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# Effect of Acute and Chronic Acidosis on Glutamine Uptake in Kidney Slices of Newborn Rats (41324)

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*Abstract.* Developmental changes in glutamine uptake, $CO_2$ and $NH_3$ production was studied in kidney slices isolated from 12-, 18-, and 65-day-old rats under normal and acidotic conditions. The kidney slices from 12- and 18-day-old animals showed lower uptake of glutamine, $CO_2$ and $NH_3$ production compared to 65-day-old animals. Chronic acidosis increased the ability of kidneys from postnatal rats to accumulate and metabolize glutamine but this increment was lower than the adults. Acute acidosis of newborn animals failed to show any significant changes in glutamine uptake and metabolism even though the degree of acidosis reached was the same between chronic and acute acidotic groups. Newborns failed to show an optimal pH for glutamine uptake and metabolism under normal and acidotic conditions. Along with other factors, the inability of newborn kidneys to increase transport of glutamine during an acid challenge can also explain the lowered ammonia production of newborn rats.

Newborn mammals are unable to appropriately increase production of ammonia, the chief urinary buffer that accepts hydrogen ions and facilitates the excretion of acid from the blood (1–4). In contrast to newborns, adult animals are able to increase production and excretion of ammonia in response to acid loading (5–8). In development, certain abrupt changes occur during the so-called "critical period" and changes in ammonia excretion have been reported to occur around 15 days postnatally (2). Renal production of ammonia in rats is primarily due to cellular metabolism of glutamine (5, 9, 12) and the glutamine may enter the kidney cells from both the luminal and antiluminal borders (13, 14).

Recently, it was found that the uptake of glutamine in kidney slices isolated from adult rats that were on a control diet and those fed ammonium chloride to induce acidosis shows dependence upon the pH and temperature of incubation (15). Uptake of glutamine by kidney slices from normal rats showed a pH optimum of 7.5 at 25° and 7.3 at 37°. In slices from acidotic rats, greatest uptake was at pH 6.8 at 25° and 6.6 at 37°. In spite of these changes, glutamine uptake in slices from normal and acidotic rats was optimal at a constant OH/H ratio of 10 and 0.4, respectively (15). One could assume that some kind of cell membrane changes underlies the shift in OH/H ratio noticed in acidotic condition. Moreover, the increased uptake of glutamine and ammonia production seen in acidosis may be due to changes in the pH-dependent characteristics of glutamine uptake and/or altered cellular metabolism of glutamine.

The present studies were performed to examine the effects of acid–base changes and medium pH on uptake and metabolism of glutamine by kidney slices from newborn rats. Our studies show that glutamine uptake and ammonia production are lower in newborns compared to adults and they are unable to appropriately increase ammoniagenesis during an acid challenge. In addition, pH optimum and the dependence of a fixed OH/H ratio for glutamine uptake were not seen in newborn rats.

**Methods.** Adult male and pregnant female Wistar rats were obtained from Simonsen Breeding Laboratories (California). Pups were born in our laboratory and they were kept with their mothers until the time of the experiment, 12 or 18 days of age.

Induction of short-term (acute) and long-term (chronic) acidosis in newborn animals were achieved by feeding 5 mmol $NH_4Cl$/kg through a stomach tube in a volume of water equal to 2.5% of their body weight for 5 and 48 hr, respectively. Chronic acidosis was induced in adult



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animals (65-day-old) by adding 0.28 *M* $NH_4Cl$ to their drinking water for one week prior to the experiment. Acute acidosis was not studied in adult animals. The degree of acidosis in animals was judged by measuring their blood and urine pH. Blood was obtained from both adults and newborns by direct cardiac puncture under ether anesthesia. Urine from adult animals was obtained using metabolism cages. Since the newborns may not urinate spontaneously, application of slight suprapubic pressure or a touch in perianal area were found useful in collecting urine from newborns. The pH measurements were made on pH meter 27 (Radiometer, Copenhagen, Denmark) adapted with a micro pH electrode.

