Kidney Glutaminase and Carbonic Anhydrase Activities and Renal Electrolyte Excretion in Rats[1]

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ABSTRACT

The plasma electrolyte composition and 24-hour urine electrolyte excretion were determined in fasting normal, K-deficient and protein and K-deficient rats following the administration of equal molar quantities of NaCl, KCl, NH_4Cl and $KHCO_3$. An attempt was made to relate any differences in the compositions of the plasma and urine to the levels of renal glutaminase and carbonic anhydrase activities, and, to the chemical compositions of the skeletal muscle and kidneys. Renal glutaminase and carbonic anhydrase activities were found increased in K-deficiency and reduced in protein and K-deficiency. Significant changes from corresponding control levels of renal glutaminase were found in normal and K-deficient animals only after the administration of NH_4Cl (increased), and, after the administration of $KHCO_3$ (reduced). The only outstanding change of the carbonic anhydrase activity of the kidneys from these animals was a reduction following NH_4Cl administration. The doubly deficient rats showed a significant increase in renal glutaminase activity after NH_4Cl administration; on the other hand, KCl administration resulted in a further reduction of the lowered renal carbonic anhydrase activity. The experimental results supported the contention that a relationship exists between urinary ammonia excretion and the level of renal glutaminase activity, while the transfer of H^+ to the urine and the level of renal carbonic anhydrase activity could not be related. No clear relationship was found between the K excretion and/or K content of the kidneys and the urine total titratable acidity and ammonia excretion, and urine pH.

It is generally held that the kidney enzymes carbonic anhydrase and glutaminase play a role in the renal electrolyte excretion (1, 2). In direct support of this belief it was recently reported (3) that the increased activities of both enzymes found in the kidneys from potassium-deficient rats, and the reduced activities of both enzymes found in the kidneys from protein and potassium-deficient rats, were directly related to changes in urinary total titratable acidity and ammonia excretion. In view of the latter observation it seemed of interest to examine the levels of renal glutaminase and carbonic anhydrase and 24-hour urine electrolyte excretion of fasting normal, potassium-deficient and protein and potassium-deficient rats following the administration of equal molar quantities of NaCl, KCl, NH_4Cl and $KHCO_3$. Any observed changes could then be related to differences in the compositions of the plasma, and to the chemical compositions of skeletal muscle and kidneys.

METHODS

Young adult male rats of the Wistar strain, of a uniform age and weighing between 260 and 290 gm, were placed on a control diet for a period of at least 7 days before the experiments were begun. The control and experimental diets were the same as those employed in previous studies (3, 4).

Separate groups of rats were employed for the kidney enzyme studies, but they were treated in exactly the same manner as those selected for the chemical analysis of plasma, urine and tissues. At the close of the specified dietary period, the animals were treated as described for the determination of the 24-hour 'endogenous' creatinine clearance (5), with the exception that the food was removed at 8:00 A.M. and stom-

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TABLE 1. PLASMA ELECTROLYTE COMPOSITION

Administered Fluid*	Diet	Body Wt.	Cl	HCO_3	Na	K
	days	*gm*	*mEq/l.*	*mEq/l.*	*mEq/l.*	*mEq/l.*
Control Diet						
Water	35	313	98.4	25.0	148.1	4.4
S.D. (10)†		15	2.2	1.3	7.2	0.5
NaCl (2)	35	331	107.0	25.7	149.7	3.7
KCl	35	325	110.5	23.5	149.2	7.4
S.D. (3)		10	0.6	0.6	3.4	2.0
NH_4Cl	35	289	124.1	6.1	134.7	5.6
S.D. (3)		8	3.2	0.5	1.0	1.1
$KHCO_3$	35	320	89.0	33.2	143.3	8.6
S.D. (3)		4	0.2	0.6	2.1	0.8
Low-Potassium Diet						
Water	35	260	83.3	35.2	152.6	2.6
S.D. (11)		13	6.2	3.7	5.3	0.9
NaCl (2)	7	280	102.9	27.1	150.8	3.7
NaCl	14	280	99.0	32.1	159.3	2.8
S.D. (3)		4	0.6	1.7	4.2	0.6
NaCl	35	284	94.7	39.0	155.8	2.4
S.D. (6)		14	3.8	2.8	3.4	0.5
KCl	35	267	114.6	24.4	150.2	8.1
S.D. (4)		7	1.0	0.7	3.8	0.7
NH_4Cl	35	277	121.4	11.2	145.9	3.6
S.D. (6)		7	1.9	0.6	3.0	0.6
$KHCO_3$	35	279	86.9	40.0	150.9	10.7
S.D. (4)		17	4.1	1.3	2.4	2.0
Low-Potassium and Low-Protein Diet						
Water	35	183	101.3	23.3	151.3	3.8
S.D. (15)		14	3.5	1.6	3.2	0.7
NaCl	35	189	104.6	23.6	154.0	4.1
S.D. (3)		9	0.4	1.4	5.0	0.2
KCl	35	180	106.1	23.7	150.5‡	5.7‡
S.D. (3)		3	1.4	0.4		
NH_4Cl	35	180	116.1	17.5	148.6	4.6
S.D. (3)		16	2.4	2.0	2.4	0.6
$KHCO_3$	35	199	98.5	26.4	153.2	5.6
S.D. (3)		10	2.0	0.4	4.0	0.3

