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Effect of a high protein diet on the renal concentrating response to vasopressin and dehydration of Aplodontia rufa and the domestic rabbit

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THE EFFECT OF A HIGH PROTEIN DIET ON THE RENAL CONCENTRATING RESPONSE TO VASOPRESSIN AND DEHYDRATION OF *APLODONTIA RUFA* AND THE DOMESTIC RABBIT

by

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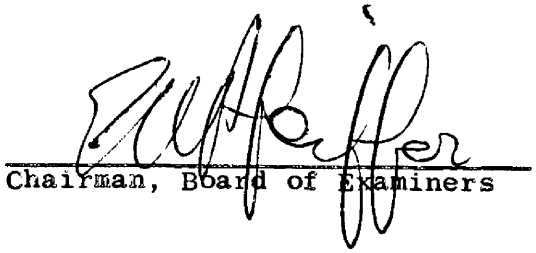

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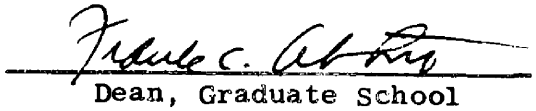

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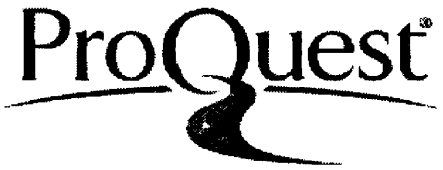

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E. W. H.

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INTRODUCTION

The mechanisms involved in the production of a urine with a concentration which is hypertonic to that of the plasma are still not completely understood. Bowman (1842) was the first to develop a theory of urine formation based on his anatomical findings. He postulated that urinary products were secreted by the tubules in the kidney and were carried away by a watery fluid formed in the glomeruli. Two years later, Ludwig (1844) formulated the "mechanical theory" of urine formation in which he hypothesized that urine formation was entirely dependent upon physical forces. According to this theory, glomerular filtration from hydrostatic pressure produced a large amount of dilute urine containing all of the urine constituents and final urine concentration was accomplished by diffusion of water from the tubules into the capillaries surrounding the tubules. Heidenhain (1883), however, opposed this theory and postulated a "secretory theory" which accounted for urine formation and concentration by glomerular secretion of water and salt and tubular secretion of special urinary products, such as urea. Starling hypothesized (1896) that fluid transfer from capillaries through a membrane impermeable to proteins into tissue spaces was dependent upon hydrostatic pressure. He applied this concept to urine formation (1899), thus supporting the "mechanical theory" held by Ludwig. Cushny (1926) incorporated both the idea of glomerular filtration and tubular reabsorption by attributing the formation of a hypertonic urine to active, tubular reabsorption of water in the tubules

from glomerular filtrate. The location of water reabsorption was ascribed to several different areas of the tubule. Walker, et al. (1941) demonstrated that approximately 80% of the filtered water had been reabsorbed during the passage of the glomerular filtrate through the proximal convoluted tubule and that only 20% of the filtered fluid passed through the rest of the nephron. Several studies showed that the reabsorption of water in the proximal tubule was a constant process and that reabsorption of the water involved in the production of a concentrated urine occurred distal to the proximal tubule. Because only mammals and some birds possess a thin loop of Henle, produce a hypertonic urine (Crane, 1927), and their tubular reabsorption of water is increased by an antidiuretic hormone (Burgess, et al., 1933), many investigators ascribed final urine concentration to active water reabsorption in the thin segment of the loop of Henle. However, because of the thinness of the epithelium of this thin segment, Walker, et al. (1941) postulated that final urine concentration was accomplished in the distal tubule by active water reabsorption. Hargitay and Kuhn (1951) proposed the now widely accepted countercurrent hypothesis which states that a hypertonic urine is produced by passive diffusion of water under the influence of an antidiuretic hormone from the collecting duct into a region of high solute concentration produced by a countercurrent flow in the loop of Henle. Therefore, the need for postulating an active transport of water out of the tubule was eliminated. This hypothesis correlates the length of Henle's loops with the ability of an animal to concentrate urine and is supported by

Sperber's data (1944) which indicated that the renal medulla is relatively larger in desert mammals than in mammals inhabiting more humid environments. Schmidt-Nielsen and O'Dell (1961) have summarized the literature which reveals that the renal concentrating ability increases as the relative size and thickness of the medulla increases.

Aplodontia rufa is considered to be the most primitive of living rodents (McGrew, 1941) and is the only living species of the family Aplodontidae which is in the suborder Sciuromorpha (Simpson, 1945). Its range is restricted to areas of heavy rainfall along the west coast of North America from southern British Columbia to central California, where it is locally known as the "Mountain Beaver" or Sewellel. It is limited to wet areas and subsists principally on ferns and succulent plants (Bailey, 1936). This animal has a relatively thin medulla with few long medullary loops of Henle (Pfeiffer, et al., 1960). The domestic rabbit, Oryctolagus cuniculus, has a relatively thick medulla and a larger number of long loops of Henle (Sperber, 1944). Comparative studies have revealed that A. rufa cannot achieve as high urine concentrations in response to vasopressin or dehydration as can the rabbit when both are maintained on a low protein diet (Dolph, et al., 1962). This is interpreted to imply that short loops of Henle in A. rufa are chiefly responsible for its limited concentrating ability. However, it has been shown that high protein diets may increase the renal concentrating ability in the dog (Levinsky and Berliner, 1959), man (Epstein, et al., 1957; Meroney, et al., 1958), rat (Radford, 1961), and desert rodents (Schmidt-Nielsen, 1958). It is possible that the limited ability

ability of A. rufa to concentrate urine may be based in part upon dietary factors and not entirely on its nephron architecture as it exists on a low protein diet in its normal environment.

Urea is a product of protein metabolism which rises in concentration in the blood as protein intake and metabolism increase. This concentration is usually accompanied by a rise in urine urea concentration and excretion. The rise in urea concentration has been correlated with an increase of the renal concentrating ability in certain mammals (Jaenike 1960 and 1961; Lassiter et al., 1961; Levinsky and Berliner, 1959; Epstein, et al., 1957; Berliner, et al., 1958).

The present study was designed to evaluate the influence of increased protein intake upon the renal concentrating response of A. rufa. If the concentrating ability were not increased in A. rufa, this would suggest that inability to utilize urea to concentrate urine is an important factor in addition to nephron structure. If the concentrating ability were increased, this would support previous studies of a similar nature. The rabbit served as a control to insure that the diet would increase urine concentration in an animal with a different renal architecture.

METHODS AND MATERIALS

Eight A. rufa, which had been livetrapped without injury from the wild, and ten rabbits, which were a cross between the White Giant and the New Zealand White strains and had been obtained from a local dealer, were the subjects for the experiments reported in this study. In general, several A. rufa and several rabbits were studied simultaneously. Identical experimental techniques were employed for both species although minor variations were adopted occasionally to facilitate urine or blood collections. An interval of at least 6 days separated various experimental periods for any one animal.

Thirteen days prior to any experimental work each animal was given a high or a low protein diet. The high protein diet consisted of Purina Rabbit Chow Checker pellets containing 15-17% protein and 0.2% sodium. The low protein diet consisted of lettuce and carrots containing 1.2% protein (Watt, et al., 1950) and 0.0012-0.0031% sodium (Bills, et al., 1949). Water was given ad libitum with both diets. The amount of each diet eaten by A. rufa was measured and the total protein intake for 24 hours was determined. This was done by giving a known amount of pellets or carrots and lettuce ad libitum to each animal. Twenty-four hours later, the amount of food left was weighed and the amount eaten was then considered to be the difference between the original amount and that which was present twenty-four hours later. These measurements were made for 3 consecutive days with 6 A. rufa.

