Renal glucose production in the intact dog

David M. Roxe, Joseph Disalvo, and Sulamita Balagura-Baruch

Department of Physiology, Cornell University Medical College, New York City 10021

RENAL GLUCOSE PRODUCTION was studied in anesthetized, fasting (18 hr) dogs, in various states of acid-base balance, by two procedures: a) by measuring the difference of glucose concentration in renal venous and arterial plasma, using the standard glucose oxidase method; b) by measuring simultaneously the rates of renal release of 14C-labeled glucose during renal intra-arterial infusion of 14C-labeled a-ketoglutaric acid, malic acid, or glutamine. No net renal glucose production was detected by chemical means in the normal state, nor in chronic metabolic acidosis, acute metabolic alkalosis, and acute respiratory acidosis or alkalosis. In contrast, in all cases renal gluconeogenesis was detected from the appearance of 14C-labeled glucose in renal venous blood during renal intra-arterial infusion of 14C-labeled precursor. With this procedure, renal gluconeogenesis was detected even in the instances in which there was net extraction of glucose from arterial blood. Acute changes of acid-base balance altered the rates of renal release of 14C-labeled glucose but did not affect renal arterio-venous concentration differences measured chemically. This study indicates that renal gluconeogenesis is a process occurring continuously at extremely low rates which fail detection by chemical means and that its contribution to the economy of the body is negligible. If changes in rates of renal gluconeogenesis occur during changes of acid-base balance, as suggested by the isotopic measurements, their magnitude is below the sensitivity of the standard glucose oxidase method.

Renal gluconeogenesis; renal metabolism; acid-base balance; 14C-labeled a-ketoglutaric acid; malic acid; glutamine

Material and Methods

We performed 23 experiments on anesthetized fasting (18 hr), mongrel dogs of either sex, weighing 17-25 kg. Following induction of anesthesia with sodium pentobarbital, 30 mg/kg, a continuous infusion into an antecubital vein was started at a rate of 5 ml/minute. In all cases the infusion contained per liter: creatinine, 5 g, and sodium p-
a filtration prepared according to the Somogyi method (9), using the glucose oxidase method (5, 8). The standard error vents glycolysis (5). Plasma glucose was determined in a single plasma sample was 0.043 mM. Recoveries of glucose collection was started. We drew simultaneous arterial and renal venous blood samples during an interval of 4 min, for this timing is presented in

vein from the jugular vein. We checked the position of this was drawn from a radiopaque catheter threaded into the renal vein from the jugular vein. We checked the position of this catheter by fluoroscopy in some cases, by direct retroperitoneal inspection in others, and at autopsy. Urine was collected from a catheter introduced into the ipsilateral ureter.

A 26-gauge needle connected to polyethylene tubing and syringe was inserted into the ipsilateral renal artery and kept patent by an infusion of heparinized isotonic sodium chloride, at 0.91 ml/min. At appropriate times, we changed this infusion to one containing 14C-uniformly labeled α-ketoglutaric acid (SA = 7 mc/mmole), malic acid (SA = 190 mc/mmmole), or glutamine (SA = 9.9 mc/mmmole). All were obtained from International Chemical and Nuclear Corporation (ICNC). Radiopurity according to ICNC was 99%. The infusions were given in heparinized isotonic sodium chloride, in a concentration of 2.5 ml/liter, and at 0.91 ml/min. In terms of loads, the infused rates were 0.33 μmoles/min of α-ketoglutarate, 0.02 μmoles/min of malate, or 0.23 μmoles/min of glutamine. These are tracer amounts.

We tried not to exceed them to avoid inducing gluconeogenesis by exogenous loading with precursors.

In 10 experiments, after 5 min of equilibration with the infusion containing radioactive substrate, a 10-min urine collection was started. We drew simultaneous arterial and renal venous blood samples during an interval of 4 min, between 3 and 7 min of the clearance period. The rationale for this timing is presented in RESULTS and Fig. 1. In each dog, we obtained two clearance periods as described, each in a different state of acid-base balance.

