EXPERIMENTAL HYPOSTHENURIA [1, 2]

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The clinical usefulness of the specific gravity test of kidney function, and the variety of conditions under which impairment of concentrating ability is encountered, furnished the incentive for this study. This test is most commonly used as an indication of the degree of renal damage in glomerulonephritis and arteriolar nephrosclerosis, in both of which there is a significant reduction in the number of nephrons. A urine of low specific gravity, however, is also encountered in some cases of acute nephritis, many acute infections, chemical poisoning, prostatic obstruction, pyelonephritis, trauma to the kidney, and severe anemia, in which there is usually no significant reduction in the number of nephrons. It seemed proper, therefore, to attempt to determine whether loss of concentrating power was due to a single mechanism, or whether it might be brought about in more than one way.

The clinical importance of loss of concentrating power has been recognized since the papers of Blackall (12) in 1820 and Bright (14) in 1827, and interest in the mechanism producing it evidenced by the many explanations that have been offered (73). Christison in 1839 (21) was apparently the first to employ a concentration test. He determined the specific gravity of morning urine, giving the average normal as 1.024 or 1.025, with a range from 1.016 to 1.030, while in patients with "granular kidneys" it fell below 1.016. He cautions that the gravity should be corrected for any protein present.

Christison (21) and Rayer (66) interpreted the polyuria as a compensatory mechanism for the loss of ability to concentrate solutes. Bartels (9) accepted Traube's hypothesis that destruction of renal mass led to an elevated blood pressure and cardiac enlargement, so that more blood was forced "through the urinary apparatus," and noted that when the heart failed, "the abnormally large amount of urine falls off, and the abnormally low specific gravity rises." Johnson (41) believed the polyuria unrelated to the arterial tension, but caused by the diuretic influence of some abnormal products in the circulation. Newman (61) suggested that the polyuria of the contracted kidney was due to obstruction of the lymphatics. Thoma (82) thought it due to increased glomerular permeability. v. Korányi (91) and his associates, who investigated hyposthenuria extensively, offered only the suggestion that with failing kidney function, the capacity of the kidney to do the work entailed in the processes of concentrating or diluting solutes withdrawn from the blood progressively diminishes. Schlayer, Hedinger, and Takayasu (74) believed the polyuria of Bright's disease to be due to hyperirritability of damaged renal vessels in response to a diuretic stimulus.

Muller (60) did not regard hyposthenuria as the necessary result of reduction in kidney mass. He could not see why a small mass could not put out a urine of normal specific gravity. He believed both the polyuria and hyposthenuria of infectious disease, urinary obstruction, glomerulonephritis, and vascular disease to be due to the vicarious secretion of water by the tubules.

Volhard (90) emphasized the wide variety of conditions in which loss of concentrating power occurs. In those in which kidney mass is reduced the remaining nephrons respond by a "compensatory" polyuria, as does the normal kidney to increased demand for elimination of waste. This polyuria exhausts the secretory apparatus (granules and vacuoles) in the tubule cells so that excretion of a concentrated urine is impossible. When there is no reduction in kidney mass, he believed the tubule secretory apparatus is primarily damaged by the poison or by increased pressure in the peritubular capillaries.

Mayrs (55) believed that while the rapid passage of fluid down the remaining tubules may contribute to the polyuria of chronic nephritis, the chief fault must be in the inability of the diseased tubule cells to overcome as great an osmotic pressure as in health.

Fremont-Smith *et al.* (27) suggested that a large volume of urine is derived from a small number of glomeruli with all their capillaries open; a small volume of concentrated urine from a larger number of glomeruli with only a few capillaries open in each. This is contrary to Verney's (89) hypothesis, based on his experimental results. Hayman and Starr (34) also found that with diuresis practically all glomeruli were open.

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[1] The results of some of these experiments were presented before the Fifty-First Annual Meeting of the Association of American Physicians, May 6, 1936 (Tr. A. Am. Physicians, 1936, **51**, 453).

[2] The expenses of this investigation were defrayed in part by a grant from the Commonwealth Fund.

[3] Dr. Shumway took part in the earlier experiments while serving as Assistant Resident; Dr. Dumke carried out most of the experiments on ureteral obstruction during his fourth year in medical school; Dr. Miller joined in the later experiments.

Rehberg (68) pictured the mechanism bringing on polyuria as follows: "When the filtrate rate is considerably decreased, nitrogen retention in the blood begins. The result is that the glomerular filtrate contains a much higher concentration of nitrogenous substances than usual, so that even with normal tubules the concentration limiting the reabsorption of water is reached at an earlier stage. Consequently, a larger amount of fluid is left which cannot be reabsorbed, a condition even more pronounced if the tubules are injured also." Hyposthenuria and isosthenuria would be explained in the same way.

Govaerts's (32) only suggestion was that in terminal nephritis the number of glomeruli may be so reduced that the volume of glomerular filtrate cannot allow for any variation in water output.

Fishberg (25) emphasized "the unitary nature" of impairment of renal function, that "in almost all diseases which cause widespread injury to the kidney there is loss of concentrating ability, which applies to each and every urinary constituent." He believed loss of concentrating ability is almost always associated with a diminution in the number of functioning renal units and increase in the amount of filtrate per unit. Åkerrén (3) offered the same explanation for the function of the Schrumpfniere. He believed the histological changes seen in the tubule cells did not necessarily have anything to do with the increase in urine volume or loss of concentrating power. This hypothesis (of Fishberg and of Åkerrén) will not account for hyposthenuria with a normal number of nephrons.

Chasis and Smith (19) suggested, from studies of inulin/urea clearance ratio, that failure of the "facultative" reabsorption of water in the distal tubule leads to the clinical condition of polyuria and hyposthenuria, while impairment of the "obligatory" reabsorption in the proximal tubule, perhaps brought about by excessive excretion of base and chloride, leads to isosthenuria.

It is apparent from this review that while some authors have been impressed by the variety of clinical and pathological conditions accompanied by hyposthenuria, most have attempted to explain it by a single mechanism in all cases, either tubular damage or the rapid passage of an abnormally large quantity of fluid down a small number of tubules.

In order to investigate whether a single mechanism is adequate to explain all cases it seemed appropriate to produce polyuria and hyposthenuria in dogs by various experimental means and then to study the ability of these animals to excrete a concentrated urine under various circumstances. If any conditions could be found which caused excretion of a urine of high specific gravity, it seemed reasonable to assume that in such cases the tubular cells were still normal (or else that the circumstances of the experiment had led to their recovery) and that the mechanism of the hyposthenuria did not lie primarily in parenchymal damage.

METHODS

The experimental methods used to produce hyposthenuria were reduction in kidney mass, uranium poisoning, and ureteral obstruction. In addition, some observations have been made on the effect of denervation, diet, anemia, vitamin B_6 deficiency, pregnancy, and constriction of the renal arteries on concentrating ability.

