Metabolism of Glutamine by the Intact Functioning Kidney of the Dog

STUDIES IN METABOLIC ACIDOSIS AND ALKALOSIS

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ABSTRACT The renal conversion of glutamine to glucose and its oxidation to CO_2 were compared in dogs in chronic metabolic acidosis and alkalosis. These studies were performed at normal endogenous levels of glutamine utilizing glutamine-^{14}C (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 CO_2. 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 CO_2. 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 CO_2 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.[1] 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, NH_3, to be available to buffer urinary hydrogen ions, the α-ketoglutaric acid must be oxidized to CO_2 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 CO_2, 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

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[1] Lyon, M. L. Personal communication.

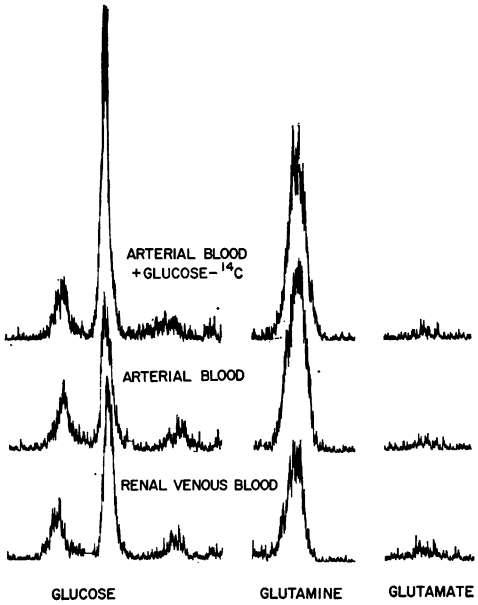

FIGURE 1 Column chromatograms of ^{14}C activity of glucose, glutamine, and glutamate of arterial and renal venous whole blood samples from an experiment. Column effluent passed first through a liquid flow scintillation counter and then returned to the amino acid analyzer. Records are traces of a recording ratemeter. The arterial samples were chromatographed in duplicate. To the upper one, a small amount of glucose-^{14}C was added just before putting it on the column to identify the glucose peak.

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 α-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 conditons 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_4Cl 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: *p*-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 μCi 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 glutamines 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, $^{14}CO_2$, 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 and 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.

$^{14}CO_2$ 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 UL were chromatographed and counted in the flow system. Other portions were treated with an excess of ninhydrin to liberate the α-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 α-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 glutathionine. 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.

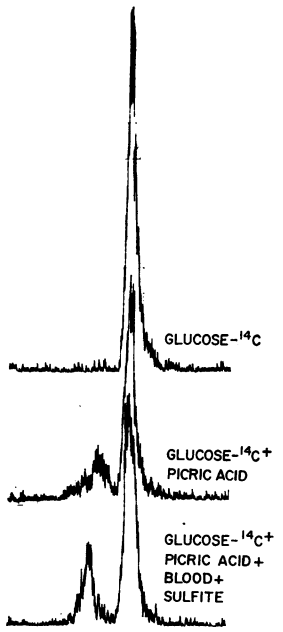

FIGURE 2 Column chromatograms of samples prepared in vitro in three ways, all containing 100 mg/100 ml cold glucose and equal amounts of glucose-^{14}C UL. *Top,* glucose-^{14}C alone; *middle,* glucose-^{14}C plus picric acid; *bottom,* glucose-^{14}C plus picric acid plus whole blood.

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

Sample chromatographed	No. of samples	cpm found per ml in A + B peaks	Per cent of Glucose-^{14}C recovered
Aqueous glucose-^{14}C	8	7831 ±58.9 SE	
Glucose-^{14}C + picric acid	8	7752 ±41.7 SE	99.0
Glucose-^{14}C + picric acid + blood + sulfite	8	7743 ±65.9 SE	98.9

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-gluconate-^{14}C. 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 sulfite. However we admit that we have no absolute proof.

