Role of acidosis in the protein wasting of fasting in the rat and the rabbit

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The purpose of these experiments was to determine if augmented renal ammoniagenesis in chronic metabolic acidosis could increase the negative nitrogen balance during prolonged fasting. To explore this question, rats and rabbits were fasted for up to 10 days because acidosis would markedly augment ammonium excretion in the rat but not in the rabbit. Since the ketoacidosis of fasting was mild in both species (<2 m*M*) and ketonuria virtually absent, a hydrochloric acid load was given to stimulate renal ammoniagenesis. Under these conditions, nitrogen balance was significantly more negative during acidosis in the rat but not in the rabbit. This increment in nitrogen excretion appeared as ammonium with no detectable difference in urea nitrogen excretion. Therefore, it appears that if more nitrogen is excreted as ammonium, net protein breakdown increases to furnish the substrate for ammoniagenesis rather than reducing the excretion of the other nitrogenous waste component urea. The implications of these findings will be discussed.

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Ces expériences avaient pour but de déterminer si, lors d'un jeûne prolongé, un accroissement d'ammoniogénèse rénale pourrait augmenter le bilan azoté négatif. Pour explorer cette question, on a fait jeûner des rats et des lapins pendant au moins 10 jours, parce qu'ainsi l'acidose ferait augmenter nettement l'excrétion d'ammonium dans le rat mais non dans le lapin. Comme, chez les deux espèces, le jeûne n'induisit qu'une faible acidocétose (<2 m*M*) et pour ainsi dire aucune cétonurie, on leur donna une charge d'acide chlorhydrique pour stimuler l'ammoniogénèse rénale. Dans ces conditions, le bilan azoté fut significativement plus négatif durant l'acidose du rat, mais non dans celle du lapin. Cette augmentation d'excrétion d'azote apparut sous forme d'ammonium sans aucune différence décelable dans l'excrétion d'azote uréique. Ainsi, il semble que si une plus grande quantité d'azote est excrétée sous forme d'ammonium, c'est que la dégradation de protéine nette augmente davantage pour produire le substrat de l'ammoniogénèse que pour réduire l'excrétion des autres déchets azotés de l'urée.
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Introduction

When human subjects were placed on a calorie-free diet, the quantity of nitrogen excreted decreased progressively over the 1st week and then tended to plateau at about 5 g of nitrogen per day (for review, see Benedict 1915; Owen et al. 1969; Cahill 1970). During this steady state, just over half the nitrogen in the urine was in the form of urea and the remainder was almost exclusively ammonium.

It is important to recall that ammonium excretion is controlled by the acid–base balance (for review, see Halperin et al. 1982). As ketoacidosis of fasting develops and persists, this would lead to an increased rate of renal ammoniagenesis to prevent severe acidemia (Sigler 1975). In support of this latter proposal, we have recently shown that sodium bicarbonate administration to prolonged fasted subjects did reduce both ammonium and urea excretions (M. C. Hannaford, L. A. Leiter, R. G. Josse, M. B. Goldstein, E. B. Marliss, and M. L. Halperin, submitted for publication).

The purpose of the present work was to evaluate the role of acidemia independent of renal ammoniagenesis as a cause for the negative nitrogen balance of fasting. In order to accomplish this aim, rats and rabbits were subjected to a chronic fast. The rabbit, in contrast with the rat, cannot excrete appreciable quantities of ammonium in the urine during chronic metabolic acidosis (Yu et al. 1976). Since ketoacidosis of starvation tends to be mild (<2 m*M*) in both species (Parrilla 1978; Goodman et al. 1980; Richardson et al. 1979), each was given a hydrochloric acid load during fasting to produce the acid load. The results to be reported suggest that an acid load leads to negative nitrogen balance primarily because of the augmented ammonium excretion in the urine.

Methods

Male Wistar rats, 400 to 700 gm, and male New Zealand white rabbits, 1.5 to 2.5 kg, were obtained from Canadian Breeding Laboratories, LaPrairie, P.Q., and Reiman's Fur Ranch, St. Agatha, Ont., respectively. Before fasting, the rat diet was Purina Rodent Laboratory Chow (chow No. 5001) and the rabbit diet was Purina Rabbit Chow (chow No. 5301, Ralston Purina Canada, Woodstock, Ont.).

