The renal conversion of glutamine to glucose and its oxidation to CO₂ were compared in dogs in chronic metabolic acidosis and alkalosis. These studies were performed at normal endogenous levels of glutamine utilizing glutamine-³⁴C (uniformly labeled) as a tracer. It was observed in five experiments in acidosis that mean renal extraction of glutamine by one kidney amounted to 27.7 µmoles/min. Of this quantity, 5.34 µmoles/min was converted to glucose, and 17.5 µmoles/min was oxidized to CO₂. Acidotic animals excreted an average of 41 µmoles/min of ammonia in the urine formed by one kidney. In contrast, in five experiments in alkalosis, mean renal extraction of glutamine amounted to 8.04 µmoles/min. Of this quantity, 0.92 µmole/min was converted to glucose, and 4.99 µmoles/min was oxidized to CO₂. Alkalotic animals excreted an average of 3.23 µmoles/min of ammonia in the urine. We conclude that renal gluconeogenesis is not rate limiting for the production and excretion of ammonia in either acidosis or alkalosis. Since 40% of total CO₂ production is derived from oxidation of glutamine by the acidotic kidney and 14% by the alkalotic kidney, it is apparent that renal energy sources change with acid-base state and that glutamine constitutes a major metabolic fuel in acidosis.
Metabolism of Glutamine by the Intact Functioning Kidney of the Dog

STUDIES IN METABOLIC ACIDOSIS AND ALKALOSIS

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A B S T R A C T The renal conversion of glutamine to glucose and its oxidation to CO₂ were compared in dogs in chronic metabolic acidosis and alkalosis. These studies were performed at normal endogenous levels of glutamine utilizing glutamine-¹⁴C (uniformly labeled) as a tracer. It was observed in five experiments in acidosis that mean renal extraction of glutamine by one kidney amounted to 27.7 μmoles/min. Of this quantity, 5.34 μmoles/min was converted to glucose, and 17.5 μmoles/min was oxidized to CO₂. Acidotic animals excreted an average of 41 μmoles/min of ammonia in the urine formed by one kidney. In contrast, in five experiments in alkalosis, mean renal extraction of glutamine amounted to 8.04 μmoles/min. Of this quantity, 0.92 μmole/min was converted to glucose, and 4.99 μmoles/min was oxidized to CO₂. Alkalotic animals excreted an average of 3.23 μmoles/min of ammonia in the urine. We conclude that renal gluconeogenesis is not rate limiting for the production and excretion of ammonia in either acidosis or alkalosis. Since 40% of total CO₂ production is derived from oxidation of glutamine by the acidotic kidney and 14% by the alkalotic kidney, it is apparent that renal energy sources change with acid-base state and that glutamine constitutes a major metabolic fuel in acidosis.

INTRODUCTION

Glutamine is the most abundant free amino acid of the plasma of man (1), dog (2), and rat. It is extracted by the kidneys of these forms in acidosis in far greater amounts than any other amino acid and constitutes the major precursor of the ammonia which buffers hydrogen ions secreted into the urine (3).

Ammonia is produced from glutamine (a neutral compound) in sequential reactions involving deamidation by glutaminase I to form glutamic acid and by oxidative deamination of the resulting glutamic acid by glutamic dehydrogenase to form α-ketoglutaric acid. For the base, NH₄⁺ to be available to buffer urinary hydrogen ions, the α-ketoglutaric acid must be oxidized to CO₂ and eliminated by the lungs or converted to some neutral product such as glucose. Thus 2 moles of ammonia and 1 mole of α-ketoglutaric acid (a relatively strong dibasic acid) are produced from each mole of glutamine degraded. Only if the α-ketoglutaric acid disappears as an acid, either by conversion to glucose or by oxidation to CO₂, does the ammonia become available as base.

