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Research Article

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DIFFUSION OF GASES OUT OF THE DISTAL NEPHRON-SEGMENT IN MAN. I. NH_3*

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Physiologic variation in rate of urine flow in mammals is ascribed principally to reabsorption from distal tubules and collecting ducts of a varying quantity of nearly solute-free water (1–4). Water reabsorption is brought about by bulk flow or diffusion (5–8) incited by the difference in osmotic pressure between the luminal contents and the hypertonic fluid of the adjacent renal medullary interstitium, a process promoted by the action of endogenous vasopressin upon the permeability of the intervening membranes (7, 9). Elaboration of urine much less than maximally dilute increases the osmolality of luminal fluid as it progresses down the most distal portion of the nephron; the result must be to raise the diffusion pressure of all substances dissolved in luminal fluid.

Two highly diffusible substances, the gases NH_3 and CO_2, are present in solution in luminal fluid together with their hydrated forms NH_4OH and H_2CO_3, in turn in equilibrium, so as to constitute two buffer systems, with the ionized species NH_4^+ and HCO_3^-, respectively. If, as a result of the reabsorption of solute-free water, these gases are reabsorbed by diffusion, the existence and magnitude of the process should be reflected in alterations in the acid-base composition of voided urine no less than by changes in the rate of excretion of total ammonia and total carbon dioxide. Examination of the effects of water diuresis in normal man upon these various parameters has provided indirect evidence that the gases NH_3 and CO_2 do in fact diffuse out of the nephron's lumen during antidiuresis (10–12).[1] This communication presents evidence that the rate of urinary ammonia excretion is largely determined by factors affecting the net diffusion of the free base NH_3 into the distal portion of the nephron; the evidence further indicates that some NH_3, having diffused into the lumen, subsequently diffuses back into the renal interstitium as a result of reabsorption of water from the lumen.

METHODS

Experimental design. Subjects were 12 healthy adult male volunteers whose rate of urinary flow was caused to vary between 1 ml per minute and the physiologic ceiling by controlled water-drinking. Serial, timed urine collections were made at intervals of about 12 minutes by voluntary emptying of the bladder. The individual experiments, generally comprising 20 or more collections, were begun about 8 a.m. in subjects who fasted overnight and remained upright during and for at least an hour before the experiment. When alkaline or mark-

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[1] "Ammonia" means total ammonia. NH_3 includes NH_4OH when ratios of the buffer species are in question, and H_2CO_3 includes anhydrous CO_2 in the analagous context. NH_4^+ is not considered a form of buffered H^+ except that "total H^+" excretion includes NH_4^+.

It has been inferred from experiments on toad bladder and other membranes (5) that vasopressin promotes osmotic movement of water out of the nephron by hydraulic flow through pores rather than by diffusion. The facts about the toad bladder are disputed (8), and there is no direct information about the nephron. It is understood that postulated movement of NH_3 out of the nephron by "diffusion" might actually be caused by solvent drag. This is immaterial to the argument as long as NH_4^+ is restrained.

Although the term is anatomically objectionable (13), "nephron" will refer to the structure consisting of one collecting duct and its tributary tubules.

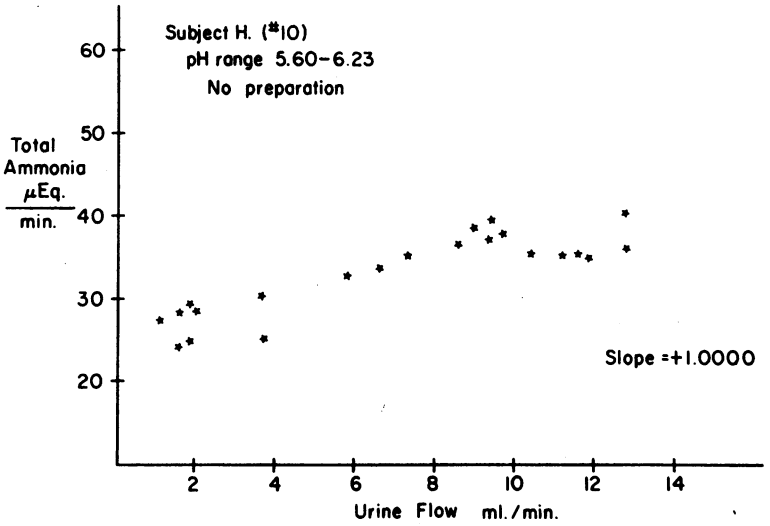

FIG. 1. DATA FROM A REPRESENTATIVE EXPERIMENT AT INTERMEDIATE RANGE OF URINARY pH. Treatment and range of pH, which varies with flow, are indicated at upper left. Points indicate ammonia excretion during a single collection period at the corresponding urinary flow rate; slope (lower right) represents the regression, in microequivalents per milliliter, calculated by the method of least squares as the straight line best fitting the data, of urinary ammonia excretion on urinary flow rate.

edly acid urines were desired, they were secured by premedication for not over 18 hours with sodium bicarbonate solution, or with ammonium chloride in gelatin capsules. Alternating 5- and 30-minute collections have also been made at flow rates of 2 to 5 ml per minute; diminished ammonia excretion was not seen in the longer collections, indicating that no appreciable ammonia is reabsorbed from the bladder under these conditions.

To assure that observed variation in the excretory rate of ammonia and other urinary constituents was related to flow rate and not to diurnal variation or other unsuspected factors connected with elapsing time, each experiment consisted of two diuretic peaks preceded and separated, and sometimes also followed, by low-flow collections. We took care to moderate proportional change in rate of flow in successive samples.

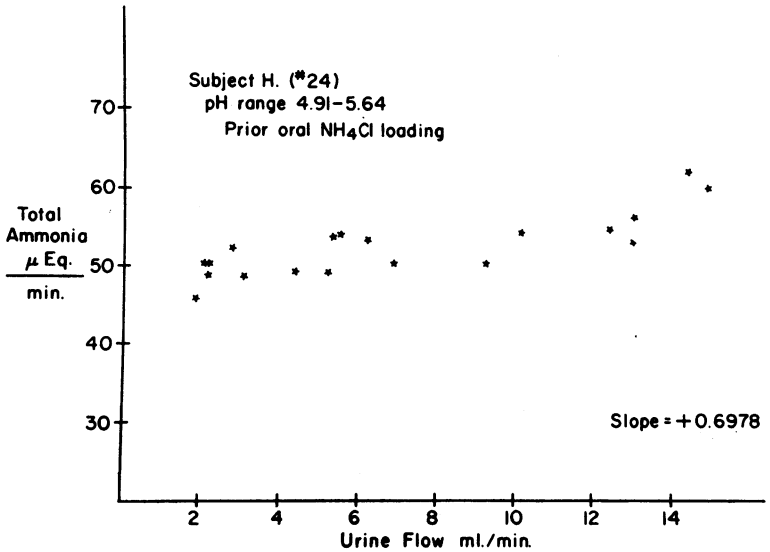

FIG. 2. DATA FROM A REPRESENTATIVE EXPERIMENT AT LOWEST RANGE OF URINARY pH. See details for Figure 1.