The weight lost by evaporation from the carrots and lettuce was determined by leaving a certain amount of this diet outside the cage while the food consumption measurements were being made. The amount of carrots and lettuce considered to have been eaten by each animal was adjusted according to the percentage of weight lost by evaporation from the sample left outside of the cage. The average amount of pellets eaten was 43 grams/ 24 hours containing approximately 7 grams of protein. The average amount of lettuce and carrots eaten was 297 grams/ 24 hours containing 36 grams of protein. These measurements indicate that a greater amount of protein was ingested by A. rufa eating pellets than by those eating carrots and lettuce.

The total urea-nitrogen and sodium excreted in 24 hours was determined by placing each animal in a metabolism cage, providing the same diet which was given the previous 13 days, and collecting urine samples in bottles which contained a small amount of thymol and mineral oil. The sodium concentration was determined on a Coleman flame photometer and the urea-nitrogen concentration by a colorimetric analysis according to Richter and Lapointe (1959).

Maximum urine concentrations were obtained by injecting exogenous antidiuretic hormone (vasopressin) into A. rufa and rabbits during water diuresis. Maximum concentrations were also obtained from A. rufa by subjecting them to dehydration.

The ratio of urine osmolality to plasma osmolality (U/P ratio) was determined because Dolph et al., (1962) found that this was the most significant and accurate measurement of renal concentrating ability.

This ratio was determined during the vasopressin-injection procedure at the time of maximum hydration and maximum urine concentration. The ratio was also determined periodically during the dehydration procedure (See page 8).

Determination of Maximum U/P Osmolality Ratios

The vasopressin injection technique closely followed the method developed by Dolph et al., (1962). Blood and urine collection techniques were identical for all procedures. Urine samples were obtained by suprapubic compression except for 6 hour collections in the dehydration procedure. Blood samples of 1-2 ml. were withdrawn with a heparinized syringe from the external jugular vein of the subclavian-innominate junction in A. rufa and the marginal ear vein in rabbits after etherizing the animal. Osmolality was determined with the Fiske Osmometer for blood plasma and urine samples. Generally, 2 ml. samples were used for urine determinations and 0.2 ml. samples for plasma determinations. Each sample was tested until readings agreed within a range of 3 milliosmols.

1. Hydration and Vasopressin Injection - After light etherization, a blood sample was obtained and a solution of chloralose in water sufficient to produce a hypnotic condition plus 50 ml. of water per kilogram of body weight was administered by means of a stomach tube. Sodium and urea-nitrogen concentrations were determined in the first blood sample. Ninety minutes later a second load of water was administered. Thirty minutes after the second hydration, blood and urine samples were collected and 1 unit of vasopressin in a volume of 1-2 ml. of water

(Parke, Davis, and Co., Lots No. AG 103-1 and Z 103-DA) was injected subcutaneously per kilogram of body weight. This dosage was required for maximum urine concentration even though this far exceeded the physiological range. Control animals were treated in the same manner as the experimental animals except that they were injected with 1.0 ml. of water instead of vasopressin. Each animal served as its own control in all experiments with A. rufa. Urine osmolality was determined every 30-40 minutes until a peak concentration was achieved and a blood sample was then taken.

In addition, 6 high-protein-fed A. rufa received 1% urea in the drinking water ad libitum 36 hours prior to the vasopressin injection procedure. This was to insure that a high urea concentration was present in the blood for this particular experiment.

2. Dehydration - Each A. rufa was placed in a metabolism cage and given a diet identical to that of the previous 13 day period. Twenty-four hours later a urine pool for the 24 hour period, a blood sample, and a urine sample were collected and the osmolality for each was determined to show the relatively normal condition before dehydration. The animal was weighed at this time and water was removed from the cage. Weight of the animal and urine osmolality were determined periodically until the urine osmolality ceased to rise or began to decline. Plasma osmolality was then determined and dehydration was ended by giving water ad libitum. Twenty-four hours later urine and blood osmolality was determined. A U/P osmolality ratio was determined each time a blood sample was taken (See table 6) but was not

determined at the time of maximum urine concentration because it was not possible to know when the peak of concentration had occurred until the urine osmolality ceased to rise or began to decline. This usually was not known until 12-18 hours after the peak had been reached.

Rabbits were not dehydrated as data of Schmidt-Nielsen and O'Dell (1961) were available.

RESULTS

Maximum U/P Osmolality Ratios Following Vasopressin Injection

Aplodontia - Urine samples collected from every A. rufa injected with vasopressin exhibited a pronounced rise in osmolality (Tables 1, 2, and 3). Concentration began to rise 30-50 minutes after injection and reached a maximum 80-175 minutes after injection (Figure 1). Osmolality declined or did not rise in the control animals. A. rufa maintained on a high protein diet, given 1% urea, or maintained on a low protein diet, achieved maximum urine concentrations which averaged 429, 456, and 421 mOsm, respectively, There was no significant difference after they were tested statistically (Table 10).

Plasma concentration fell after hydration and further declined after vasopressin treatment. The average osmolality at the time of maximum urine concentration was 292 mOsm in A. rufa fed a high protein diet, 290 mOsm in those given 1% urea, and 274 mOsm in those fed a low protein diet. Generally, plasma osmolality in control animals fell after hydration, but usually did not decline again after 1.0 ml. injection of water.

The urine-plasma osmolality ratio (U/P) at the time of maximum urine concentration averaged 1.47 in A. rufa that had been fed a high protein diet, 1.57 in those given 1% urea, and 1.48 in those fed a low protein diet. There was no statistically significant difference between these ratios (Table 10).

Rabbits - Rabbits injected with vasopressin achieved a maximum urine concentration 60-260 minutes after injection and the osmolality rose in a manner similar to that shown by A. rufa (Figure 2). High-protein-fed rabbits achieved a maximum urine concentration which averaged 883 mOsm, while low-protein-fed rabbits achieved a concentration which averaged 715 mOsm (Tables 4 and 5). Osmolality declined or did not rise in rabbits injected with 1.0 ml. of water.

Plasma osmolality changes were similar to those in the A. rufa (Tables 1, 2, 3, 4, and 5). Osmolality at the time of maximum urine concentration was 285 mOsm in those fed a high protein diet and 288 mOsm in those fed a low protein diet.

The U/P osmolality ratio at the time of maximum urine concentration averaged 3.28 in high-protein-fed rabbits and 2.59 in low-protein-fed-rabbits. There was a statistically significant difference between these two ratios (Table 10).

