Journal of Clinical Investigation
Vol. 45, No. 3, 1966

The Functional Adaptation of the Diseased Kidney. III. Ammonium Excretion *

E. J. DORHOUT-MEES,† M. MACHADO,‡ E. SLATOPOLSKY, S. KLAHR, AND N. S. BRICKER §

(From the Renal Division, Department of Internal Medicine, Washington University School of Medicine, St. Louis, Mo.)

Ammonium excretion by the mammalian kidney is a rate-limited process that involves the *de novo* synthesis of NH_3 by the renal tubular epithelial cells and the subsequent transfer of the ammonia from cell water to tubular fluid. Biologically, this process serves to increase markedly the capacity of the kidney to excrete hydrogen ions, for NH_3 is a strong base, and in an acidic urine virtually all of the NH_3 molecules will combine with hydrogen ions to form NH_4^+. This in turn will contribute to the maintenance of a gradient favoring further secretion of protons.

In health, ammonium excretion accounts for a significant fraction of the total hydrogen ion excreted in the urine, and in acidosis the rate of NH_4^+ excretion increases strikingly. However, if acidosis is sustained, ammonium excretion will reach a plateau within a few days, and the excretion rate will not increase spontaneously thereafter (1). On the other hand, if amino acid precursor substrates, such as alanine and glutamine, are infused after the excretion rate has reached its apparent maximum, a substantial increase in excretion will occur (2).

In chronic renal disease, ammonium excretion rates are diminished despite the presence of chronic acidosis (3). Uncertainty persists, however, about the genesis of this decrease. Obviously the upper limit of ammonium excretion achieved by any kidney cannot exceed the average maximal rate of excretion per nephron times the number of urine-forming nephrons, and it is implicit in the nature of the progressive renal diseases that the total nephron population diminishes with time. It is not clear, however, whether ammonia synthesis in the surviving nephrons is impaired, normal, or, as Elkinton (4) has suggested, supernormal.

We have attempted to examine this question using an experimental model. Ammonium excretion was measured in dogs with chronic renal disease (i.e., pyelonephritis). Experiments were performed initially when the diseased kidney functioned in concert with a contralateral kidney that was free of disease and hence retained its full complement of nephrons. The control kidney then was removed, and the ammonium excretion rate of the same diseased kidney was measured again. Measurements also were made of the response to the infusion of amino acid precursors of ammonia synthesis before and after removal of the control kidneys. We believe that the data support the view that the average rate of ammonium excretion per nephron increased adaptively to supernormal levels in the residual nephrons of the pyelonephritic kidneys.

Methods

Thirty-seven experiments were performed on ten female mongrel dogs ranging in weight from 10 to 20 kg. In each animal, the urinary bladder was divided into two permanent hemibladders (5) as a preliminary procedure. The terminology used to designate the three successive stages of study has been presented previously (6); a summary is included below.

* Submitted for publication June 25, 1965; accepted November 12, 1965.

Supported by U. S. Public Health Service research grant AM-02667; Department of the Army, Research and Development Branch contract DA-49-007-MD-772; and a U. S. Public Health Service training grant.

† Present address: Academisch Ziekenhuis, Utrecht, Holland.

‡ Formerly research fellow, Rockefeller Foundation. Present address: Hospital das Clinicas, Faculdade de Medicina, São Paulo, Brazil.

§ Career Award recipient, U. S. Public Health Service. Performed during the tenure of an Established Investigatorship of the American Heart Association.

Address requests for reprints to Dr. Neal S. Bricker, Renal Division, Washington University School of Medicine, 660 South Euclid, St. Louis, Mo. 63110.

Stage I refers to the initial studies performed in each dog before the induction of renal disease. These experiments were done to establish the patterns of function of the two kidneys before there was any reduction of nephron population.

Stage II refers to the studies performed after chronic pyelonephritis had been induced in the experimental kidney. The mechanism of induction of the lesion and the interval between induction and study have been described in a previous publication (6). In the stage II studies the diseased and the contralateral control organs were investigated simultaneously.