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Balance studies

Animals were studied in individual metabolic cages. Urine was collected daily under oil with thymol and chloroform as preservative as previously described by Richardson et al. (1979). Urine samples were frozen and stored at −4°C until the time of individual determinations.

Rat study

Three separate groups of animals were studied. (1) Seven rats were fasted for 10 days while receiving hydrochloric acid daily via orogastric tube (150 m*M* HCl, 5 mequiv./kg body weight). With this protocol, the acid load given was different in individual rats because of the variations in body weight. (2) Five rats were fasted for 10 days and given sodium bicarbonate daily via orogastric tube (150 m*M* $NaHCO_3$, 2−5 mequiv./kg body weight). (3) Three rats were fasted for 10 days and not given any supplements; two additional rats received 150 m*M* potassium chloride in a similar manner to the rats receiving hydrochloric acid; as the nitrogen excretion data of the rats in group 3 did not differ significantly, the data from these rats were combined.

Rabbit study

Two groups were studied. (1) Six rabbits were fasted for 10 days. (2) Six additional rabbits were fasted for 10 days and given hydrochloric acid (150 m*M*, 2 mequiv./kg body weight). The dose of hydrochloric acid was only given if the plasma bicarbonate concentration exceeded 18 mequiv./L.

Blood samples were obtained from rats anaesthetized with ether via heparinized capillary tube from the orbital vein prior to fasting and on alternate days during the study. Blood samples were collected from rabbits sedated with chlorpromazine (20 mg im, 30−60 min prior to sampling).

Analytical methods

The pH and P_{CO_2} were measured on a Radiometer PHM-72 acid-base analyzer (Radiometer, Copenhagen, Denmark), sodium and potassium on a Radiometer FLM-3 flame photometer, chloride on a Radiometer CMT-10 chloride titrator, ammonium by an Orion ammonium electrode (Orion Research Inc., Cambridge, MA), inorganic phosphorus and urea by standard autoanalyzer techniques, and creatinine by a Beckman creatinine analyzer 2 (Beckman Instruments, Inc., Palo Alto, CA) all as previously described (Richardson et al. 1979). Bicarbonate was calculated from pH and P_{CO_2}, also as previously described (Richardson et al. 1979). β-Hydroxybutyrate was assayed by enzymic microfluorimetric methods modified from published techniques to enable assay of replicates on 5 to 10 μL of blood at a 1:2 dilution in the perchloric acid supernatant (Marliss et al.1978).

Results

When rats were fasted for up to 10 days, the mean daily weight loss was 4.2 g/day and there was no significant difference in weight loss in the rats fed the acid, base, or potassium supplements. When the urine excretions were examined (Table 1) the urine volume tended to be higher in the group which received the acid load, and this is probably secondary to the volume of solution administered by orogastric tube. As expected, ammonium excretion was generally highest in the rats fed the acid load and lowest in the rats fed the alkali load. Urea nitrogen excretion was not significantly different in the acid-fed rats but total nitrogen excretion was highest in these rats. The rise in total nitrogen excretion was directly related to the rate of ammonium excretion, as shown graphically in Fig. 1. Animals were divided according to their actual rate of ammonium excretion, regardless of their experimental group. It is obvious that the greater the rate of ammonium excretion, the greater the negative nitrogen balance, since urea excretion was not depressed by any of the treatments. There was no significant change in blood acid−base parameters in the acid-fed rats because the daily acid load was almost equivalent to the rate of ammonium excretion (Table 2).

When rabbits were fasted for 10 days, the mean daily weight loss was 57 g/day and there was no significant difference in the rabbits fed the acid supplement. In contrast with the rats, ammonium excretion was a very small component of total nitrogen excretion and it did not rise to be a significant component of nitrogen excretion in the acid-fed rabbits. As in the rats, an acid load did not influence the daily urea nitrogen excretion (Table 1). Hence, total nitrogen excretion was not significantly altered when hydrochloric acid was given. When blood parameters were examined, the rabbits developed and sustained a metabolic acidosis. The acid load which was initially the same per kilogram body weight in the two species had to be withheld for several days in the rabbits to prevent severe acidemia.

