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Ammonia Excretion and Renal Glutaminase Activity During Administration of Strong Acid and Buffer Acid.* (22947)

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Excretion of urinary ammonia rises during administration of strong acid loads as a consequence of increased ammonia production owing to activation of the ammonia-producing enzymes of the renal tubular cell(1,2,3) and accelerated ammonia transport because of enhanced diffusion of ammonia into an acid urine. Activation of renal glutaminase and other ammonia-producing enzymes by administration of a strong acid (NH_4Cl) could result from the impact of the acid load on intracellular buffers, effect of acid tubular fluid, or rapid removal of ammonia from the tubular cell.

Theoretically, a buffer acid load should have the same effect on the intracellular pH as a physiologically equivalent load of strong acid; in the kidneys, however, the presence of the buffer anion permits secretion of large amounts of H^+ without a drastic fall in urine pH. Ingestion of NaH_2PO_4, therefore, accelerates excretion of titratable acid without notably altering ammonia excretion(4,5). In the present study, therefore, the effects of physiologically equivalent loads of strong acids and buffer acids on ammonia excretion and renal glutaminase activity were compared to determine whether activation of glutaminase results from prerenal or renal effects of an acid load.

Procedure. Three groups of male Sprague-Dawley rats, weighing 200-300 g, were fed 10 cc of standard electrolyte deficient diet (SEDD) twice daily and distilled water *ad lib.* for 14 days (Table I). Group 1 received only the SEDD, Group II the SEDD plus 2 mM KCl and 3 mM NH_4Cl daily, Group III the SEDD plus 2 mM KCl and 3.75 mM NaH_2PO_4.† Daily 24 hour urine samples were collected under mineral oil using phenyl mercuric nitrate and toluene as preservatives. The ammonia of urine was determined by modification of the microdiffusion method of Conway(6). Urine pH was measured with a Beckman pH meter using external glass electrodes, and corrected to 37.5°C by a factor of 0.01 pH unit per degree. To determine titratable acid, urine was titrated to a corrected pH 7.4 with 0.1 N NaOH using Beckman automatic titrator. At the end of

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† Since the pK of NaH_2PO_4 is 6.8, the ratio $\frac{Na_2HPO_4}{NaH_2PO_4}$ at pH 7.4 is $\frac{4}{1}$. Therefore, 3.75 mM of NaH_2PO_4 would have the same impact on the buffer systems of the body as 3 mM NH_4Cl, since only 3 mM of the NaH_2PO_4 would be converted to Na_2HPO_4.

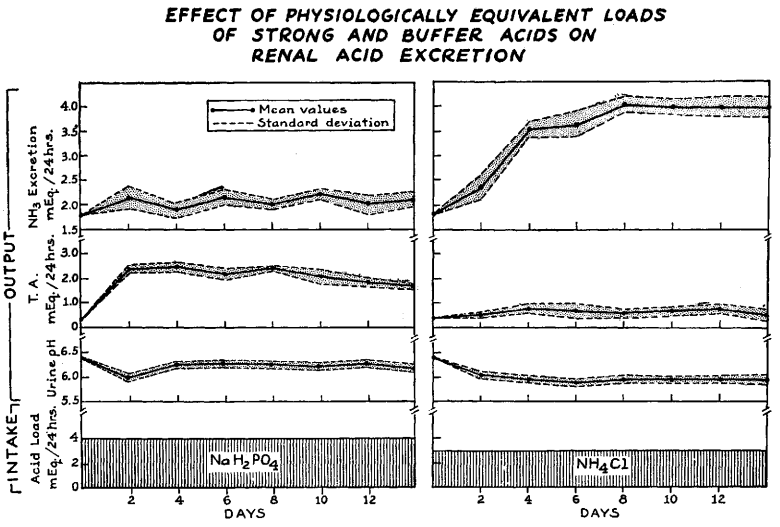

FIG. 1.

the experimental period the rats were anesthetized by intraperitoneal injection of sodium amytal and arterial blood collected anaerobically from the abdominal aorta. Kidneys were removed and immediately frozen. Maximal glutaminase activity was measured in a cold homogenate of kidney using the method previously described(2). The CO_2 content of blood was measured on 0.2 ml serum(7).