One of the theories of control of renal ammonia production which is currently most popular relates it to renal glucose production. According to this concept (4–6), the key rate-limiting enzyme of the gluconeogenic pathway, phosphoenolpyruvate carboxykinase, is induced in acidosis. This enzyme is required for the conversion of oxaloacetate to phosphoenolpyruvate. As a consequence of an increase in enzyme concentration within tubular cells, the concentrations of intermediates of the Krebs' cycle back to and including α-ketoglutaric acid are reduced. This has the effect of promoting the conversion of glutamic acid to α-ketoglutaric acid, lowering the renal concentration of glutamic acid. A reduction of renal glutamic acid deinhibits glutaminase I, thus increasing conversion of glutamine to glutamic acid (7). Since the conversion both of glutamine to glutamic acid and of glutamic acid to α-ketoglutaric acid liberates ammonia, the production of ammonia increases in proportion to the enhanced conversion of glutamine to glucose. An inference which is implied rather than
explicitly stated in most descriptions of this sequence is that gluconeogenesis is rate limiting for the production of ammonia by the kidney. Of this some doubt has been expressed (8-11).

Since the extraction of glutamine by the kidney in acidosis far exceeds that which occurs in alkalosis (2, 12), it follows in acidosis that the oxidation of \( \alpha \)-ketoglutaric acid to CO\(_2\) and/or its conversion to glucose or other neutral products must exceed the rates in alkalosis. Thus it is possible that the metabolic fuel of the kidney is different in acidosis and in alkalosis (8, 13).

The present paper is directed to these two questions: (a) is the production of glucose rate limiting for the production of ammonia from glutamine by the kidney, and (b) is the metabolic fuel of the kidney altered in acidosis in comparison with alkalosis? Our results indicate that gluconeogenesis is not rate limiting in renal production of ammonia from glutamine and that a much greater proportion of the metabolic fuel of the kidney is represented by glutamine in acidosis than in alkalosis.

For such experiments to have any physiological meaning, two conditions must be met. They must be performed on the intact functioning kidney in vivo, not on slices or homogenates. They must be performed at normal endogenous blood concentrations of glutamine. This latter factor is especially significant, for most of the studies of renal gluconeogenesis have been performed on cortical slices incubated in artificial media containing glutamine as the single substrate at a concentration 10-20 times normal. Those studies performed on the intact kidney in vivo at normal endogenous levels of glutamine have for the most part shown variable and relatively insignificant rates of gluconeogenesis (10, 11, 14).

METHODS

Our experiments have been performed on 10 mongrel dogs, 5 in chronic metabolic acidosis, 5 in chronic plus acute metabolic alkalosis, mild in degree. These dogs, which weighed between 18 and 26 kg, were lightly anesthetized with intravenous pentobarbital, supplemented as needed. Chronic acidosis was induced by incorporating 10-15 g of NH\(_4\)Cl in the food for 3 or 4 days before an experiment. Chronic alkalosis was induced by incorporating 10-20 g of NaHCO\(_3\) in the food for a similar period of time, and then on the day of the experiment adding a small amount of bicarbonate to the infusion to insure the formation of alkaline urine.

The principle of our experiments is the following: \( \beta \)-aminohippurate (PAH) and creatinine (Cr) were infused intravenously for the measurement of renal blood flow (RBF) and glomerular filtration rate (GFR), respectively. GFR was used for no purpose other than to assess normalcy of renal function. Another infusion containing a total of 83 \( \mu\)Ci of glutamine-\(^{14}C\) uniformly labeled (UL) of high specific activity was given as a prime and thereafter at a constant rate for 20 min before and through the 45 min of a three period clearance experiment. No cold glutamine was given. Blood glutaminse thus represent normal endogenous levels. Since the excretion of glutamine is negligible even at greatly elevated plasma levels (15), glutamine and radioactivity of glutamine in urine were not measured. From arterial and renal venous blood glutamine concentrations and renal arterial and renal venous blood flows, the extraction of glutamine by the kidney was calculated in terms of micromoles/minute. From counts per minute/minute of glutamine extracted, calculated in a similar fashion, the specific activity was calculated. Dividing counts per minute/minute of any product added to renal venous blood by the specific activity of the glutamine extracted yields micromoles/minute of product produced by the kidney. The products studied included glucose, \(^{14}C\)-CO\(_2\), and glutamate, \(^{14}C\). Total \( CO_2 \) produced by the kidney was measured by the Van Slyke manometric technique on whole blood (16). Dividing micromoles of \( CO_2 \) derived from glutamine by total \( CO_2 \) produced by the kidney yielded the proportion which originated in glutamine.