Analytical methods. The determinations of blood and urine pH, plasma Pco₂ and bicarbonate, creatinine in plasma and urine, and β-aminohippurate in blood and urine were done as described previously (1). Plasma for glucose analysis was obtained by centrifuging blood in Vacutainer tubes containing potassium oxalate and sodium fluoride; the latter prevents glycolysis (5). Plasma glucose was determined in filtrates prepared according to the Somogyi method (9), using the glucose oxidase method (5, 8). The standard error (SE) of multiple determinations of glucose concentration in a single plasma sample was 0.043 mm. Recoveries of glucose added to plasma were between 95 and 105%. Glucose was analyzed in plasma, not in blood, because the RV-A values are approximately 1.5 times greater when determined in plasma than in blood (9), a fact that increases the likelihood of observing RV-A differences. McCann and Jude (9) reported that no significant change occurs in plasma glucose concentration after the blood had remained in an ice bath for 30–55 min before centrifugation and that glucose added to blood does not enter red cells appreciably over several minutes. Since in our study, blood samples were collected in tubs with sodium fluoride and centrifuged immediately, it is unlikely that significant penetration of glucose into red cells and degradation occurred.

For radioactivity measurements, glucose was isolated as the phenylglucosazone (10). Following two recrystallizations, its purity was checked by the characteristic melting point, and by microscopic observation of the uniformly colored and shaped crystals. The samples were placed in planchets and counted twice for 15 min in a Nuclear-Chicago low-background gas-flushing Geiger-Müller detector system. Background counting was 3 counts/min. The samples ranged from equal to background (arterial plasma) to 70 times higher. All analyses were done in duplicate. For calculations of SA (counts/min per μmole), the amount of glucose in each planchet was determined from the weight of the phenylglucosazone. The SE of multiple determinations of the specific activity of glucose in a single plasma sample was 0.51 counts/min per μmole.

Calculations. Creatinine clearance was taken as a measure of glomerular filtration rate. Renal blood flow was calculated using the equation of Wolf (14), from PAI concentrations in arterial and renal venous blood. Renal plasma flow (RPF) was calculated from RBF and hematocrit. In addition, RPF was calculated according to Wolf (14), from creatinine concentrations in arterial and renal venous plasma. Glucose concentration (μm) and SA in renal venous plasma were corrected for abstraction of urinary water by
multiplying the measured values by the fraction: (RPF - urine flow)/RPF.

Rates of renal release of 14C-labeled glucose were calculated as follows, using the values determined in renal venous plasma:

\[
\text{SA} \text{ (counts/min per } \mu \text{ mole) } \times \text{ concentration (} \mu \text{mole/ml) } \times \text{ RPF} \text{ (ml/min) } = \text{ counts/min per min released into renal vein.}
\]

Due to recirculation, some labeled glucose returns to the kidney in arterial plasma. The rates of this arterial inflow are calculated as explained above, using the corresponding values measured in arterial plasma.

The net rate of renal release of 14C-labeled glucose (counts/min per min), equals the difference between renal venous and arterial rates, as follows:

\[
\text{renal venous outflow (counts/min per min) - renal arterial inflow (counts/min per min) = net release of labeled glucose (counts/min per min)}
\]

The expression in counts/min per min is preferred to that of SA because it allows valid comparisons of values during an experiment even if RPF and glucose concentrations were to change between two determinations.

Statistical analysis of the significance of the difference between mean values was determined using the t test to obtain P values (3).

RESULTS

Table 1 summarizes the mean values for RPF, urine flow, and RV-A differences of plasma glucose, determined with the glucose oxidase method, in 67 clearance periods from 23 dogs in various states of acid-base balance. Arterial plasma glucose levels averaged 5.81 μmole/ml and ranged from 3.20 to 7.72 μmole/ml. In 15 normal animals, renal venous and arterial plasma glucose did not differ (P > .05), indicating no net renal production or extraction of glucose. Similarly, there was no statistically significant net extraction or production of glucose by the kidneys of dogs in chronic metabolic acidosis, acute metabolic alkalosis, and acute respiratory acidosis or alkalosis.