All experiments were made on healthy female mongrel dogs of unknown age, weighing from 5 to 25 kgm. They were kept in well ventilated cages and fed a stock diet of Ralston's Purina Dog Chow. All were observed at least four weeks before being used.

When hyposthenuria had been produced, attempts to obtain a concentrated urine fell into several groups.

1. *Diet.* Concentrating ability on low and high protein diets were observed in normal dogs and after subtotal nephrectomy. Jolliffe and Smith (42) showed that creatinine and urea clearances were reduced on a low protein diet, but did not study concentrating ability. Their original cracker meal diet was used for low protein periods in some experiments, in others Pitts' modification (64) was used. One pound of ground lean meat, alone or plus 5 grams NaCl daily, furnished the high protein diets. One animal was given Whipple and Robscheit-Robbins' (92) bread diet and salmon.

2. *Hormones.* Pituitrin in doses of 40 to 80 international units was given over a period of 2 to 8 hours after water had been withheld for 24 hours and the highest specific gravity obtained on several catheter specimens during a 12-hour period recorded. The effect of adrenal cortical extract (eschatin, 2 to 10 cc.) was studied in a like manner.

3. *Increased concentration of salts in the glomerular filtrate.* Sodium sulphate, or a mixture of sodium sulphate and bisodium phosphate, in doses of 0.5 to 1.0 gram per kgm. was injected slowly, intravenously, at the end of a 24-hour concentration test and the maximum specific gravity obtained during the following 7 to 12 hours recorded. These salts were used since Alving and Van Slyke (5), and Addis and Foster (2) have called attention to the fact that sulphates and phosphates have a greater effect on urinary specific gravity, for a given concentration, than any of the other salts or urea.

4. *Attempts to increase the plasma colloid osmotic pressure, and so reduce the effective filtration pressure in the glomerular capillaries.* The means used were intravenous injections of acacia, dehydration by croton oil (or magnesium sulphate) and arica nut catharsis, injection of dog plasma concentrated by freezing and drying, or by Thalhimer's (80) method of evaporation in cellophane tubing, or by intravenous injections of sucrose. When sucrose was used, 35 to 50 cc. of a 50 per cent solution was given in the morning and the dog put in a metabolism cage. A second dose was usually

given in the afternoon. The next morning, the animal was catheterized, and this added to the cage specimen. These specimens all contained large amounts of sucrose, the specific gravity of course depending on the total volume. The animal was catheterized again after 2 to 3 hours. This specimen usually had less than 1 per cent sucrose and the gravity given is corrected for sucrose. This was done because our interest is in the ability of the kidney to concentrate normal urinary constituents, and we have no data on the ability of the diseased human kidney to concentrate sucrose under similar conditions for comparison.

5. *Decreased rate of filtration in order to allow more time for reabsorption in the tubule.* Under sodium pentobarbital anesthesia, sufficient spinocaine was injected subdurally (after laminectomy) to lower blood pressure to 80 to 100 mm. Hg, recorded from a cannula in carotid artery. In one animal (Dog 64), Dr. Goldblatt put a clamp around the aorta above the renal arteries and constricted it sufficiently to lower femoral pressure to about 80 mm. Hg.

The ability of an animal to excrete a concentrated urine under control conditions and after injury was judged by a "concentration test." After trial of several techniques, that adopted consisted of emptying the bladder by catheter, and then placing the animal in a metabolism cage without food or water. After 24 hours, the animal was catheterized again, cage and catheter specimens combined, and the volume and specific gravity, corrected for any protein present, recorded. This technique naturally raises the question of the propriety of using the specific gravity of the whole 24-hour specimen. In most of the clinical concentration tests the specific gravity of the urine passed during the latter part of a period of dehydration is used as a measure of concentrating ability. It might seem that it would have been better to have followed a similar procedure with the dogs, and recorded only the gravity of the urine passed during the latter part of a 24-hour period. The relative volumes of cage and catheter specimens varied tremendously; frequently no cage specimen was obtained, the catheter specimen representing the entire 24-hour excretion. The gravity of urine passed during the latter part of a 24-hour period of water deprivation was not significantly higher than that passed during the earlier part with a sufficient frequency to justify a more elaborate technique. In 50 concentration tests, the specific gravity of cage and catheter specimens were determined separately; in 31 the catheter specimen was of higher specific gravity, in 19 lower. In 30, or 60 per cent of the determinations, the specific gravity of the catheter specimen was within 0.005 of the cage specimen. The mean specific gravity of the catheter specimen exceeded that of the cage specimen by 0.0024, its standard deviation being 0.0071.

A single concentration test may at times fail to give a reliable estimate of an animal's ability to concentrate. That is, animals fed on the same diet and subjected to repeated concentration tests by the above technique will occasionally show gravities distinctly lower than the range on other tests. We have no satisfactory explanation for this, and the possible contributing factors have not been investigated. Figure 1 shows the distribution of specific gravities in 122 tests on 46 normal female dogs. On repeated tests all could excrete urine of higher specific gravity than 1.030; 41, or 89 per cent, better than 1.040; 21, or 46 per cent, better than 1.050; and 5, or 11 per cent, better than 1.060. For this reason, it has been necessary to carry out several tests on any animal both before and after any experimental procedure. While in general the urine volumes tended to be lower with higher specific gravities, the correlation was poor. There was no relation between size of animal and maximum specific gravity over the size range used. Withholding water for 48 hours as a rule yielded a urine of somewhat higher gravity than that obtained after 24 hours. The average increase was 0.007 (Table I).

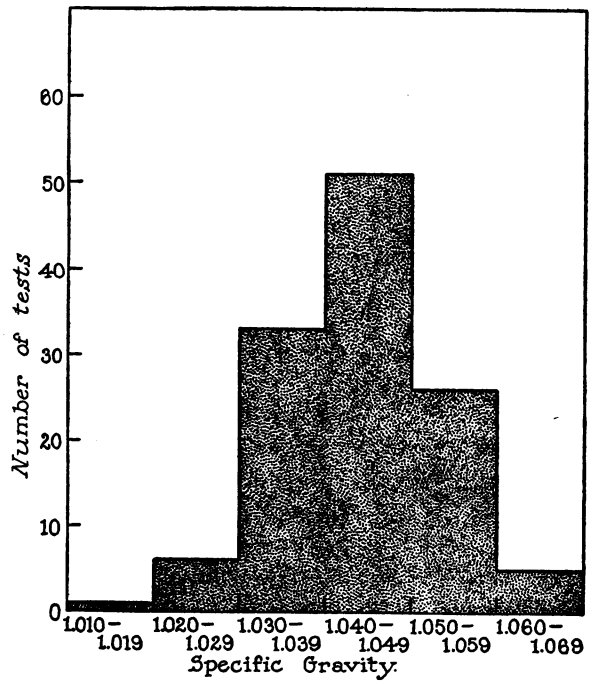

FIG. 1. DISTRIBUTION OF SPECIFIC GRAVITIES IN 122 TESTS ON 46 NORMAL FEMALE DOGS

When urine volumes were adequate, specific gravities were determined to the fourth place by a Westphal balance with a 10 cc. plummet at 20° C. and compared to water at 4° as unity. Where volumes were small, gravities were determined by pyknometer at room temperature and corrected to 20° assuming a linear relation between the specific gravity of urine and water over this small range of temperature differences. All gravities were corrected for protein, using Lashmet and Newburgh's factor (47). Proteins were determined by Shevky and Stafford's method (76) after calibration of the volume of precipitate per gram of protein by macro-Kjeldahl analyses. The specific gravity figures have been rounded off to three places to save space.