The experimental technique included catheterization of the ureters separately through a low abdominal incision, introduction of a retention needle into a femoral artery, and the insertion of a radiopaque catheter into the right renal vein under fluoroscopic guidance. Other veins were catheterized for administration of the two intravenous infusions. Arterial and renal venous blood samples were collected over timed intervals of 2 min at the middle of each 15 min urine collection period. All analyses except those of creatinine were performed on whole blood. Creatinine was analyzed in plasma. All data are presented for one kidney only, the right one.
Renal arterial blood inflow to the kidney was calculated from clearance and extraction of PAH by the Wolf equation (17). Renal venous blood outflow from the kidney has been estimated as arterial inflow minus urine flow. The product of concentration in micromoles/milliliter or counts per minute/milliliter of any component of arterial blood and the rate of arterial inflow is equal to the quantity entering the kidney each minute. Similarly micromoles/milliliter or counts per minute/milliliter of any component of renal venous blood multiplied by renal venous outflow is equal to the quantity leaving the kidney each minute. The difference between the quantities entering and leaving constitutes the quantity extracted by or produced in the kidney each minute.

Glutamine and other neutral and acidic amino acids were separated by column chromatography of picric acid filtrates of whole blood by the Moore and Stein method (18) and analyzed in a Phoenix automatic amino acid analyzer (Phoenix Precision Instrument Div., Virtis Co., Gardiner, N. Y.). A Nuclear-Chicago liquid flow scintillation analyzer was interposed between the column and the ninhydrin portion of the amino acid analyzer to quantify \(^{14}C\) activity of glutamine and certain of its metabolites. Although considerable activity was observed in the early portions of the chromatogram, significant activity in the neutral and acidic amino acid portions of the chromatogram was observed only in glutamine and glutamate. Our evidence that the first two peaks, at immediately following column volume, represent glucose-\(^{14}C\) will be presented under Results. Although graphic traces of \(^{14}C\) activity were obtained from a recording rate meter in many instances, counts per minute were uniformly quantified from a rapid print-out record of successive 2-min counts corrected for background activity during each chromatogram. These analyses of concentrations of amino acids/milliliter and counts per minute/milliliter in the amino acid peaks were the only ones not performed in duplicate. To perform these analyses once on single samples required a minimum of seven days, assuming that everything went well. \(^{14}C\)O\(_2\) activity in whole blood was measured in a Packard liquid scintillation counter by a modification of the method of Passmann, Radin, and Cooper (19). In order to convert counts per minute in the Packard liquid scintillation system to that in the Nuclear-Chicago liquid flow scintillation system, portions of a mixture of cold and glutamate-\(^{14}C\) U.L were chromatographed and counted in the flow system. Other portions were treated with an excess of ninhydrin to liberate the \(\alpha\)-carboxyl CO\(_2\) and analyzed by the Passmann et al. (19) method in the Packard counter. On the assumption that one-fifth of the carbons of glutamate appeared as CO\(_2\) when treated with an excess of ninhydrin, a conversion factor relating the two systems was calculated. Glutamine-\(^{14}C\) was not used because liberation of \(\alpha\)-carboxyl carbon as CO\(_2\) by ninhydrin is incomplete.

RESULTS

Quantification of glucose. Early in the course of our experiments we observed that 100 mg/100 ml glucose to which a trace of glucose-\(^{14}C\) had been added, when chromatographed, yielded a sharp peak of counts coincident with a peak we had observed just following column volume in an experiment in which we had administered glutamine-\(^{14}C\). Fig. 1 illustrates this peak, identified in the filtrate of arterial blood by the addition of a trace of glucose-\(^{14}C\) just before chromatography. This figure also illustrates the extraction of glutamine-\(^{14}C\) from arterial blood as well as the addition of glucose-\(^{14}C\), glutamate-\(^{14}C\), and other \(^{14}C\) metabolites of glutamine to renal venous blood by the intact functioning kidney of the dog. When we first observed this glucose-\(^{14}C\) peak, we did not consider that the preceding peak at column volume might represent a glucose oxidation product. Fig. 2 illustrates the chromatograms of three samples compounded in vitro as follows: the top one containing glucose-\(^{14}C\) only, the middle one containing glucose-\(^{14}C\) in 1\% picric acid, and the bottom one containing glucose-\(^{14}C\) in 1\% picric acid to which 10 ml of whole blood had been added. The picric acid was removed by passing the filtrates through a Dowex 2-10x column. The blood filtrate was subsequently treated with sodium sulfite to remove interference by glutathione. This was the standard procedure to which all blood filtrates were normally subjected. It is obvious that \(^{14}C\) derived from glucose appears in both peaks in this experiment in vitro. It is possible that the peak at column volume is the peak of an oxidation product of glucose formed to a slight extent in picric acid and to a greater extent in the sulfite-treated blood filtrates in vivo experiments.