Preparation and incubation of kidney slices, measurement of glutamine uptake, $CO_2$ and $NH_3$ production were described in detail previously (15, 16). In short, four to six slices of kidney cortex, approximately 0.4–0.5 mm thickness, were cut from each kidney and placed in Warburg incubation flasks equipped with two side arms and a central well. The incubation medium was 15 m*M* phosphate buffer (12 m*M* $Na_2HPO_4$ and 3 m*M* $NaH_2PO_4$) containing 110 m*M* NaCl, 5.0 m*M* KCl, 12 m*M* $MgSO_4$, 1.0 m*M* $CaCl_2$, and 5.0 m*M* sodium acetate. The reaction chamber of Warburg flasks contained 3.0 ml incubation medium and 0.1 ml [$^3$H]inulin (New England Nuclear) and one of the side arms contained 0.1 ml L-[U-$^{14}$C]glutamine (Amersham/Searle) and 0.1 ml stock L-glutamine (Sigma Chemicals). The concentration of glutamine in the medium was 1 m*M*. At the start of the incubation period, the medium contained [$^3$H]inulin and [$^{14}$C]glutamine to give specific activities of approximately 0.1 and 0.01 $\mu$Ci/ml medium, respectively. Small pieces of accordion-type, folded filter paper moistened with 0.2 ml of 20% KOH were placed in the central well of the flasks to trap the evolved $^{14}CO_2$. The flasks were attached to manometers and equilibrated at 25° in the water bath of a Warburg apparatus. At time zero, glutamine was tipped into the reaction chamber to begin the experiment and the kidney slices were incubated for 30 min at 25°. After incubation, slices were removed from the medium, rinsed in glutamine-free medium for about 2 sec, and blotted on filter paper. A small portion of the slices was removed, weighed, and dried overnight at 100°, to determine the total water content of the slices. The remainder of the slices was transferred to small test tubes containing distilled water and kept overnight to leach [$^3$H]inulin and [$^{14}$C]glutamine from the tissue. The filter paper and the remaining KOH from the central well of the flasks were transferred to counting vials and the activity due to the liberated $^{14}CO_2$ was determined. This determination of evolved $CO_2$ is only a partial measurement of glutamine metabolism since all permeating glutamine may not be completely oxidized to $CO_2$. Radioactivity of the incubation medium and the supernatant fluid from test tubes containing the leached tissues were determined. Since the only source of $^{14}$C was the added glutamine, the radioactivity in the intracellular fluid plus that trapped as $CO_2$ approximated the uptake of glutamine. Accordingly, glutamine taken up and left in the cells was calculated by multiplying the cell-to-medium ratio (cpm/ml intracellular fluid: cpm/ml medium) by the concentration of glutamine in the medium. The fraction of glutamine metabolized to $CO_2$ was added to the equivalent amount remaining in the cells to obtain total uptake.

For ammonia measurements, slices were placed in 25-ml Erlenmeyer flasks containing 6.4 ml incubation medium and 0.2 ml of glutamine. The final concentration of glutamine in the medium was 1 m*M*. The incubations were carried out for 30 min at 25°. Ammonia in the medium was determined using an ammonia electrode (Orion Research Inc.) after the samples were alkalanized by adding saturated NaOH.

A two-sample, two-tailed, student's *t* test was used to test significance levels and *P* values less than 0.05 were regarded as indicating significance.

**Results.** Table I shows the measurements of blood and urine pH in 12-, 18-, and 65-day-old animals. In all age groups, chronic acidosis lowered pH of blood and urine significantly ($P < 0.05$). In neonates,

TABLE I. MEASUREMENTS OF BLOOD AND URINE pH IN NEWBORNS AND ADULTS

| | 12 day old animals | | 18 day old animals | | 65 day old animals | |
|---|---|---|---|---|---|---|
| | Blood pH | Urine pH | Blood pH | Urine pH | Blood pH | Urine pH |
| Controls, nontreated (8) | 7.40 ± 0.02 | 7.0 ± 0.02 | 7.38 ± 0.02 | 6.7 ± 0.01 | 7.39 ± 0.01 | 6.8 ± 0.01 |
| $NH_4Cl$-treated, acute acidosis (7) | 7.35 ± 0.03 | 5.6 ± 0.03* | 7.31 ± 0.04 | 5.7 ± 0.01* | — | — |
| $NH_4Cl$-treated, chronic acidosis (6) | 7.29 ± 0.05* | 5.45 ± 0.02* | 7.25 ± 0.03* | 5.54 ± 0.01* | 7.20 ± 0.03* | 5.5 ± 0.09* |