The results in Table 1 derive from data pooled from the dog population as a whole. The wide range of values observed in each condition listed is reflected in the large SEM. In a few instances, we observed net extraction and net production of glucose (these cases are also included in Table 1), not related to a specific state of acid-base balance, to glomerular filtration rates, nor to renal plasma flow rates. The extreme RV-A values were −1.07 μmole/ml, corresponding to extraction of 146 μmoles/min in a dog in acute respiratory acidosis, and +0.70 μmole/ml, corresponding to production of 94 μmoles/min in a dog in chronic metabolic acidosis. However, there was no consistent pattern in any condition listed.

Initially we had planned to study the effects of acid-base balance on renal gluconeogenesis induced by precursor administration, following the experimental design of in vitro studies. Accordingly, α-ketoglutarate was infused intravenously at 500 μmoles/min, into a normal dog and into a chronically acidic dog; and citrate was infused intravenously at 250 μmoles/min, into one normal and two chronically acidic dogs. α-Ketoglutarate was used because it is one of the best glucose precursors in renal slices (6), and citrate because it increases renal tissue α-ketoglutarate more than infusion of the latter (1). Neither precursor induced net glucose production detectable by chemical analysis.

Tracer amounts of α-ketoglutaric acid-14C, malic acid-14C, or glutamine-14C were infused into a renal artery in 12 of the 23 dogs whose results are summarized in Table 1. Aliquots of the same samples of plasma were used for chemical analysis and for determinations of SA. Table 2 summarizes the results of this study. All figures arc group data for each condition listed. The results in counts/min per min, corrected for recirculation of 14C-labeled glucose, appear at the far right. In 23 out of 23 clearance periods, renal gluconeogenesis was detected from the appearance of 14C-labeled glucose in the renal venous outflow, despite the fact that no net renal production of glucose was determined chemically. Furthermore, with isotope, renal gluconeogenesis was detected even in the few instances in which net glucose extraction occurred.

The differences in mean counts/min per min among the acid-base conditions listed in Table 2 are not statistically significant (P > .05). The wide ranges of counts/min per min and large values of SEM point out a great deal of overlapping among the various conditions, based on marked differences in counts/min per min observed from dog to dog. We could not relate these differences to the precursor used, to glomerular filtration rates, nor to the rates of renal plasma flows. To illustrate, infusion of α-ketoglutaric acid-14C yielded 978 net counts/min per min of glucose-14C in one dog in chronic metabolic acidosis, and 15,500 counts/min per min in another dog in the same condition. For this reason, it is not possible to conclude from this group data whether acid-base changes affect renal release of labeled glucose.

Thus, to study whether changes of the acid-base state affect renal release of glucose-14C in the same dog, counts/

---

### Table 1. Renal glucose production determined with glucose oxidase method in 23 dogs in normal and altered acid-base balance

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of Dogs</th>
<th>No. of Observations</th>
<th>RPF, ml/min</th>
<th>Urine Flow, ml/min</th>
<th>Plasma Glucose RV-A, μmole/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>15</td>
<td>21</td>
<td>102 ± 13</td>
<td>1.46 ± 0.31</td>
<td>+0.10 ± 0.08</td>
</tr>
<tr>
<td>Chronic metabolic acidosis</td>
<td>8</td>
<td>17</td>
<td>198 ± 200.80</td>
<td>0.96 ± 0.38</td>
<td>+0.12 ± 0.05</td>
</tr>
<tr>
<td>Acute metabolic alkalosis</td>
<td>7</td>
<td>15</td>
<td>189 ± 23</td>
<td>2.5 ± 1.20</td>
<td>+0.11 ± 0.09</td>
</tr>
<tr>
<td>Acute respiratory acidosis</td>
<td>6</td>
<td>10</td>
<td>153 ± 200.90</td>
<td>0.75 ± 0.13</td>
<td>−0.13 ± 0.09</td>
</tr>
<tr>
<td>Acute respiratory alkalosis</td>
<td>4</td>
<td>4</td>
<td>132 ± 22</td>
<td>0.80 ± 0.32</td>
<td>−0.10 ± 0.08</td>
</tr>
</tbody>
</table>