A question of real significance is, do we lose any counts during the procedures involved in the preparation of blood filtrates for chromatography? Table I indicates that we do not. Thus essentially all the counts, which were present in the second peak when glucose-\(^{14}C\)
TABLE I

Recovery of Counts in Glucose Peaks of 24 Samples Prepared as Described in Fig. 2

<table>
<thead>
<tr>
<th>Sample chromatographed</th>
<th>No. of samples</th>
<th>cpm found per ml in A + B peaks</th>
<th>Per cent of Glucose-14C recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous glucose-14C</td>
<td>8</td>
<td>7831 ± 58.9 SE</td>
<td></td>
</tr>
<tr>
<td>Glucose-14C + picric acid</td>
<td>8</td>
<td>7752 ± 41.7 SE</td>
<td>99.0</td>
</tr>
<tr>
<td>Glucose-14C + picric acid + blood + sulfate</td>
<td>8</td>
<td>7743 ± 65.9 SE</td>
<td>98.9</td>
</tr>
</tbody>
</table>

was chromatographed alone without further treatment, were variably divided between the first and second peaks in the other samples. No statistical difference in the sums of the counts existed among samples treated in these diverse manners. On the supposition that the first peak represents gluconic acid, we chromatographed a saline solution containing a small amount of D-glucuronate-14C. Counts appeared as a single sharp peak in the second position, namely where true glucose appeared. Since the first peak is an artifact dependent on our methods of preparation of blood filtrates, we do not feel that identification of its nature is imperative.

Unfortunately these several facts do not prove that the two peaks in our experimental samples represent only glucose. There is nothing very specific about peaks which occur at column volume and just subsequent to column volume. In fact we have seen identical peaks in experiments in which glycine has been infused to measure synthesis of serine (20). However, both of these latter amino acids are potential sources of glucose; hence this fact provides no contradiction of our view. We shall therefore assume that these peaks represent glucose and the products of its oxidation formed during treatment of blood with picric acid and sulfate. However we admit that we have no absolute proof.

Calculation of specific activity of glutamine. A second major problem arose early in our experiments. We observed that the specific activity of the glutamine of arterial blood decreased as it passed through the kidney to become renal venous blood. At first glance this seems to indicate that glutamine-14C is preferentially extracted by the kidney in comparison with cold glutamine or that the kidney produces cold glutamine and adds it to renal venous blood at some site distal to the point where it is extracted. We could accept neither view, not the first, because it negates the use of 14C in any tracer study and not the second, because it negates the well established fact that the kidney of the dog cannot synthesise glutamine (21, 22). Table II demonstrates that this phenomenon is the consequence of still another factor, namely the very slow rate of attainment of equilibrium of glutamine across the membranes of the red cell mass.

Unfortunately these several facts do not prove that the two peaks in our experimental samples represent only glucose. There is nothing very specific about peaks which occur at column volume and just subsequent to column volume. In fact we have seen identical peaks in experiments in which glycine has been infused to measure synthesis of serine (20). However, both of these latter amino acids are potential sources of glucose; hence this fact provides no contradiction of our view. We shall therefore assume that these peaks represent glucose and the products of its oxidation formed during treatment of blood with picric acid and sulfate. However we admit that we have no absolute proof.

![Figure 3 Comparisons of mean values of arterial blood CO2 concentrations, rates of ammonia excretion (one kidney), and arterial blood glutamine concentrations in five dogs in chronic metabolic acidosis and five dogs in chronic metabolic alkalosis.](image)

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the renal vein. This necessitated analysis of whole blood and calculation of specific activity on the basis of micro-
moles/minute and counts per minute/minute of glutamine extracted, a procedure which we had fortunately and
arbitrarily adopted from the start.

**Acid-base parameters, significant and nonsignificant.**

Fig. 3 illustrates the mean of the CO₂ contents of 15
samples of arterial blood (5 experiments) in acidosis
and a similar number in alkalosis. The mean in acidosis
was 12.5 μmoles/ml, and the range extended from 13.9
to 9.5 μmoles/ml. The mean in alkalosis was 25.0 μmoles/
ml, and the range extended from 30.7 to 21.3 μmoles/ml.
The difference between the means was highly significant,
\( P < 0.0005 \).