* Rats on the control and low-K diets received, under fasting conditions by stomach tube in 24 hr., a total of 20 ml water, NaCl (3.6%), KCl (4.6%), NH_4Cl (3.295%) and $KHCO_3$ (6.166%); while rats on the low-K and low-protein diet received, similarly, water, NaCl (0.9%), KCl (1.148%), NH_4Cl (0.824%), and $KHCO_3$ (1.542%), plus water drunk ad libitum.

† Numbers within the parentheses represent number of pairs of animals.

‡ Single determination.

ach tubings were performed at 10:00 A.M., 3 P.M., 8:00 P.M. and 8:30 A.M., the next day. The urine collection period was from 10:00 A.M. to 10:00 A.M. the following day. The control and low-potassium animals received 5-ml quantities of either water, NaCl (3.6%), KCl (4.592%), NH_4Cl (3.295%), or $KHCO_3$ (6.166%) at the times specified. The doubly deficient rats were unable to tolerate these concentrated salt solutions and therefore were given either water, NaCl (0.9%), KCl (1.148%), NH_4Cl (0.824%), or $KHCO_3$ (1.542%). Hence, during the 24 hours, the control and low-potassium rats were administered 20 ml of water or the same volume of a salt solution containing a total of 12.32 mM of salt, while the doubly deficient rats received 20 ml of water or the same volume of a salt solution containing a total of 3.08 mM of salt. Water was allowed ad libitum to all animals.

At the close of the period of urine collection, the animals selected for the kidney enzyme studies were killed by decapitation and exsanguinated. Their kidneys were quickly removed and subjected to the analyses of carbonic anhydrase and glutaminase activities according to the procedures previously described (3). The remaining animals were placed under sodium pentobarbital anesthesia for the collection of blood, kidneys and skeletal muscle. The plasma was analyzed for 'true' creatinine, chloride, carbon dioxide content, sodium and potassium; the kidneys and skeletal muscle for water, sodium, potassium, chloride and total fat; and, the urine for 'true' creatinine, pH (determined at 25°C and values reported were not corrected to 38°C), chloride, carbon dioxide content, total titratable acidity, ammonia, sodium and potassium. Methods of chemical analysis were the same as previously reported (3–5, 18).

RESULTS

Plasma Electrolyte Content. The results of the chemical analysis of the plasma from fasting control and experimental rats 24 hours following the administration by stomach tube of water and various salt solutions of equivalent concentrations are presented in table 1. It will be seen that the administration of equivalent amounts of Cl as the salts of NaCl, KCl and NH_4Cl resulted in increases of the plasma chloride concentration, when compared with the corresponding plasma chloride concentration found in the animals given water. The increases of the plasma chloride concentration were progressively greater following the administration of the salt solutions in the order listed. The plasma bicarbonate concentration remained practically unchanged upon administering Cl as NaCl. The administration of Cl as KCl resulted in a small decrease of the plasma bicarbonate content in the normal rats, no change in the rats fed the low-potassium and low-protein diet (the amount of chloride delivered to these rats was less) and a decrease to control levels in the case of the potassium-deficient rats. A striking decrease of the plasma bicarbonate content was found in each group of rats receiving Cl as NH_4Cl. Attention should be called to the slight increase of the plasma potassium concentration which was encountered in each group of animals receiving

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NH_4Cl. Hartmann, Harris, Martin, Rolf and White (6) also noted a tendency for the plasma potassium concentration to rise in dogs given NH_4Cl by stomach tube. The extent of the participation of a probable contraction of the extracellular volume in the rise of the plasma potassium concentration found in these experiments cannot be clearly evaluated.