Figure 1. Urine Concentration (mOsm) With Vasopressin in A. rufa #5

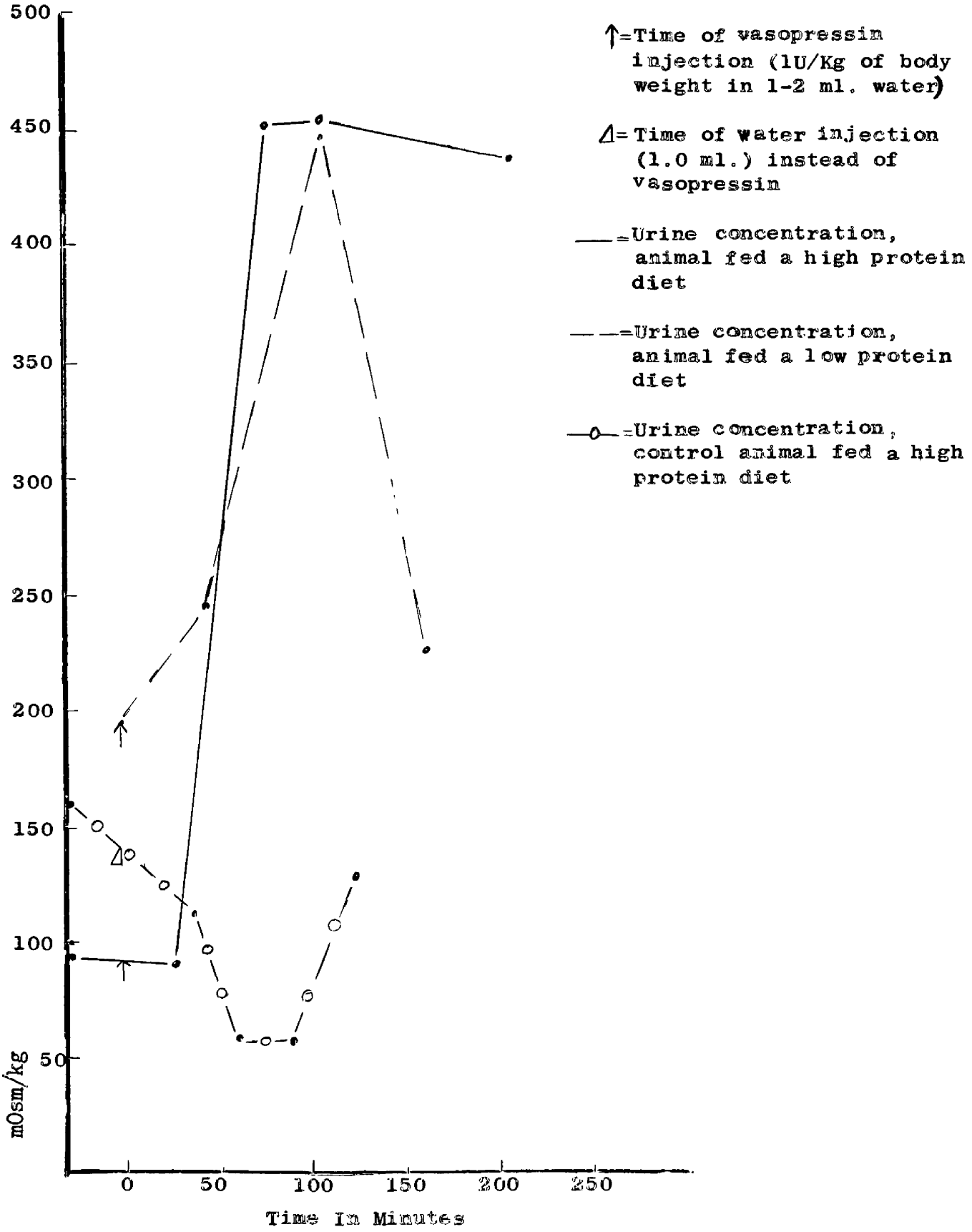


Table 1. Summary of Experimental Results With Vasopressin Injection

Aplodontia rufa - Urea loading (1%)

	Prior to Initial Hydration	Before 1U/Kg of Vasopressin, 30 min. after 2nd Hydration			At Maximum Urine Concentration		
Animal	Plasma mOsm	Urine mOsm	Plasma mOsm	U/P	Urine mOsm	Plasma mOsm	U/P
1	313	-	292	-	447	288	1.55
3	307	292	291	1.00	477	292	1.63
4	302	110	283	.39	466	287	1.62
7	311	200	291	.69	396	294	1.35
8	307	102	292	.35	471	288	1.64
6	-	162	295	.55	481	288	1.67
**6	314	**147	**295	** .50	⊥** 81	⊥**313⊥**	.39
	*309	*169	*291	*.58	*456	*290	*1.57

* = Mean

** = Control, not included in the mean

⊥** = Sample taken at approximately the time of maximum urine concentration in vasopressin-injected animals.

Table 2. Summary of Experimental Results with Vasopressin Injection

Aplodontia rufa - High Protein Diet

	Prior to Initial Hydration	Before 1U/Kg of Vasopressin, 30 min. after 2nd Hydration			At Maximum Urine Concentration		
Animal	Plasma mOsm	Urine mOsm	Plasma mOsm	U/P	Urine mOsm	Plasma mOsm	U/P
1	303	162	295	.55	380	292	1.30
1	315	190	293	.65	373	294	1.27
1	297	109	280	.39	463	260	1.78
1	**311	** 73	**287	**.25	**354	**281	**1.26
2	**314	-	-	-	-	-	-
3	**290	** 51	**280	**.18	**352	**280	**1.26
4	310	-	295	-	451	305	1.48
4	310	102	292	.35	435	292	1.49
5	311	98	312	.31	462	308	1.50
5	305	180	293	.62	441	326	1.35
6	306	96	315	.31	484	287	1.68
6	310	218	290	.75	511	274	1.86
7	301	114	301	.38	-	-	-
7	302	122	286	.43	323	300	1.08
8	309	252	286	.88	394	278	1.42
	*307	*149	*295	*.51	*429	*292	*1.47
Controls		Before 1.0 ml. of water, 30 min. after 2nd Hydration			@	@	@
1	-	-	291	-	97	300	.32
2	298	85	283	.30	83	286	.29
3	312	58	301	.19	28	292	.10
3	296	101	292	.34	54	307	.18
4	322	124	308	.40	70	312	.22
5	304	170	289	.59	153	296	.52
6	303	97	298	.33	91	-	-
7	311	123	292	.42	92	295	.31
8	303	169	299	.57	49	310	.16
	*306	*116	*294	*.39	* 80	*300	*.26
	***306	***128	***294	***.43			

* = Mean

** = Not included in determining the mean because of inadequate adjustment to diet.

*** = Mean, including all animals

@ = Samples obtained at approximately the time of maximum urine concentration in vasopressin-injected animals.

Table 3. Summary of Experimental Results with Vasopressin Injection

Aplodontia rufa - Low Protein Diet

	Prior to Initial Hydration	Before 1U/Kg of Vasopressin, 30 min. after 2nd Hydration			At Maximum Urine Concentration		
Animal	Plasma mOsm	Urine mOsm	Plasma mOsm	U/P	Urine mOsm	Plasma mOsm	U/P
3	313	299	290	1.03	493	291	1.69
4	310	148	-	-	484	301	1.64
5	305	199	308	.65	443	-	-
6	305	160	298	.54	450	-	-
7	303	185	283	.65	390	295	1.32
8	305	365	294	1.24	402	296	1.32
**	**	**	**	**	**	**	**
S	299	127	289	.40	433	279	1.60
T	283	134	-	-	318	235	1.40
Y	286	172	275	.60	372	274	1.40
O	-	122	-	-	452	-	-
I	-	261	-	-	569	-	-
I	-	56	-	-	390	-	-
Q	-	168	-	-	329	-	-
Q	-	167	-	-	366	-	-
	*301	*183	*291	*.73	*421	*282	*1.48
Controls		Before 1.0 ml. of water 30 min. after 2nd Hydration			@	@	@
1	312	152	315	.48	110	323	.34
**	**	**	**	**	**	**	**
I	285	117	261	.50	37	275	.10
X	300	155	275	.60	70	283	.30
J	-	104	-	-	100	-	-
N	-	130	-	-	61	-	-
O	-	108	-	-	35	-	-
P	-	257	-	-	162	-	-
S	-	75	-	-	55	263	.20
	*299	*137	*284	*.52	*74	*274	*.23
	***301	***166	***289	***.67			

* =Mean
** =Dolph et al., (1962)
*** =Mean, including all animals
@ =Samples taken at approximately the time of urine concentration in vasopressin-injected animals.