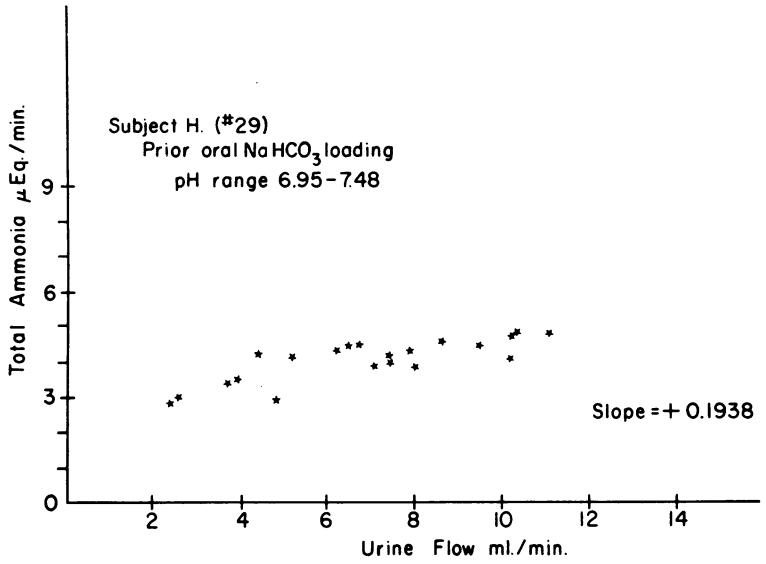

FIG. 3. DATA FROM A REPRESENTATIVE EXPERIMENT AT HIGHEST RANGE OF URINARY pH. See details for Figure 1.

The rate of total acid excretion in the more acid urines, estimated as the sum of the excretory rates of ammonia and titratable acid, varied little and inconsistently with flow (Table II). There was usually also no consistent variation of total acid excretion with time, although a few experiments showed a definite trend, also discernible in comparison of urines voided early and late at comparable flow rates. A trend downward of total acid excretion with time was considered to reflect a decreasing rate of H^+-secretion, related either to withdrawal of prior acid loading, or to some undetected cause, perhaps gastric secretion of HCl. To counteract this trend in experiments in which subjects had been premedicated with NH_4Cl, we gave a small amount of acid (40 μEq per minute) as NH_4Cl throughout the experiment in divided oral doses per period. Results were satisfactory as judged by near-constancy of total acid excretion throughout the experiment. Slightly greater amounts of alkali (100 μEq per minute) were usually given as $NaHCO_3$ throughout experiments conducted upon withdrawal of alkali-loading, with satisfactory results.

Analytical methods. Urinary pH and P_{CO_2} were determined under anaerobic conditions at 37° C immediately upon collection, with, respectively, a Metrohm capillary glass electrode [2] and the electrode [2] of Severinghaus and Bradley (14). All other analyses were made in duplicate and were discarded and repeated if agreement was unsatisfactory. Ammonia was determined promptly by microdistillation (15) from samples acidified and chilled immediately upon collection. Creatinine content (16) of all urine samples was determined, and regression of creatinine excretion on flow was always examined;

[2] Blood parameter analyzer, Epsco, Inc., Cambridge, Mass.

TABLE I

*Effect of urinary pH on slope of linear regression of urinary ammonia excretion on urinary flow rate**

Urine pH at max. diuresis	No. of experiments	Mean slope of regression of ammonia excretion on urine flow rate		Mean increment of ammonia excretion at maximal diuresis†	
		μEq/ml	*SEM*	*μEq/min*	%
<5.9	5	+1.0437	±.1164	13.4	28.4
	[8]	[+1.0090]	[±.1673]	[12.4]	[26.2]
5.9 to 6.5	7	+0.9952	±.0794	12.7	45.8
	[17]	[+1.0286]	[±.0774]	[13.5]	[51.2]
>6.5	8	+0.2890	±.0364	3.3	78.8
	[20]	[+0.3285]	[±.1774]	[3.9]	[71.9]

* The 20 experiments represented by unbracketed figures are considered technically above reproach. Estimates based on these differ little from estimates in brackets, based on all 45 experiments; recognized, minor deficiencies in a number of experiments (see footnote to Table II) were too trivial to affect the results materially.

† Derived from slope of regression and from range of flow rate in each experiment.

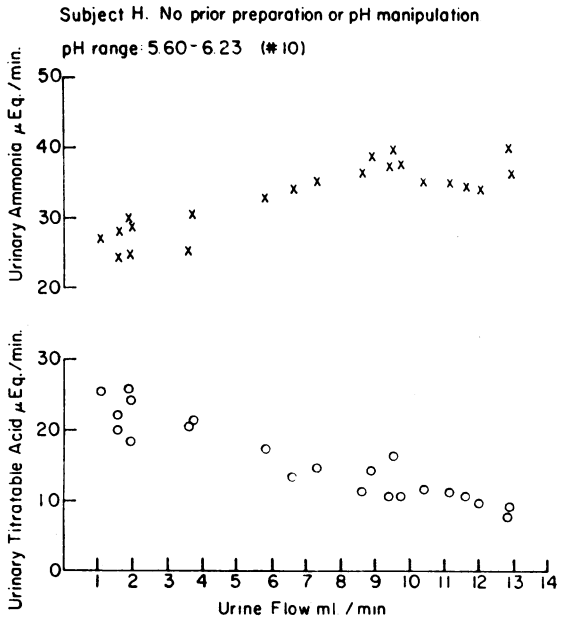

FIG. 4. EFFECT OF WATER DIURESIS ON RATE OF URINARY EXCRETION OF TOTAL AMMONIA AND OF TITRATABLE ACID. See also Table II.

there was no significant or consistent variation of creatinine excretion as a function of flow. Creatinine content of urine samples was used as an index of suboptimal bladder emptying, and correction to constant mean creatinine excretion was applied to the data relating excretion of ammonia and titratable acid to urinary flow rate where there was appreciable random variation of apparent creatinine excretion rate, and where comparison of corrected and uncorrected data showed that correction to constant creatinine appreciably reduced the variance of regression on urinary flow rate of urinary titratable acid (3 experiments) or ammonia excretion (12 experiments). Slopes of these regressions were neither markedly nor consistently affected by such correction. In the remaining experiments, no correction was applied to the regressions, except that any individual point was discarded if creatinine excretion deviated from the mean for the experiment by more than 20%.

Urinary "titratable acidity" refers here to residual titratable acid excretion (or titratable acidity minus CO_2), and was determined by titration to pH 7.4 after acidification and aeration on urine samples diluted *in vitro* to compensate for variable *in vivo* dilution; the end point was estimated by visual colorimetry, or by the Sargent model D automatic recording titrator, which also gave titration curves of urinary nonvolatile buffer.