Creatinine and urea clearances were determined on the

TABLE I

Urine specific gravity for first and second 24 hours of a 48-hour concentration test in normal animals and after subtotal nephrectomy

Dog	Normal		Dog	Subtotal nephrectomy	
	First 24 hours	Second 24 hours		First 24 hours	Second 24 hours
33	1.044	1.055	3	1.028	1.025
36	1.042	1.052	4	1.020	1.023
37	1.034	1.037	8	1.016	1.021
38	1.042	1.045	15	1.029	1.037
39	1.050	1.064	17	1.010	1.012
40	1.051	1.044	19	1.025	1.022
	1.044	1.061	41	1.026	1.034
			44	1.023	1.023

majority of animals, inulin on a few. Creatinine was estimated by Rehberg's (67) method. Two to three grams of creatinine were given by stomach tube one and one-half hours before the test. Usually blood samples were taken at the beginning and end of each collection period, occasionally in the middle of each period. The length of collection periods varied from 10 minutes to one hour, depending on the urine flow. The bladder was emptied by catheter at the end of each period. We have not obtained more complete emptying of the bladder by washing with saline or injection of air than by a properly done catheterization. The adequacy of this method has been shown (*1*) by recovering only insignificant amounts of the test substance in washings, and (*2*) by laparotomy after careful catheterization. Urea in urine was estimated by Van Slyke's (85) gasometric urease method, in blood by the same method in the earlier experiments, in the later ones by the hypobromite method (86). Inulin was administered intravenously in doses of 1 to 2 grams per kgm. and estimated in an iron filtrate (77) of plasma and urine by the Shaffer-Somogyi (75) method, before and after acid hydrolysis (72). Blood samples were centrifuged immediately after being drawn.

Blood pressure was determined by Jensen and Apfelbach's (39) direct method. Glomeruli were counted by a modification of Kunkel's (45) method, which permits a correction for uninjected glomeruli. It is assumed, for the purpose of estimating the original equipment of an animal, that the number of glomeruli in each of the kidneys was approximately equal.

Subtotal nephrectomy

Polyuria and hyposthenuria have been repeatedly produced experimentally by reduction in kidney mass. The literature is reviewed by Chanutin and Ferris (17). Reduction in functioning mass has been accomplished by surgical removal of renal tissue, ligation of branches of the renal arteries, injection of non-absorbable particles into the renal artery, and exposure of the kidney to x-ray. The results have not been entirely consistent, due to the survival of varying amounts of kidney tissue and presumably to the various experimental methods used. Some of the earlier investigations were more concerned with the relation of the kidneys to metabolism and the existence of an internal secretion of the kidney than they were with functional disturbances.

The first partial nephrectomy was done in 1889 by Tuffier (84) who removed one kidney and then part of the other in dogs. He noted no change in the elimination of urine or urea, and that 1.5 grams of kidney per kgm. was compatible with life. De Paoli (22) in similar experiments on cats, dogs, and rabbits believed one-half of one kidney the minimal amount for survival. Bradford (13) found that removal of approximately two-thirds of the renal tissue was followed by a marked and persistent polyuria, and that the greater the amount of tissue removed, the greater was the polyuria. There was an accompanying reduction in specific gravity from the normal of 1.030 to 1.050 to from 1.010 to 1.020. He states that the dogs were unable to concentrate their urine or to put out a high concentration of urea. Passler and Heinecke (62) noted the polyuria, but did not record specific gravities. Polyuria was also noted by Janeway (38), Allen, Scharf, and Lundin (4), Lundin and Mark (50), Hartman (33), Apfelbach and Jensen (7), and Chanutin and Ferris (17). No change in urine volume was found by Bainbridge and Beddard (8), Pilchèr (63), Anderson (6), Mark (52), Mark and Geisendörfer (53), and Cash (15). A fixed low specific gravity, with inability to concentrate was recorded by Anderson; Mark; Lundin and Mark; Hartman; Apfelbach and Jensen; and Chanutin and Ferris; no change in specific gravity by Bainbridge and Beddard; Karsner, Bunker, and Grabfield (43); and by Cash.

Arterial hypertension also was recorded by Passler and Heinecke; Janeway; Allen, Scharf, and Lundin; Mark and Geisendörfer; Lundin and Mark; Hartman; Chanutin and Ferris; and Wood and Ethridge (93); only in the postoperative period by Cash and by Ferris and Hynes (24). No effect on blood pressure was found by Anderson, by Apfelbach and Jensen, and by Adams, Egloff, and O'Hare (1). The elevation of blood pressure in dogs, when present, was only slight or moderate, 10 to 35 mm. Hg, and not of the same order as that obtained by Goldblatt *et al.* (30) by constricting the renal arteries. Cash believed two factors necessary for the production of hypertension, reduction of renal tissue to 50 per cent of normal and the presence of necrotic renal tissue, a conclusion challenged by Chanutin and Ferris who found the hypertension to persist in rats after all necrotic tissue had been absorbed. By ligation of both poles of one kidney and removal of the other in rats, Chanutin and Ferris (17) produced a chronic renal insufficiency characterized by polyuria, low fixed specific gravity, albuminuria, nitrogen retention, hypertension, and

cardiac hypertrophy. The polyuria seen early, without hypertension, was thought to be due to increased glomerular permeability. When pathological changes in the renal rest had taken place, as indicated by albuminuria and elevation of the nonprotein nitrogen, there was generally a hypertension. This was assumed to be a compensatory mechanism to maintain an increased volume of urine, and the polyuria was believed dependent to a great extent on the increased blood pressure. The pathological changes were presumably due to the protein in the diets (16) since it was more marked on high protein diets. Mark also produced a rapidly fatal insufficiency in subtotal nephrectomized dogs by feeding meat. Chanutin and Ludewig (18) believed the urea clearance a good indicator of the degree of renal damage, while the concentration test showed only qualitative reduction in function, since it might be low with normal clearances, while reduced clearances were always accompanied by low specific gravity. This conclusion is at variance with that of Alving and Van Slyke in man (5).