The mean rate of urinary excretion of ammonia by
one kidney was 41.4 μmoles/min in 15 periods in acidosis
and 3.23 μmoles/min in a similar number of periods in
alkalosis. The difference between the means again was
highly significant, \( P < 0.0005 \).

In contrast, the means of the concentrations of gluta-
mine in arterial blood scarcely differed in the two series.
One dog in acidosis had a much higher level of glutama-
tine than the other four. Eliminating this animal from
the series reduced the difference between the means of
acidotic and alkalotic animals to a nonsignificant level,
\( P = 0.10 \). This dog was included in all other calcula-
tions (experiment 3, acidosis) and did not differ in any
consistent fashion from the remaining four. This con-
irms the generally accepted view that the blood concen-
tration of the major precursor of renal ammonia, namely
glutamine, is not different in acidosis and alkalosis de-
spite the marked difference in ammonia excretion.

Columns 2 and 3 of Tables III and IV demonstrate
that the filtration rates and renal arterial blood infows
also did not differ in these two acid-base states. There-
fore the fact that the glutamine extracted by the kidney
(columns 4 of Tables III and IV) is so much greater
in acidosis (27.7 μmoles/min) than in alkalosis (8.04
μmoles/min) gains added significance. It strongly sug-
gets that some change in the interior milieu of tubular
cells accounts for the increased renal metabolism of glutamine in acidosis.

**Renal gluconeogenesis from glutamine in acidosis and
alkalosis.** Tables III and IV and Fig. 4 demonstrate
that although the formation of glucose from glutamate
by the kidney in acidosis exceeds that in alkalosis, it
is in no sense rate limiting for the production of am-
onia. It is important to distinguish between micromoles
of glutamine converted to glucose and micromoles of glu-
cose formed from glutamine. The latter is five-sixths of
the former as defined by our means of calculation. Thus

![Figure 4](image_url)

**Figure 4** Comparisons of mean values of glutamine ex-
tracted, glutamine converted to glucose, glutamine used in
other reactions, and ammonia excreted by five dogs in chronic
metabolic acidosis and by five dogs in chronic metabolic
alkalosis. All data from one kidney.

**Metabolism of Glutamine by the Intact Functioning Kidney of the Dog** 561
### Table III

Renal Metabolism of Glutamine by the Kidney of the Dog in Chronic Metabolic Acidosis