Figure 2. Urine Concentration (mOsm) With Vasopressin in Rabbit B

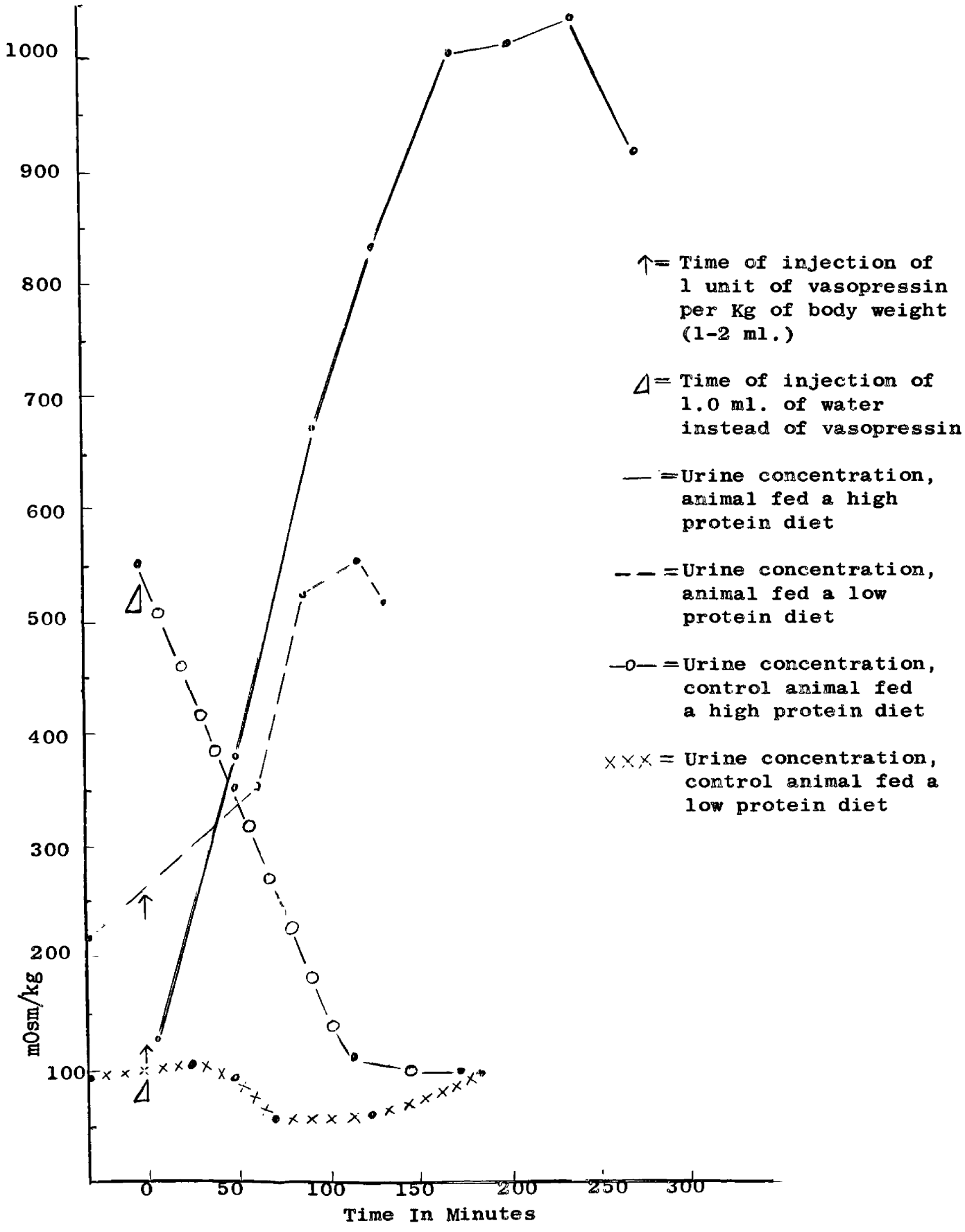


Table 4. Summary of Experimental Results With Vasopressin Injection

Rabbit - High Protein Diet

	Prior to Initial Hydration	Before 1U/Kg of Vasopressin, 30 min. after 2nd Hydration			At Maximum Urine Concentration		
Animal	Plasma mOsm	Urine mOsm	Plasma mOsm	U/P	Urine mOsm	Plasma mOsm	U/P
A	317	245	279	.88	1400	272	5.15
A	306	-	285	-	787	-	-
A	312	-	276	-	1153	274	4.20
A	324	170	279	.61	825	-	-
B	310	304	277	1.10	658	294	2.24
B	316	128	291	.44	1057	290	3.64
B	334	240	279	.86	456	-	-
C	308	298	280	1.06	1155	276	4.18
D	327	357	286	1.25	846	284	2.98
D	310	202	294	.69	929	290	3.20
D	315	149	292	.51	1083	285	3.80
D	334	355	295	1.20	1068	302	3.54
E	311	248	292	.85	636	316	2.01
E	304	264	302	.87	1088	286	3.80
E	328	54	292	.18	700	289	2.42
E	316	137	298	.46	1045	297	3.52
E	316	-	291	-	528	-	-
F	316	275	291	.95	794	275	2.88
F	324	170	293	.58	810	286	2.83
G	321	166	285	.58	1051	270	3.89
G	299	223	266	.84	672	272	2.47
H	322	272	294	.93	925	278	3.33
I	324	355	316	1.12	980	271	3.62
J	317	168	298	.56	550	284	1.94
	*317	*228	*289	*.78	*883	*285	*3.28
Controls		Before 1.0 ml. of water 30 min. after 2nd Hydration			@	@	@
A	325	245	303	.81	86	310	.28
B	307	445	279	1.60	85	277	.31
C	304	141	280	.50	82	278	.30
F	307	424	281	1.51	93	292	.32
	*311	*314	*286	*1.10	*87	*290	*.30
	**316	**241	**288	**.83			

* =Mean

** =Mean, including all animals

@ =Samples taken at approximately the time of maximum urine concentration in vasopressin-injected rabbits.

Table 5. Summary of Experimental Results With Vasopressin Injection

Rabbit - Low Protein Diet

	Prior to Initial Hydration	Before 1U/Kg of Vasopressin, 30 min. after 2nd Hydration			At Maximum Urine Concentration		
Animal	Plasma mOsm	Urine mOsm	Plasma mOsm	U/P	Urine mOsm	Plasma mOsm	U/P
A	306	185	299	.60	758	299	2.54
A	316	105	278	.40	866	289	3.00
B	297	99	286	.35	441	-	-
B	313	214	283	.75	565	282	2.00
D	311	118	289	.40	790	282	2.79
D	322	41	295	.14	512	284	1.80
E	315	94	286	.33	897	312	2.86
F	303	254	293	.86	810	280	2.89
G	317	135	280	.50	711	278	2.56
H	297	80	283	.28	-	-	-
J	306	93	296	.31	804	284	2.83
	*309	*129	*288	*.45	*715	*288	*2.59
Controls		Before 1.0 ml. of water, 30 min. after 2nd Hydration			@	@	@
J	299	52	286	.18	59	302	.20
B	301	91	275	.33	66	287	.23
D	313	162	278	.58	46	287	.16
	*304	*102	*280	*.36	*57	*292	*.19
	**308	**123	**286	**.42			

* =Mean

** =Mean, including all animals

@ =Sample taken approximately at the time of maximum urine concentration in vasopressin-injected rabbits.

Maximum U/P Osmolality Ratios - Dehydration

Each A. rufa subjected to dehydration exhibited changes in the osmolality of the plasma and urine, 24 hour urine output, and weight. Rises in urine concentration were accompanied by rises in plasma concentration, a fall in urine volume, and a fall in weight (Table 6). The average time to which any animal was subjected to dehydration was 50 hours with a maximum of 54 hours and a minimum of 42 hours. The average weight loss was 10.7%.

The urine osmolality averaged 648 mOsm prior to water removal from the cage, reached an average maximum of 814 mOsm 30-42 hours after water removal, and then declined 12-24 hours later to an average of 679 mOsm. The highest urine osmolality achieved was 894 mOsm. Twenty-four hours after water was returned the osmolality declined to 482 mOsm. This was considerably lower than that noted before dehydration.