*Note.* Values are means ± SE. The number of animals used in each group are given in parentheses. The data for the 65-day-old animals for Tables I, II, and III are taken from a previous publication (15).
* Significantly different from nontreated controls ($P < 0.05$).

even though acute acidosis decreased urine pH significantly ($P < 0.05$), blood pH was lower but not statistically different from their nontreated controls. Acute acidosis was not studied in adult animals.

Total glutamine uptake in kidney slices isolated from newborns and adults as a function of varying medium pH is shown in Table II. The general pattern is clear in that the ability of kidney slices to accumulate glutamine increases with the age of the animal. Acute acidosis failed to increase glutamine uptake in newborns and is not different from the nontreated group. In 12-day-old animals, chronic acidosis increased glutamine uptake significantly ($P < 0.001$) and is maximum around a pH of 7.2. In 18-day-old animals, chronic acidosis increased glutamine uptake significantly at pH 7.2 ($P < 0.05$), but no significant change was observed at pH 6.8 and 7.6. In adults, chronic acidosis increased their ability to transport glutamine at a lower incubation medium pH, but we did not find any change at high pH values.

Glutamine metabolized as $CO_2$ expressed in micromoles per kilogram wet tissue per minute for different groups under control and acidotic conditions is shown in Table III. The ability of newborn kidney slices to metabolize glutamine to $CO_2$ was significantly lower than those of adults under normal and acidotic conditions. Even though acute acidosis of 12- and 18-day-old rats failed to increase $CO_2$ production from glutamine, slices from chronically acidotic rats show significantly higher $CO_2$ production ($P < 0.001$). In adults, both acidosis

TABLE II. GLUTAMINE UPTAKE IN NEWBORNS AND ADULTS

| | 12 days old | | | 18 days old | | | 65 days old | |
|---|---|---|---|---|---|---|---|---|
| Incubation pH | Controls (7) | Acute acidosis (7) | Chronic acidosis (8) | Controls (7) | Acute acidosis (7) | Chronic acidosis (8) | Controls (7) | Chronic acidosis (6) |
| 6.8 | 21.6 ± 0.99 | 23.7 ±1.81 | 31.6* ±2.9 | 33.1 ±3.17 | 32.3 ±3.46 | 35.7 ±4.01 | 35.9 ±4.37 | 66.7* ±2.03 |
| 7.2 | 25.0 ± 2.88 | 28.0 ±1.36 | 40.6* ±2.79 | 31.0 ± 2.00 | 35.1 ±2.12 | 43.1** ±4.30 | 44.8 ±3.05 | 53.5 ±4.08 |
| 7.6 | 25.0 ± 1.95 | 26.2 ±1.56 | 33.9* ±2.06 | 33.4 ±3.28 | 33.5 ±2.77 | 40.4 ±4.17 | 62.2 ±5.36 | 53.6 ±3.44 |

*Note.* Values are expressed in μmol/kg wet tissue/min, mean ± 1 SE. The number of incubations carried out for each condition are given in parentheses. All incubations were done at 25°.
* Significantly different from controls ($P < 0.001$).
** Significantly different from controls ($P < 0.005$).

TABLE III. $CO_2$ PRODUCTION FROM GLUTAMINE IN NEWBORNS AND ADULTS

| Incubation pH | 12 days old | | | 18 days old | | | 65 days old | |
|---|---|---|---|---|---|---|---|---|
| | Controls (7) | Acute acidosis (7) | Chronic acidosis (8) | Controls (7) | Acute acidosis (7) | Chronic acidosis (6) | Controls (7) | Chronic acidosis (6) |
| 6.8 | 1.3 ±0.19 | 1.7 ±0.28 | 3.2* ±0.49 | 1.8 ±0.27 | 2.1 ±0.22 | 3.5* ±0.42 | 8.3 ±0.98 | 23.4* ± 2.53 |
| 7.2 | 1.4 ±0.19 | 1.9 ±0.32 | 3.2* ±0.67 | 2.2 ±0.09 | 2.7 ±0.32 | 3.8* ±0.34 | 6.4 ±0.57 | 14.0* ± 1.37 |
| 7.6 | 1.2 ±0.07 | 1.5 ±0.18 | 2.2* ±0.20 | 1.9 ±0.32 | 1.8 ±0.42 | 3.0* ±0.37 | 5.6 ±0.91 | 15.5* ± 1.00 |