Stage III refers to the studies performed after the control organ had been removed surgically, when the residual nephrons of the diseased kidney were charged with the entire responsibility for the renal regulation of acid-base balance. Stage III studies were performed from 4 to 27 days after removal of the control organs.

At each stage of study, metabolic acidosis was induced by the exogenous oral administration of ammonium chloride for at least 3 days. In stage I, the dose ranged from 4 to 8 g per day depending upon body weight; in stage II the same or a slightly smaller dose was employed; in stage III the dose of ammonium chloride required to achieve the same degree of metabolic acidosis generally was substantially less. The dose was adjusted to maintain CO_2 content at about 15 mmoles per L or pH at 7.2 ± 1.0 or both. The animals were fed the same diet throughout the studies.

The dogs were studied in the postabsorptive state without anesthesia. Blood was obtained through an indwelling femoral arterial catheter, and samples were drawn continuously during each urine collection into heparinized tubes containing mineral oil. Urine also was collected anaerobically under oil into sterile tubes.

Glomerular filtration rate was measured by the exogenous creatinine clearance. The creatinine was infused in a 5% mannitol vehicle containing sodium phosphate (30 mmoles per L) buffered to pH 7.4. The rate of infusion was 4.8 ml per minute in stages I and II, but was decreased usually by 50% in stage III (6). At least three clearance periods of 10 to 20 minutes were obtained before infusion of the amino acid precursor, and an additional three or more periods were obtained to assess the effects of the amino acids. Creatinine was determined according to the method of Bonsnes and Taussky (7). Ammonia was determined in duplicate or triplicate on all samples by the method either of Nathan and Rodkey (8) or of Conway (9). Analyses of ammonia were begun immediately after the samples had been collected, and the measurements were made with acid-cleaned glassware in a room containing no stored urine. Titratable acidity also was measured on the urine samples according to the technique previously described (10) using a Radiometer automatic titrator. Arterial pH was determined with an IL meter, model 113, with a 37° C water bath.

In studying ammonium excretion rates by animals with permanent hemibladders, attention must be given to the possibility that ammonia may be added to the urine as it passes through the bladders by virtue of bacterial degradation of urea. To minimize this potential source of contamination, we maintained a program of rigorous bladder care. This involved frequent rinsing of the hemibladders, particularly before each experiment. In stages I and II, the equality of ratios of urinary ammonium and titratable acid excretion to glomerular filtration rate ($U_{NH_3}V$/GFR and $U_{TA}V$/GFR) between the two kidneys of individual animals made the possibility of random NH_3 contamination very unlikely. However, the primary questions asked in these studies made it particularly important to exclude contamination in the stage III experiments. Therefore, an increase in ammonium excretion rate between stages II and III was accepted in any given animal only if the following criteria were met: *1)* $U_{NH_3}V$ showed no tendency to decrease with consecutive clearance periods; *2)* the increase in NH_4^+ excretion in stage III did not occur at the expense of a decrease in $U_{TA}V$ as would occur if NH_3 were added distal to the nephron; and *3)* the urine pH did not increase by more than 0.8 U between the stage II and III studies.

TABLE I

*Values for GFR, $U_{NH_3}V$, and $U_{TA}V$ in experimental and control kidneys in stages I and II**

	GFR				$U_{NH_3}V$/GFR				$U_{TA}V$/GFR			
	Stage I		Stage II		Stage I		Stage II		Stage I		Stage II	
	E	C	E	C	E	C	E	C	E	C	E	C
	ml/min				*μEq/100 ml*				*μEq/100 ml*			
Mean	41.5	40.3	14.7	44.7	132	130	163	159	106	96	124	141
Range	(25.6–57)	(22.4–59)	(4.3–32.3)	(27.5–52.2)	(81–184)	(72–195)	(105–210)	(106–219)	(46–171)	(21–149)	(37–187)	(70–191)