The plasma osmolality averaged 313 mOsm prior to dehydration, 356 mOsm at the end of dehydration, and 310 mOsm 24 hours later after water was returned to the cage. The highest plasma osmolality obtained was 371 mOsm (Table 6).

The U/P osmolality ratio averaged 2.07 prior to dehydration, 1.91 at the end of dehydration and 1.55 24 hours later.

Table 6. Summary of Experimental Results With Dehydration - Aplodontia rufa

Immediately Prior to Dehydration

Animal	Urine mOsm	Plasma mOsm	U/P	Weight Grams	Urine Volume ml/24 hours
1	641	323	1.98	1000	96
3	681	320	2.13	1045	148
4	635	304	2.09	995	52
6	712	306	2.33	1215	150
7	675	304	2.22	1335	85
8	545	319	1.71	1100	145
	*648	*313	*2.07		*113

Results of Dehydration

Animal	Maximum Urine Concentration mOsm	At Termination of Dehydration					
		Urine mOsm	Plasma mOsm	U/P	Weight Grams	% Weight Lost	Urine Volume ml/24 hours
1	708	523	366	1.43	867	13.3	24
3	894	738	351	2.10	960	8.2	16
4	842	757	353	2.14	895	10.1	15
6	823	710	352	2.02	1125	7.5	30
7	798	640	371	1.72	1165	12.8	30
8	819	706	345	2.04	965	12.3	70
	*814	*679	*356	*1.91		*10.7	*31

Twenty-four Hours After Termination of Dehydration

Animal	Urine mOsm	Plasma mOsm	U/P	Weight Grams	Urine Volume ml/ 24 hours
1	471	329	1.43	931	61
3	330	283	1.17	1065	66
4	413	314	1.32	1038	69
6	650	313	2.08	1265	286?
7	470	309	1.52	1320	265?
8	557	314	1.77	1075	254?
	*482	*310	*1.55		*167

* = Mean

? = May have been some contanination by the drinking water.

Twenty-four Hour Urine Samples

Aplodontia - Twenty-four hour urine samples had an average urea-nitrogen and sodium concentration of 420 mg% and 25 mEq/liter from A. rufa on a high protein diet, 151 mg% and 51 mEq/liter on a low protein diet. Total output of urea-nitrogen and sodium averaged 498 milligrams/kilogram of body weight/ 24 hours (mg/kg/ 24 hr.) and 3.15 mEq/kilogram of body weight/ 24 hours (mEq/kg/ 24 hr.) respectively on a high protein diet, and 477 mg/kg/ 24 hr. and 12.96 mEq/kg/ 24 hr. respectively on a low protein diet (Table 7). There was no statistically significant change in the urine urea-nitrogen total output per kilogram per 24 hours with a change in diet. Figures 3 and 4 show the distribution of individual samples for urea-nitrogen concentration and total output per 24 hours. Total urine output averaged 147 ml/ 24 hr. from animals on a high protein diet and 349 ml/ 24 hr. from those on a low protein diet.

Rabbits - Twenty-four hour urine samples had an average urea-nitrogen and sodium concentration of 738 mg% and 106 mEq/liter respectively on a high protein diet, and 156 mg% and 39.1 mEq/liter respectively on a low protein diet. Total output of urea-nitrogen and sodium averaged 345 mg/kg/ 24 hr. and 5.30 mEq/kg/ 24 hr. respectively from those animals on a high protein diet and 315 mg/kg/ 24 hr. and 7.13 mEq/kg/ 24 hr. respectively from those on a low protein diet (Table 8). There was no statistically significant change in total output of urea-nitrogen or sodium with a change in diet (Table 10). Figures 3 and 4 show the distribution of the individual samples. Total urine output was 153 ml/ 24 hr. from animals on a high protein diet and 623 ml/ 24 hr. from those on a low protein diet.

A. rufa produced urine which had a higher sodium concentration and a total sodium output than that of the rabbit which was given a low protein diet. A. rufa also produced a greater total output of urea-nitrogen/kilogram of body weight than the rabbit regardless of diet.

Table 7. Twenty-four Hour Urine Collection

Aplodontia rufa - High Protein Diet

Animal	Weight Kg.	24 Hour Urine Volume in ml.	U-N mg%	U-N mg/Kg/24 hr.	Na mEq/L	Na mEq/Kg/24 hr.
1	1.0	150	490	735	15.2	2.28
1	1.0	140	372	521	42.0	5.88
2	0.9	380	515	572	35.2	5.25
3	0.8	152	324	615	12.5	2.38
3	0.8	115	370	533	17.0	3.19
4	0.9	126	300	420	7.0	.98
4	0.9	118	302	397	47.0	6.16
5	1.3	160	460	557	32.5	4.00
5	1.3	230	404	715	29.6	5.24
6	1.2	73	686	394	45.0	2.73
6	1.2	150	420	525	20.6	2.58
7	1.4	75	666	356	9.4	.51
7	1.4	142	400	407	27.0	2.74
8	1.0	106	410	435	16.0	1.70
8	1.0	94	310	292	18.0	1.69
		*147	*420	*498	*24.9	*3.15
Low Protein Diet						
1	1.0	250	212	530	49.3	12.30
3	0.8	193	150	363	47.6	11.50
4	0.9	360	190	760	58.5	23.40
5	1.3	415	130	415	41.5	13.40
6	1.2	343	140	399	40.0	11.40
7	1.4	495	148	523	17.5	6.20
8	1.0	390	90	351	32.0	12.50
		*349	*151	*477	*40.9	*12.96

* Mean

Table 8. Twenty-four Hour Urine Collections

Rabbit - High Protein Diet

Animal	Weight Kg.	24 Hour Urine Volume in ml.	U-N mg%	U-N mg/Kg/24 hr.	Na mEq/L	Na mEq/Kg/24 hr.
A	3.3	178	686	370	92	4.98
A	3.3	246	480	358	75	5.57
B	3.2	88	750	206	85	2.34
B	3.2	86	650	175	134	3.61
C	3.3	254	492	396	72	5.64
C	3.3	220	540	361	154	10.23
D	3.0	123	1280	525	147	6.03
D	3.0	84	1280	360	82	2.30
E	2.6	168	686	444	137	8.85
E	2.6	98	820	309	127	4.80
F	2.7	127	592	250	77	3.62
F	2.7	130	828	399	130	6.25
G	3.0	155	694	345	90	4.65
G	3.0	175	560	326	83	4.85
		*152	*738	*345	*106	*5.30
Low Protein Diet						
A	3.3	480	151	220	61	8.90
B	3.2	313	219	214	62	6.06
D	3.0	708	186	439	18.2	4.30
E	2.6	756	140	407	25.3	7.35
F	2.7	790	113	330	10.3	3.02
G	3.0	690	132	303	57.5	13.20
		*623	*156	*319	*39.1	*7.13