Since other factors such as necrotic tissue, fibrosis, possible tubular damage, and inflammation are present when functioning kidney mass is reduced by ligation of arteries, radiation, or injection of foreign material, surgical removal was selected as the method best adapted to yield an uncomplicated picture of the effects of reduction in kidney mass.

After a preliminary period of observation, healthy female mongrel dogs were anesthetized with ether and approximately one-third of the right kidney was removed through a lumbar incision. Two to six weeks later the left kidney

TABLE II

Summary of data before and after operation on animals subjected to subtotal nephrectomy†

Dog	Weight	Before operation						After operation						Kidney weight and glomerular count		Survival
		Concentration test		Mean clearance		Blood urea nitrogen	Mean blood pressure	Concentration test		Mean clearance		Blood urea nitrogen	Mean blood pressure			
		Volume, Mean, Range	Specific gravity, Mean, Range	Creatinine	Urea			Volume, Mean, Range	Specific gravity Mean, Range	Creatinine	Urea			Left	Right	
	kgm.			*cc. per minute*	*cc. per minute*	*mgm. per cent*	*mm. Hg*			*cc. per minute*	*cc. per minute*	*mgm. per cent*	*mm. Hg*	*grams; thousands*	*grams; thousands*	*months*
3	12.5	152 82–270	1.038(3) 1.037–41				150(2)	273 160–418	1.023(7) 1.019–28			18.5	175(2)	28.0	23.5 36	1.6
4	10.5	95 43–135	1.038(3) 1.024–48				145(2)	169 110–244	1.021(4) 1.020–23				172(2)	28.0 214	15.5 60	3.5
6	8.0	91 90–92	1.040(2) 1.035–45	31.0	13.5	24.1	114(2)	204 144–275	1.017(5) 1.013–28			62.6	165(2)	28.0 346	12.0 98	2.5
8	13.3	195 81–242	1.030(4) 1.026–34	45.0	33.0	8.7	125	545 235–1016	1.015(4) 1.013–16	10.7	5.4	63.0	175(3)	43.5 395	16.3 86	1.0
9	17.6	177 100–235	1.034(3) 1.033–35	56.5	27.5	15.2	115	397 220–650	1.021(5) 1.015–25	20.7	9.1	32.4	153(3)	38.5 212	28.0 140	1.0
10	12.0	108 88–128	1.042(2) 1.037–46	46.3	27.2	24.0	114(3)	248	1.017	4.7	3.1	71.0	145	30.0 442	16.5 64	1.0
15	17.8	164 85–340	1.037(4) 1.025–44	62.7	32.7	11.0	125(2)	248 59–570	1.025(7) 1.018–29	34.4	16.2	35.5	142(2)	53.0 376	62.5 254	2.5
17	8.4	113 87–135	1.052(3) 1.040–66	51.9	15.6	13.4	130(2)	427 330–620	1.011(4) 1.009–14	5.4	3.0	107.0	140(5)	31.5 201.0(?)	14.8 62	3.0
19	10.7	141 56–267	1.040(3) 1.012–57	39.1	19.9	22.2	140(2)	252 223–290 416 222–730	1.021(8) 1.016–25 1.014(9)* 1.011–16	22.5 10.7*	13.8 4.2*	21.9 67.0*	155(9) 164(8)	77.5 556	30.0 68	27.5
41	11.0	111 39–140	1.038(5) 1.021–57	53.6‡ 51.6	21.4	12.0	142(4)	86 62–130 188 130–225	1.036(2) 1.035–37 1.021(5) 1.016–27	41.5‡ 39.0 16.2‡ 15.6	20.0 12.4	13.2 34.0	168(9)	29.5 384	21.5 125	7.0
44	8.8	60 40–84	1.044(5) 1.036–48	29.6‡ 29.7	13.1	14.4	128(3)	103 63–144 173 113–360	1.036(2) 1.032–39 1.017(5) 1.014–21	24.4‡ 23.4 10.1‡ 10.1	13.6 8.5	15.6 41.8	157(13)	22.0 394	17.5 100	7.0

† Figures in parentheses indicate the number of observations averaged.
* After March 1, 1938.
‡ Inulin clearance.

was removed. Observations were begun about a week after the second operation. In two animals (41, 44) concentration tests and clearances were determined between the first and second operations. The dogs were sacrificed after from 1 to 28 months. As is shown in Table II, and Figure 2, after operation the urine volume on concentration tests was increased and the specific gravity reduced. The specific gravity did not increase significantly after deprivation of water for 48 hours (Table I) except in two animals (Numbers 15 and 41) which had the greatest amount of remaining renal tissue. This is in sharp contrast to the moderate rise in normal animals for the last half of a 48-hour test. The creatinine and urea clearances were significantly reduced after operation. In Dogs 41 and 44, the inulin and creatinine clearances remained equal as kidney mass was reduced. None of the animals showed the marked rise in blood pressure which Goldblatt obtains by constricting the renal arteries. The rise in pressure varied from about 10 to 15 mm. Hg in three animals to approximately 50 in two others. All of the animals showed some nitrogen

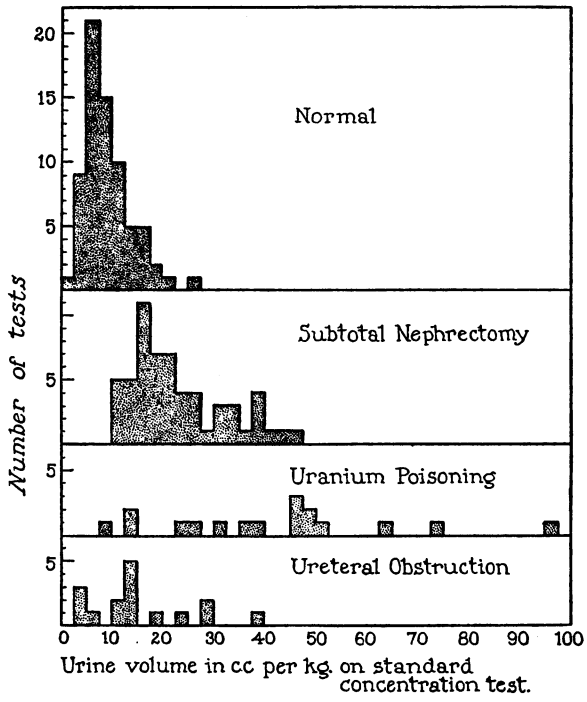

FIG. 2. HISTOGRAM OF URINE VOLUMES ON CONCENTRATION TESTS IN NORMAL DOGS AND AFTER VARIOUS PROCEDURES

TABLE III

Effect of diets on kidney function tests in normal dogs and after subtotal nephrectomy