*Note.* Values are expressed in μmol/kg wet tissue/min, mean ± 1 SE.
* Significantly different from controls ($P < 0.001$).

and incubation of slices in low medium pH increased $CO_2$ production.

Glutamine-stimulated $NH_3$ production, calculated as the difference between basal ($NH_3$ produced in slices in the absence of added glutamine) and total $NH_3$ production ($NH_3$ produced in the presence of added 1 m*M* glutamine) for the three different age groups under normal and acidotic conditions is shown in Table IV. Chronic acidosis of all three age groups increased $NH_3$ production significantly compared to their nontreated controls. However, acute acidosis failed to increase $NH_3$ production in newborns.

**Discussion.** Previous studies of the process whereby neonatal rats develop the ability to produce and secrete adequate amounts of ammonia would indicate that it is probably multifactorial. Definitive evidence exists that glutaminase I increases in rat pups after 15 days of age (2, 17, 18). Another factor which plays a role is the inability of the kidney to adequately lower urinary pH (2, 3): Since ammonia moves into the tubular fluid by "non-ionic diffusion trapping" (19) the limitation in lowering pH will also result in reduced excretion of ammonia. More recently preliminary results have been presented from this laboratory showing that mitochondria from kidney of infant rats have a high glutamate content as compared to mature animals (20). Since glutamate inhibits the action of mitochondrial glutaminase, the high glutamate concentration could also act to limit ammonia production.

The present results indicate that changes in membrane permeability to glutamine induced by acidosis do not occur in the neonate. In a previous study (15), we have shown that the optimal OH/H ratio shifts

TABLE IV. GLUTAMINE-STIMULATED $NH_3$ PRODUCTION IN NEWBORNS AND ADULTS

| Incubation pH | 12 days old | | | 18 days old | | | 65 days old | |
|---|---|---|---|---|---|---|---|---|
| | Controls (7) | Acute acidosis (7) | Chronic acidosis (8) | Controls (7) | Acute acidosis (7) | Chronic acidosis (6) | Controls (7) | Chronic acidosis (6) |
| 6.8 | 6.3 ±1.33 | 10.9 ± 1.83 | 12.4* ± 1.17 | 10.0 ± 1.21 | 15.3 ± 2.25 | 18.7** ± 2.30 | 13.8 ± 1.66 | 31.3* ± 2.45 |
| 7.2 | 11.2 ±1.19 | 12.9 ± 1.43 | 17.5*** ± 2.23 | 10.7 ± 0.77 | 13.9 ± 1.79 | 19.1* ± 1.86 | 17.4 ± 1.38 | 33.6* ± 3.01 |
| 7.6 | 11.8 ±1.20 | 16.3 ± 2.00 | 17.7*** ± 2.35 | 14.2 ± 0.74 | 23.0** ± 2.83 | 22.6** ± 2.47 | 24.2 ± 1.88 | 42.0* ± 2.93 |

*Note.* Values are expressed in μmol/kg wet tissue/min, mean ± 1 SE.
Significantly different from controls: *$P < 0.005$; **$P < 0.01$; ***$P < 0.05$.

from 10 in animals of normal acid base balance down to 0.6 in acidosis for uptake of glutamine in rat kidney slices. At the optimum, uptake is also greater in the acidotic than normal (15). These results have been interpreted to indicate that chronic acidosis produces an adaptive membrane change which results in an increased permeability of glutamine at lower plasma pH. It was also indicated that membrane permeability does not become a limiting factor for utilization of glutamine for ammonia production. No optimal pH could be detected in kidney slices of infant rats in normal acid–base balance. When acidosis was induced, a marked optimum pH for uptake was not well established, and more significantly the suggested optimum was not indicated to be a low pH and to be adaptive in promoting glutamine uptake. These results are suggestive that with regard to the penetration of glutamine into cells of the infant kidney, the membranes are different and do not show the adaptive changes as do membranes from renal cells of mature animals.