* In stage I, both the experimental and the control kidneys (designated as E and C, respectively) were free of disease. In stage II the experimental kidney was diseased. The data in the Table represent values from nine of the ten dogs included in this series. The tenth dog (dog 8, Tables II and III) was excluded from these calculations because the ammonium and titratable acid values for the control kidney in stage II were lost due to a laboratory accident. In order not to bias the comparison between the diseased and control kidneys, we also omitted the data for the diseased kidney. The values for stage I for dog 8 were as follows: For the experimental kidney: GFR 43.6, $U_{NH_3}V$/GFR 139, $U_{TA}V$/GFR 139; for the control kidney: GFR 45.5, $U_{NH_3}V$/GFR 150, $U_{TA}V$/GFR 134. The values for the diseased kidney in stage II are included in the subsequent Tables. GFR = glomerular filtration rate; $U_{NH_3}V$ = rate of ammonium excretion (microequivalents per minute); $U_{TA}V$ = titratable acid excretion (micromoles per minute).

TABLE II

*Value for GFR, $U_{NH_3}V$, $U_{TA}V$, and blood and urine pH in the three successive stages of study in the experimental (diseased) kidneys only**

Dog	GFR					$U_{NH_3}V$								$U_{TA}V$			Blood pH			Urine pH		
	I	II	III		II to III	I	II	III	II to III	I	II	III	II to III	I	II	III	I	II	III	I	II	III
	ml/min			*days*	% Δ	*μEq/min*			% Δ	*μEq/100 ml GFR*			%Δ	*μEq/min*								
1	48.7	32.3	45.4 37.3	(4) (11)	15.4	47	45	56.3 51.7	15	97	138	124 139	0	39	55.4	54.4 88.3	7.32	7.13	7.18† 7.30	5.90	5.39	5.19 5.95
2	25.6	11.4	16.1	(5)	41.2	29.1	13.6	43.3	218	112	120	270	+125	11.5	9.6	12.6	7.27	7.19	7.23	6.05	5.83	6.53
3	33.1	6.8	12.3	(5)	80.9	53.4	21.1	46.1	119	162	242	374	+ 55	32	3.0	27.8	7.28	7.13	7.22	4.88	6.89	6.69
4	40.4	19.1	27.3 27.4	(18) (27)	43.1	50.8	20.1	70 44	119	126	105	253 160	+52	28.8	13.1	37.0 28.6	7.24	7.30†	7.22 7.27	5.33	5.40	6.24 6.25
5	28.5	8.8	10.1	(12)	14.8	52.4	18.1	19.6	8.3	184	206	195	−5.4	48.7	15.2	30.8	7.23	7.03†	7.14	5.22	5.39	5.73
6	57.4	8.1	10.6 18.7	(6) (29)	131	72.9	15.6	29.6 23.5	50	124	193	279 125	−35	50.7	11.2	23.3 29.4	7.29	7.26	7.15 7.30	5.70	6.10	5.80 5.66
7	37.0	17.0	14.8 14.8 16.7	(4) (5) (17)	−1.8	56.9	25.6	27.3 21.8 53.6	109	154	140	194 147 321	+123	58.3	22.0	32.7 33.6 23.7	7.27	7.31	7.17 7.22 7.11	4.81	5.77	5.40 5.16 6.18
8	43.6	20.2	26.0 25.8	(12) (16)	27.7	60.5	48.5	51.1 58.1	19.8	139	240	196 225	−6.3	60.5	35.8	49.1 40.7	7.34	7.16	7.20 7.18	4.86	5.88	5.27 5.36
9	56.4	4.3	10.5 10.5	(9) (12)	144	45.8	9.31	16.6 17.6	89.2	81.2	210	157 167	−20.4	63.3	1.63	22.5 26.7	7.39	7.22	7.18 7.23	5.0	6.6	5.37 5.47
10	43.9	24.8	27.8	(11)	12.1	62.0	30.5	81.5	167	151	123	287	+134	61.0	47.6	76	7.31	7.31	7.29	5.60	5.16	5.98
Mean	41.5	15.3	20.3		50.8	53.1	24.7	43.9	91.4	133	172	226	42.2	45.4	21.5	38.5	7.29	7.20	7.23	5.34	5.84	5.98

* The Roman numerals refer to stages I, II, and III as defined in the text. The figures in parentheses after the stage III values for GFR refer to the days that elasped after removal of the control kidney. The percentage change represents the increase (or decrease) between stages II and III. When more than one stage III study was performed, the last study in the series was used to compute this percentage. The last stage III study also was used to compute the mean values.