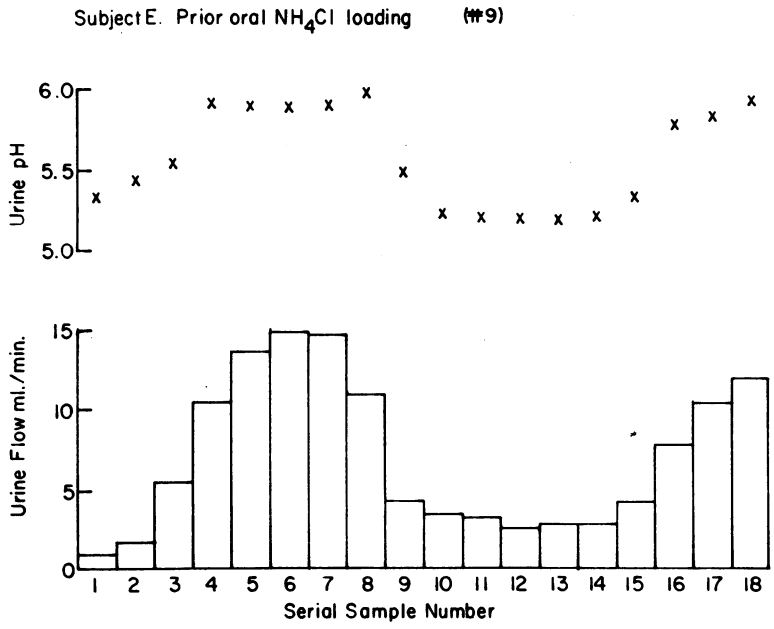

FIG. 5. EXPERIMENT IN A SUBJECT WITH MODERATELY ACID URINE BEFORE WATER-DRINKING AND WITH URINARY pH DEPRESSED BY PRIOR INGESTION OF AMMONIUM CHLORIDE. The parallelism between urinary flow rate and pH is representative for all experiments with similar initial pH. The graph also illustrates general design of all experiments reported here; serial samples along abscissa represent consecutive, quantitative urine collections at intervals of about 12 minutes.

TABLE II

*Effect of urinary flow rate on urinary excretion of titratable acid**

Experiment no.	pH range	A Slope of regression of ammonia excretion on urine flow rate*	B Slope of regression of titratable acid excretion on urine flow rate*	Algebraic sum of slopes A + B
10 H	5.5 to 6.1	+1.0000 (+0.716 to 1.284)	−1.3025 (−1.071 to −1.534)	−.3025
12 H	5.6 to 6.2	+1.0245 (+0.190 to 1.860)	−0.9505 (−0.017 to −1.735)	+.0740
15 S	5.3 to 5.9	+0.7053 (+0.122 to 1.288)	−0.9113 (−0.688 to −1.135)	−.2060
22 M	5.4 to 6.3	+1.7232 (+1.229 to 2.217)	−0.8784 (−0.081 to −1.749)	+.8448
24 H	4.8 to 5.6	+0.6978 (+0.613 to 0.782)	−0.8874 (−0.530 to −1.244)	−.1896
26 H	4.7 to 5.6	+1.6925 (+0.488 to 2.897)	−1.6061 (−1.084 to −2.128)	+.0864
27 E	5.6 to 6.3	+1.4131 (+1.096 to 1.729)	−0.8485 (−0.591 to −1.105)	+.5646
38 R	5.1 to 5.8	+0.9965 (+0.361 to 1.632)	−1.1771 (−0.619 to −1.735)	−.1806
40 R	4.7 to 5.3	+0.7113 (+0.394 to 1.029)	−0.4125 (−0.094 to −0.731)	+.2988
43 R	5.1 to 5.9	+1.1545 (+0.877 to 1.432)	−0.8360 (−0.515 to −1.157)	+.3185
44 R	5.9 to 6.3	+0.9797 (+0.273 to 1.686)	−1.2807 (−0.709 to −1.898)	−.3010
45 Z	4.9 to 5.7	+1.6583 (+0.918 to 2.398)	−1.9040 (−1.191 to −2.616)	−.2453
				Mean +.0635 (−.181 to +.308)

* 97% confidence limits are given in parentheses.

Data are summarized from all experiments with urinary pH not over 6.4 during maximal diuresis and with data on the excretion of titratable acid as well as ammonia. Experiments 22, 26, and 27 were technically substandard, either because there was a small downward trend with time of the sum of ammonia and titratable acid excretion, or because of nonuniform distribution of urine flows. If these experiments are excluded from the statistical analysis, the mean of the algebraic sums is −0.0815, with 95% confidence limits of −.212 to +.109.

RESULTS

Effect of urinary flow rate on urinary ammonia excretion

In 45 successive experiments, without exception, the rate of ammonia excretion has varied directly with urinary flow rate. Figures 1, 2, and 3 depict this relation as it was observed in three representative experiments in different pH ranges. The slope of the best-fitting straight line was invariably positive and almost always significantly so. A tendency was sometimes noted for the rate of increase of ammonia excretion with increasing flow to fall off as flow approached its ceiling, producing a small upward convexity of the curves. Transformations of the data did not approximate linearity, so comparison was made with the straight lines best fitting the data. Some nonuniformity may arise from this procedure and may affect comparison if points are not evenly distributed along the abscissa; nonuniformity of this distribution was therefore one criterion of exclusion from the 20 most satisfactory experiments analyzed (see footnote to Table II).

Table I shows the influence of urinary pH range on the slope of the linear regression of urinary ammonia excretion on urinary flow rate. Also shown is the mean increment of ammonia excretion as urine flow rate rises to its physiological ceiling (mean 13.0 ml per minute from about 1 ml per minute). This increment is relatively very small (3.3 μEq per minute) in the highest range of urinary pH, and much greater in both the intermediate and the lowest pH range