Dog	Stock diet				Cracker meal				Meat diet				Meat+NaCl			
	Specific gravity on concentration test	Mean clearance		Blood urea nitrogen	Specific gravity on concentration test	Mean clearance		Blood urea nitrogen	Specific gravity on concentration test	Mean clearance		Blood urea nitrogen	Specific gravity on concentration test	Mean clearance		Blood urea nitrogen
		Creatinine	Urea			Creatinine	Urea			Creatinine	Urea			Creatinine	Urea	
		cc. per minute	*cc. per minute*	*mgm. per cent*		*cc. per minute*	*cc. per minute*	*mgm. per cent*		*cc. per minute*	*cc. per minute*	*mgm. per cent*		*cc. per minute*	*cc. per minute*	*mgm. per cent*
NORMAL DOGS																
50	1.055	37.8	22.4	10.5	1.035	26.7	17.7	7.4	1.054	43.7	26.1	12.0	1.049	58.7	31.9	11.7
53	1.061	42.5	24.9	9.8	1.026	26.8	17.3	8.5	1.055	41.7	24.2	20.9	1.044	45.6	26.5	14.6
SUBTOTAL NEPHRECTOMIZED DOGS																
9	1.019	19.1	11.5	29.8	1.021	21.8	10.5	17.9	1.023	19.0	5.1	50.0	1.025	22.4	10.4	32.9
15	1.029	25.6	11.6	22.0	1.022	26.1	11.7	18.7	1.018	36.7	18.4	18.9	1.029	52.5	18.6	14.1
19	1.028	23.3	16.1	23.3	1.027	25.7	14.0	11.5	1.027	19.1	11.1	27.0	1.027	22.0	11.2	26.0

retention. All were in good condition when sacrificed except Dog 19. This animal remained in excellent condition from January, 1936, to August, 1937. After this time, she began to lose weight and developed a progressive decrease in concentrating ability and clearances, and increasing nitrogen retention, but no further elevation in blood pressure and no anemia.

The ability to excrete a dilute urine was preserved in the four animals in which it was tested (Dogs 3, 10, 41, 44) as shown by gravities of less than 1.002 after administration of water by stomach tube.

Table III shows the effect of low and high protein diets on function tests in two normal animals (Dogs 50 and 53) and in three after subtotal nephrectomy (Dogs 9, 15, and 19). In the normal animals, the change in clearances is in the same direction, though less marked than those described by Jolliffe and Smith. There is also a failure of the normal dog to excrete a concentrated urine on the cracker meal diet. The partially nephrectomized dogs, on the other hand, show no consistent variation in clearances, and no variation in concentrating ability. In one animal (Dog 9) there is a hint that addition of salt to a high protein diet may have been followed by a decrease in blood urea nitrogen (28, 46).

Table IV shows the effect of pituitrin, eschatin, intravenous injection of hypertonic sulphate, increase in plasma colloid, and low blood pressure on urinary specific gravity. After pituitrin there was a slight increase in specific gravity in four of six animals above that of the maximum concentration test after operation. In no case did it reach the mean concentration test gravity before operation, and in only one instance did it exceed 0.002. In five of six normal dogs given large doses of pituitrin at the end of a concentration test, higher gravities were obtained than after any 24 hours without water, but in only one animal did the difference exceed 0.009. In two of four of these dogs deprived of water for 48 hours, higher gravities were obtained than after pituitrin; in one the gravity was 0.003 and in the other 0.011 lower than after pituitrin. This agrees with the well known fact that in normal dogs pituitrin diminishes urine volume and with this increases specific gravity, but as a rule it does not go significantly higher than after water deprivation alone. With

TABLE IV

Effect of various procedures on urinary specific gravity after subtotal nephrectomy, uranium poisoning, and ureteral obstruction

Dog	Specific gravity on test		After pituitrin	After salts	Increased plasma colloid			Low pressure	
	Before operation	After injury			Specific gravity	Change in plasma protein	Method	Specific gravity	Mean blood pressure
	Mean	Maximum							
SUBTOTAL NEPHRECTOMY									
3	1.038	1.028	1.029	1.042				1.040	70
4	1.038	1.023	1.018	1.032	1.026		Acacia		
6	1.040	1.015		1.031	1.022		Acacia	1.036	57
8	1.030	1.016	1.018	1.026				1.027	74
9	1.034	1.021			1.041	6.3-9.9†	Croton oil Arica nut	1.033	58
10	1.042	1.016	1.018	1.026					
15	1.037	1.029	1.026*		1.058	5.9–6.7	Croton oil Arica nut		
19	1.040	1.027 1.016‡	1.015 1.019*	1.024 1.024	1.045	5.8–7.2	Croton oil Arica nut	1.036	57
41	1.038	1.027			1.039	5.5–5.8	Sucrose	1.046	100
44	1.044	1.021	1.028		1.040	5.9–7.8	Concentrated plasma		
17	1.052	1.014	1.011*						
URANIUM POISONING									
7	1.048	1.019	1.010	1.019				1.015	100
11	1.051	1.020	1.003						
12	1.041	1.009		1.015					
13	1.044	1.019		1.023					
14	1.038	1.017	1.021		1.013	7.8–8.3	Croton oil Arica nut	1.015	76
45	1.040	1.010		1.018					
46	1.055	1.021		1.022	1.022	6.0–7.1	Sucrose		
64	1.041	1.015			1.017	7.0–7.7	Sucrose	1.012	87
URETERAL OBSTRUCTION									
20	1.043	1.013	1.017						
21	1.043	1.027	1.029	1.013	1.019	4.8–5.9†	Sucrose	1.013	65
22	1.033	1.015		1.018					
25	1.044	1.016		1.014					
26	1.052	1.023		1.031	1.013	6.5–8.9	Sucrose	1.012	75
31	1.054	1.013	1.030	1.018	1.018	7.0–10.6	Sucrose Arica nut		
34	1.051	1.026	1.031		1.024	5.8–6.3	Concentrated plasma	1.020	90
35	1.047	1.014	1.018		1.016	7.7–8.4	Sucrose	1.018	40

* After eschatin.
† Red count.
‡ Maximum of 9 tests after March 1, 1938.

reduced kidney mass, pituitrin does not lead to any increase in urinary specific gravity above that obtained by water deprivation. Yet in these urines, the concentration of salts was not high, so that this cannot be the limiting factor that Motzfeldt (59) has shown it to be in the normal animals. Apparently, lack of this hormone is not an important cause of this type of hyposthenuria. Nor did adrenal cortical hormone, which has been shown to affect sodium reabsorption, have a detectable effect on specific gravity in these dogs.

Intravenous injection of large doses of sodium sulphate after 24 hours without water led to a urine exceeding the maximum postoperative concentration test by 0.01 or more in four of six experiments. In one (Dog 3) the gravity was as high as the maximum preoperative test, and in two others (Dogs 4 and 8) was 0.006 and 0.004 below the mean preoperative value. These urines were extremely high in sulphates, 75 to 85 per cent of the elevation of the specific gravity above that of water being accounted for by the sulphate present.