† Venous blood.

TABLE III

*$U_{NH_3}V$ and $U_{TA}V$ rates in diseased kidneys of three dogs before (stage II) and after (stage III) removal of the contralateral kidneys**

Clearance period	V	C_{Cr}	Urine pH	U_{NH_3}	$U_{NH_3}V$	U_{TA}	$U_{TA}V$
	ml/min	*ml/min*		*μEq/ml*	*μEq/min*	*μEq/ml*	*μEq/min*
			Stage II				
Dog 6							
1	1.7	8.6	5.98	9.1	15.2	6.7	11.2
2	1.8	8.6	6.15	9.4	16.9	6.7	12.0
3	1.7	7.3	6.17	8.8	14.8	6.2	10.5
Mean		8.1			15.6		
			Stage III				
1	2.2	11.3	5.65	13.4	29.0	11.5	24.9
2	2.2	10.0	5.8	13.2	28.6	9.9	21.4
3	2.5	10.6	5.95	12.8	31.3	9.7	23.7
Mean		10.6			29.6		23.3
			Stage III				
1	2.3	17.6	5.62	9.6	21.7	12.0	27.1
2	3.0	19.9	5.68	8.9	26.3	10.4	20.7
3	3.0	18.6	5.68	7.5	22.6	10.1	30.5
Mean		18.7			23.5		29.4
			Stage II				
Dog 7							
1	2.9	17.4	5.40	8.6	29.4	7.5	21.8
2	3.5	17.7	5.65	7.5	26.3	6.4	22.4
3	4.0	15.9	5.82	5.9	20.3	6.3	21.7
Mean		17.0			25.6		22.0
			Stage III				
1	2.1	16.6	6.28	26.3	55.7	9.6	20.3
2	2.4	17.4	6.12	23.2	54.7	11.0	25.9
3	2.3	16.2	6.15	22.1	50.6	10.5	24.0
Mean		16.7			53.6		23.4
			Stage II				
Dog 5							
1	1.7	8.7	5.45	10.3	17.8	8.1	14.0
2	1.9	9.0	5.42	10.2	19.3	8.0	15.1
3	1.9	8.8	5.30	9.2	17.4	8.7	16.5
Mean		8.8			18.1		15.2
			Stage III				
1	1.9	9.6	5.62	9.1	17.0	14.8	27.7
2	2.2	10.5	5.70	9.8	21.9	14.4	32.2
3	2.3	10.2	5.78	8.8	20.1	14.2	32.5
Mean		10.1			19.6		30.8

* V_a = urine flow; C_{Cr} = creatinine clearance; U_{NH_3} and U_{TA} = urinary concentration of ammonium and titratable acid.

Results

The results of the studies performed in stages I and II are summarized in Table I. In stage I, the mean values for GFR, $U_{NH_3}V$/GFR, and $U_{TA}V$/GFR were closely comparable in the two sets of normal kidneys. In stage II the mean values for GFR for the experimental (i.e., pyelonephritic) kidneys decreased by an average of 65%, whereas GFR for the control kidneys increased by 11%. The values for $U_{NH_3}V$ averaged 163 μEq per 100

ml GFR for the experimental kidneys and 159 μEq per 100 ml GFR for the contralateral control organs. The corresponding values for $U_{TA}V$ per 100 ml GFR were 124 and 141 μEq. The absolute rate of $U_{NH_3}V$ in the control kidneys increased by 36% between stage I and stage II. This is reflected in Table I by a 22% increase in the ratio $U_{NH_3}V/GFR$ despite the 11% increase in GFR.