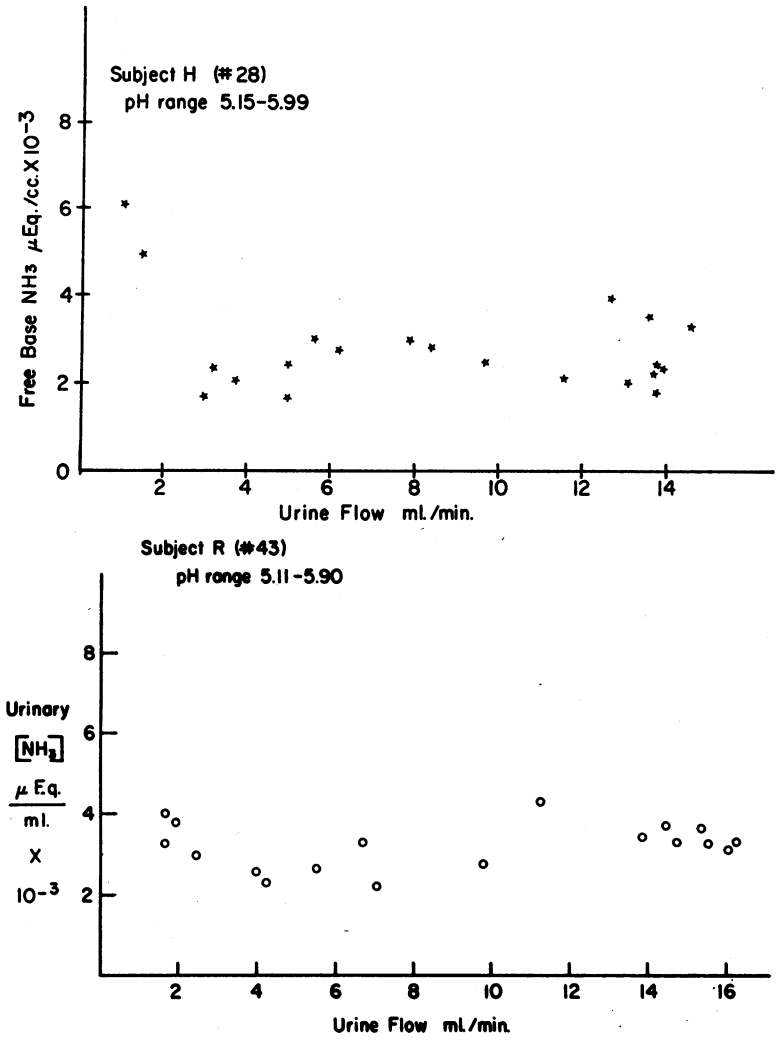

FIG. 6. EFFECT OF WATER DIURESIS ON URINARY FREE-BASE [NH_3]. Data are from representative experiments at lowest range of urinary pH.

(12.7 and 13.4 μEq per minute, respectively). The percentile increase of ammonia as flow rises, however, is greatest in alkaline and neutral urine (78.8%), where the absolute rate of ammonia excretion is very low, falling progressively to 45.8 and 28.4% in moderately and very acid urine, respectively, as increasing amounts of ammonia are excreted per minute.

Effect of urinary flow rate on urinary excretion of titratable acid and on urinary pH

Titratable acidity. Figure 4 shows data from an experiment representative of those in which maximal urinary pH is less than 6.4. Titratable acidity rises, as urine flow falls, by an amount approximately equivalent to the decrease of total ammonia excretion over the same range of urine flow. Table II presents values for the slopes of the regressions of ammonia and of titratable acid on flow in twelve successive experiments of this kind. The paired values for the slopes of the two regressions in each experiment are of similar magnitude if sign is ignored (see algebraic sums of slopes, Table II), and no paired value for any experiment differs significantly from its fellow, whereas all slopes differ significantly from zero. Three experiments are regarded as quantitatively less reliable than the other nine (see Table II).

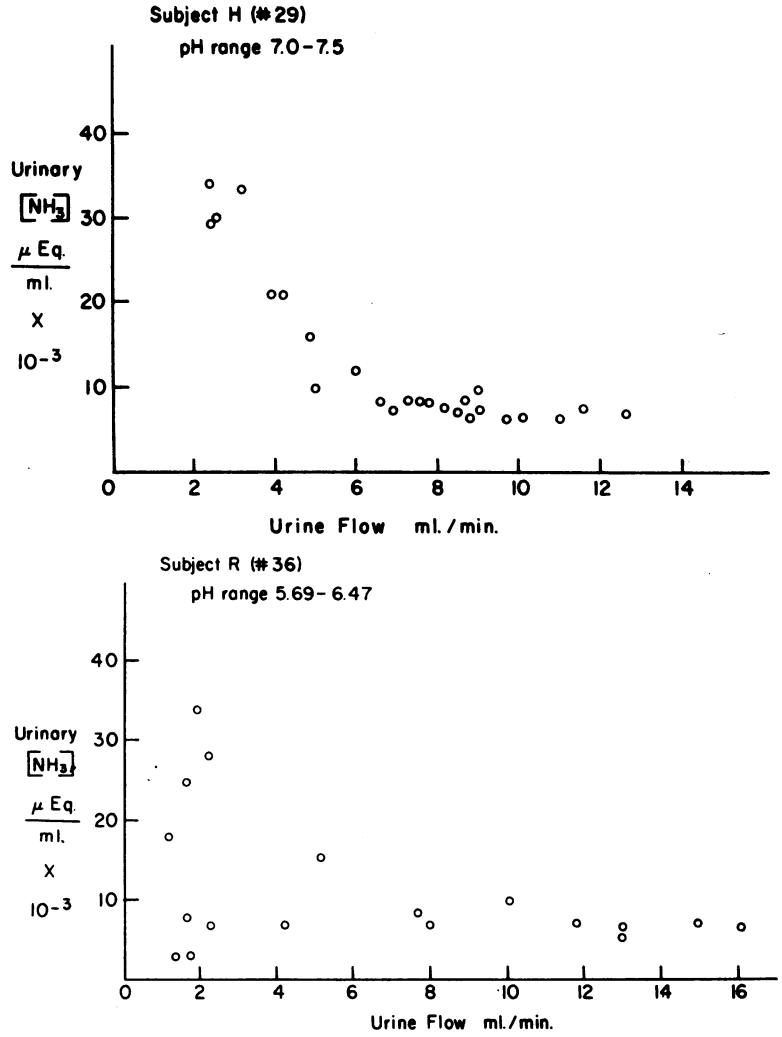

FIG. 7. EFFECT OF WATER DIURESIS ON URINARY FREE-BASE [NH_3]. Data are from experiments representative of those at intermediate and high ranges of urinary pH.

Urinary pH. In all of 26 experiments where urinary pH at high flow was below 6.6 at 37° C, [H^+] of urine, like the rate of excretion of titratable acid, varied as an inverse function of urinary flow rate. Change of urinary pH, with variation of urinary flow rate between 1 to 2 ml per minute and the ceiling, was of considerable magnitude, generally 0.5 to 1 pH unit; a typical experiment is represented in Figure 5. Observations recorded from time to time over the past 15 years (17–19) show that urine tends, irrespective of starting pH, to approach pH 6.9 at 25° C (or 6.6 at 37° C); the phenomenon has not been explained.

Effect of urinary flow rate on urinary NH_3 concentration

The concentration of the free base NH_3 in voided urine at 37° C has been calculated with the aid of the Henderson-Hasselbalch equation from concentration of total ammonia and pH at 37° C, deriving pK'_a from pK_a, 8.890 at 37° C (20), by applying the theoretical correction term for ionic strength (21) as confirmed experimentally by Bank and Schwartz (22). When the flow rate of urine nearly free of bicarbonate rises to 2 ml per minute, [NH_3] appears to approach a low, limiting value and is little affected by further rise