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>GFR (ml/min)</th>
<th>Renal Arterial inflow</th>
<th>Glutamine extracted (μmoles/min)</th>
<th>Glutamine converted to glucose (μmoles/min)</th>
<th>Glutamine converted to CO₂ (μmoles/min)</th>
<th>Total CO₂ produced (μmoles/min)</th>
<th>Glutamine Converted to CO₂ (μmoles/min)</th>
<th>Per cent of total CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>23.8</td>
<td>135</td>
<td>15.6</td>
<td>292,643 (18,795)</td>
<td>50.8</td>
<td>158</td>
<td>154,313</td>
<td>8.21</td>
</tr>
<tr>
<td>25.0</td>
<td>152</td>
<td>31.3</td>
<td>478,912 (15,286)</td>
<td>67.1</td>
<td>4.39</td>
<td>137</td>
<td>225,500</td>
<td>14.75</td>
</tr>
<tr>
<td>25.1</td>
<td>149</td>
<td>28.0</td>
<td>549,400 (19,614)</td>
<td>55.075</td>
<td>2.81</td>
<td>122</td>
<td>246,069</td>
<td>12.54</td>
</tr>
<tr>
<td>4</td>
<td>24.5</td>
<td>328</td>
<td>30.3</td>
<td>505,314 (16,683)</td>
<td>94.88</td>
<td>4.69</td>
<td>269,025</td>
<td>16.13</td>
</tr>
<tr>
<td>26.9</td>
<td>285</td>
<td>27.8</td>
<td>499,008 (17,959)</td>
<td>67.727</td>
<td>3.77</td>
<td>130</td>
<td>338,317</td>
<td>18.84</td>
</tr>
<tr>
<td>25.1</td>
<td>226</td>
<td>24.6</td>
<td>452,716 (18,373)</td>
<td>108.328</td>
<td>5.90</td>
<td>220</td>
<td>351,443</td>
<td>19.13</td>
</tr>
<tr>
<td>30.4</td>
<td>343</td>
<td>31.3</td>
<td>458,498 (14,648)</td>
<td>44.108</td>
<td>3.01</td>
<td>171</td>
<td>267,586</td>
<td>18.3</td>
</tr>
<tr>
<td>37.5</td>
<td>315</td>
<td>20.5</td>
<td>445,335 (21,692)</td>
<td>96.858</td>
<td>4.46</td>
<td>246</td>
<td>341,756</td>
<td>23.1</td>
</tr>
<tr>
<td>38.0</td>
<td>274</td>
<td>23.4</td>
<td>491,385 (20,981)</td>
<td>124.090</td>
<td>5.91</td>
<td>246</td>
<td>341,756</td>
<td>23.1</td>
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<tr>
<td>36.0</td>
<td>317</td>
<td>27.4</td>
<td>575,681 (21,041)</td>
<td>176.649</td>
<td>8.40</td>
<td>245</td>
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<td>14.2</td>
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<td>39.3</td>
<td>305</td>
<td>27.1</td>
<td>559,114 (20,601)</td>
<td>198.115</td>
<td>9.62</td>
<td>287</td>
<td>367,643</td>
<td>17.9</td>
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<td>32.2</td>
<td>274</td>
<td>18.5</td>
<td>545,338 (29,510)</td>
<td>300.190</td>
<td>10.17</td>
<td>196</td>
<td>415,874</td>
<td>14.1</td>
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<td>40.7</td>
<td>475</td>
<td>38.8</td>
<td>658,483 (16,967)</td>
<td>82.497</td>
<td>4.86</td>
<td>392</td>
<td>442,049</td>
<td>26.1</td>
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<td>41.5</td>
<td>427</td>
<td>35.7</td>
<td>672,308 (18,819)</td>
<td>56.788</td>
<td>3.02</td>
<td>333</td>
<td>509,737</td>
<td>34.1</td>
</tr>
<tr>
<td>41.1</td>
<td>400</td>
<td>35.5</td>
<td>723,621 (20,407)</td>
<td>108.838</td>
<td>5.33</td>
<td>327</td>
<td>482,715</td>
<td>23.7</td>
</tr>
<tr>
<td>Mean</td>
<td>32.5</td>
<td>294</td>
<td>19,425</td>
<td>5.34</td>
<td>4.45</td>
<td>230</td>
<td>17.5</td>
<td>87.5</td>
</tr>
<tr>
<td>±SE</td>
<td>1.80</td>
<td>25.7</td>
<td>0.62</td>
<td>0.52</td>
<td>1.04</td>
<td>0.26</td>
<td>1.31</td>
<td>6.53</td>
</tr>
</tbody>
</table>

Counts per minute/minute of glucose = counts per minute/micro mole of uniformly labeled glutamine extracted defines the glucose as uniformly labeled. This is independent of the mechanism of conversion of glutamine to glucose, which has been shown to be:

2 Glutamine = Glucose + 4CO₂ + 4NH₃

10C  6C  4C

In acidosis, a mean of 5.34 μmoles/min of glutamine was converted to glucose; 4.45 μmoles/min of glucose was formed. In alkalosis, corresponding values were 0.92 and 0.78 μmoles/min. In acidosis, glucose was added to the renal vein in all 15 periods of 5 experiments. In alkalosis, in four periods glucose was extracted. Whether this indicates the limits of error of the method or is true is unknown. If true, it indicates that glucogenesis varies from moment to moment especially in alkalosis, a fact claimed by others (10, 11). It must be emphasized that our method traces only glutamine into glucose. It does not measure total glucose production, excluding that derived from lactate, pyruvate, and other non-glutamine-derived precursors. Indeed it is just this fact which enables us to say that the conversion of glutamine to glucose is not rate limiting for the production of ammonia.

The equation at the start of the second paragraph above enables one to calculate the CO₂ produced in the conversion of glutamine to glucose as well as the CO₂ produced from glutamine in other reactions.

(a) CO₂ from conversion of glutamine to glucose = micromoles glutamine converted × 5/4.10.

(b) CO₂ produced from glutamine oxidized in other reactions = total CO₂ from glutamine — CO₂ from conversion of glutamine to glucose.