In Table II, composite data are shown for the experimental kidneys in each of the three stages of study. This Table includes values for GFR, $U_{NH_3}V$ (both in microequivalents per minute and in microequivalents per 100 ml GFR), $U_{TA}V$, blood pH, and urine pH. The mean adaptive increase in GFR in the diseased kidneys between stages II and III was 50.8%. This value compares rather closely with a mean value of 62.2% previously reported for a larger group of animals (6). The absolute rate of $U_{NH_3}V$ (in microequivalents per minute) increased unequivocally in the diseased kidney in nine of the ten animals after removal of the control organ (i.e., between stages II and III), and a small, but equivocal, increase occurred in one dog (dog 5). The mean increment for all ten dogs was 91.4%.

Detailed protocols are shown in Table III for three animals including dog 5. In dog 7, the $U_{NH_3}V$ in stage III was markedly increased in relation to stage II despite the fact that urine flow rates were less, urine pH was somewhat greater, and GFR was not increased.

The group average for $U_{NH_3}V$ per unit of GFR also increased between stages II and III by 42% (Table II); however, there was no consistent relationship between the percentage increase in GFR and that in $U_{NH_3}V$. Consequently the values for $U_{NH_3}V/GFR$ increased in five animals, decreased in four, and remained unchanged in one. The absolute values for $U_{TA}V$ were greater in stage III than in stage II. Thus the sum of $U_{NH_3}V$ plus $U_{TA}V$ was considerably greater in stage III. The

TABLE IV

*Effects of amino acid infusion on maximal rates of $U_{NH_3}V$ in experimental kidneys at each of the three successive stages of study**

Dog	Stage I Diseased kidneys	Stage II Diseased kidneys			Stage II Control kidneys	Stage III Diseased kidneys		
		Base line	$U_{NH_3}V$ substrate infusion			Base line	$U_{NH_3}V$ substrate infusion	
	% Δ†	*μmoles/min*		% Δ	% Δ	*μmoles/min*		% Δ
1	102	45	75	67	54	51.7	135	161
2	103	13.6	23.6	73.5	66	43.3	53.3	23.1
5	81	18.1	25.5	40.9	52	19.6	25.0	27.6
6	35	15.6	18.7	19.9	31	29.6	29.6	0.0
						23.5	27.0	11.5
7	47.8	25.6	32.6	27.3	10	27.3	38.2	39.9
						21.8	28.3	29.8
						53.6	56.6	5.6
8	43	48.5	62.3	28.5		51.1	71.3	39.5
						58.1	62.1‡	6.9
9	82	9.3	14.7	57.9	62	16.6	22.4	34.9
						17.6	23.4‡	32.9
10	60.5	30.5	47.0	54.1	64	81.5	99.9	22.6
Mean	69			48.8§	48.4			40.7‖

* The response of the control kidneys is included for the stage II studies.

† Per cent change refers to the per cent increment in $U_{NH_3}V$ between base-line periods and substrate infusion periods.

‡ Alanine was infused at a rate of 200 μmoles per minute and glutamine (dogs 8 and 9, indicated by the double dagger, stage III) at a rate of 400 μmoles per minute. The infusions were begun after the completion of three to five control clearance periods referred to in the Table as "base line." In stage III, two separate studies were performed in dogs 6, 8, and 9, and three studies were performed in dog 7.

§ Average does not include value for dog 8 so as to make averages for control and diseased kidneys comparable.

‖ Mean is comprised of final alanine experiments.

mean values for blood and urine pH were closely comparable during stages II and III.