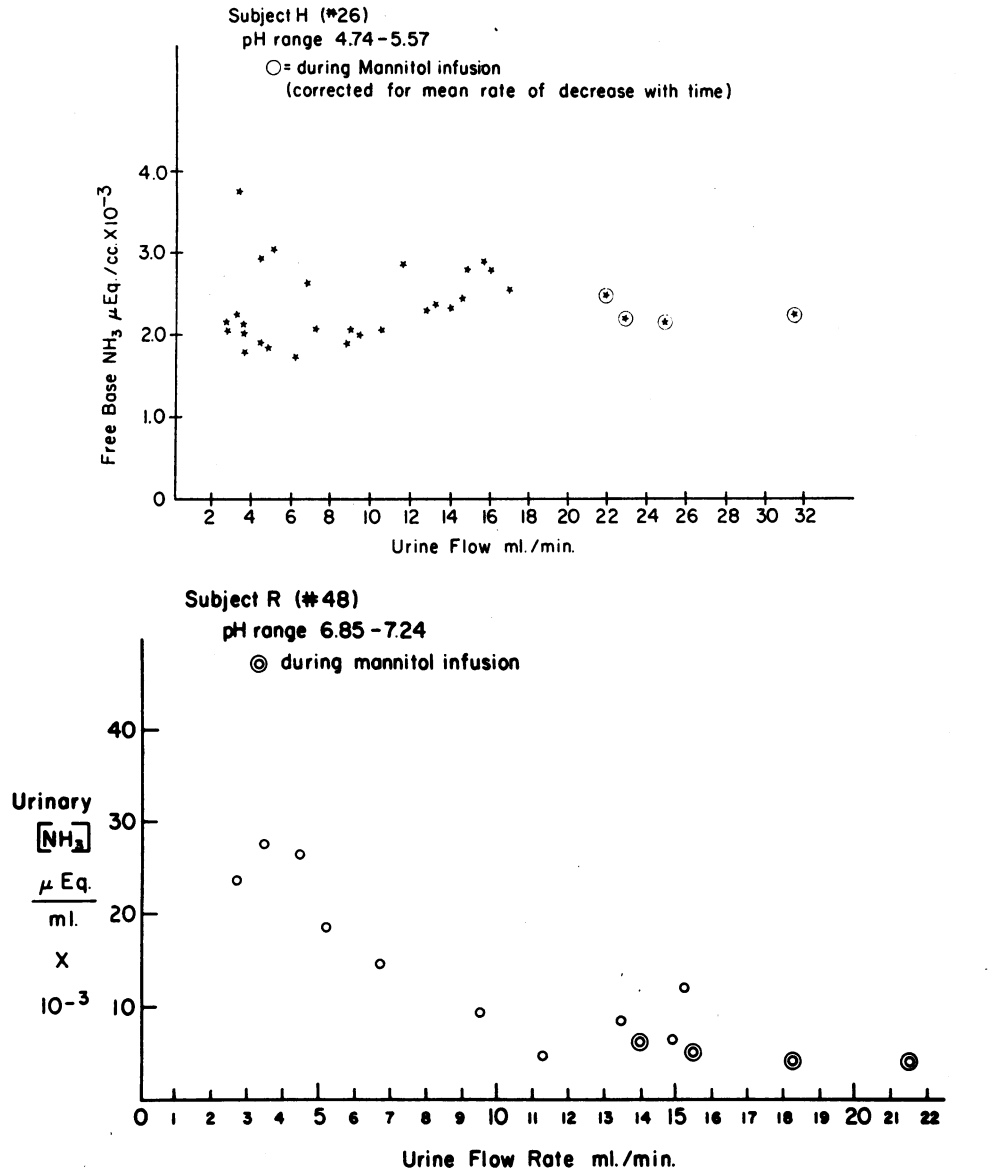

FIG. 8. CALCULATED FREE-BASE [NH_3] IN VOIDED URINE. Mannitol was infused intravenously after a second peak of water diuresis in order to push urinary flow rate well above the physiologic ceiling. Subject H was pretreated with NH_4Cl. During the 6-hour experiment (no. 26), a small decrease of [NH_3] occurred as a function of time, a decrease apparent in later low-flow as well as high-flow values. It was thought to reflect prior stimulation of renal deaminating activity, and has been corrected for.

in flow rate; two experiments representative of seven such observations are depicted in Figure 6.

In urine containing more bicarbonate, [NH_3] is higher, and the rise occurring at low flows is greater and begins at higher flow rates than with more acid urines; still, there appears to be little change of [NH_3] as flow rate rises above 4 to 7 ml per minute, depending upon urinary pH. Figure 7 shows two experiments representative of seven made at higher urinary pH.

In two of the experiments with acid urine, after attainment of maximal water diuresis, mannitol was infused intravenously in order to produce higher flows by osmotic diuresis. Little effect on

urinary [NH_3] was observed in either case as flow rose above the physiologic ceiling, to over 30 ml per minute in one experiment (no. 26, Figure 8). In another experiment (no. 48, Figure 8), done after the 45 experiments analyzed here and similar to the former experiment except that urinary pH was higher, osmotic diuresis again had no apparent effect on urinary [NH_3].

DISCUSSION

Effect of urinary flow rate on ammonia excretion

The data show that in normal man increasing urine flow rate by water-drinking increases urinary ammonia excretion, which confirms the most systematic studies previously reported of the effect of water diuresis on ammonia excretion in unprepared, healthy, fasting humans, those made 40 years ago by Hubbard and Munford (23, 24). Nevertheless, the uniform effect of flow on ammonia excretion, irrespective of starting urinary pH, is not at present generally recognized; possible reasons for seeming discrepancies between our findings and those of more recent investigators will be considered.

Prior reports in man. The most extensive of more recent relevant studies in man is that of Nutbourne and de Wardener (19), who concluded that water diuresis does not increase ammonia excretion. As will be noted, the rate of H^+-secretion influences urinary ammonia excretion and to a much greater extent than flow rate. If ammonia excretion is studied before and after induction of a single diuretic peak under experimental conditions that result simultaneously in a steady decrease of renal tubular H^+-secretion, the effect of flow on ammonia excretion is likely to be obscured (although its effect on H^+-excretion will be overestimated). Such was the case in the experiments of Nutbourne and de Wardener, in which renal tubular H^+-secretion must have been falling from two causes as urinary flow increased. One cause was doubtless the feeding, 1 hour before commencing observations, of a meal, which, because it stimulates gastric secretion of H^+, must cause temporary inhibition of renal tubular H^+-secretion to a variable degree in different subjects. Another cause was presumably related to the 6-day premedication with NH_4Cl up to 1 hour before the experiments; its withdrawal should occasion a progressive decline of total H^+ claiming renal excretion. Data for a representative experiment of Nutbourne and de Wardener show that a decrease of tubular H^+-secretion was indeed occurring during the course of the experiment; they show a decline amounting to fully 30 μEq per minute in the rate of elimination of H^+ (as NH_4^+ and residual titratable acid) over the 7 hours of observation of rising and falling flow. Similar declines occurred in the series of experiments as a whole. The same data show, nevertheless, that urinary flow increased on both control and experimental days and that the increase was attended both times by increasing urinary ammonia excretion during the period of rising flow, provided data are corrected for falling tubular H^+-secretion.