The administration of $KHCO_3$, of a millimolar strength equal to that of the chloride-containing solutions, to the normal and the doubly deficient animals resulted in an increase of the plasma bicarbonate content which was accompanied by an equivalent fall of the plasma chloride concentration. In contrast, when the potassium-deficient rats were given $KHCO_3$, the average plasma chloride concentration was found to be slightly higher than the value found in the animals given water even though the plasma bicarbonate content was further increased.

Electrolyte Excretion in Urine. The results of the analysis of the 24-hour urine samples of fasting control and experimental rats given by stomach tube either water or various salt solutions are summarized in table 2. Comparisons were made with the results obtained when the animals were given water.

NaCl Administration. The administration of 12.32 mEq Cl as NaCl to the normal rats resulted in the excretion of a urine with a slightly higher pH, a slightly lower total titratable acidity and an increased ammonia content. On the basis of the amount administered and the 24-hour urinary excretion, the normal rats retained chloride in excess of sodium, and lost potassium.

The same number of equivalents of Cl as NaCl when administered to rats fed the low-potassium diet for 7, 14 and 35 days resulted in an increased ammonia excretion which seemed to be of a greater magnitude as the period of potassium deprivation was prolonged. It will be noted that the pH of the 7-day urine was elevated and that the total titratable acidity excretion was reduced. Thereafter, there was a tendency for the urinary pH to fall and the total titratable acidity to increase as the low-potassium diet was continued. These animals retained sodium in excess of chloride and although the potassium excretion was small, the administration of NaCl caused a slightly greater loss of potassium.

TABLE 2. TWENTY-FOUR-HOUR RENAL ELECTROLYTE EXCRETION

Administered Fluid*	Diet	Creat. Clear.†	pH	Excreted Total Acidity	NH_3	HCO_3	Cl	Na	K
	days	*ml/24 hr.*		*mEq*	*mEq*	*mEq*	*mEq*	*mEq*	*mEq*
				Control Diet					
Water	35	2910	6.46	0.35	0.89	0.05	0.36	0.41	0.60
S.D. (10)		284	0.07	0.06	0.12	0.02	0.12	0.10	0.10
NaCl (2)	35	3990	6.60	0.31	1.28	0.13	9.80	10.52	1.03
KCl	35	3160	5.96	0.38	0.99	0.02	11.02	1.57	9.63
S.D. (3)		304	0.09	0.04	0.17	0.01	0.18	0.19	0.44
NH_4Cl	35	2140	5.57	1.04	5.01	0.01	9.87	3.22	3.72
S.D. (3)		257	0.13	0.01	0.09	0.01	0.31	0.45	1.53
$KHCO_3$	35	2450	8.16	−0.79	0.29	4.10	1.96	2.35	9.71
S.D. (3)		140	0.15	0.05	0.27	0.43	0.25	0.17	0.83
				Low-Potassium Diet					
Water	35	2090	6.33	0.38	1.85	0.04	0.29	0.34	0.03
S.D. (11)		231	0.09	0.04	0.31	0.01	0.09	0.07	0.01
NaCl (2)	7	3410	6.93	0.19	2.37	0.36	9.89	9.81	0.25
NaCl	14	3670	6.71	0.33	2.66	0.26	10.43	9.41	0.16
S.D. (3)		183	0.06	0.06	0.23	0.03	0.48	0.34	0.02
NaCl	35	3110	6.35	0.51	2.72	0.12	9.86	9.11	0.16
S.D. (6)		229	0.09	0.11	0.28	0.09	0.25	0.39	0.05
KCl	35	2780	5.72	0.49	1.68	0.03	9.92	3.22	6.03
S.D. (4)		288	0.28	0.06	0.24	0.03	0.45	0.53	1.00
NH_4Cl	35	2170	6.14	0.51	6.49	0.06	8.41	2.48	0.16
S.D. (6)		142	0.12	0.06	0.41	0.03	0.23	0.07	0.02
$KHCO_3$	35	1230	8.16	−0.42	0.21	3.08	0.73	3.66	2.50
S.D. (6)		325	0.15	0.04	0.06	0.58	0.21	0.60	0.41
				Low-Potassium and Low-Protein Diet					
Water	35	1570	6.46	0.14	0.60	0.03	0.35	0.39	0.05
S.D. (15)		269	0.16	0.02	0.07	0.02	0.12	0.12	0.02
NaCl	35	2080	6.75	0.10	0.66	0.13	3.10	2.92	0.07
S.D. (3)		310	0.06	0.01	0.14	0.06	0.11	0.14	0.02
KCl	35	1470	6.55	0.08	0.43	0.10	3.32	1.53	1.79
S.D. (3)		81	0.13	0.01	0.07	0.03	0.27	0.16	0.08
NH_4Cl	35	1390	6.24	0.11	0.93	0.02	2.63	0.81	0.10
S.D. (3)		208	0.05	0.02	0.09	0.01	0.24	0.10	0.01
$KHCO_3$	35	1630	7.33		0.15	0.83	1.23	1.38	2.16
S.D. (3)		51			0.03	0.09	0.16	0.09	0.04

* See the footnotes at the bottom of table 1.
† Twenty-four-hour 'endogenous' creatinine clearance.