Both species developed ketoacidosis with fasting but the blood β-hydroxybutyrate was less than 2 m*M* in both species (1.1 ± 0.2 m*M* in the rat and 1.5 ± 0.3 m*M* in the rabbit). There was no significant degree of ketonuria in either species.

Discussion

Negative nitrogen balance of chronic fasting leads to the loss of a considerable bulk of lean body mass. For example, the loss of 5 g of nitrogen per day in the fasted human is equivalent to the loss of 31 g of protein (16% of protein is nitrogen). Since lean body mass has approximately 180 g of protein per kilogram wet weight (water is almost 80% of the bulk), subjects should lose 170 g (~1/3 lb (1 lb = 0.454 kg)) of these tissues per day during the steady state of chronic fasting. The oxidation of a similar quantity of fat would supply approximately 1500 kcal (1 kcal = 4.1855 kJ); therefore, the loss of lean body mass accounts for approximately one-half of the daily weight loss during fasting. The critical question to be addressed is, Does this proteolysis occur primarily for acid−base considerations or to supply substrates for gluconeogenesis?

Obese rats and rabbits can fast for at least 2 weeks with only a relatively small weight loss (see Results section). However, ketoacidosis is mild in both species,

TABLE 1. Effect of an acid load on urine nitrogen excretion in fasted rats and rabbits

Parameter	Rat		Rabbit	
	−HCl	+HCl	−HCl	+HCl
Volume, mL/day	9±1	13±2*	296±88	211±49
NH_4^+, mg N/day	7±2	31±6*	15±2	39±12
Urea, mg N/day	91±13	107±14	624±75	663±129
Total N, mg/day	97±12	140±13*	639±72	702±102

NOTE: For details, see Methods section. The daily excretions in each animal were averaged over the last 4 to 8 days of fasting, since the animals had reached a steady state at this time. Results are reported as the mean ± SEM.

*$p < 0.05$ as compared to the control group.

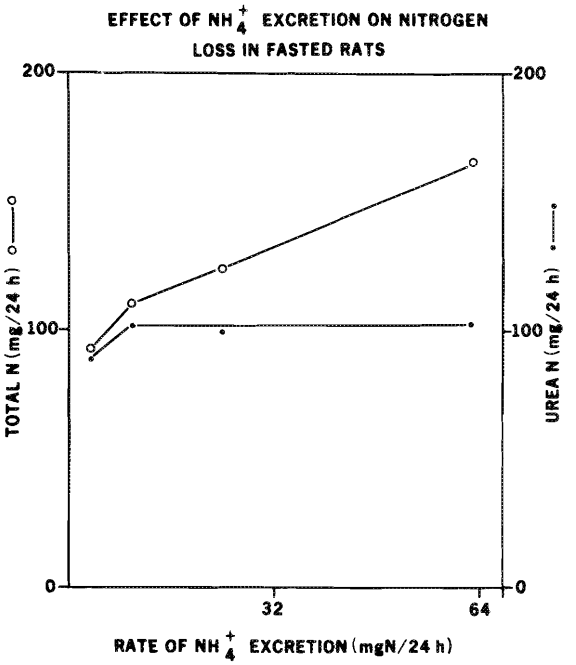

FIG. 1. Rats were fasted as described in the Methods section and then divided into four groups depending on the rate of ammonium excretion. The number of rats in each excretion group was four except the third group which had five rats. The mean ± SEM for ammonium excretions in the four groups were 3.4 ± 0.7, 9.9 ± 0.6, 24 ± 2, and 63±6 mg ammonium N/24 h. Urea nitrogen excretion (solid symbols) was not significantly different in all groups whereas total nitrogen excretion (open symbols) increased significantly as the rate of ammonium excretion increased.

as the blood β-hydroxybutyrate concentration was less than 2 m*M* at this time (see Results section). Thus, ketonuria is avoided during fasting in these animals, and consequently, there is little augmentation of ammonium excretion. When an acid load (hydrochloric acid) was administered during fasting, the rat increased its ammonium excretion but there was little change in the very low rates of ammonium excretion in the rabbit (Table 1). This difference can be used to evaluate the role of acidemia in causing protein wasting during fasting by comparing results from a species which either will or will not have a large amount of ammonium excretion.