MacKnight, MacKnight, and Robinson (25) found urinary ammonia excretion consistently increased during water diuresis in alkaline urine, but were unable to demonstrate any effect of flow in acid urine except when flow fell below 1 ml per minute. Data summarizing individual experiments with acid urine are confined to one graph derived from an unprepared subject and one from a subject premedicated with NH_4Cl. In the first experiment, there is a continuous and unexplained fall of urinary pH, amounting to 2 pH U throughout the experiment; in the other, ammonia excretion appears to rise and fall with urinary flow. These investigators divided their data on more acid urines into two groups according to pH range and showed a single mean plot for each group consisting of pooled data for four or five experiments, each averaging about five individual urine collections. It seems not unlikely that the considerable variation of these mass plots may be ascribed to pooling of data for urine of varying pH, and this variation may have obscured an actual relation between flow and ammonia excretion.

The data reported by Richterich (26) are still scantier; this author reports that the rate of ammonia excretion is affected by urinary flow, but believes that in acid urine this effect occurs only in the range of low flow. Supporting data, however, are confined to part of one experiment. In the reports of Eggleton (27), Munck and Lassen (28), and Ryberg (29), the effect of water diuresis on ammonia excretion was of secondary

concern; in general, a relation to flow is discernible, but the data do not permit unequivocal interpretation.

Reports in other species. Recourse may of course be had to species differences to account for discrepancies with other data, but the published evidence for water diuresis exerting little or no effect on the rate of ammonia excretion in acid urine is not really convincing. Richterich (26) interpreted his data to mean that herbivores (rabbits and guinea pigs) differ markedly from man in their relation of urinary ammonia excretion to pH, and also that the effect of flow rate on ammonia excretion is slight in acid urine. Nevertheless, he states (26) that in the guinea pig "urine flow had a limiting effect at any urinary pH investigated." Orloff and Berliner (30) claimed that variations in the flow rate of acid urine in dogs (pH 6.2 or less) exerted no influence on the rate of urinary ammonia excretion "irrespective of the means of inducing diuresis," but their data did not include an examination of the effect of water diuresis on ammonia excretion.[3]

Interpretations of the present report. A. Urinary flow rate as a determinant of urinary ammonia excretion—NH_3 diffusion hypothesis. Changes in the rate of osmotic water reabsorption appear unlikely to entail directly any major change in the rate of "active transport" of ions or other solutes into or out of the nephron; indeed, the current view that urine is concentrated by reabsorption of "solute-free water" reflects the fact that no considerable change is generally recognized in the rate of excretion of urinary solutes, with the exception of urea, as urinary water excretion varies physiologically (1, 4). The first significance, then, of our finding, in 45 successive experiments, that urinary ammonia excretion varies with urine flow in man is that ammonia transport by diffusion is probably concerned. Evidently, as water is osmotically abstracted from the distal part of the nephron, either the solutes will be carried out with the reabsorbed water, or else, being restrained, their diffusion pressure must rise; in either case, any highly diffusible substance would probably be reabsorbed passively.

Urinary ammonia is known to have arisen *de novo* in the kidney as a result of deamination, principally of glutamine and, to a lesser extent, of certain amino acids brought to it by the circulation (31–33). The great diffusibility of the free base NH_3—greater than that of urea and nearly equal to CO_2 (34)—has already given rise to the diffusion hypothesis (35) of urinary ammonia excretion. According to this hypothesis, NH_3 diffuses through the renal parenchyma and penetrates into both the renal venous blood and into the acidified contents of the fluid within the lumen of the distal portion of the nephron. The strongest evidence for the diffusion hypothesis has been the urinary pH-dependency of urinary ammonia excretion: the more acid the urine, the greater the rate of urinary ammonia excretion (23, 24, 30, 36–40). More than 98% of the total ammonia present in fluid at any physiologic pH is in the form NH_4^+; most of the NH_3 diffusing into the nephron must therefore be instantaneously converted to NH_4^+, but the extent of this conversion will increase as urinary acidity increases, so that the total ammonia transported by the time $[NH_3]$ is about the same inside and outside the lumen will be directly proportional to $[H^+]$ of luminal fluid. The bulk of the ammonia is assumed to be transported at a site in the nephron at or below that where luminal contents are acidified; this process has been supposed to consist of exchange of the H^+ secreted for Na^+ reabsorbed (41) and assigned by most investigators to the distal tubule (42–47). That certain organic weak bases introduced into the body are eliminated in urine at rates varying directly with urinary $[H^+]$ supports the diffusion hypothesis of urinary ammonia excretion (30, 35).

B. *Further support for the diffusion hypothesis.* The equivalence between increased ammonia excretion and decreased titratable acid excretion that is observed uniformly (Table II) as urinary flow rate rises to its ceiling strongly supports the diffusion hypothesis. Since 99% or more of the ammonia present in any fluid of pH less than 7 is in the form of NH_4^+, it follows that as NH_3 diffuses into the lumen, it will be converted almost quantitatively to NH_4^+ by combining with H^+

[3] Orloff and Berliner (30) induced "water diuresis" in experiments with acid urines by administration of a "3 to 5 per cent glucose solution delivering 100 to 200 μM per min. of sodium sulfate." In the case of alkaline urines (where an effect of diuresis on ammonia excretion was recognized), "the sustaining infusion contained small amounts of $NaHCO_3$, Na_2HPO_4, or K_2HPO_4."

according to the reaction: $H^+ + NH_3$ diffusing in $\rightarrow NH_4^+$. Where the bicarbonate content of urine is low enough to be neglected,[4] change of the rate of H^+-excretion with changing urinary flow (NH_4^+ taken not to be a form of H^+) can be measured as titratable acid, and equivalence between increasing total ammonia excretion and decreasing titratable acid excretion should be seen as urine flow rises, provided transport of all the ammonia into the nephron is occurring by means of diffusion of NH_3. If ammonia were transported by active secretion of NH_4^+, as has also been suggested (48, 49), no such equivalence would be expected, nor would there be evident reason for the enhancing effects of either increased acidity or increased flow rate of urine upon urinary ammonia excretion.

Kinetic aspects of diffusion of NH_3 into the nephron

NH_3 might diffuse from the medullary interstitium into the nephron's lumen until the concentrations inside and outside approach equilibrium; conversely, accumulation of ammonia in the lumen might be limited because the rate of flux of NH_3 from the sites of its production in the renal parenchyma to the luminal fluid is insufficient to allow diffusion to proceed to equilibrium in the time required for luminal fluid to traverse the distal nephron segment. It has also been suggested (30) that accumulation of ammonia in luminal fluid might be limited by the rate of ammonia production when urinary pH is 6.2 or less.

From the parallelism between the rate of excretion of urinary ammonia and the flow of urine at higher pH (> 6.2), recent proponents of the diffusion hypothesis have assumed (26, 30, 35) that under these circumstances equilibrium conditions prevail across the nephron wall in regard to NH_3. The assumption is doubtless correct, although, as will be shown, the conclusion need not follow from the premise. If equilibrium conditions are assumed, it follows that water reabsorption distal to the locus of urine acidification by H^+-secretion would cause NH_3 to diffuse back out of the nephron's lumen unless there were a simultaneous, comparable rise of interstitial [NH_3] radially toward the papilla, or the nephron-segment distal to the site of NH_3 equilibration became relatively impermeable to NH_3. Evidence against the former assumption will be presented.