The animals fed the low-potassium and low-protein diet received only 3.08 mEq Cl as NaCl. The 24-hour urine from these animals exhibited changes which were similar in direction to those found in normal animals, namely, a slightly higher pH, a lower total titratable acidity and a slightly elevated ammonia content.

It will be observed that in the three groups of animals, the administration of sodium chloride was accompanied by an increase of the 24-hour 'endogenous' creatinine clearance.

Potassium Chloride Administration. An

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examination of the data will reveal that the composition of the urine differed in several respects when KCl rather than NaCl was administered. Thus, when given 12.32 mEq Cl as KCl, the normal and the low-potassium groups of rats excreted a urine with a lower pH and a slightly increased total titratable acidity. Further, the urinary ammonia excretion did not change significantly. In both groups of animals there was a loss of sodium and a retention of both chloride and potassium. In the normal rats the retention of chloride was slightly greater than the retention of potassium minus the loss of sodium. Approximately one-half of the administered potassium was retained by the low-potassium group of rats. Here the loss of sodium and the retention of chloride were greater than found in the normal rats given KCl, but the retention of chloride was less than the retention of potassium minus the loss of sodium.

There was also found to be a retention of potassium and a loss of sodium in the low-potassium and low-protein group of rats given 3.08 mEq Cl as KCl. In this case, however, the chloride excreted in the urine exceeded the amount administered. In these animals the urine exhibited a slight increase of pH and small reductions of total titratable acidity and ammonia.

The 24-hour 'endogenous' creatinine clearance was found to be increased in the normal and low-potassium groups of rats, but the increases were not as great as those found when NaCl was given. The doubly deficient rats exhibited an insignificant reduction of the 24-hour 'endogenous' creatinine clearance.

It will be noted that the administration of KCl to the potassium-deficient rats corrected the extracellular alkalosis (table 1). Simultaneously, there was the excretion of urine with a lowered pH and containing only a small amount of bicarbonate. This confirms the observations of Cooke, Segar, Cheek, Coville and Darrow (7) who performed similar experiments employing potassium-deficient rats. It is generally held (8) that increasing excretion of potassium is associated with a marked tendency for the urine to become alkaline. This does not seem to be substantiated by the present experiments wherein the urine pH of normal rats became reduced following the oral administration of KCl and a large fraction of the administered K was excreted. Perhaps species difference, or the manner of administration, may be involved in the response of the urine pH to the administration of KCl. For example, Liddle, Bennett and Forsham (9) reported the urine pH became increased following the oral administration of KCl to human subjects, and, it has been reported by a number of authors (10, 11) that the urine pH of dogs becomes alkaline following the infusion of KCl.

Ammonium Chloride Administration. The administration of 12.32 mEq Cl as NH_4Cl to the normal rats resulted in a fall of the urine pH, which was accompanied by a striking increase of the total titratable acidity and ammonia excretions. However, the milliequivalent increased excretion of total titratable acidity was far less than that of ammonia. Simultaneously, there were considerable losses of sodium and potassium and a retention of chloride. Likewise, there was a fall of the urine pH in the potassium-deficient animals given the same quantity of NH_4Cl, but the decrease of pH was not as great as when similar animals were administered KCl. It is interesting to note that the total titratable acidity was only relatively slightly increased while the ammonia excretion was markedly elevated. Compared with the normal rats, the potassium-deficient animals exhibited a lesser sodium loss and a greater chloride retention. Further, even though the potassium excretion was small, there was a slightly increased potassium loss.

No striking changes were encountered when the doubly deficient animals were given 3.08 mEq Cl as NH_4Cl. The urine pH decreased slightly, which was accompanied by a small reduction of the total titratable acidity and a significant increase in ammonia excretion. These animals also exhibited some losses of sodium and potassium, but retained chloride.

The 24-hour 'endogenous' creatinine clearance was found to be conspicuously altered only in the control rats where the value was reduced.