In the rat, a dose of hydrochloric acid was chosen to cause a similar degree of ammonium excretion (per kilogram body weight) as that seen in fasted human subjects (Hannaford et al., submitted for publication). The daily acid load was virtually completely excreted, as the blood acid−base parameters were similar to those in rats which did not receive the acid load (Table 1). Despite similar blood values, the rate of ammonium excretion was higher during acid loading but there was no change in the rate of urea excretion. This was true over a wide range of ammonium excretions (Fig. 1). Therefore, while the acid load caused a greater degree of protein catabolism in the fasted rat, the increment seemed to be related to the requirement for ammonium excretion. The lack of reduction in urea excretion suggests that sufficient aminoacid nitrogen destined for urea production cannot be shunted completely into the ammoniagenesis pathway, thus obligating augmented protein breakdown (Fig. 2). In contrast, in previous studies where a low protein diet was ingested by animals with metabolic acidosis caused by hydrochloric acid intake, nitrogen excreted as ammonium was markedly increased to about half of total urine nitrogen (Pitts 1936). However, this increase was at the expense of urea, the two nitrogenous wastes varying reciprocally under conditions of low protein intake (Steenbock 1914; Keaton 1921).

When studies were carried out in the fasted rabbit, this species excreted the acid load much more slowly; hence, a significant degree of acidemia persisted in these animals (Table 2). The rabbit, unlike man and the rat, does not excrete large quantities of ammonium in the urine during acidosis (Yu et al. 1976). Therefore, the effects of acidosis independent of ammonium excretion could be evaluated. Urea nitrogen excretion was

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TABLE 2. Effect of chronic acid loading on blood acid–base and electrolyte values in animals fasted for 7–10 days

	Rat		Rabbit	
Parameter	−HCl	+HCl	−HCl	+HCl
pH	7.39±0.02	7.38±0.02	7.44±0.01	7.30±0.01*
P_{CO_2}	38±2	37±3	35±1	30±1*
HCO_3	23±1	21±1	23±1	15±0.6*
Na	148±2	149±2	142±1	152±5
K	4.4±0.4	3.7±0.2	3.3±0.1	3.5±0.1
Cl	110±1	112±1	108±2	115±5

NOTE: For details, see Methods section and Table 1. The blood values are reported as the mean ± SEM.
*$p < 0.01$.

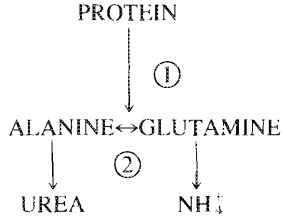

FIG. 2. Net protein catabolism (process 1) results in the formation of the principal circulating aminoacids alanine and glutamine. The primary nitrogenous end product of alanine catabolism is urea which is synthesized in the liver whereas ammonium is produced from glutamine in the kidney. It appears that when an acid load is administered to fasted rats, insufficient quantities of circulating aminoacids (alanine) can be converted to glutamine and thus be precursors for urine ammonium (via process 2) requiring that there be an increased rate of net protein breakdown under these conditions.

fairly constant over the entire period of fasting in the rabbit. Metabolic acidosis caused a small increase in ammonium excretion but there was no significant change in urea nitrogen excretion; hence, there was little change in the nitrogen balance induced by acid loading in this species.

In summary, in the fasted rat, acid loading is associated with an increased negative nitrogen balance due to increased urine ammonium excretion. In comparison with the rabbit, it appears that the increased negative nitrogen balance in the rat is obligated by the ammonium excretion and not by chronic acidemia per se. It is interesting to speculate that the absence of ketonuria is a teleological advantage to the fasting rat, as excretion of an organic anion would induce additional ammonium excretion (as ammonium β-hydroxybutyrate) and hence more nitrogen loss. In quantitative terms, a chronic acid load of 1 mmol/kg body weight will oblige an ammonium nitrogen loss of 14 mg/day; this latter quantity is equivalent to almost 0.5 g of lean body mass (14 mg × 6.25 (protein 16% N) × 5 (tissues are 80% water)). Continued loss at this rate should cause the loss of appreciable quantities of lean body mass in the rat over a longer period of time.

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