metabolism in the isolated perfused rat kidney. *Biochem. J.,* **128**, 421—426, 1972.

5) Schurek, H.J., Brecht, J.P., Lohfert, H. *et al.* The basic requirements for the function of the isolated cell free *perfused rat kidney. Pflügers Arch.,* **354**, 349—365, 1975.

6) Bowman, R.H. Gluconeognesis in the isolated perfused rat kidney. *J. Biol. Chem.,* **245**, 1604—1612, 1970.

7) Krebs., H.A., Bennett, D.A.H., De Gasquet, P. *et al.* Renal gluconeogenesis. The effect of diet on the gluconeogenic capacity of rat-kidney-cortex slices. *Biochem. J.,* **86**, 22—27, 1963.

8) Ross, B.D., Hems, R. and Krebs, H.A. The rate of gluconeogenesis from various precursors in the perfused rat liver. *Biochem. J.,* **102**, 942—951, 1967.

9) Goodman, A.D., Fuisz, R.E. and Cahill, G.F., Jr. Renal gluconeogenesis in acidosis, alkalosis and pottassium deficiency: Its possible role in regulation of renal ammonia production. *J. Clin. Invest.,* **45**, 612—619, 1966.

10) Goorno, W.E., Rector, F.C., Jr. and Seldin, D.W. Relation of renal gluconeogenesis to ammonia production in the dog nd rat. *Am. J. Physiol.,* **213**, 969—974, 1967.

11) Hanson, R.W. and Garber, A.J. Phosphoenolpyruvate carboxykinase. I. Its role in gluconeogenesis. *Am. J. Clin. Nutr.,* **25**, 1010—1021, 1972.

12) Pogson, C.I., Longshaw, I.D., Roobol, A. *et al.* Phosphoenolpyruvate carboxykinase and renal gluconeogenesis. In gluconeogenesis (ed. Hanson, R.W. and Mehlman, M.A.), John Wiley & sons, New York, pp. 335—368, 1976.

13) Iynedjian, P.B., Ballard, F.J. and Hanson, R.W. The regulation of phosphoenolpyruvate carboxykinase (GTP) synthesis in rat kidney cortex. The role of acid-base balance and glucocorticoids. *J. Biol. Chem.,* **250**, 5596—5603, 1975.

14) Alleyne, G.A.O. and Scullard, G.H. Renal metabolic response to acid base change. I. Enzymatic control of ammoniagenesis in the rat. *J. Clin. Invest.,* **48**, 364—370, 1969.

15) Hems, D.A. and Brosnan, J.T. Effect of metabolic acidosis and starvation on the content of intermediary metabolites in rat kidney. *Biochem. J.,* **123**, 391—397, 1971.

16) Goldstein, L. Relation of glutamate to ammonia production in the rat kidney. *Am. J. Physiol.,* **210**, 661—666, 1966.

17) Steiner, A.L., Goodman, A.D. and Treble, D.H. Effect of metabolic acidosis on renal gluconeogenesis *in vivo*. *Am. J. Physiol.,* **215**, 211—217, 1968.

18) Preuss, H.G. Pyridine nucleotides in renal amonia metabolism. *J. Lab. Clin. Med.,* **72**, 370—382, 1968.

19) Preuss, H.G., Vivatsi-Manos, O. and Vertuno, L.L. Effect of glutamine deamination on glutamine deamidation in rat kidney slices. *J. Clin. Invest.,* **52**, 755—764, 1973.

20) Preuss, H.G. and Weiss, F.R. Rate-limiting factor in rat kidney slice ammoniagenesis. *Am. J. Physiol.,* **221**, 458—464, 1971.

21) Pitts, R.F. Metabolism of amino acids by the perfused rat kidney. *Am. J. Physiol.,* **220**, 862—867, 1971.

22) Boyd, T.A. and Goldstein, L. Kidney metabolite levels and ammonia production in acute acid-base alterations in the rats. *Am. J. Physiol.,* **236**, E289—E294, 1979.

23) Goldstein, L. Glutamine transport by mitochondria isolated from normal and acidotic rats. *Am. J. Physiol.,* **229**, 1027—1033, 1975.

24) Goldstein, L. α-Ketoglutarate regulation of glutamine transport and deamidation by renal mitochondria. *Biochem. Biophy. Res. Commun.,* **70**, 1136—1141, 1976.

25) Teng, C.T. Studies on carbohydrate metabolism in rat kidney slices. II. Effect of alloxan diabetes and insulin administration on glucose uptake and glucose formation. *Arch. Biochem. Biophys.,* **48**, 415—423, 1954.

26) Landau, B.R. Gluconeogenesis and pyruvate metabolism in rat kidney, *in vitro*. *Endocrinology,* **67**, 744—751, 1960.

27) Anderson, J.W. and Stowring, L. Glycolytic and gluconeogenic enzyme activities in renal cortex of diabetic rats. *Am. J. Physiol.,* **224**, 930—936, 1973.

28) Joseph, P.K. and Subrahmanyan, K. Evaluation of the rate-limiting steps in the pathway of glucose metabolism in kidney cortex of normal, diabetic, cortisone-treated and growth hormone-treated rats. *Biochem. J.,* **128**, 1293—1301, 1972.

29) Kamm, D.E., Strope, G.L. and Kuchmy, B.L. Renal cortical and hepatic phosphoenolypruvate carboxylase in the diabetic rat: Effect of acid-base status. *Metabolism,* **23**, 1073—1079, 1974.

30) Goldstein, L., Boylan, J.M. and Schröck, H. Adaptation of renal ammonia production in the diabetic ketoacidotic rat. *Kidney Int.,* **17**, 57—65, 1980.