Analysis of the changes in $CO_2$ production from glutamine produced by acidosis show that acidosis results in a shift in metabolic fate of glutamine, so that more of it is oxidized to $CO_2$. This is shown in Fig. 1 wherein the data from Tables II and III have been combined. It is clear that in chronic acidosis, $CO_2$ produced from added glutamine is always increased at all medium pHs. In acute acidosis the increase is less and not always found (18-day-old rats at pH 7.6). A similar effect is noticed if one calculates the ratio of $NH_3$ produced to glutamine uptake (Fig. 2). The effect is most marked in slices from both 12- and 18-day-old animals at medium pH of 6.8. At higher pHs, the effect of acidosis is seen consistently only in slices from the 18-day-old animals, although a marked increase is shown by the 12-day-old animals in acute acidosis at medium pH 7.6. It seems that in

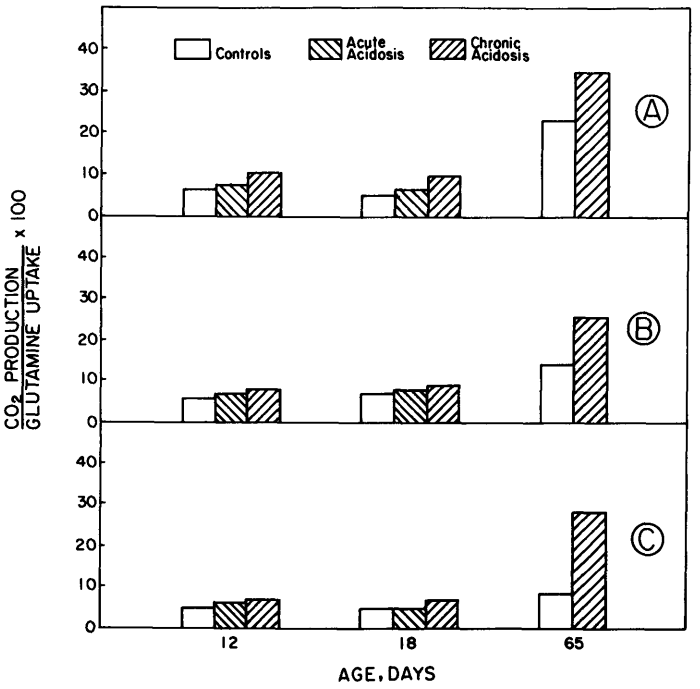


FIG. 1. Relation between $CO_2$ production and glutamine uptake in kidney slices isolated from 12-, 18-, and 65-day-old rats. (A) Data when the incubation medium pH was 6.8; (B) when pH was 7.2; and (C) when pH was 7.6. Different hatchings on bars indicate different experimental conditions.

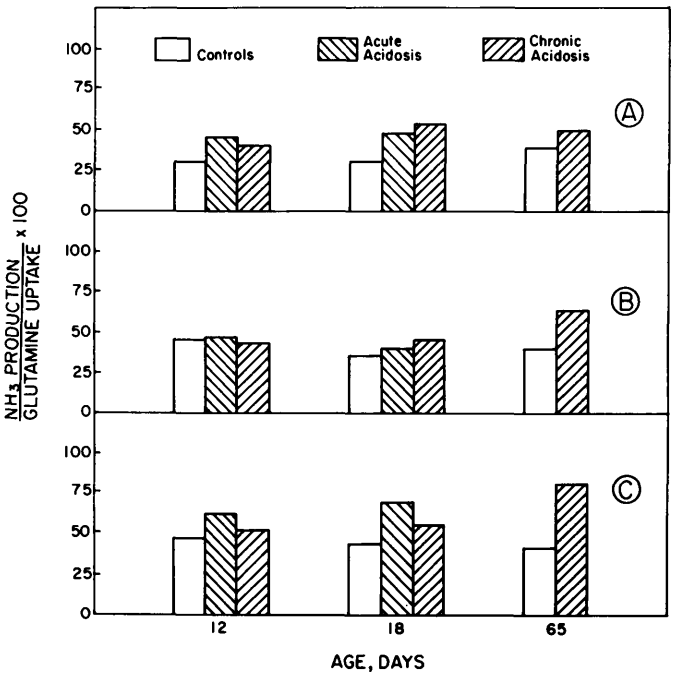


FIG. 2. Relation between $NH_3$ production and glutamine uptake. All symbols are same as in Fig. 1.

the pups, acidosis induces a shift in glutamine utilization so that oxidation and ammoniagenesis are more efficient. These changes are comparable to changes brought about by maturation.