* = Mean

Figure 3. Twenty-four Hour Urine Urea-Nitrogen Concentration

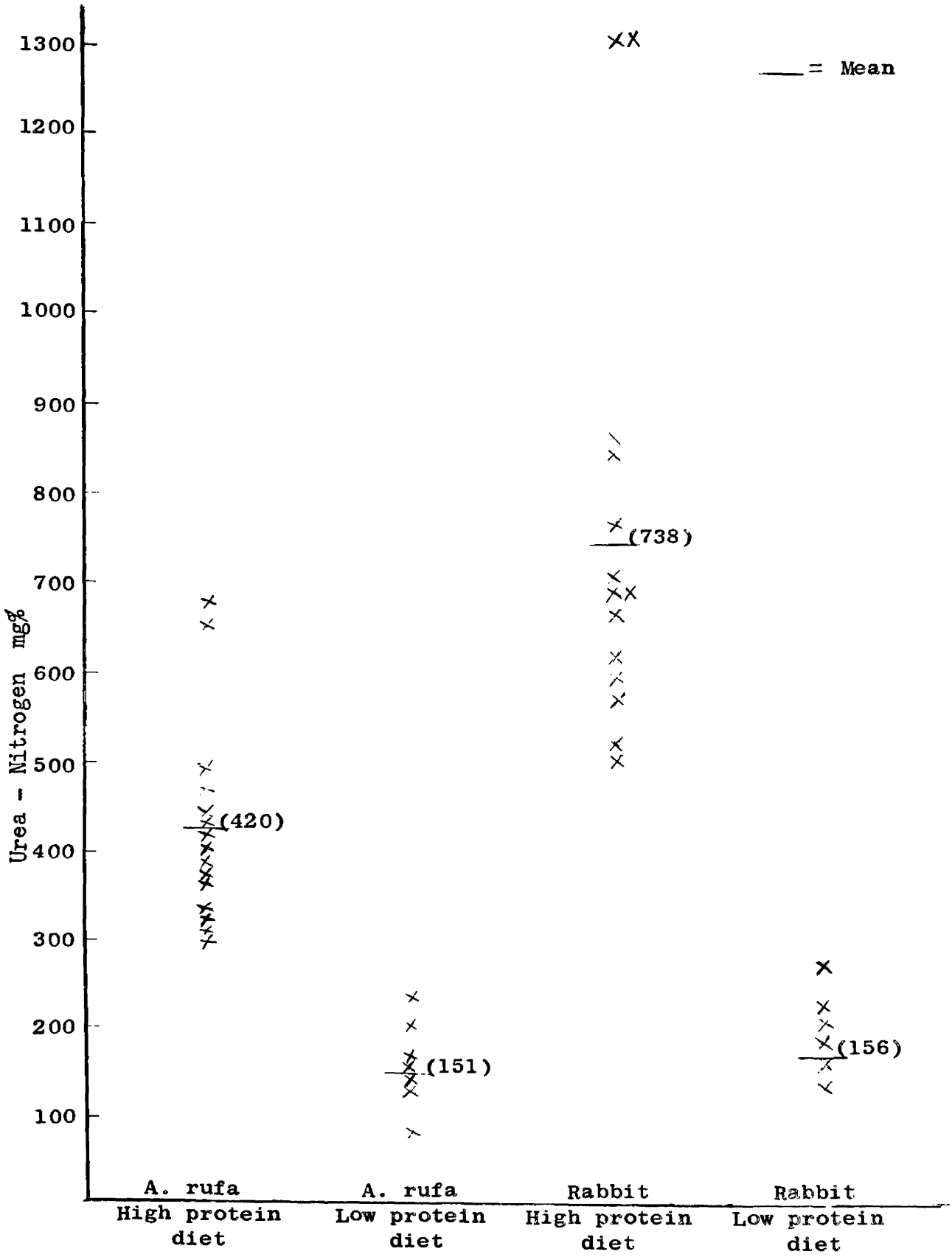
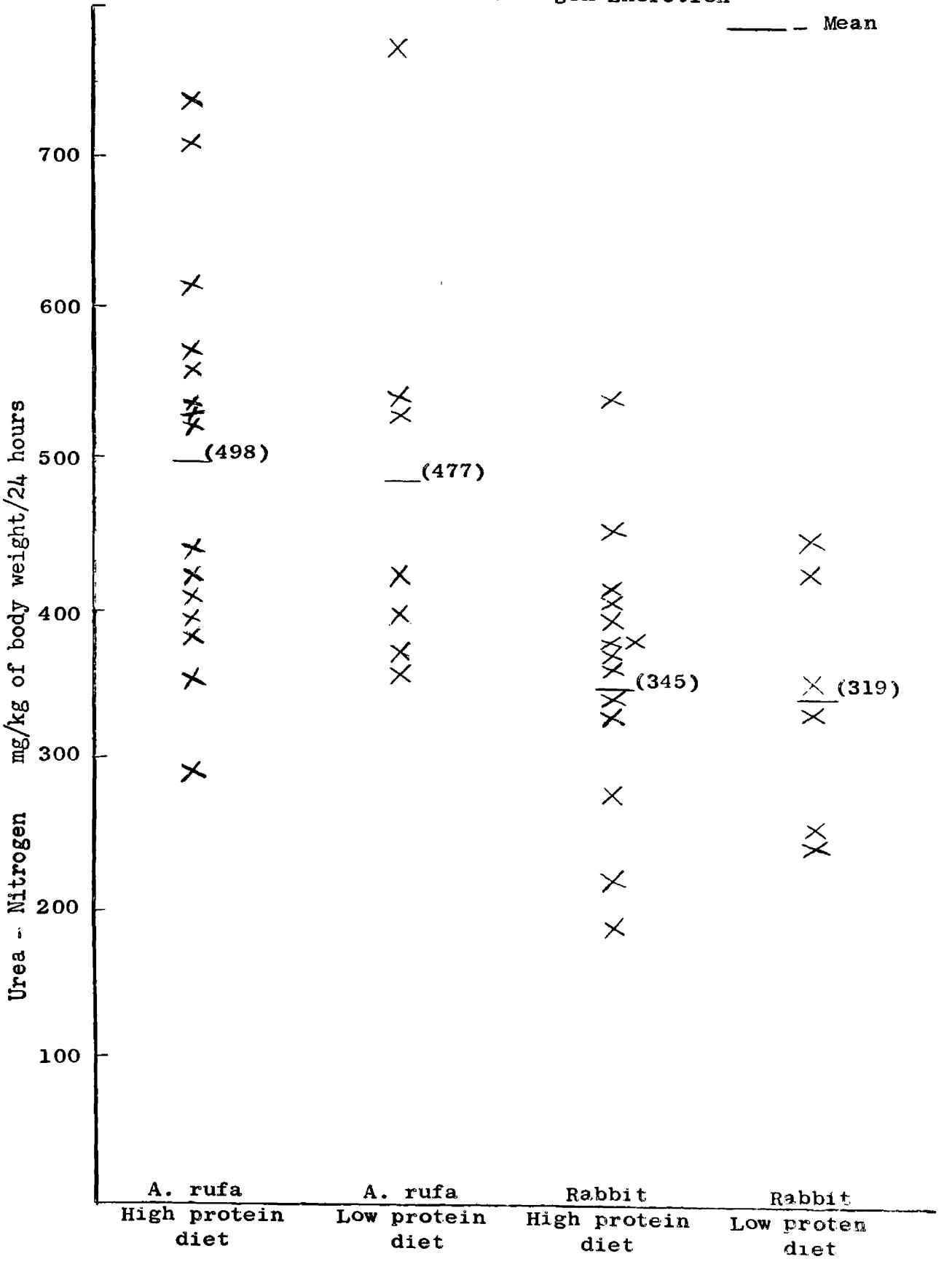


Figure 4. Twenty-four Hour Urine Urea-Nitrogen Excretion



Plasma Urea-Nitrogen and Sodium Concentration

Aplodontia - The plasma urea-nitrogen concentration averaged 11.4 mg% in high-protein-fed A. rufa, 7.7 mg% in those fed a low protein diet, and 20.5 mg% in those given 1% urea (Table 9). The plasma sodium concentration did not change with a change in diet.

Rabbits - The plasma urea-nitrogen concentration averaged 16.4 mg% with the highest being 24 mg% in rabbits maintained on a high protein diet and 12.6 mg% with the highest being 17.5% in those given a low protein diet. The plasma sodium concentration did not change with a change in diet (Table 9).

Statistical Treatment of Data

The Mann-Whitney U test (Siegel, 1956) was used for testing these data. This was chosen because there were too few samples obtained to assume that the observations made were drawn from normally distributed populations which is necessary for the more common parametric tests. Table 10 is a summary of the non-parametric treatment of the data at the .05 level of significance.