Administration of Potassium Bicarbonate. The administration of 12.32 mEq K as $KHCO_3$ to the normal and the potassium-deficient rats, and of 3.08 mEq K to the doubly deficient animals, elicited responses of urinary electrolyte excretion which were similar in direction. Thus, the urine became alkaline with striking

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reductions of total titratable acidity and ammonia excretion. The bicarbonate content of the urine was increased. There were losses of sodium in excess of chloride, and a retention of potassium. The loss of sodium encountered in the potassium-deficient animals was appreciably greater than the loss of chloride. It might be noted that the retention of potassium by these animals was found to be considerably in excess of the amount retained by similar animals given an equal equivalent of K as KCl.

Following $KHCO_3$ administration, the 24-hour 'endogenous' creatinine clearance was found to be reduced in the normal and potassium-deficient rats, while it appeared to remain unchanged in the double deficient animals.

Kidney Glutaminase and Carbonic Anhydrase Activity. From a perusal of the data presented in table 3, it will be seen that the activities of both glutaminase and carbonic anhydrase were increased in the kidneys from potassium-deficient rats and both enzyme activities were reduced in the kidneys from rats made deficient in protein as well as potassium. Significant changes from corresponding control levels of glutaminase activity were found in the normal and potassium-deficient rats only after the administration of NH_4Cl and $KHCO_3$. It will be seen that in these instances the glutaminase activity was increased after NH_4Cl was given and was reduced after $KHCO_3$ administration. Considered similarly, the only outstanding change of the carbonic anhydrase activity was found to be a reduction after the administration of NH_4Cl. However, small decreases in carbonic anhydrase activity were also found in the normal animals given NaCl and KCl, and in the potassium-deficient rats following the administration of NaCl, KCl and $KHCO_3$.

The activities of glutaminase and carbonic anhydrase in the kidneys from the doubly deficient animals remained uniformly reduced. However, the glutaminase activity was significantly higher after NH_4Cl was given and somewhat increased after KCl administration. The carbonic anhydrase activity of the kidneys from these animals seemed to be further reduced after the administration of KCl.

Tissue Electrolyte Composition. The results of the chemical analysis of kidney and skeletal muscle from certain members of the control and experimental rats are presented in table 4.

TABLE 3. KIDNEY GLUTAMINASE AND CARBONIC ANHYDRASE ACTIVITY*

Administered Fluid†	Body Wt., gm	Kidney Wt., gm/100 gm BW	Nitrogen, mg/gm Kidney	Glutaminase, U/gm N	Carbonic Anhydrase, U/gm N
Control Diet					
None	309	0.686	31.2	2970	427
S.D.‡	12 (16)	0.04 (16)	1.2 (16)	188	104
Water	301	0.694	31.6	2900	360
S.D.	3	0.04	1.6	188	49
NaCl	317	0.710	32.5	3040	301
S.D.	17	0.03	1.1	68	38
KCl	304	0.666	32.1	2884	330
S.D.	7	0.03	1.7	198	37
NH_4Cl	308	0.636	32.3	5556	231
S.D.	14	0.03	1.2	238	46
$KHCO_3$	304	0.737	32.0	2488	375
S.D.	7	0.03	1.8	110	60
Low-Potassium Diet					
None	266	1.186	30.8	7977	668
S.D.	12 (16)	0.06 (16)	2.6 (16)	313	99
Water	282	1.177	32.1	6690	555
S.D.	16	0.06	1.3	196	90
NaCl	297	1.090	32.3	8000	438
S.D.	18	0.07	0.2	218	16
KCl	272	0.978	31.2	7290	446
S.D.	17	0.05	0.9	246	49
NH_4Cl	270	1.147	31.3	8360	311
S.D.	18	0.09	0.3	196	33
$KHCO_3$	265	1.030	31.8	4020	434
S.D.	10	0.05	1.0	190	33
Low-Potassium and Low-Protein Diet					
None	170	0.691	29.6	1739	270
S.D.	9 (16)	0.04 (16)	1.3 (16)	143	42
KCl	184	0.700	29.6	2170	186
S.D.	14	0.05	0.7	297	32
NH_4Cl	181	0.785	33.8	2690	262
S.D.	10	0.03	1.1	87	47
$KHCO_3$	195	0.736	31.8	1750	249
S.D.	6	0.03	0.4	195	35

* Values for enzyme activity represent an average of 2 assays; each assay being conducted on the pooled kidneys of 4 rats.

† See the footnotes at the bottom of table 1.

‡ Numbers within the parentheses indicate the number of rats. Where this is not indicated the number is 8.