Values are means ± SEM. RV-A = renal venous-arterial glucose concentration. Renal venous values are corrected for abstraction of urine water. The mean values for renal plasma flow (RPF), urine flow, and RV-A in each condition are not significantly different from the corresponding in the normal state (P > .05). In the normal state, renal venous glucose concentrations were not significantly different from arterial concentrations (P > .05).
min per min were measured during the initial state, and again after induction of an acute alteration of acid-base balance. Due to the high cost of labeled precursors, we did not infuse them throughout the entire experiment. Rather, the labeled precursor was given only for 12 min in each of the two conditions studied in each dog. Eight minutes were allotted for equilibration, and 4 min for withdrawal of blood samples. The choice of this procedure is based on results obtained in three preliminary experiments in which radioactivity of renal venous glucose was determined between 45 and 28 min after beginning the renal intra-arterial infusion of radioactive \( \alpha \)-ketoglutaric acid. The results are shown in Fig. 1.

As seen in Fig. 1, radioactivity of renal venous glucose increases steeply in the 1st 3 min, then slowly until at about 5 min plateau levels are attained and maintained for as long as 28 min. In experiments 4 and 5, blood samples were drawn in less than 1 min, whereas in experiment 7, they were drawn over a period of several minutes, with midpoints at 10 and 20 min. The difference in SA of two samples separated by a 10-min interval is less in experiment 7 than in experiments 4 and 5. Accordingly, in all subsequent experiments time-integrated samples were drawn. If one subtracts the SA of arterial glucose from the SA of renal venous glucose, the difference also decreases.

From the results in Fig. 1 we assumed that determinations of SA of glucose \(^{14}\text{C}\) in samples of renal venous and arterial plasma drawn between 8 and 12 min of inflow of \(^{14}\text{C}\)-labeled precursor represent values in steady-state conditions. Thus, to minimize the amounts of precursor used, a 10-min clearance period was started 5 min after beginning the radioactive infusion. Blood samples were drawn between 8–12 min of infusion. Then, it was replaced by one of isotonic NaCl at equal rate. After the clearance period was over, the desired change of acid-base balance was induced. After 40–49 min, the whole procedure was repeated. Since PAH secretion is depressed by \( \alpha \)-ketoglutaric acid (2), it is conceivable that measurements of RBF and RPF in the conditions of these experiments, using PAH, are not valid. Thus, in all cases RPF was also determined using the creatinine concentrations in arterial and renal venous plasma and in urine, according to the Wolf equation (14). The values for RPF obtained using both methods did not differ (\( P > .05 \)), a fact predictable from the very low doses of \( \alpha \)-ketoglutaric acid infused.

Figure 2 shows the results of 10 experiments performed as described above on 10 different dogs. In each, the results of the two clearance periods are arranged so that the left bar corresponds to measurements at the lowest plasma Pco\(_2\) levels of each pair, and with one exception (experiment 21), at the highest blood pH level of each pair. In the first three experiments, the initial determinations (left bars) were during spontaneous respiratory acidosis in experiment 15, mild chronic metabolic acidosis in experiment 13, and acute metabolic alkalosis induced by sodium bicarbonate infusion in experiment 9. The infusion was discontinued after the initial period. Breathing CO\(_2\) (right bars) enhanced the rates of net release of glucose-\(^{14}\text{C}\).

In experiments 10, 11, and 14 the initial measurements (right bars) were during chronic metabolic acidosis in experiments 10 and 14 and in combined respiratory acidosis and mild metabolic acidosis in experiment 11. Mechanical hyperventilation (left bars) depressed the rates of release of glucose-\(^{14}\text{C}\). Thus, in all the first six experiments, the highest rates occurred during the period in which plasma Pco\(_2\) was higher and blood pH lower, irrespective of the chronological sequence of events. The mean rate of net release of \(^{14}\text{C}\)-labeled glucose at the lower plasma Pco\(_2\) level in these six experiments is 36% of the maximal obtained at the higher Pco\(_2\) level. This difference is statistically significant (\( P < .005 \)).