Table 9. Plasma Urea-Nitrogen and Sodium Concentration

Aplodontia rufa

Animal	High Protein Diet U-N mg%	Na mEq/L	Low Protein Diet U-N mg%	Na mEq/L	1% Urea U-N mg%
1	13.0	143	9	148	24.6
1	11.6	143	-	-	-
1	13.8	152	-	-	-
2	14.0	155	-	-	-
2	@6.5	@137	-	-	-
2	@7.0	@155	-	-	-
3	@5.0	@135	10.4	154	22.0
3	@5.0	@150	-	-	-
3	12.4	148	-	-	-
4	12.1	157	11.6	152	17.4
5	11.5	151	5.5	155	-
5	9.0	154	-	-	-
5	8.5	143	-	-	-
6	11.0	162	8.5	154	17.4
6	10.0	150	-	-	30.3
7	11.4	161	-	140	17.4
7	@8.6	@148	-	-	-
8	9.6	148	10.0	143	18.0
	*11.4	*151	*7.7	*149	*20.5
			Rabbit		
A	16.0	-	10.0	153	
A	16.5	155	-	-	
A	20.0	163	-	-	
B	10.0	-	12.0	144	
B	14.0	152	**20.8	155	
C	14.8	-	-	-	
C	13.6	149	-	-	
C	13.6	148	-	-	
D	22.5	154	15.0	152	
E	14.4	152	13.0	160	
F	24.0	157	15.5	144	
G	18.5	155	10.2	141	
H	-	-	16.5	-	
J	-	-	17.5	-	
	*16.4	*153	*12.6	*150	

* = Mean

** = Not included in determing the mean because of an error in chemical analysis.

@ = Not included in determining the mean because of inadequate preliminary adjustment to diet.

Table 10. Summary of Statistical Analysis Using Mann-Whitney U Test

Type of Sample	Means of Samples to be Compared		P
Urine U-N mg/kg/24 hr.	A. rufa-H.P. 498 (15)	A. rufa-L.P. 477 (7)	$>.05$
	Rabbit-H.P. 345 (14)	Rabbit-L.P. 319 (6)	$>.05$
	A. rufa-H.P. 498 (15)	Rabbit-L.P. 345 (14)	$<.05$
Plasma U-N mg%	A. rufa-H.P. 11.4 (13)	A. rufa-L.P. 7.7 (6)	$<.025$
	Rabbit-H.P. 16.4 (12)	Rabbit-L.P. 12.6 (8)	$>.05$ but $<.10$
	A. rufa-H.P. 11.4 (13)	Rabbit-H.P. 16.4 (12)	$<.001$
Urine Maximum Concentration mOsm (Vasopressin)	A. rufa-H.P. 429 (11)	A. rufa-L.P. 421 (14)*	$>.05$
	A. rufa-1% urea 456 (6)	A. rufa-L.P. 421 (14)*	$>.05$
	Rabbit-H.P. 883 (24)	Rabbit-L.P. 715 (10)	$<.05$
U/P ratios (Vasopressin)	A. rufa-H.P. 1.47 (11)	A. rufa-L.P. 1.48 (7)*	$>.05$
	A. rufa-1% urea 1.57 (6)	A. rufa-L.P. 1.48 (7)*	$>.05$
	Rabbit-H.P. 3.28 (20)	Rabbit.L.P. 2.59 (9)	$<.025$

* = Mean which includes samples from Dolph et al. (1961)
( ) = Number of observations
H.P.=High Protein Diet
L.P.=Low Protein Diet

DISCUSSION

According to the countercurrent hypothesis, the hairpin-like loop of Henle functions as a countercurrent multiplier system which produces an increase in osmotic concentration in the kidney from the cortex toward the papilla (Hargitay and Kuhn, 1951). Production of hypertonic urine is then accomplished under the influence of anti-diuretic hormone (ADH) by the passive diffusion of water into the region of increasing concentration as the urine flows through the collecting ducts toward the papilla. Gottschalk and Mylle (1959) believe the countercurrent multiplier system probably operates in the following manner. Sodium by an unknown mechanism, is actively transported out of the ascending limb of the loop of Henle, which is relatively impermeable to water, into the medullary interstitium. Chloride follows the sodium as a result of the electrochemical gradient. An osmotic gradient is established between the fluid in the ascending limb and the interstitium as a consequence of this movement. This effect is multiplied as the fluid in the descending limb comes into equilibrium with the interstitum by water diffusing out and probably sodium chloride diffusing into the lumen of the tubule. This raises the osmolality of the fluid entering the tip and ascending limb of the loop and produces an increasing osmotic gradient in the direction of the tip of the papilla. Longer loops of Henle therefore increase this multiplier effect and consequently increase renal concentrating ability (Schmidt-Nielsen and O'Dell, 1961). The movement of the sodium out of the ascending limb causes a hypotonic urine to enter into the distal

convoluted tubule. ADH increases the permeability of the distal tubule which allows water to diffuse into the interstitium of the cortex which results in an isosmotic urine entering the collecting duct. ADH also increases the permeability of the collecting duct which allows water to diffuse into the hyper-osmotic medullary interstitium producing a concentrated urine. The medullary capillaries (vasa recta) maintain a high solute concentration in the medulla by removing the water which is reabsorbed from the descending loop of Henle and the collecting duct.

The vasa recta also add to the efficiency of the system by trapping sodium, urea, and other solutes in the medulla. Wirz (1953) demonstrated that the tonicity of blood in the vasa recta is equal to that of the interstitial or tubular fluid at the same level in the medulla. This is caused by the diffusion of solute into the descending limbs of the vasa recta and out of the ascending limbs while water diffuses in the opposite direction. Therefore, water is removed from the medulla, but solute is retained.

Gamble, McKhann, Butler, and Tuthill (1934) discussed a correlation between urea and "water economy" in renal function. Recent studies have confirmed the unique role of urea in the concentrating mechanism. Ullrich and Jarausch (1956) have shown that there is an increase in urea concentration from cortex to papilla and that the urea concentration in the urine is equal to that in the tip of the papillary interstitium during dehydration. Several investigators have demonstrated that the urea passes out of the collecting ducts into the medullary interstitium and adds to the solute concentration (Klumper, et al.,1958; Bray, 1960; Ullrich, 1960, Lassiter et al., 1961). According to Berliner (1958 and 1960), this reab-

sorption of urea is by passive diffusion out of the collecting ducts and Jaenike (1961) supports this hypothesis by showing that the collecting ducts are made more permeable to urea by ADH. Schmidt-Nielsen (1958), however, attributes increased urea concentration in the medulla to an active transport mechanism based on studies with desert rodents (Schmidt-Nielsen, B., et al., 1948; Schmidt-Nielsen, K., et al., 1948).