By comparing the results which were obtained when water was administered, it will be observed that the skeletal muscle from the potassium-deficient animals exhibited, typically, a loss of potassium and an unequivalent gain of sodium. The skeletal muscle from the doubly deficient rats also exhibited similar changes of potassium and sodium, but they were far less severe. It is interesting to point out that the kidneys from the potassium-deficient rats exhibited a comparatively small, but significant, reduction of potassium which was more than compensated by a gain of sodium. That the slight fall of kidney potassium may be real is found in the animals placed on the

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TABLE 4. ELECTROLYTE CONTENT OF SKELETAL MUSCLE AND KIDNEY*

Administered Fluid†	Diet	Skeletal Muscle				Kidney			
		H_2O	Cl	Na	K	H_2O	Cl	Na	K
	days	*gm*	*mEq*	*mEq*	*mEq*	*gm*	*mEq*	*mEq*	*mEq*
Control Diet									
Water	35	326.0	4.9	8.4	47.0	353.3	19.2	21.8	34.2
S.D. (6)		1.3	0.5	0.7	1.6	7.0	0.7	1.3	0.1
NaCl (2)	35	321.2	6.0	10.3	45.3	359.2	24.0	31.1	33.9
$KHCO_3$	35	325.1	4.4	6.8	49.5	353.9	19.2	23.8	44.6
S.D (3)		1.3	0.1	0.6	1.3	3.0	0.1	1.6	2.3
Low-Potassium Diet									
Water	35	326.2	4.9	17.6	32.7	409.5	17.7	26.0	31.6
S.D. (11)		1.9	0.4	2.3	2.4	11.1	1.4	1.9	0.5
NaCl (2)	7	325.2	5.4	11.1	42.6	359.0	23.7	27.8	34.9
NaCl	14	318.2	5.2	14.9	38.5	353.2	21.3	29.0	33.7
S.D. (3)		1.8	0.3	1.1	2.0	2.1	0.4	1.0	0.3
NaCl	35	317.8	5.2	19.3	31.8	386.6	23.5	35.8	31.6
S.D. (6)		4.7	0.7	1.3	1.7	11.3	2.3	3.7	0.7
NH_4Cl	35	303.0	5.6	13.3	31.6	384.7	25.4	24.6	33.3
S.D. (6)		4.2	0.2	0.9	0.9	12.9	1.0	0.3	1.0
$KHCO_3$	35	325.9	4.5	8.5	51.8	369.1	17.0	22.1	49.4
S.D. (6)		4.5	0.2	0.9	2.4	9.3	1.4	1.0	0.9
Low-Potassium and Low-Protein Diet									
Water	35	340.6	6.5	10.4	41.8	375.0	20.1	25.2	35.4
S.D. (3)		5.0	0.2	1.0	0.7	‡	‡	‡	‡
NaCl	35	330.1	6.3	10.8	46.3	362.1	20.1	26.2	35.9
S.D. (3)		1.8	0.4	1.3	0.6	2.0	0.8	0.7	1.1
KCl	35	327.8	6.2	8.5	51.7	350.1	21.3	24.2	40.3
S.D. (3)		2.9	0.3	0.4	1.4	1.1	2.3	0.9	1.4
NH_4Cl	35	325.1	7.3	10.0	47.8	358.0	21.1	24.2	34.4
S.D. (3)		4.5	0.9	1.1	1.1	5.9	0.4	0.2	2.2
$KHCO_3$	35	331.9	5.0	8.1	50.3	351.7	17.9	23.8	39.9
S.D. (3)		1.6	0.1	0.5	1.7	1.7	0.4	0.9	0.1

* Values are expressed in terms of 100 gm fat-free solids.
† See the footnotes at the bottom of table 1.
‡ Average from a group of 2 pairs of rats.

low-potassium diet for 7, 14 and 35 days, and administered NaCl. As the dietary period was prolonged in these animals, there was a progressive decrease of skeletal muscle potassium and a gain of sodium. After 7 days on such a diet, the kidney potassium content was unchanged, but after 35 days it was found to be reduced to the figure encountered in the potassium-deficient rats given water.

The potassium content of the kidneys from normal and potassium-deficient rats was found to be increased following $KHCO_3$ administration. Likewise, the potassium content of the kidneys from the doubly deficient rats was found to be increased following loading with both KCl and $KHCO_3$. Unfortunately, the tissues from the normal and potassium-deficient rats following KCl loading were not analyzed. However, in other experiments carried out in our laboratory, the analysis of skeletal muscle from potassium-deficient rats receiving KCl in amounts comparable to those employed in the present study, revealed a striking increase of potassium. Since the potassium contents of the skeletal muscle and the kidneys from the three groups of rats were found to be increased after $KHCO_3$ administration, and also to be increased when KCl was administered to the doubly deficient animals, it does not seem too unrealistic to believe that the kidney potassium content would also be increased in normal and potassium-deficient animals after KCl administration.