More significant physiologically are the results of an increase in plasma colloids and of a low blood pressure. In four of seven experiments the urinary specific gravity obtained with increased plasma colloid was as high or higher than the mean concentration test before operation. In another, the gravity was 0.019 higher than the maximum after operation and only 0.004 lower than the mean preoperative value. In the remaining two, in which there was no significant increase in gravity, an attempt had been made to increase plasma colloids by intravenous injections of acacia solutions, and there was a reasonable doubt whether the doses given were large enough. Similarly, the specific gravity of the urine excreted at very low blood pressure, while small in volume, exceeded the maximum postoperative concentration test by 0.011 to 0.021 (average 0.016) and in two animals was as high as the mean preoperative concentration test gravity, while in the others it fell 1 to 4 points below this level.

The reduction in clearance is not directly proportional to the reduction in kidney mass, nor to the percentage of glomeruli remaining. Figure 3 shows the relation between the per cent of the original glomerular equipment of the animal remaining after operation and the per cent reduction in creatinine and urea clearances below the control level. The clearances are reduced less rapidly than the number of glomeruli, the difference being most marked with the smaller kidney fragments. This might be due to opening up and the more continuous activity of an increasing percentage of the total number of remaining glomeruli, until with extreme reduction in the

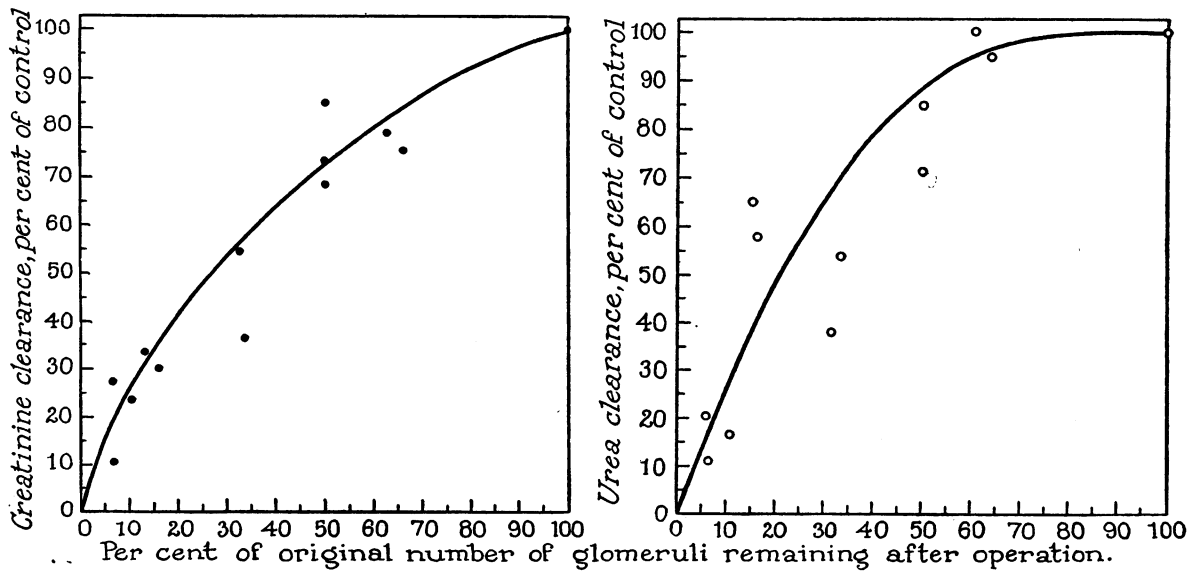

FIG. 3. RELATION BETWEEN REDUCTION IN THE NUMBER OF GLOMERULI AND THE REDUCTION IN CLEARANCES AFTER SUBTOTAL NEPHRECTOMY

total number all which remained were continuously open, or to an increased amount of filtrate per open glomerulus. It is probable that both mechanisms are active. The former offers a reasonable explanation for the slight reduction in clearances when the number of glomeruli are reduced to 50 per cent of the original, with no increase in blood nitrogen. When, however, the kidney mass has been reduced to a point (about 35 per cent of glomeruli in these animals) where blood urea nitrogen is increased, it seems not unreasonable to assume that the diuretic, vasodilator effect of the urea would lead to the constant perfusion of all the remaining glomeruli. Yet with still further reduction in the number of glomeruli the difference between the degree of glomerular reduction and decrease in clearances is greater. When the glomeruli were reduced to about 35 per cent, the average creatinine clearance was 46 per cent of control level, while when the glomeruli were reduced to 5 to 15 per cent (average 9.4 per cent) the creatinine clearance was only reduced to an average of 23.8 per cent. This is similar to Verney's structural and functional reserve. The smaller reduction in urea than in creatinine clearance is consistent with this interpretation since with diuresis—increased volume flow down each tubule—there would be less back diffusion. The difference in creatinine and urea clearances is more difficult to explain on the basis of a simple perfusion of more glomeruli. Rhoads, Alving, Hiller, and Van Slyke (69), and Levy and Blalock (48), found a relative increase in renal blood flow after unilateral nephrectomy, but this might be due either to perfusion of more glomeruli or to a greater blood flow per glomerulus. Medes and Herrick (56) found that the creatinine clearance paralleled the blood flow as measured by the Stromuhr. If applied to these animals, it would indicate a 25 to 85 per cent greater flow per glomerulus in the kidney remnant than in the kidney of the normal animal, if it is assumed that in the latter all the glomeruli were open. The data do not support the hypothesis that the increase in systemic pressure is an important factor in producing the polyuria, for there is no relation between the degree of blood pressure elevation and the percentage increase in urine volume on concentration test. On the other hand, changes in glomerular capillary pressure resulting from local vascular adjustments within the kidney, perhaps influenced by increased concentrations of various substances in the plasma, may well be of extreme importance.

Histologically, the kidney fragments failed to show the tubular dilatation and flattening of epithelium described by Mark (52), or the degenerative lesions found by Anderson (6) in rabbits. Except in Dog 19, comparison of sections from the kidney remnant and the normal kidney did not show any striking difference. In the dogs allowed to survive several months, there was apparently some increase in the size of the glomeruli. None showed any glomerulitis with hematoxylin and eosin stain. Since all of the kidneys had been injected, the Heidenhain-Mallory stains were unsatisfactory. In some kidneys, there was slight tubular degeneration but this was evident in the intact kidney as often as in the kidney remnant. Sections of the kidney remnant from Dog 19 showed definite glomerular fibrosis and glomerulitis. There was also some widening of the tubules, but no definite flattening of epithelium.

Summary. Removal of sufficient renal mass thus leads to the excretion of an increased volume of dilute urine. The animals show a low specific gravity on concentration test, reduced urea and creatinine clearances, with variable elevation in blood pressure and blood urea nitrogen. The picture, as Volhard has emphasized, closely resembles that of nephrosclerosis, and differs from that of chronic glomerular nephritis chiefly in the absence of anemia and hematuria. Under certain conditions, especially increase in concentration of plasma proteins and reduction in blood pressure, which there is no reason to believe lead to improvement in the condition of the tubule cells, urine can be obtained which equals or approaches in specific gravity that of the intact animal. The deduction seems logical that abnormality of renal epithelium is not the primary cause of the hyposthenuria.