In experiments 12, 21, and 22, metabolic changes of acid-base balance were induced by acid infusion in experiment 22 (left bar), and by bicarbonate infusion in experiments 21 (right bar) and 12 (left bar). Due to the respiratory compensations that occurred, namely, hyperventilation in experiment 22 and hyperventilation in experiment 21, it is difficult to interpret the results. Bicarbonate infusion

### Table 2. Renal glucose production determined simultaneously using glucose oxidase method, with determinations of rates of net renal release of glucose \(^{14}\text{C}\) during renal intra arterial infusion of \(^{14}\text{C}\)-labeled \( \alpha \)-ketoglutaric acid, malic acid, or glutamine in 17 dogs in normal and altered acid-base balance

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of Dogs</th>
<th>No. of Observations</th>
<th>RPF, ml/min</th>
<th>Urine Flow, ml/min</th>
<th>Plasma Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>4</td>
<td>6</td>
<td>180 ± 15</td>
<td>1.40 ± 0.45</td>
<td>+0.03 ± 0.08</td>
</tr>
<tr>
<td>Chronic metabolic acidosis</td>
<td>5</td>
<td>6</td>
<td>189 ± 25</td>
<td>0.51 ± 0.2b</td>
<td>+0.20 ± 0.16</td>
</tr>
<tr>
<td>Acute metabolic acidosis</td>
<td>3</td>
<td>3</td>
<td>180 ± 23</td>
<td>4.84 ± 2.50</td>
<td>-0.07 ± 0.05</td>
</tr>
<tr>
<td>Acute respiratory acidosis</td>
<td>6</td>
<td>6</td>
<td>147 ± 18</td>
<td>0.75 ± 0.38</td>
<td>0.28 ± 0.19</td>
</tr>
<tr>
<td>Acute respiratory alkalosis</td>
<td>4</td>
<td>4</td>
<td>132 ± 22</td>
<td>0.80 ± 0.86</td>
<td>0.08 ± 0.13</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Numbers given in parentheses indicate range. RV A = renal venous arterial glucose concentration, rv-a - counts/min per min in renal venous outflow - counts/min per min in arterial inflow. Glucose concentration and SA in renal venous plasma have been corrected for abstraction of urine water. The mean values for renal plasma flow (RPF), urine flow rates, and RV-A in each condition are not significantly different from the corresponding in the normal state (\( P > .05 \)). The differences in mean counts/min per min among the conditions listed are not statistically significant (\( P > .05 \)).
depicted the rates of release of glucose-\textsuperscript{14}C in experiment 12 (left bar) but had little effect in experiment 21 (right bar). The increase of plasma \textit{Pco}_\textsubscript{2} in this case was greater than in any of the others, but the rates were not stimulated to any appreciable extent. Thus it seems that bicarbonate infusion prevented stimulation by \textit{CO}_\textsubscript{2} retention and that \textit{CO}_\textsubscript{2} retention prevented depression by bicarbonate infusion. In experiment 22, acid infusion (left bar), which lowered plasma bicarbonate from 19.8 to 12.2 mm Hg, caused hyperventilation and decrease of plasma \textit{Pco}_\textsubscript{2} from 35.5 to 20.5 mm Hg. The depression of release of glucose-\textsuperscript{14}C observed, resembles that in experiment 10, in which mechanical hyperventilation produced similar lowering of plasma \textit{Pco}_\textsubscript{2}.

Experiment 23 is the only case in which highest rates occurred at the more alkaline blood pH and at the lower plasma \textit{Pco}_\textsubscript{2}. Following the initial measurements (right bar), mechanical hyperventilation lowered plasma \textit{Pco}_\textsubscript{2} from 53.0 to 10.0 mm Hg. In contrast with the other experiments in which lowering of \textit{Pco}_\textsubscript{2} was associated with depression of release of glucose-\textsuperscript{14}C, in this case marked enhancement occurred. We have no explanations for this finding.

**DISCUSSION**

The results obtained in this study on anesthetized dogs in various states of acid-base balance indicate that, on an average, renal extraction or production of glucose is nil (Table 1), although in isolated instances, either may amount to considerable proportions. These results are hardly surprising since glucose was analyzed using the standard glucose oxidase method, with a sensitivity of 1 mg/100 ml and with a variability of about 4\% of the mean in replicate measurements in a single sample (5).