DISCUSSION

The results of the present experiments indicate that the activities of rat kidney glutaminase and carbonic anhydrase can be altered independently and with some rapidity. This was especially evident with NH_4Cl loading where there occurred a prompt increase in glutaminase activity and a fall in carbonic anhydrase activity. In support of the work of others employing rats (2, 3, 12, 13) it would appear that potassium deficiency with accompanying alkalosis and an acid load for excretion represent stimuli for an increase of renal glutaminase. Lowered renal glutaminase activities have been reported after the oral administration of $NaHCO_3$ (2) and after the feeding of a diet low in protein as well as potassium (3). The renal glutaminase activity was also found to be lowered in the present experiments after $KHCO_3$ loading.

The importance of the renal excretion of ammonia in the regulation of the acid-base balance is generally recognized (14, 15). Recently, Rector and co-workers (12, 13) have reviewed the various possible factors which are believed to participate in the control of ammonia excretion. Among the various factors, it would appear that the hydrogen ion concentration of the urine has an important influence on the rate of ammonia excretion. Then there is the mechanism, and the factors concerned in its regulation, of ammonia formation by the tubular cells. Finally, there are the factors involved in the transfer of ammonia, or ammonium ion, from the tubular cells into the urine. It is evident that when so many factors are involved the extent of ammonia excretion may be variable, depending upon the experimental conditions.

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Rector and co-workers (12, 13) in experiments on rats found the ammonia excretion to be related in a significant manner to the renal glutaminase activity. Thus, the inhibition of carbonic anhydrase with Diamox was found to lead to the excretion of a urine with an increased pH and a lowered titratable acidity. The urinary ammonia, after a slight initial fall was then found to increase to levels in excess of control values along with an increased activity of renal glutaminase. In other experiments, animals were given a constant NH_4Cl load resulting in a progressive, stepwise increase to a plateau in renal glutaminase activity, which was closely paralleled by a similar increase in ammonia excretion. The results of the present experiments offer further support to the conclusion that a relationship exists between urinary ammonia excretion and renal glutaminase activity. Thus, when compared with normal rats receiving water, similarly treated potassium-deficient rats (with an elevated renal glutaminase activity) and similarly treated doubly deficient animals (with a reduced renal glutaminase activity) exhibited an increased and a lowered ammonia excretion, respectively, in the presence of comparable urinary pH values and essentially the same excretions of Na and Cl. Further, the increases in ammonia excretion encountered following NaCl administration seemed to be related to the level of renal glutaminase activity. Finally, the administration of NH_4Cl induced a striking increase in ammonia excretion and a prompt rise in renal glutaminase activity.

There admittedly are a number of factors which can affect the amount of ammonia transferred to the urine at a given level of renal glutaminase activity. The urinary pH and the acid load are obviously important. In the present experiments, for example, the administration of $KHCO_3$ resulted in the excretion of an alkaline urine with marked reductions of total titratable acidity and ammonia. Even though $KHCO_3$ administration caused the renal glutaminase activity to be reduced, its level in the potassium-deficient rats was still found to be far in excess of the value encountered in the kidneys from untreated normal animals. It must be pointed out, however, that there did not appear to be a strict relationship between the urinary pH and ammonia excretion. For example, the urines from potassium-deficient rats given equal milliequivalent of Cl as KCl and NH_4Cl exhibited pH values of 5.72 and 6.14, respectively, with respective urinary ammonia excretions of 1.68 mEq and 6.49 mEq/rat/24 hours. As a further illustration, an increased amount of ammonia was excreted by normal and potassium-deficient rats following the administration of NaCl. On the other hand, there was an absence of a similar response after the administration of KCl, even though there was a fall in urine pH. Acid loading with NH_4Cl was found to result in a prompt and marked increase in urinary ammonia excretion, which was related to the level of renal glutaminase activity. Following the administration of equal milliequivalents of Cl as NH_4Cl, the normal and the potassium-deficient rats were found to have transferred into the urine, respectively, 6.05 and 7.00 mEq H^+/rat/24 hours as titratable acidity and ammonia. Of the H^+ transfer, 5.01 and 6.49 mEq, respectively, could be attributed to ammonia. However, this increased H^+ transfer could not be related to the level of renal carbonic anhydrase since the activity of this enzyme was found to be significantly reduced after NH_4Cl administration.