Uranium poisoning

The histological and functional changes produced by uranium have been reviewed by MacNider (51).

There is an initial polyuria. With large doses this is followed by a decrease in urine formation and finally anuria; with small doses recovery gradually takes place. The essential and dominant lesion in the kidney is injury to the epithelium of the tubules, especially to the distal portion of the proximal convolutions, according to Suzuki (79). In animals which recover, the tubular epithelium

is replaced by a flattened abnormal type. The urine is dilute and frequently contains sugar. Some workers report increased excretion of chloride and nitrogen, others a decrease. Phenolsulphonphthalein excretion is diminished. There is a retention of nitrogen, creatinine, *etc.*, in the blood. Various changes in the glomeruli and in the reaction of their arterioles have been described, but the perfusion experiments of Ghoreyeb (29) and the blood flow measurements of Tribe, Hopkins, and Barcroft (83) indicate that there is no decrease in renal blood flow.

The dose of uranium acetate used in these experiments varied from 1 to 3 mgm. per kgm. With the larger doses, some of the animals died before all the desired observations could be made. Table V and Figure 2 show the increase in urine

TABLE V

Summary of data on animals subjected to uranium poisoning and ureteral obstruction†

Dog	Weight	Before injury							After injury							Kidney weight and glomerular count	
		Concentration test		Mean clearance			Blood urea nitrogen	Mean blood pressure	Concentration test		Mean clearance			Blood urea nitrogen	Mean blood pressure		
		Volume, Mean, Range	Specific gravity, Mean, Range	Inulin	Creatinine	Urea			Volume, Mean, Range	Specific gravity, Mean, Range	Inulin	Creatinine	Urea			Left	Right
	kgm.			*cc. per minute*	*cc. per minute*	*cc. per minute*	*mgm. per cent*	*mm. Hg*			*cc. per minute*	*cc. per minute*	*cc. per minute*	*mgm. per cent*	*mm. Hg*	*grams; thousands*	*grams; thousands*
URANIUM POISONING																	
7	10.3	58 45–70	1.048(2) 1.040–55		43.8	33.2	7.4		663 500–980	1.017(3) 1.013–19		2.1	1.4	140		30.5 406	
13	10.8	97 93–104	1.044(3) 1.044–44		45.2	25.9	11.9	124	410 101–800	1.017(3) 1.013–19		21.7	3.4	57.5	110	66.5 344	55.0
14	13.2	123 86–160	1.038(2) 1.031–45		35.4	22.4	15.0	122	558 500–615	1.015(3) 1.013–17		12.3	3.6	70.5	122	34.8 322	35.0
28	9.6	87 63–105	1.042(4) 1.036–47	38.9	37.7	20.8	13.6	130	210 160–260	1.016(2) 1.013–18	3.05	2.3	2.1	68.2	140	29.3 259	33.0 266
39	7.0	51 17–91	1.048(5) 1.042–53	38.2	37.6	24.1	15.2	125	440	1.012	20.6	14.6	11.0	27.0	110	19.0	19.1
45	8.0	106 92–120	1.040(2) 1.040–41	44.4	43.2	18.2	23.0	135	388 360–415	1.009	2.7	2.2	1.7	61.0	140	18.0 369	19.0
46	8.2	64 62–65	1.055(2) 1.054–56	45.5	45.3	24.4	21.4	143	148 120–175	1.019(2) 1.013–22	3.0	2.3	1.9	96.6	150	23.5	21.8 278
64	12.7	149 142–156	1.036(2) 1.030–41	48.9	51.2			130	525 450–600	1.013(2) 1.011–15	5.2	4.2			130		
URETERAL CLAMP																	
22	12.4	75 69–81	1.033(2) 1.032–33		57.5	32.1	9.2	142	150 one lost	1.019(2) 1.015–23		3.0	1.7	130.0	145	48	46
26	7.5	90 60–120	1.052(2) 1.050–54		41.9	18.8	13.3		192 104–285	1.023(2) 1.022–23		9.7	7.5	31.6	150	45	63
34	7.0	50 32–78	1.051(5) 1.041–67		36.2	24.1	11.2	133	134 106–168	1.023(4) 1.022–26		22.7	16.7	20.6	135	40 238	42.5 152
35	9.1	42 35–48	1.047(2) 1.046–48		30.9	17.4	12.3	157	100 48–126	1.015(3) 1.014–17		18.3	8.3	34.0	135	43 350	44 365
38	7.7	65 52–86	1.041(4) 1.035–47		21.2	10.5	19.5		150	1.015		2.7	1.9	93.8			
28	10.0	57 50–62	1.052(3) 1.047–56		31.8	14.2	13.6	135	38 35–44	1.026(2) 1.024–28 1.032(3) 1.031–33 1.042(3) 1.036–47		0.87 24.4* 37.6*	0.78 13.7 16.3	39.9 14.2	162 130 130	29.3 259	33.0 266
39	5.4	46 22–67	1.044(3) 1.041–50		43.7	28.5	14.4		218 210–225 91	1.015(2) 1.010–19 1.042		16.1 44.5*	15.6 19.1	28.4 25.1	125	19.1	19.0

† Figures in parentheses indicate the number of observations averaged.
* After removal of clamp.

volume and decrease in specific gravity on concentration tests, and the reduction in clearances after poisoning. There was no rise in blood pressure in any of these animals. Table IV shows the general failure of the methods yielding a more concentrated urine after subtotal nephrectomy to do so after uranium poisoning. No means have been found which will enable even the moderately poisoned kidney to put out a urine of high gravity. Some of the animals shown in Table IV have been omitted from Table V to save space, since clearances were not done after poisoning.