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TABLE IV
Renal Metabolism of Glutamine by the Kidney of the Dog in Chronic Metabolic Alkalosis

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>GFR ml/min</th>
<th>Renal Art. inflow</th>
<th>Glutamine extracted μmoles/min</th>
<th>Glutamine converted to glucose μmoles/min</th>
<th>Ammonia excreted μmoles/min</th>
<th>Total CO2 produced μmoles/min</th>
<th>Glutamine converted to CO2 μmoles/min</th>
<th>Per cent of total CO2 of total glutamine produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>26.6</td>
<td>2.24</td>
<td>214.962</td>
<td>23.26</td>
<td>29990</td>
<td>1.29</td>
<td>196</td>
<td>6.91</td>
</tr>
<tr>
<td>24.7</td>
<td>242</td>
<td>8.60</td>
<td>203.660</td>
<td>23.68</td>
<td>38812</td>
<td>1.64</td>
<td>13.7</td>
<td>5.23</td>
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<td>30</td>
<td>292</td>
<td>10.08</td>
<td>134.944</td>
<td>13.37</td>
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<td>1.38</td>
<td>1.15</td>
<td>4.86</td>
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<td>11</td>
<td>29.2</td>
<td>9.05</td>
<td>149.216</td>
<td>16.488</td>
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P refers to the differences in the means in acidosis and in alkalosis. NS means not significant.

Fig. 5 illustrates the moieties of CO2 produced in these diverse ways in acidosis and in alkalosis. These data again emphasize the fact that the conversion of glutamine to glucose is not rate limiting in the production of ammonia. Since in acidosis conversion of glutamine to glucose accounts for only 12% of the total oxidized and in alkalosis for only 7%, it is obvious that the ammonia freed as a consequence of the oxidation of 88% and 93% of the glutamine by other pathways plays a much greater role in production of renal energy as well as ammonia than does gluconeogenesis.

DISCUSSION
A variety of factors have been described as increasing ammonia production and excretion in acidosis. These include an induction of glutaminase I (23), a reduction of concentration of intracellular potassium (24), α-ketoglutarate (25) or glutamate (7), an increase in renal gluconeogenesis (6) and in tubular cell pNH2 (26), and an increase in the ratio of oxidized to reduced pyridine nucleotides (27). In the opinion of the authors, none of these has been shown to be rate limiting for renal production of ammonia.

Since neither blood glutamine concentration nor glomerular filtration rate is different in acidosis and alkalosis, it is obvious that at least that fraction of glutamine filtered and reabsorbed cannot account for differences in extraction and metabolism to form ammonia. Renal arterial inflow is also similar in acidosis and alkalosis. In contrast, net uptake of glutamine does occur across peri-
tubular membranes of tubular cells in acidosis (15, 2).

However, it is doubtful that increased uptake by this
route could alone account for increased production of ammonia. If there is some single rate-limiting factor which controls ammonia production it would seem most logical to ascribe it to an altered passive or active transport of glutamine into tubular mitochondria. Both glutaminase I and glutamic dehydrogenase are intramitochondrial enzymes. If glutamine were excluded from mitochondria in alkalosis but were allowed to enter in acidosis, this fact alone could account for most of the known facts concerning control of ammonia production. Unfortunately there is no evidence for or against this hypothesis. At present it seems most reasonable to view the control of ammonia production as the sum of the partial effects of all the factors listed at the start of the discussion. Such multiple factor control is philosophically more attractive to the authors than single factor control. This is especially true of gluconeogenesis, for the control of ammonia production is a highly significant factor in the renal regulation of acid base balance whereas the renal production of glucose is a very insignificant factor for supplying the glucose needs of the body. This is not meant to imply that renal gluconeogenesis from glutamine does not contribute a small fraction of ammonia production. It obviously does in the dog.

A criticism of our work which may be valid is that it applies only to the dog and only to NHCl acidosis and NaHCO3 alkalosis. It may also be true that gluconeogenesis plays a larger role in control of renal production of ammonia in the rat than it does in the dog. However we feel that differences between these two species are probably more quantitative than qualitative. Indeed we know of no study which truly demonstrates that renal gluconeogenesis from glutamine is rate limiting for the production of ammonia.