Although the mechanism by which urea is concentrated in the medullary interstitium has not been proven, it is known that high protein diets increase the renal concentrating ability in the dog (Levinsky and Berliner, 1959; Bray, 1960; Jaenike, 1960 and 1961), rat (Radford, 1959) and man (Epstein, et al., 1957; Meroney, et al., 1958). Our data show the same effect for the rabbit, but not for A. rufa (Tables 1, 2, 3, 4, and 5). Higher plasma and urine urea concentration induced by feeding a high protein diet or 1% urea indicate that both species served adequately for comparative study (Tables 7, 8, 9, and Figure 3). The different response of the two species to a high protein diet, suggests that there is some mechanism in the kidney of the rabbit which tends to retain urea more efficiently than that of A. rufa. This mechanism may be explained by different morphology of the vasa recta. The vasa recta of the rabbit extend into the tip of the papilla and form hairpin-like loops (Plate 1, Figure 5) but in A. rufa they appear to form a loose rete with many anastomoses and then rejoin to become the venulae rectae (Plate 1, Figure 6). Looped vasa recta, serving as countercurrent exchangers, are considered to be extremely important in trapping solutes in the papillae (Berliner, 1958). The absence of looped vasa recta in A. rufa may reduce the ability to concentrate solute in the medullary

interstitium and thus prevent an increase in concentrating ability even though plasma and urine urea concentration are raised. This is supported by our data which show higher plasma and urine urea concentrations in the rabbit than in A. rufa, although total urea excretion per 24 hours was higher in A. rufa (Figure 4). The failure to increase the renal concentrating ability by feeding a high protein diet in A. rufa is quite similar to that shown in the pig, beaver, and Psammomys (Schmidt-Nielsen, et al., 1961). The nephron structure is quite similar in these animals with the exception of Psammomys. Although the anatomy of the renal medullary vasculature has not been described in the pig and beaver, it is possible that their failure to increase the renal concentrating ability with a high protein diet could also be explained by a lack of looped vasa recta.

The total urea-nitrogen excretion per 24 hours may have been higher in these animals fed a high protein diet even though the results do not reveal a significant difference in excretion between animals on the two diets (Table 10). A rapid diffusion of urea out from hypertonic urine in the ureter and bladder occurs at low urine flows lowering the urea concentration and total output obtained from 24 hour urine samples (Levinsky and Berliner, 1959). This loss of urea from the urine may explain the lack of a statistical significant difference in total urine urea output in 24 hours.

The high sodium excretion by low-protein-fed A. rufa (Table 7) was probably caused by the increased urine flow through the loops of Henle, washing sodium out of the medulla into the urine and preventing an accumulation of sodium to occur (Malvin and Wilde, 1959). Lack of looped vasa recta, which would result in a less efficient countercurrent

system, may account for a greater urine concentration of sodium in A. rufa than in the rabbit.

The maximum urine concentration achieved during dehydration when maintained on a high protein diet in A. rufa (Table 6) approximated the average of 810 mOsm achieved in the animals studied by Dolph (1961) and were slightly higher than the average of 725 mOsm reported by Nungesser, et al. (1960) who fed the animals desiccated carrots.

Schmidt-Nielsen and O'Dell (1961) report a nearly maximal urine concentration of 1390 mOsm in dehydrated rabbits fed a normal laboratory diet. This is higher than the maximum of 1087 reported by Dolph (1961) who fed the rabbit desiccated carrots. Although few samples have been obtained from the rabbit, it seems likely that a high protein diet increases the renal concentrating ability in response to dehydration as it does to vasopressin.

Dolph (1961) also found higher urine concentration in response to dehydration than to vasopressin during water diuresis. The possible reasons for this were reviewed by him.

SUMMARY

Several investigators have shown that high blood and urine urea concentration increases the renal concentrating ability. A similar investigation was carried out with A. rufa and rabbits.

During the vasopressin-injection procedure, 24 experiments were performed on high-protein-fed A. rufa, 7 on low-protein-fed A. rufa, 7 on urea-loaded A. rufa, 28 on high-protein-fed rabbits, and 15 on low-protein-fed rabbits. All hydrated animals which received vasopressin showed significant rises in urine concentration. High-protein-fed rabbits achieved urine concentration and U/P osmolality ratios which were significantly higher than those achieved by low-protein-fed rabbits. There was no significant difference in maximum urine concentration and U/P osmolality ratios achieved by A. rufa regardless of diet or urea loading.

A total of 6 high-protein-fed A. rufa were subjected to dehydration. The maximum urine concentration achieved was no higher than that achieved by dehydrated low-protein-fed A. rufa.

Fifteen 24 hour urine collections were obtained from high-protein-fed A. rufa, 7 from low-protein-fed A. rufa, 14 from high-protein-fed rabbits and 6 from low-protein-fed rabbits. The urea-nitrogen concentration was higher in those fed a high protein diet and the urine urea-nitrogen concentration was higher in rabbits which were fed a high protein diet than in A. rufa fed the same diet. However, A. rufa excreted more urea-nitrogen in 24 hours than did rabbits which were fed either diet. Low-

protein-fed A. rufa excreted more sodium than high-protein-fed A. rufa or rabbits fed either diet. Total 24 hour urine volume from high-protein-fed animals was less than that excreted by those fed a low protein diet.

The plasma urea-nitrogen concentration was determined in 18 high-protein-fed A. rufa, 6 low-protein-fed A. rufa, 7 urea-loaded A. rufa, 14 high-protein-fed rabbits, and 9 low-protein-fed rabbits. The highest urea-nitrogen concentration was in the urea-loaded A. rufa. High-protein-fed animals exhibited a higher urea-nitrogen concentration than those fed a low protein diet.

The plasma sodium concentration was determined in 18 high-protein-fed A. rufa, 7 low-protein-fed A. rufa, 9 high-protein-fed rabbits, and 7 low-protein-fed rabbits. There was no significant change in concentration with a change in diet.

It is concluded that a high protein diet does not increase the renal concentrating ability in A. rufa, but it does increase it in rabbits. Therefore, other factors as well as the structure of the loops of Henle play an important role in urine concentration. The lack of looped vasa recta in A. rufa may explain this species' inability to increase urine concentration when it is fed a high protein diet. The presence of looped vasa recta, which could act as countercurrent exchangers and thus increase the efficiency of trapping urea in the medulla, may be responsible for increasing the renal concentrating ability in rabbits which were fed a high protein diet.

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PLATE I

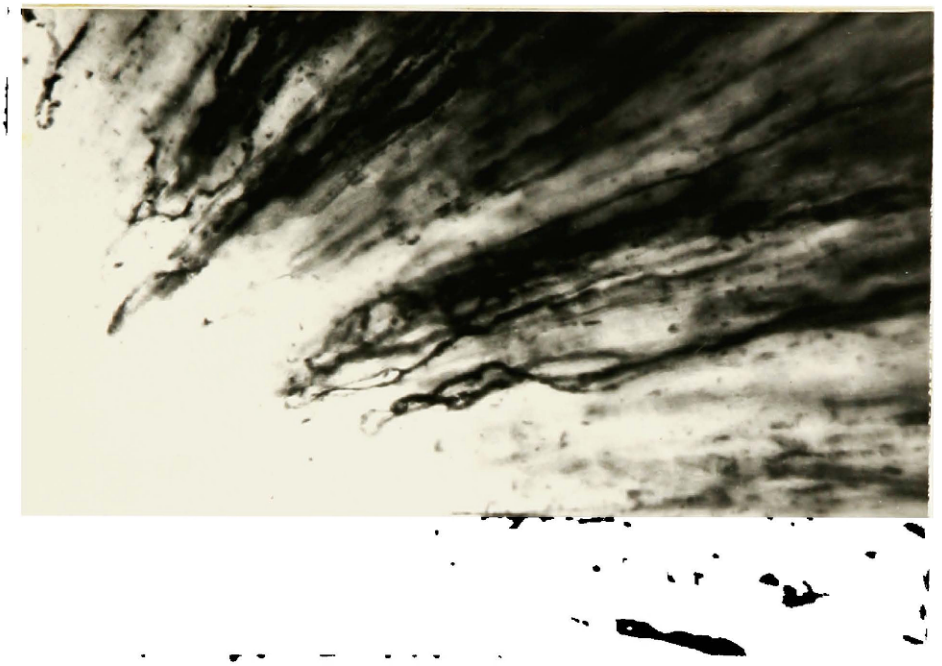

Figure 5. Looped Vasa Recta in the Renal Papilla of the Rabbit (India ink injection via the renal artery).

Figure 6. Vasa Recta Ending in a Rete in the Renal Papilla of A. rufa (India ink injection via the renal artery).