Diminished permeability of the terminal nephron-segment to NH_3? This assumption was adopted by Milne, Scribner, and Crawford (35) in their mathematical treatment of the diffusion hypothesis, principally on the basis of data reported by others as showing that the flow rate of acid urine has no effect on ammonia excretion rate. Our data confirm prior evidence (23, 24) that this is untrue, at least in man. The assumption that the terminal nephron-segment is poorly permeable to NH_3 is also incompatible with the near-constancy of urinary [NH_3] at all flow rates studied greater than 2 to 7 ml per minute (Figures 6–8), from which it also follows that urinary [NH_3] reflects interstitial [NH_3] in the region of the terminal nephron-segment, unless urinary [NH_3] changes beyond the papilla.

Limitation on accumulation of NH_3 in the lumen? The idea that accumulation of ammonia in the nephron is limited during elaboration of urine of pH less than 6.2 by the rate of renal ammonia production was also based on the hypothesis that water diuresis will not increase ammonia excretion under such conditions (30). There was never any sound experimental basis for this hypothesis, which would be very hard to reconcile with the large increase of urinary ammonia excretion resulting promptly when pH falls well below pH 6.2.

Limitation of diffusion? Accumulation of ammonia in the lumen might be restricted, however, if the rate of flux of NH_3 into the lumen were too slow to permit establishment of equilibrium conditions within the time available for luminal fluid to pass through the distal nephron-segment.

[4] All urine contains bicarbonate, but the amount is small in urine of pH 6.0 and below, and rises ever more rapidly with rising urinary pH. Below what pH urinary HCO_3^- is negligible depends on the issue under consideration. For demonstrating equivalence of change of the excretion rates of ammonia and titratable acid, as in Table II, urine up to about pH 6.3 at maximal flow can be considered to contain negligible HCO_3^-; for qualitative rise of pH with diuresis, experiments will serve in which the pH of high-flow urines is 6.5 or less at 37° C. For exact calculations of the nephron's "H^+ balance" with varying flow, HCO_3^- is negligible only when urinary pH at maximal flow is below 6.0.

There would then exist an appreciable NH_3 concentration gradient between the renal parenchyma and the luminal fluid. The parenchymal $[NH_3]$ would then be higher than under equilibrium conditions, and this would favor removal of ammonia through diffusion of NH_3 into renal effluent blood, thus assisting the renal veins in their competition with the urine for removal of the ammonia produced in the kidney.

Limitation of the rate of diffusion of NH_3 into the nephron sufficient to prevent attainment of equilibrium must become more and more conspicuous as the quantity of ammonia being transported into the nephron's lumen per minute rises. No significant limitation of transport of available NH_3 exists unless $[NH_3]$ inside and outside the lumen are not close to equilibrium by the time luminal fluid has reached the terminal nephron-segment. Any NH_3 concentration gradient developing from renal parenchyma to lumen must be maximal during excretion of very acid urine, and limitation of diffusion could not be said to be very important for renal ammonia excretion unless under these conditions of maximal ammonia transport, $[NH_3]$ of the interstitial water were well above that of the urine at the papilla.

Diuresis could increase ammonia excretion under such conditions by increasing the volume of luminal fluid per minute into which diffusion can occur; for practical purposes, this volume is given by the urinary flow rate. Since according to Fick's law of diffusion the rate of flux of a diffusing solute is proportional to the concentration difference across the area of diffusion, one would expect ammonia transport to be increased to the extent that the lowering of luminal $[NH_3]$ during diuresis increases the integrated NH_3 concentration difference across the nephron's wall. The lowering of urinary $[NH_3]$ by diuresis, however, is slight at most in urine of any pH. The largest proportional decrease of urinary $[NH_3]$ that might be concealed in the scatter of the points as the flow rate of acid urine rises progressively above 2 to 4 ml per minute (Figures 6–8) could represent an important increase in the concentration difference across the luminal wall only if urinary $[NH_3]$ were already very close to interstitial $[NH_3]$.[5] This would mean, however, that limitation of diffusion was of little or no significance under the conditions where it would have to be most conspicuous. The scant effect of raising urinary flow rate above 2 to 4 ml per minute upon urinary $[NH_3]$ therefore argues strongly against any important limitation on diffusion of NH_3 from its sources to a concentration inside the lumen approximating that outside it. Equilibrium conditions must certainly prevail at high rates of urine flow, at least during elaboration of urine of intermediate to maximal pH; the argument for back-diffusion of NH_3 at lower flows becomes increasingly compelling as the quantity of NH_3 that must be transported over the same gradient into the lumen diminishes sharply with rising urinary pH.

Evidence for back-diffusion of NH_3. A. *Topography of urine acidification by H^+-secretion in relation to final water reabsorption.* The argument for diffusion of NH_3 out of the terminal nephron-segment depends upon the validity of the view that urine acidification by H^+-secretion is effected mainly at a site proximal to completion of urine concentration by water reabsorption. The latter process occurs throughout the collecting duct, at least during antidiuresis (4), whereas acidification by H^+-secretion proper has been as-

[5] The following sample calculations clarify this point. Suppose interstitial $[NH_3]$ to be 1.5 μEq per L and luminal $[NH_3]$ to be 1.0 μEq per L at the papilla when urine is maximally acid. Suppose $[NH_3]$ of the luminal fluid of the terminal nephron-segment drops 10% when the urinary flow rate of very acid urine rises from 2 to 14 ml per minute; no more is compatible with the effect on urinary $[NH_3]$ of such an increase in flow rate as the experimental series represented by Figures 6 and 8A show. It is easily calculated that there will result a maximal increase of 20% over the initial value of the concentration difference across the luminal wall. (Since urinary $[NH_3]$ scarcely changes, urinary NH_3 excretion increases almost 7-fold, but urinary ammonia excretion increases much less, Table I shows, owing to the associated rise of urinary pH). Conversely, suppose interstitial $[NH_3]$ under the same conditions to be four times luminal $[NH_3]$, and suppose the same effect (a 10% decrease) of rising flow rate on luminal $[NH_3]$. The initial NH_3 concentration difference across the luminal wall is now 3 μEq per L, and it will increase 3.3% to 3.1 μEq per L). The evidence therefore indicates that the maximal decrease of urinary $[NH_3]$ possible to conceal in the scatter of urinary $[NH_3]$ of acid urine, as flow rises above 2 ml per minute (Figures 6 and 8A), could reflect a sufficient increase of NH_3 concentration difference across the luminal wall to yield the observed increase in urinary ammonia excretion only if equilibrium conditions had already been approximated.

signed mainly to the distal tubules (42–44, 46, 47).