A word of explanation concerning extraction of glutamine, excretion of ammonia, and our conclusion that gluconeogenesis is not rate limiting for the production of ammonia may be necessary. From column 4 of Tables III and IV, the mean extractions of glutamine in acidosis were 27.7 μmoles/min and in alkalosis 8.04 μmoles/min. If the full complements of ammonia had been produced from these quantities of glutamine extracted, they would equal 55.4 μmoles/min in acidosis and 16.08 in alkalosis. Instead 41.4 μmoles/min of ammonia was excreted in acidosis, and 3.23 μmoles/min was excreted in alkalosis. The differences from the theoretical, namely 14.0 μmoles/min in acidosis and 12.85 μmoles/min in alkalosis, represent ammonia added to renal venous blood (28) and disappearing as a consequence of transamination with ketoacids within the kidney. These amounts represent a total of 27% of the theoretical in acidosis and 20% in alkalosis. Other studies have shown these latter figures to vary between 25% and 33% in acidosis (3), somewhat less in alkalosis. Had these same quantities of glutamine been utilized solely through the gluconeogenesis pathway, glucose production should have been 13.9 μmoles/min in acidosis (27.7/2) rather than 4.45 μmoles/min, and 4.01 μmoles/min in alkalosis (8.04/2) rather than 0.78 μmoles/min.

Although we believe that the first two peaks of the rate meter trace of 14C activity (Figs. 1 and 2) represent glucose-14C, our argument that renal gluconeogenesis is not rate limiting for production of ammonia does not stand or fall on the verity of this assumption. If none of the activity in these peaks represents glucose, no problem exists. The kidney makes no glucose. If all of the activity represents glucose, the figures stand as presented. If only a portion of these peaks represent glucose, then depending on A-V differences of true glucose (magnitude and direction), the calculated glucose production could either be more or less than that reported. However the maximum amount of glucose which could possibly be produced from glutamine would be less than 37% of the total glutamine extracted. Thus from Table III (column 4), a total of 27.7 μmoles/min was extracted. From column 13 of this Table, 17.5 μmoles/min of glutamine was oxidized to CO2. The difference (namely 10.2 μmoles/min) is the maximum which could be converted to glucose under any circumstance.

One might consider the rate of oxidation of glutamine to CO2 through the Krebs cycle to constitute one or a series of rate-limiting steps in the production of ammonia. The problem of this interpretation is that ammonia is produced before entry of α-ketoglutarate into the Krebs cycle, and therefore the immediate rate-limited steps must include the glutaminase I and the glutamate dehydrogenase reactions. If, as has been proposed (25), the renal tissue concentration of α-ketoglutarate is rate limiting, it must act either at the glutaminase and/or glutamate dehydrogenase steps. On the other hand if tissue concentration of glutamate (7) is rate limiting, it must act at the glutaminase I step. It is possible that some intermediate further along the Krebs cycle than α-ketoglutarate might also be rate limiting in this same manner by acting on the glutaminase or glutamic dehydrogenase steps.

The fact that glutamine constitutes a major source of energy of the kidney in acidosis is reasonable. This follows from the fact that it is necessary for the resulting α-ketoglutaric acid to be converted to a neutral substance (glucose) or to CO2 for the base (NH3) to become available to buffer urinary acid. Since little of the α-ketoglutaric acid derived from glutamine is converted to glucose, it must be oxidized through the Krebs cycle.
Pilkington and O'Donovan (8) have shown that slices of the cortex of the dog kidney made acidotic in vitro convert much more glutamine to CO₂ than to glucose. Gluconeogenesis is thus not rate limiting for utilization of glutamine nor for formation of ammonia in slices. Both Simpson and Sherrard (13) and Pilkington and O'Donovan (8) have demonstrated that acidic slices oxidize glutamine to CO₂ at much faster rates than do alkaliotic slices. The latter investigators have accordingly suggested that glutamine may be a major fuel of respiration in the kidney of the acidic animal.

Gold and Spitzer (29) and Nieth and Schollmeyer (30) maintain that free fatty acids and lactate are major metabolic fuels of the kidney in normal acid-base balance. We have demonstrated in the acidotic kidney that glutamine is probably the equivalent of the more significant of these fuels. It is therefore apparent that the metabolic fuels of the kidney, far from being constant, vary with the acid-base state of the animal.

ACKNOWLEDGMENTS

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REFERENCES