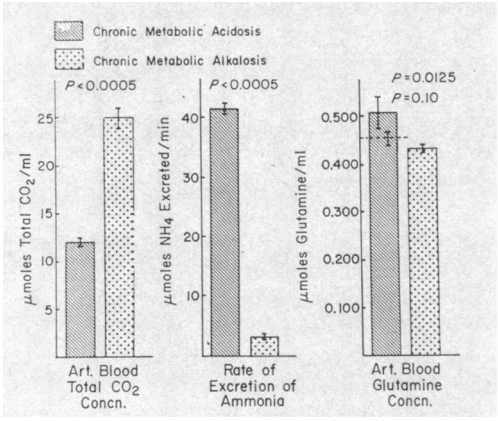

FIGURE 3 Comparisons of mean values of arterial blood CO_2 concentrations, rates of ammonia excretion (one kidney), and arterial blood glutamine concentrations in five dogs in chronic metabolic acidosis and five dogs in chronic metabolic alkalosis.

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-^{14}C 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 ^{14}C in any tracer study and not the second, because it negates the well established fact that the kidney of the dog cannot synthesize 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. In this experiment glutamine and counts per minute in the glutamine peak were measured separately in both whole blood and plasma from each sample of arterial and renal venous blood. The hematocrits of arterial and renal venous blood samples were also measured, and from this data the glutamine and counts per minute in the glutamine peaks of cells and of plasma in 1.0 ml of whole blood were calculated separately. Thus in period 1, the fractions of cells and plasma of 1.0 ml of arterial whole blood were 0.451 and 0.549, respectively, sum = 1.0. The glutamine content of the cell fraction of 1.0 ml of arterial blood was 0.216 μmole and that of the plasma fraction was 0.197. The sum of these two figures, namely 0.413 μmole, is the concentration per milliliter of whole blood. Similar calculations of distribution of micromoles and countes per minute between cells and plasma of all samples of arterial and renal venous blood were made. It is apparent from arterial-renal venous differences that most of the glutamine and counts per minute in glutamine which were extracted by the kidney were derived from the plasma fraction. Little was derived from the cell fraction. The cell fraction of the glutamine of arterial blood exceeded somewhat the plasma fraction whereas the cell fraction of the glutamine of renal venous blood greatly exceeded the plasma fraction. Accordingly, the specific activity of whole blood glutamine decreased as it passed from the artery through renal tissue into

TABLE II

Renal Extraction of Glutamine from Cell Fractions and Plasma Fractions of Whole Blood Perfusing the Kidney of the dog. Explanation of the Cause of the Decrease in Specific Activity of Blood as It Flows through the Kidney

Period	Fraction Art.	Fraction R. Ven.	Phase	Art. Glu-NH_2 μmoles	Art. Glu-NH_2 cpm	R. Ven. Glu-NH_2 μmoles	R. Ven. Glu-NH_2 cpm	Art.-R. Ven. Δμmoles	Art.-R. Ven. Δcpm	Glutamine extracted μmoles/min	Glutamine extracted cpm/min	Calc. SA
				in 1 ml whole blood								
1	0.451	0.458	Cells	0.216	650	0.212	515	0.004	135	27.36	575,681	21,041
	0.549	0.542	Plasma	0.197	3811	0.121	2181	0.076	1630			
2	0.447	0.455	Cells	0.203	822	0.192	859	0.011	−37	27.14	559,114	20,601
	0.553	0.545	Plasma	0.184	4059	0.112	2250	0.072	1809			
3	0.432	0.439	Cells	0.186	941	0.182	884	0.004	57	18.48	545,338	29,510
	0.568	0.561	Plasma	0.174	4454	0.116	2584	0.058	1870			

the renal vein. This necessitated analysis of whole blood and calculation of specific activity on the basis of micromoles/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 CO_2 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 glutamine in arterial blood scarcely differed in the two series. One dog in acidosis had a much higher level of glutamine 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 calculations (experiment 3, acidosis) and did not differ in any consistent fashion from the remaining four. This confirms the generally accepted view that the blood concentration of the major precursor of renal ammonia, namely glutamine, is not different in acidosis and alkalosis despite the marked difference in ammonia excretion.