The effects of precursor amino acid infusion on $U_{NH_3}V$ are shown in Table IV. In seven of the eight dogs studied, data were available for the simultaneous response of both diseased and control kidneys in stage II. The mean increment in $U_{NH_3}V$ during alanine infusion was 49% for the diseased kidneys and 48% for the control organs. The changes in the diseased kidneys between stages II and III are shown for all eight dogs in Table IV. In stage II the increment averaged 46.1%. In stage III, the spontaneous excretion rates (i.e., in the presence of acidosis but before amino acid infusion), as already discussed, were greater than they were in the same kidneys in stage II; indeed, in five of the eight animals, the values before alanine administration in stage III exceeded the values during alanine infusion in stage II. The effects of alanine on the adapted kidneys (i.e., in stage III) were as follows: The increments ranged from 0 to 39.9% in seven animals, and the value was 161% in the eighth animal (dog 1). The average increase for the total group was 41%. If the data from dog 1 are excluded, the increment for the group averaged 23.6%. Despite the decrease in the percentage increment, however, the absolute increments in $U_{NH_3}V$ rates after alanine infusion were closely comparable in stages II and III in most of the animals.

In two of the animals in which alanine was infused in stage III, the effects of glutamine were measured in separate experiments. These data are also included in Table IV. In dog 9, the percentage increment was approximately the same with glutamine as with alanine; in dog 8 the response to glutamine was not so marked.

Discussion

The residual nephrons of the diseased kidney of the dog have been found to increase $U_{NH_3}V$ adaptively in association with a marked reduction in the available nephron population. Ammonium reduction was measured during sustained metabolic acidosis before (stage II) and after (stage III) the removal of the contralateral control kidney. The rate of $U_{NH_3}V$ increased unequivocally in nine of the ten dogs studied, and the increment for the group averaged 90.4%.

In studies performed before nephrectomy, $U_{NH_3}V$ was found to be diminished in the diseased kidneys in relation to the contralateral nondiseased organs; however, the ratios of $U_{NH_3}V$/GFR in the diseased versus control kidneys were indistinguishable. Thus, in the absence of a marked decrease in total (i.e., bilateral) nephron population, the residual nephrons of the diseased kidney maintain the same level of glomerulotubular balance (with ammonia as the index of tubular function) as do the nephrons of the contralateral kidney. These observations confirm previous studies in dogs (10) and man (11, 12) with unilateral or predominantly unilateral renal disease.

In further evaluating the significance of the clearance ratios in the stage II studies, consideration must be given to the fact that $U_{NH_3}V$ by the control kidneys increased by an average of 36% between stages I and II. GFR also increased in the control kidneys by 11%. It is of interest, therefore, that the values for $U_{NH_3}V$/GFR in the diseased kidneys in stage II remained equal to those in the control kidneys despite the occurrence not only of an increase in both $U_{NH_3}V$ and GFR in the control organ, but an unequal increase in the two functions. Presumably the changes in the control kidneys reflect a limited adaptive increase in both tubular and glomerular function in consequence of the induction of disease (and loss of nephrons) in the experimental organs. The equality of clearance ratios, therefore, could indicate that a similar adaptive increase occurred in the residual urine-forming nephrons of the pyelonephritic organs. In any event, the data would favor the view that whatever factors controlled the relationship between GFR and $U_{NH_3}V$ in the control kidney exercised control over these same relationships in the surviving nephrons of the diseased kidney. The response to amino acid infusion in stage II also is of interest in this context. The percentage increase in $U_{NH_3}V$ for the diseased kidneys did not differ consistently from that of the control organs, and the mean values for the two groups of kidneys were virtually identical.

It is hard for us to reconcile the foregoing data with randomly distributed structural lesions producing a spectrum of defects in ammonia synthetic ability in the involved tubular segments. However, the data do not rule out the existence of iso-

lated defects in a portion of the nephron population. If an appreciable number of nephrons sustained a significant reduction in ammonia synthetic capacity, the equality of clearance ratios would require that GFR be reduced to a proportional degree in these same units (or else in uninvolved nephrons), or that $U_{NH_3}V$ be increased in a highly precise manner in the uninvolved segments or units, or both. The response to alanine would require, furthermore, that the noninvolved segments of these nephrons respond in a quantitatively normal fashion to precursor substrate. Finally, these units presumably would contribute to the marked adaptive increase in $U_{NH_3}V$ observed between stages II and III.