Direct analysis has recently shown that luminal fluid is further acidified within the collecting ducts (45, 46, 48), but the process does not necessarily consist entirely or principally of H^+-secretion proper. Ullrich and his colleagues found that H^+ and ammonia were added to, and Na^+ reabsorbed from, the lumen of the nephron between the farthest zone to which the catheter could be passed and a site in the collecting duct located closer to the papilla. Urine collected through the bladder from the uncatheterized kidney, however, was regularly more acid (in 23 of 24 experiments) than fluid simultaneously collected through a catheter with its tip placed at locations nearer the papilla (45), whereas ammonia concentration at these latter sites was higher than that of bladder urine in 2 out of 8 experiments and of the same order of magnitude in 3 to 4 of the remaining 6 experiments (48). Water was being reabsorbed from the collecting duct, although the experiments were made under diuretic conditions (48). H^+-generation resulting from diffusion of NH_3 out of the luminal fluid most directly explains about half of these data, although the authors did not so conclude; the other half would be expected if both H^+-generation owing to back-diffusion of NH_3 and H^+-secretion occur distally to the region sampled.

These experiments were performed on the golden hamster, whose exceptionally well-developed collecting ducts and single papilla permit passage of catheters deep into the inner medulla. In this species, and perhaps in others with anatomically similar kidneys, the prominent collecting duct may take over a considerable portion of H^+-secretion reserved to the distal tubule in other species. The experiments described were conducted under conditions of forced diuresis and usually of acidosis as well, so reabsorption of NH_3 might possibly or even probably begin considerably higher in the collecting duct during antidiuresis in hamsters. The postulated generation of H^+ through back-diffusion of NH_3 can fully account for the apparent discrepancies in the locus of H^+-secretion between the reports of Ullrich and his colleagues (45, 48) and those of investigators working with other mammals and with amphibia (42–44, 47).

Gottschalk, Lassiter, and Mylle (46) have recently reported that the pH of luminal fluid obtained by micropuncture from nephrons of rats declines in the collecting duct, and that the fall is much greater during antidiuresis. Taken together with the evidence presented here that variation of urinary flow rate in man affects neither total H^+-elimination nor H^+-secretion proper, the latter observation suggests that acidification of collecting duct fluid is due to H^+-generation resulting from diffusion of NH_3 out of the collecting duct rather than to H^+-secretion proper.

B. *Summarized evidence for back-diffusion of NH_3.* We have rejected the hypothesis of limitation of diffusion in favor of the view immediately suggested by the near-constancy of values for urine $[NH_3]$ above flow rates of 2 to 7 ml per minute, namely, that NH_3 diffuses to equilibrium in the nephron's lumen at and below the site of H^+-secretion proper, and that equilibrium conditions are thereafter maintained as the luminal fluid proceeds to the papilla. The constancy of the sum of titratable acid and ammonia excretion in nearly bicarbonate-free urine will also be maintained as NH_3 diffuses back: $NH_4^+ \rightarrow H^+ + NH_3$ (diffuses off).

Water could be reabsorbed from the NH_3-permeable terminal nephron-segment without causing back-diffusion of NH_3, provided the interstitial $[NH_3]$ rose toward the papilla so as to equal or exceed the rise in luminal $[NH_3]$ that would be effected by reabsorption of truly "solute-free" water in the amount reabsorbed from that segment. Our data cannot exclude this possibility, nor can they explain the progressive rise of urinary $[NH_3]$ occurring, more sharply in alkaline urine, as urinary flow falls below a critical value, which is influenced by urinary pH (Figures 6–8). Quantitative kinetic analysis of these and other data (50) is required to explain why $[NH_3]$ rises conspicuously in low-flow urine.

Additional evidence indicates back-diffusion of NH_3 during the excretion of nearly bicarbonate-free urine, and more of it as urine flow falls. By the equilibrium back-diffusion model, there must be a point in the distal nephron beyond which H^+ simultaneously appears in luminal fluid as an equivalent amount of ammonia disappears from it; also, back-diffusion of NH_3 in proportion to water reabsorption should result in increased

acidity of collecting duct fluid during antidiuresis. We have cited evidence indicating that both these phenomena occur (45, 46, 48). This evidence is difficult to reconcile either with limitation of diffusion or with a rise toward the papilla of interstitial [NH_3] sufficient to prevent back-diffusion, since in both cases H^+ should be consumed instead of generated in the collecting duct as NH_3 continued to diffuse into the luminal fluid.

Relation between change of residual titratable acid and change of urinary pH with changing urinary flow rate

Nutbourne (51) has drawn attention to the nature and magnitude of the rise of urinary pH that is ascribable to the purely physicochemical effect of dilution; this phenomenon is caused by change in the activity coefficient of phosphatic and other urinary buffer ions as predicted by the limiting equation of Debye and Hückel. To ascertain the contribution of this physicochemical effect to the rise of urinary pH produced by water diuresis, we diluted low-flow urine samples, from five experiments with very acid urine, immediately after voiding with water *in vitro* so as to simulate the difference in the volume voided per minute over the range of flow observed in the corresponding experiments. The resulting increase in *in vitro* pH was calculated for each experiment from pooled, multiple, linear regressions of pH on the logarithm of sample dilution; it averaged 0.095 pH U (range, 0.072 to 0.117). The *in vitro* change averaged 14% and never exceeded 18% of the difference in pH between low-flow and high-flow urine.

SUMMARY

1. Urinary excretion of total ammonia regularly rises, irrespective of initial urinary pH, when urine flow of healthy men is caused to rise from around 1 ml per minute to the physiologic ceiling by water-drinking. The absolute increase during diuresis is considerably greater in acid than in alkaline urine; the percentile increase is greatest for the highest urinary pH range (neutral to alkaline), decreasing progressively as urinary pH declines.

2. When urinary pH is less than 6.4 during water diuresis, the increase of ammonia excretion is accompanied by a decrease, approximately chemically equivalent, in the rate of excretion of residual titratable acid. The equivalence supports the view that the rate of elimination of ammonia in the urine is governed by the amount of net diffusion of NH_3 out of the renal parenchyma into the fluid passing through the lumen of the distal portion of the nephron, since enhanced net diffusion of NH_3 into the lumen as urinary flow increases will diminish H^+-excretion by converting H^+ to NH_4^+ while simultaneously increasing total ammonia excretion.

3. Urinary pH varies with urinary flow rate, tending always to approach 6.6 at 37° C as flow rises. When the pH of low-flow urine is 6.0 or less, it increases about 0.5 to 1.0 pH U as flow rate rises from 1 ml per minute to its ceiling. Less than 20% of the change can be ascribed to the physicochemical effect of dilution; the remainder appears to be due entirely to increased net diffusion of NH_3 into luminal fluid.

4. Urinary concentration of free-base NH_3 is little affected when the flow rate of neutral to alkaline urine rises above about 4 to 7 ml per minute, or the flow rate of acid urine above about 2 ml per minute, to and well beyond the physiologic ceiling of water diuresis. The findings are incompatible with impermeability to NH_3 of the terminal nephron-segment, where water reabsorption is completed, and indicate equilibration under these conditions of [NH_3] of the luminal fluid with that of the adjacent interstitial water. Semiquantitative treatment of the data provides evidence, reinforced by more direct published data, indicating that NH_3, after diffusing to equilibrium in the acidified luminal fluid of the distal nephron-segment, subsequently diffuses out of the terminal nephron-segment as a result of and in proportion to osmotic reabsorption of "solute-free water."

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