Columns 2 and 3 of Tables III and IV demonstrate that the filtration rates and renal arterial blood inflows also did not differ in these two acid-base states. Therefore 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 suggests 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 glutamine by the kidney in acidosis exceeds that in alkalosis, it is in no sense rate limiting for the production of ammonia. It is important to distinguish between micromoles of glutamine converted to glucose and micromoles of glucose formed from glutamine. The latter is five-sixths of the former as defined by our means of calculation. Thus

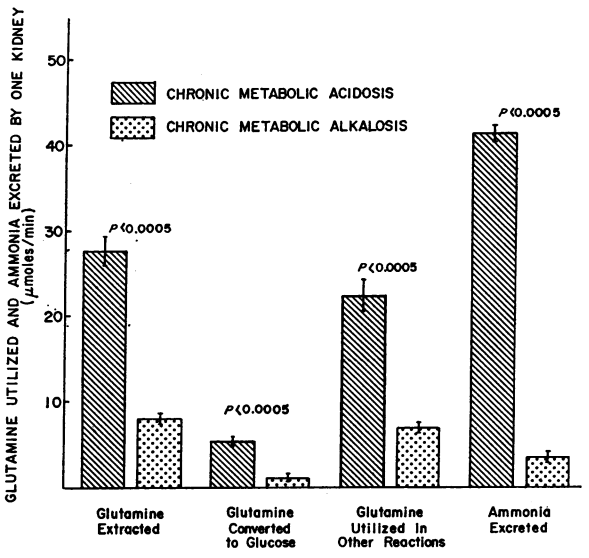

FIGURE 4 Comparisons of mean values of glutamine extracted, glutamine converted to glucose, glutamine used in other reactions, and ammonia excreted by five dogs in chronic metabolic acidosis and by five dogs in chronic metabolic alkalosis. All data from one kidney.

TABLE III

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

Exp. No.	GFR	Renal Art. inflow	Glutamine extracted			Glutamine converted to glucose			Ammonia excreted	Total CO_2 produced	Glutamine Converted to CO_2			Per cent of total
			μmoles/ min	cpm/ min	SA	cpm	Glu-NH_2	Glucose			cpm/ min	Glu-NH_2	CO_2	
	ml/min					*μmoles/min*			*μmoles/min*			*μmoles/min*		
3	23.8	135	15.6	292,643	18,795	50,660	2.70	2.25	35.7	158	154,313	8.21	41.1	26.0
	25.0	152	31.3	478,912	15,286	67,140	4.39	3.66	39.5	137	225,500	14.75	73.8	53.8
	25.1	149	28.0	549,400	19,614	55,075	2.81	2.34	40.2	122	246,069	12.54	62.7	51.4
4	24.5	328	30.3	505,314	16,683	94,882	5.69	4.74	39.0	130	269,025	16.13	80.7	62.0
	26.9	285	27.8	499,608	17,959	67,727	3.77	3.14	40.6	230	338,317	18.84	94.2	41.0
	25.1	226	24.6	452,716	18,373	108,328	5.90	4.92	39.1	220	351,443	19.13	95.7	43.5
	30.4	343	31.3	458,498	14,648	44,108	3.01	2.51	41.0	171	267,586	18.3	91.4	53.4
5	37.5	315	20.5	445,335	21,692	96,858	4.46	3.72	40.6	254	331,995	15.3	76.5	30.1
	38.0	274	23.4	491,385	20,981	124,090	5.91	4.93	40.4	246	341,756	16.3	81.4	33.1
	36.0	317	27.4	575,681	21,041	176,649	8.40	7.00	40.2	245	298,443	14.2	70.9	28.9
7	39.3	305	27.1	559,114	20,601	198,115	9.62	8.02	39.8	287	367,643	17.9	89.3	31.1
	32.2	274	18.5	545,338	29,510	300,190	10.17	8.48	39.6	196	415,874	14.1	70.5	35.9
	40.7	475	38.8	658,483	16,967	82,479	4.86	4.05	51.1	392	442,649	26.1	130.5	33.3
8	41.5	427	35.7	672,388	18,819	56,788	3.02	2.52	49.4	333	509,737	27.1	135.4	40.7
	41.1	400	35.5	723,621	20,407	108,838	5.33	4.44	44.8	327	482,715	23.7	118.3	36.2
Mean	32.5	294	27.7		19,425		5.34	4.45	41.4	230		17.5	87.5	40.0
±SE	1.80	25.7	1.68		904		0.62	0.52	1.04	20.8		1.31	6.53	2.78