In addressing himself to the question of $U_{NH_3}V$ in renal disease, Relman (3) has pointed out that the equality of $U_{NH_3}V/GFR$ ratios between diseased and contralateral control kidneys does not necessarily exclude specific tubular defects in ammonia synthesis in that blood flow to the tubules might decrease roughly in proportion to GFR; therefore, GFR might be inversely related to the degree of structural damage in functioning nephrons. If future investigations establish the fact that partially damaged nephrons contribute to urine formation in detectable numbers in the usual forms of chronic progressive renal disease, some mechanism, such as the one proposed by Relman, may well subserve the regulation of glomerulotubular balance among the constituent nephrons. However, we believe that a regulatory system, whatever its nature, must be so designed as to maintain a high level of functional organization among the residual nephrons.

One of the intriguing questions raised by these studies relates to the mechanisms of adaptation in ammonium excretion that occurred to a limited degree in the control kidneys between stages I and II and to a more marked degree in the diseased kidneys between stages II and III. The degree of metabolic acidosis was comparable under the different conditions, and urine pH was higher in stage III than in stage II in several experiments; hence these variables probably do not provide the explanation. Urine flow rates generally were greater in stages III and II, but this was not invariable (*cf.* dog 7, Table III). The unequivocal response to amino acid precursor infusion in both kidneys of the stage II models would suggest that substrate subserved a rate-limiting function, and perhaps the somewhat diminished response to substrate in stage III would imply that in the adapted states this role was diminished, although the absolute rate of increase in $U_{NH_3}V$ (in contrast to the percentage increase) was similar in stages II and III. The possibility that endogenous substrate delivery is increased in stage III in consequence of an adaptive rise in renal blood flow per nephron (6) may be worthy of consideration. So also may be the possibility that enzyme activity becomes rate limiting in stage III. In dogs without renal disease, Rector and Orloff found that glutaminase activity does not increase as $U_{NH_3}V$ rises after exogenous acid administration (13); moreover Goldstein (14) has recently shown in acidotic rats, free of renal disease, that $U_{NH_3}V$ may increase at a time when the expected increase in glutaminase activity is inhibited by actinomycin A. Nevertheless *in vitro* measurement of glutaminase activity in stage II versus stage III animals would be of interest. Definitive resolution of this question very likely must await a clearer understanding both of the rate-limiting events in $U_{NH_3}V$ and the events underlying the functional adaptations that occur in the remaining nephrons when the total population of functioning units is diminished.

One final point merits brief consideration. In the process of adaptation, there was no constant relationship between the percentage increment in $U_{NH_3}V$ and the percentage increment in GFR. Thus although $U_{NH_3}V$ increased in all ten dogs and GFR increased in nine of the ten, the ratio $U_{NH_3}V/GFR$ rose in five, decreased in four, and remained unchanged in one. Therefore, in these animals, the stage III ratios did not serve as an accurate index of the presence or absence of the adaptive increase in $U_{NH_3}V$. The best example of this is seen in dog 6, Table II. Ammonium excretion rate increased by 50% between stages II and III; however, GFR increased in the same kidney by 141%. Hence the change in $U_{NH_3}V/GFR$ was -35%. In man, with chronic renal disease, Wrong and Davies (15) have shown that the values for $U_{NH_3}V$ tend to decrease in proportion to the decrement in GFR. It is possible, therefore, that with greater chronicity, any adaptive change

in $U_{NH_3}V$ would parallel the simultaneous adaptation in GFR. It is also possible that the experimental model does not accurately reflect the situation in man, and in this context it must be noted that these animals all had a nonglomerular disease. One conclusion that may be permissible from the variable change in ratios is that it may not be possible to exclude an adaptive increase in $U_{NH_3}V$ on the basis of a normal or even diminished $U_{NH_3}V/GFR$ ratio in advanced renal disease in any given patient.