The other observations made in this study are that kidney slices of infant rats have a limited production of ammonia and they do not respond to acute acidosis, and only very poorly to chronic acidosis. Since these observations have been previously made by others and discussed in other publications (2, 17, 18), they will not be considered further.

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1. Cort JH, McCance RA. The renal response of puppies to an acidosis. J Physiol **124**:358–369, 1954
2. Goldstein L. Renal ammonia and acid excretion in infant rats. Amer J Physiol **218**:1394–1398, 1970.
3. Goldstein L. Ammonia metabolism in the kidneys of suckling rats. Amer J Physiol **220**:213–217, 1971.
4. McCance RA. Renal function in early life. Physiol Rev **28**:331–348, 1948.
5. Owen EE, Robinson RR. Amino acid extraction and ammonia metabolism by the human kidney during the prolonged administration of ammonium chloride. J Clin Invest **42**:263–276, 1963.
6. Pitts RF. Renal production and excretion of ammonia. Amer J Med **36**:720–742, 1964.
7. Pitts RF, Pilkington LA, McLeod MB, Leal-Pinto E. Metabolism of glutamine by the intact functioning kidney of the dog. Studies in metabolic acidosis and alkalosis. J Clin Invest **51**:557–565, 1972.
8. Welbourne TC. Mechanism of renal ammonia production adaptation to chronic acidosis. Med Clin North Amer **59**:629–648, 1975.
9. Pilkington LA, O'Donovan DJ. Metabolism of glutamine in cortex slices from dog kidney during acid–base alterations. Amer J Physiol **220**: 1634–1639, 1971.
10. Preuss HG. Renal glutamate metabolism in acute metabolic acidosis. Neprhon **6**:235–246, 1969.
11. Shalhoub R, Webber W, Glabman S, Canessa Fischer M, Klein J, DeHass J, Pitts RF. Extraction of aminoacids from and their addition to blood plasma. Amer J Physiol **204**:181–186, 1963.

12. Vanslyke DE, Phillips RA, Hamilton PB, Archibald RM, Futcher PH, Hiller A. Glutamine as source material of urinary ammonia. J Biol Chem 150:481–482, 1943.
13. Lemieux G, Vinay P, Cartier P. Renal hemodynamics and ammoniagenesis. Characteristics of the antiluminal site for glutamine extraction. J Clin Invest 53:884–894, 1974.
14. Pilkington LA, Young TK, Pitts RF. Properties of renal luminal and antiluminal transport of plasma glutamine. Nephron 7:51–60, 1970.
15. George JP, Solomon S. pH and temperature dependence of glutamine uptake, carbon dioxide and ammonia production in kidney slices from acidotic rats. J Physiol 316:251–261, 1981.
16. George JP, Solomon S. Dependence of glutamine metabolism and ammonia synthesis in rat kidney slices. J Endocrinol 79:271–276, 1978.
17. Benyajati S, Goldstein L. Renal glutaminase adaptation and ammonia excretion in infant rats. Amer J Physiol 228:693–698, 1975.
18. Wacker GR, Zarkowsky HS, Burgh HP. Changes in kidney enzymes after birth. Amer J Physiol 200:367–369, 1961.
19. Pitts RF. Production and excretion of ammonia in relation to acid–base regulation. In: Orloff J, Berliner RW, eds. Handbook of Physiology, Sect 8, Renal Physiology. Washington, D.C., American Physiological Society, p.455, 1973.
20. Zamet CN, Solomon S. Developmental changes of mitochondria of rat kidneys in relation to ammonia production. Fed Proc 39:513, 1981.

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