counts per minute/minute of glucose ÷ counts per minute/micromole of uniformly labeled glutamine extracted defines the glucose as uniformly labeled. This is independent of the mechanism of conversion of glutamine to glucose, which in abbreviated form has been shown to be:

$$\underset{10C}{2\ \text{Glutamine}} = \underset{6C}{\text{Glucose}} + \underset{4C}{4CO_2} + 4NH_3.$$

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 gluconeogenesis 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 nonglutamine-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.

Alteration of renal energy sources in acidosis as compared with alkalosis. Tables III and IV and Fig. 5 summarize our evidence that oxidation of glutamine to CO_2 is a major source of energy in the acidotic kidney, 40% of the total to be exact. Oxidation of glutamine is a minor source of energy in the alkalotic kidney, 14.5% of the total. In the calculation, counts per minute/minute of CO_2 formed ÷ counts per minute/micromole of uniformly labeled glutamine extracted yields micromoles of glutamine oxidized to CO_2. Each μmole of glutamine oxidized produces 5 μmoles of CO_2. Thus in acidosis, 17.5 μmoles of glutamine oxidized results in the production of 87.5 μmoles of CO_2. In alkalosis the oxidation of 4.99 μmoles of glutamine produces 25 μmoles of CO_2. The total production of CO_2 is probably the least accurate measurement made, not because the Van Slyke and Neill method (16) is inaccurate, but because the V-A differences are so small. This no doubt accounts for the wide variations in CO_2 production from one period to the next. This inaccuracy is even greater in the experiments in alkalosis in which the V-A differences become even smaller in proportion to the excretion of bicarbonate in the urine. In these experiments total CO_2 excreted per minute and $^{14}CO_2$ excreted per minute were both added to the rates of renal production of CO_2 and $14CO_2$, respectively.

The equation at the start of the second paragraph above enables one to calculate the CO_2 produced in the conversion of glutamine to glucose as well as the CO_2 produced from glutamine in other reactions.

(*a*) CO_2 from conversion of glutamine to glucose = micromoles glutamine converted $\times 5 \times 4/10$.

(*b*) CO_2 produced from glutamine oxidized in other reactions = total CO_2 from glutamine − CO_2 from conversion of glutamine to glucose.