Summary

Maximal rates of ammonium excretion were measured in the chronically diseased kidney of the dog. Studies were performed on both kidneys before disease was induced (stage I). Measurements then were repeated after experimental pyelonephritis had been induced in one kidney, but not in the other (stage II). Finally, the functional patterns were re-evaluated after removal of the control organ under conditions in which the diseased kidney was responsible for the entire contribution to acid-base preservation (stage III). Chronic acidosis of comparable degree and duration was induced at each phase of study by exogenous administration of regulated doses of ammonium chloride. The response to amino acid precursors of ammonia synthesis also was determined before and after the removal of the control organ. In stage I, ammonium excretion values were equal in the two sets of normal kidneys. In stage II, ratios of ammonium excretion to glomerular filtration rate remained equal bilaterally, and the percentage increment after amino acid infusion also was equal bilaterally. In stage III, the spontaneous rate of ammonium excretion increased in the diseased kidneys by an average of 91.6%. The response to amino acid infusion persisted in stage III, but was somewhat less than in stage II. The data suggest that in response to a diminution in the total number of functioning nephrons, ammonium excretion per nephron increases adaptively in the residual nephrons.

References

1. Pitts, R. F. Renal excretion of acid. Fed. Proc. 1948, **7**, 418.
2. Canessa-Fischer, M., R. Shalhoub, S. Glabman, J. DeHaas, and R. F. Pitts. Effects of infusions of ammonia, amides, and amino acids on excretion of ammonia. Amer. J. Physiol. 1963, **204**, 192.
3. Relman, A. S. Renal acidosis and renal excretion of acid in health and disease. Advanc. intern. Med. 1964, **12**, 295.
4. Elkinton, J. R. Hydrogen ion turnover in health and in renal disease. Ann. intern. Med. 1962, **57**, 660.
5. Desautels, R. E. Hemisection of bladder for the collection of separate urine samples. Surg. Gynec. Obstet. 1957, **105**, 767.
6. Bricker, N. S., S. Klahr, and R. E. Rieselbach. The functional adaptation of the diseased kidney. I. Glomerular filtration rate. J. clin. Invest. 1964, **43**, 1915.
7. Bonsnes, R. W., and H. H. Taussky. On the colorimetric determination of creatinine by the Jaffe reaction. J. biol. Chem. 1945, **158**, 581.
8. Nathan, D. G., and F. L. Rodkey. A colorimetric procedure for the determination of blood ammonia. J. Lab. clin. Med. 1957, **49**, 779.
9. Conway, E. J. Microdiffusion Analysis and Volumetric Error. London, Crosby Lockwood, 1950.
10. Morrin, P. A. F., N. S. Bricker, S. W. Kime, Jr., and C. Klein. Observations on the acidifying capacity of the experimentally diseased kidney in the dog. J. clin. Invest. 1962, **41**, 1297.
11. Steinmetz, P. R., R. P. Eisinger, and J. Lowenstein. The excretion of acid in unilateral renal disease in man. J. clin. Invest. 1965, **44**, 582.
12. Bricker, N. S., E. J. Dorhout-Mees, S. Klahr, Z. B. Orlowski, R. E. Rieselbach, and L. E. Todd. The rate limiting and adaptive events in tubular and glomerular functions of the chronically diseased kidney *in* Proceedings of the Second International Congress of Nephrology, Prague, Excerpta Medica Foundation, International Congress Series no. 78, 1964, p. 39.
13. Rector, F. C., Jr., and J. Orloff. The effect of the administration of sodium bicarbonate and ammonium chloride on the excretion and production of ammonia. The absence of alterations in the activity of renal ammonia-producing enzymes in the dog. J. clin. Invest. 1959, **38**, 366.
14. Goldstein, L. Actinomycin D inhibition of the adaptation of renal glutamine-deaminating enzymes in the rat. Nature (Lond.) 1965, **205**, 1330.
15. Wrong, O., and H. E. F. Davies. Excretion of acid in renal disease. Quart. J. Med. 1959, **28**, 259.