Cooke *et al.* (7) suggested that the alkalosis of the extracellular fluids typically encountered in potassium-deficient rats occurs as the result of a passage of H^+ into the muscle cells in partial replacement of a cellular potassium loss. Cooke *et al.* (7) suggested further that if the electrolyte composition of the cells of the distal tubule of the kidney parallels that of muscle, the failure of renal adjustment of the alkalosis could be explained according to the views of Berliner, Kennedy and Orloff (16). These authors presented evidence to support the view that when the body is depleted of potassium the renal exchange of H^+ for Na^+ is favored and an acid urine is excreted despite the extracellular alkalosis. In converse, when the potassium concentration of the renal tubule cells is elevated, the exchange of K^+ for Na^+ is favored, and that of H^+ for Na^+ is suppressed, with the result of the excretion of an alkaline urine despite an extracellular acidosis. However, Darrow, Cooke and Coville (17) failed to find any changes in the electrolyte composition of the kidneys from potassium-deficient rats exhibiting alkalosis and, likewise, Iacobellis, Muntwyler and Griffin (18) found

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no significant changes of sodium and potassium in the kidneys from potassium-deficient dogs, to support this thesis. In the present study an analysis of the whole kidneys from potassium-deficient rats receiving water by stomach tube revealed a slight, but significant, reduction of potassium and a greater than equivalent gain of sodium. It might be argued that only certain of the renal tubule cells more specifically concerned with ion exchange are depleted of potassium and the excretion of an acid urine in the presence of an elevated plasma bicarbonate could thereby be explained. However, a similar argument fails to explain the excretion of a urine with a lowered pH in the normal and potassium-deficient rats given a KCl load. These animals excreted abundant potassium in the urine and the potassium content of the kidney was presumably increased. According to theory the exchange of hydrogen for sodium should have been suppressed and an alkaline urine should have resulted. The hypothesis of a H^+ and K^+ competition in the renal excretion of these ions (16) would appear to require further scrutiny since it is evident that many factors may be involved in renal electrolyte excretion. The results of acute and chronic experiments may differ markedly.

REFERENCES

1. Pitts, R. F. and R. S. Alexander. *Am. J. Physiol.* 144: 239, 1945.
2. Davies, B. M. A. and J. Yudkin. *Biochem. J.* 52: 412, 1952.
3. Iacobellis, M., E. Muntwyler and G. E. Griffin. *Am. J. Physiol.* 178: 477, 1954.
4. Muntwyler, E. and G. E. Griffin. *Proc. Soc. Exper. Biol. & Med.* 89: 349, 1955.
5. Muntwyler, E. and G. E. Griffin. *Am. J. Physiol.* 173: 145, 1953.
6. Hartmann, A. F., Jr., F. D. Harris, H. F. Martin, D. Rolf and H. L. White. *Am. J. Physiol.* 167: 563, 1951.
7. Cooke, R. E., W. E. Segar, D. B. Cheek, F. E. Coville and D. C. Darrow. *J. Clin. Investigation* 31: 798, 1952.
8. Berliner, R. W., T. J. Kennedy, Jr. and J. G. Hilton. *Am. J. Physiol.* 162: 348, 1950.
9. Liddle, G. W., L. L. Bennett and P. H. Forsham. *J. Clin. Investigation* 32: 1197, 1953.
10. Winkler, A. W. and P. K. Smith. *Am. J. Physiol.* 138: 94, 1942–43.
11. Roberts, K. E., M. G. Magida and R. F. Pitts. *Am. J. Physiol.* 172: 47, 1953.
12. Rector, F. C., Jr., D. W. Seldin, A. D. Roberts, Jr. and J. H. Copenhaver. *Am. J. Physiol.* 179: 353, 1954.
13. Rector, F. C., Jr., D. W. Seldin and J. H. Copenhaver. *J. Clin. Investigation* 34: 20, 1955.
14. Pitts, R. F. *Federation Proc.* 7: 418, 1948.
15. Pitts, R. F. *Am. J. Med.* 9: 356, 1950.
16. Berliner, R. W., T. J. Kennedy, Jr. and J. Orloff. *Am. J. Med.* 11: 274, 1951.
17. Darrow, D. C., R. E. Cooke and F. E. Coville. *Am. J. Physiol.* 172: 55, 1953.
18. Iacobellis, M., E. Muntwyler and G. E. Griffin. *Am. J. Physiol.* 183: 395, 1955.

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