Although in normal dogs we found renal glucose production of 0.1 mm or 1.8 mg/100 ml in plasma (Table 1), in agreement with that reported by McCann and Jude (9) for unanesthetized dogs, of 1.9 mg/100 ml in plasma, the value has no statistical significance in our study due to the variability in the dog population. McCann and Jude's (9) finding of renal glucose production of 0.97 mg/100 ml in blood, is also higher than that of Steiner et al. (13) for anesthetized normal dogs of 0.09 mg/100 ml in blood, even though they used a modified procedure to increase sensitivity. The latter authors also report increased production in chronic metabolic acidosis to 0.34 mg/100 ml in blood, a finding that we failed to confirm in plasma using the standard method. If renal production of glucose increases in chronic acidosis, or during administration of precursors such as \textit{a}-ketoglutarate, the increment in rates is less than about 16 \textmu moles/min from the considerations that follow. If one assumes RPF of about 200 ml/min, and increased renal glucose production to raise renal venous concentration by 1.5 mg/100 ml or 0.08 mm, i.e., levels detectable by chemical means, net rates of glucose release correspond to about 16 \textmu moles/min.

The appearance of glucose-\textsuperscript{14}C in renal venous blood during intra-arterial infusion of \textsuperscript{14}C-labeled precursors (Fig. 1) indicates that the kidney is synthesizing and releasing newly formed glucose from its pool of precursors, since the exogenous doses given were tracer amounts. Inferentially, the rates of renal gluconeogenesis must be very small, since net production is not detectable (Table 2).
Even in the few cases in which net renal extraction of glucose occurred, 14C-labeled glucose appeared in renal venous blood. To exemplify, in one dog, (experiment 9 in Fig. 2), when RV-A values were -0.3 mm, corresponding to extraction of 47 μmoles/min during acute metabolic alkalosis, renal release of 14C-glucose was 9,759 counts/min per min. During subsequent CO2 breathing, the latter increased to 10,453 counts/min per min, despite increased extraction of 146 μmoles/min. It thus seems that renal gluconeogenesis is a continuous process occurring even when the kidney extracts glucose in net amounts from arterial blood.

In cortical slices of dog kidney, Goorno et al. (4) showed enhancement of gluconeogenesis by chronic metabolic acidosis, and depression by chronic metabolic alkalosis. In rat kidney slices, Kamm et al. (6) showed stimulation of glucose production by increasing Pco2 levels in the incubating media or by chronic respiratory acidosis, and depression by decreasing external Pco2 levels. The results of the studies shown in Fig. 2, in which the rates of renal release of glucose-14C were determined in two states of acid-base balance in each dog, confirm in vivo stimulation of gluconeogenesis by raising plasma Pco2 (experiments 9, 13, 15), and depression by lowering plasma Pco2 (experiments 10, 11, 14).

From the studies in vitro (4) mentioned above, decreased rates during bicarbonate infusion and increased rates during acid infusion might have been predicted. However, only in one of two cases (experiment 12) did bicarbonate infusion depress the rates. In the other (experiment 21), slight enhancement occurred. Furthermore, acid infusion (experiment 22) decreased, not increased, the rates. We attribute these results to the concomitant respiratory compensations: hypoventilation and CO2 retention during bicarbonate infusion in experiment 21 conceivably prevented depression of gluconeogenesis; hyperventilation and CO2 loss in experiment 22 not only prevented acidosis and the expected rise in release of 14C-labeled glucose, but depressed it. These results suggest that renal gluconeogenesis is probably affected by more than one factor in acid-base balance.

To summarize, in the group of dogs studied, we did not find by chemical means net renal glucose extraction nor production, although each may be considerable in a few individual dogs. By isotopic means, however, renal gluconeogenesis was detected in 92% of cases. This indicates that renal gluconeogenesis is a continuous process occurring even during net renal extraction of glucose from arterial blood. Changes in acid-base balance may affect renal gluconeogenesis, as suggested by determinations of the rates of release of 14C-labeled glucose. However, their influence on renal gluconeogenesis, if existent, is below the limits of sensitivity of the standard glucose oxidase method.

REFERENCES