TABLE IV

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

Exp. No.	GFR	Renal Art. inflow	Glutamine extracted: µmoles/min	Glutamine extracted: cpm/min	Glutamine extracted: SA	Glutamine converted to glucose: cpm	Glutamine converted to glucose: Glu-NH_2	Glutamine converted to glucose: Glucose	Ammonia excreted	Total CO_2 produced	Glutamine converted to CO_2: cpm/min	Glutamine converted to CO_2: Glu-NH_2	Glutamine converted to CO_2: CO_2	Per cent of total
	ml/min						*µmoles/min*		*µmoles/min*			*µmoles/min*		
	23.7	253	7.75	197,568	25,493	46880	1.84	1.53	0.57	103	106,845	4.19	21.0	20.4
10	26.6	285	9.24	214,962	23,264	29990	1.29	1.07	0.43	296	160,682	6.91	34.6	11.7
	24.7	242	8.60	203,660	23,681	38812	1.64	1.37	0.26	109	160,033	6.76	33.8	31.1
	30.8	292	10.08	134,944	13,387	18501	1.38	1.15	0.74	108	64,998	4.86	24.3	22.5
11	29.2	287	9.05	149,216	16,488	11536	0.70	0.58	0.69	69	76,697	4.65	23.3	33.9
	30.6	240	7.15	130,497	18,251	−5623	−0.31	−0.26	0.73	161	82,503	4.52	22.6	14.0
	39.6	399	11.72	154,692	13,199	13821	1.05	0.87	6.02	262	129,053	9.78	48.9	18.7
12	39.7	361	9.70	181,898	18,752	9671	0.52	0.43	7.46	351	147,213	7.85	39.3	11.2
	38.6	301	9.43	178,652	18,945	−9096	−0.48	−0.40	7.18	288	165,716	7.64	38.2	13.3
	29.7	211	4.65	136,411	29,336	25410	0.87	0.72	2.75	157	75,528	2.57	12.9	8.21
13	28.1	192	5.41	153,659	28,403	21296	0.75	0.62	2.40	175	109,666	3.86	19.3	11.0
	28.3	226	10.52	230,770	21,936	120681	5.50	4.58	2.44	259	156,922	7.15	35.8	13.8
	63.9	302	5.26	75,238	14,304	−12528	−0.88	−0.73	5.97	255	14,974	1.05	5.23	2.05
14	64.9	312	6.62	80,916	12,223	477	0.04	0.30	5.66	358	25,348	2.07	10.4	2.90
	78.7	290	5.38	80,877	15,033	−2509	−0.17	−0.14	5.15	250	15,543	1.03	5.15	2.06
Mean	38.5	280	8.04		19,513		0.92	0.78	3.23	213		4.99	25.0	14.5
±SE	4.37	14.2	0.57		1,444		0.39	0.32	0.70	24.2		0.68	3.41	2.47
P	NS	NS	<0.0005		NS		<0.0005	<0.0005	<0.0005	NS		<0.0005	<0.0005	<0.0005

P refers to the differences in the means in acidosis and in alkalosis. NS means not significant.

Fig. 5 illustrates the moieties of CO_2 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 pNH_3 (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 peritubular membranes of tubular cells in acidosis (15, 2). However, it is doubtful that increased uptake by this

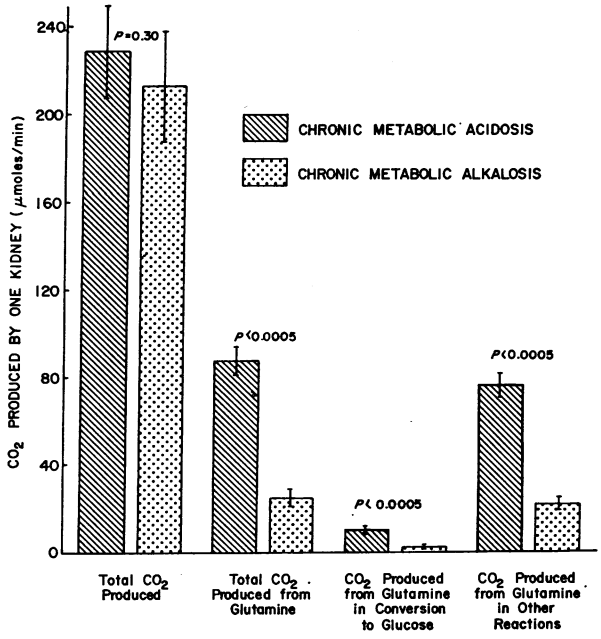

FIGURE 5 Comparison of mean values of total CO_2 produced, total CO_2 from glutamine, CO_2 from conversion of glutamine to glucose, and CO_2 from glutamine in all other reactions by five dogs in chronic metabolic acidosis and by five dogs in chronic metabolic alkalosis. All data from one kidney.

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 NH_4Cl acidosis and $NaHCO_3$ 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 ^{14}C activity (Figs. 1 and 2) represent glucose-^{14}C, 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 CO_2. 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 CO_2 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 CO_2 for the base (NH_3) 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 CO_2 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 acidotic slices oxidize glutamine to CO_2 at much faster rates than do alkalotic slices. The latter investigators have accordingly suggested that glutamine may be a major fuel of respiration in the kidney of the acidotic 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.

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