Results. Daily urinary acid excretion in control rats maintained on SEDD alone remained constant throughout a 14 day period, amounting to 1.7 ± 0.2 mEq for ammonia, 0.4 ± 0.1 mEq for titratable acid and 6.4 ± 0.2 for pH.

During administration of NH_4Cl loads, excretion of urinary ammonia rose slowly, reaching a peak in 4 to 6 days (Fig. 1). Urine pH fell during the first two days to approximately 5.9, but titratable acid did not change significantly. The rise in urinary ammonia was accompanied by an increase in renal glutaminase activity (Table I).

Physiologically equivalent loads of NaH_2PO_4, on the other hand, produced no change in ammonia excretion, while titratable acid rose sharply despite a very slight fall in urine pH (Fig. 1). Renal glutaminase activity remained unchanged (Table I).

Despite the marked difference in their effects on the pattern of acid excretion and the activity of renal glutaminase, NH_4Cl and NaH_2PO_4 loads produced approximately the same slight depression of serum bicarbonate (Table I).

TABLE I. Effect of NaH_2PO_4 Loads on Serum Bicarbonate Concentration and Renal Glutaminase Activity.

Treatment	Serum HCO_3^-	Renal glutaminase, μM NH_3/100 mg dry kidney/hr
Standard electrolyte deficient diet (SEDD)	23.9 ± 1.2	788 ± 59
SEDD + 2 mM KCl + 3 mM NH_4Cl daily	19.6 ± 1.4	1850 ± 100
SEDD + 2 mM KCl + 3.75 mM NaH_2PO_4	20.5 ± 1.6	675 ± 69

Conclusions. Activation of renal glutaminase during administration of NH_4Cl does not appear to result from pre-renal effects of the acid load, since physiologically equivalent loads of NaH_2PO_4 produced the same slight depression of serum bicarbonate but affected neither excretion of ammonia nor activity of renal glutaminase. Rather, it appears that activation of glutaminase is related to changes, as yet unidentified, within the kidney.

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A New Simple Method for Indirect Determination of Blood Pressure in the Rat.* (22948)

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Technics generally used for indirect determination of blood pressure in the rat's tail have one or more of the following disadvantages: 1) they are cumbersome, 2) they require complex apparatus, or 3) they are relatively inaccurate. The older plethysmographic methods(1,2,3) have been further complicated by adapting them to electronic devices in order to increase their sensitivity (4). The photoelectric tensometer depends upon change in transmission of a light beam incident on a small area of the rat's tail(5) or foot(6). Friedman and Freed(7) described what is called a microphonic manometer which, according to these observers, operates by amplifying the pulsatile vibrations of the caudal artery in the tail similar to that noted in the indirect recording of blood pressure in the human brachial artery. A graphic method of recording these tail vibrations has been reported to yield greater accuracy than that obtained with the microphonic manometer(8).

The present report is concerned with the relationship of these pulsatile vibrations, as seen by the naked eye, to blood pressure in the rat's tail as measured by other standard procedures. Thus, by simply observing the characteristic pattern of vibration in the tail, the indirect systolic pressure may quickly be determined without the use of expensive or complex equipment. This report also deals with comparative measurements of blood pressure after whole body warming versus tail warming, the influence of cuff length on blood pressure readings, and difficulties encountered following the use of vaso-constrictor agents.

Materials and method. 1. Description of the Visual Method. When the rat's tail is warmed for 5-10 minutes or the rat itself is placed in a ventilated incubator at 39°C for 15 minutes and a 20 mm cuff attached to a manometer is placed on proximal part of tail, pulsatile vibrations can readily be seen along the entire length of the tail. These movements are of maximal amplitude at the distal end of the tail which must be allowed to hang freely. If the cuff is then inflated to a pressure exceeding the systolic pressure, the vibrations cease. With slow deflation of the cuff the vibrations suddenly reappear and increase in amplitude as the cuff pressure approaches zero. The pressure at which the pulsations make their first reappearance is taken as the systolic endpoint. This point is sharp, reproducible within narrow limits, and easily seen by the inexperienced observer. Often the endpoint is heralded by an arching upward of the end of the tail, after which the vibrations immediately appear. Observations of minimal vibrations are facilitated by use of darkened room with a lamp placed so that a sharp shadow of the tail is cast on a white background one or two cm behind the tail. To avoid struggling and to limit extraneous movements, the animals are confined in a

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