The histological evidence of damage to tubular epithelium permits the assumption that the polyuria and low specific gravity are due to impairment of water reabsorption, though whether this is chiefly in proximal or distal tubule is not indicated. The mechanism of the low clearances, however, requires further scrutiny. Diminished clearance might be due to back diffusion of the test substance through the damaged tubular epithelium, to diminished renal blood flow, or to decrease in the permeability of the glomerular epithelium. The lack of histological evidence of glomerular thrombi, the marked increase in urine volume, and the increase in albuminuria make the last explanation unlikely. The experiments of Ghoreyeb and Tribe mentioned above, and those of Dunn, Dible, Jones, and McSwiny (23) in oxalate poisoning, indicate that there is no decrease in renal blood flow. However, to confirm this point blood flow was measured by Barcroft's method in two animals after poisoning. These showed flows within the normal range for this method, but did not permit measurements in the same animal before and after poisoning. In two other animals, blood flow was measured by the method of Van Slyke, Rhoads, Hiller, and Alving (88) using a modification of their technique (31) of explanting the kidney so as to be certain that the samples of renal vein blood were not contaminated by arterial blood or urine. Creatinine and inulin were used as test substances. Samples of blood were drawn from femoral artery and renal vein before the beginning of urine collections, and at the end of each period. From analyses of blood samples curves were drawn for arterial and renal vein concentrations during the time of the experiment, and the values at the middle of each collection period estimated. Plasma flow was calculated as clearance ÷ per cent extraction, blood flow from the hematocrit value. Table VI shows the results obtained. In control experiments, inulin and creatinine clearances agree reasonably well. There is more discrepancy in the values for blood flow as calculated from inulin and creatinine. Small errors in extraction ratios make large differences in calculated blood flows. The agreement seems sufficient, however, to furnish acceptable evidence that there was no decrease in glomerular blood flow after poisoning. The marked decrease in A–V difference in Dog 64 after poisoning is striking. This must be due either to diminished glomerular permeability or to much of the filtered substance having re-entered the blood stream through the tubule cells. Reasons have been given for believing that the former is not the mechanism; another reason for this belief is that the average extraction ratio for inulin is greater than for creatinine, so that the glomerular membrane would have had to become more permeable to the large inulin molecule than to the smaller creatinine. After poisoning there is also a marked drop in the creatinine/inulin clearance ratio. Dog 65 was first given 0.5 mgm. per kgm. of uranium acetate to see if with the smaller dose there would be a decrease in creatinine clearance without much change in inulin clearance. This did occur, but the difference was slight. Two weeks later, she received an additional 1.0 mgm. per kgm. and another experiment was carried out after two days. This showed some further depression in creatinine clearance and in creatinine/inulin clearance ratio, but again the creatinine clearance is not markedly altered. It seems that if the tubules are sufficiently damaged to permit any considerable back diffusion of creatinine, the large inulin molecule will also regain the blood stream by the same mechanism but to a lesser extent than creatinine (Dogs 28, 39, 45, 46; Table V). These experiments indicate that with tubular damage a diminished extraction ratio may account for reduced clearances, a mechanism suggested by Van Slyke *et al.* (87), but for which they had no direct evidence at the time.

The kidneys from these animals showed histological changes similar to those repeatedly described after uranium poisoning. There was no obstruction to perfusion flow, the glomeruli were well injected, and showed no consistent abnormality. The tubules, especially the proximal convoluted segments, were the site of degeneration and necrosis. The severity of the tubular lesion varied with the dose, length of survival, and in different animals receiving the same treatment.

Summary. A predominantly tubular lesion, with no reduction in blood flow, can result in reduced clearances, and the excretion of an increased volume of dilute urine. In contrast to animals in which a similar decrease in renal function had been brought about by a reduction in renal mass, no conditions could be found under which these animals excreted a more concentrated urine. The most obvious explanation is that the damaged tubular epithelium was not only unable to reabsorb glucose, and to establish the normal osmotic gradient between lumen and capillary by reabsorption of water, but also permitted an abnormally great back diffusion not only of urea and creatinine, but even of the large inulin molecule. How far this process is simple diffusion, and how far it is influenced by the osmotic pressure of the plasma proteins in the peritubular capillaries, cannot be analyzed further from the data at hand.

Ureteral obstruction

This method was used to simulate the conditions encountered in prostatic hypertrophy, stone, and hydronephrosis. Urethral obstruction would have been better, but would have precluded catheterization.

Suter (78) believed two factors contribute to the polyuria in such cases, tubular damage and "nervous reflex." Hinman and Hepler (36) believed that while excretory back pressure is the essential factor in producing hydronephrosis, its effect is closely linked with nutritional disturbances, and that the tubular atrophy is due more to anemia than to pressure. When the ureter is obstructed, constriction of renal artery gives a more rapidly developing hydronephrosis and atrophy than ureteral constriction alone (37). Through the kindness of Dr. Goldblatt, his clamps and instruments were available. Under ether anesthesia a small midline incision was made just above the symphysis, and a clamp applied to each ureter close to the bladder. These were adjusted so as to constrict the ureter markedly, but not to occlude it. Since a slowly developing or marked hydronephrosis is associated with reduction in the number of glomeruli (46, 57) the effort was made to produce a lesion and carry out the observations as rapidly as possible.

After 5 to 11 days, these animals showed a definite impairment of concentrating ability, and reduction in clearances. There was no elevation in blood pressure. The degree of impairment was similar to that after uranium poisoning. Urine volumes, however, were not so uniformly increased (Figure 2). At times the volume of urine during a concentration test would be as low as during the control period, although the specific gravity was always lower. Table IV shows the results of attempts to obtain a concentrated urine by the means previously employed. After pituitrin a significant increase in specific gravity was obtained in one animal (Dog 30), increases of 2 to 5 points in four others. In no instance, however, was urine obtained of a concentration approaching the mean specific gravity on concentration test before obstructing the ureters. Administration of sulphate, increasing plasma protein concentration, and lowering the blood pressure were likewise without significant effect.

The kidneys showed some dilatation of the pelvis, but save in the left kidney of Dog 21 no marked atrophy of the renal cortex. Histologically, the glomeruli appeared normal. The tubular cells showed more or less evident cloudy swelling. In about half the kidneys, there was a secondary pyelonephritis.

That the tubular damage is a reversible process, from which recovery can take place, is shown in Dogs 28 and 39, in which the clamps were removed after reduction in clearances and low specific gravities had been secured. Functional recovery was complete or nearly so in a month.

TABLE VI

Renal blood flow before and after uranium poisoning

Dog	Inulin			Creatinine			Cell volume	Blood flow		Creatinine / Inulin	Remarks
	Clearance	A-V difference		Clearance	A-V difference			From inulin	From creatinine		
	cc. per minute	mgm.	per cent	cc. per minute	mgm.	per cent	per cent	cc. per minute	cc. per minute		
64	49.5			53.0						1.07	Control
	45.7	30	31.5	45.4	2.3	24.7	20.0	181	230	0.99	
	51.6	21	29.0	57.2	2.6	29.1	20.0	223	246	1.11	
	5.2	16	4.0	4.4	0.7	2.8	23.8	170	201	0.84	After uranium
	5.3	10	2.8	4.2	0.6	2.5	24.0	246	224	0.80	
	5.0	8	2.4	4.2	0.4	1.7	25.5	276	324	0.84	
65	37.4	50.0	31.2	36.0	4.0	26.3	38.2	245	221	0.97	Control
	39.7	33.5	29.5	38.8	3.2	23.6	37.1	211	256	0.98	
	36.2	63.0	26.2	33.2	4.7	25.3	36.1	216	206	0.92	Twenty-two
	32.5	44.0	26.5	30.2	4.4	25.3	38.4	198	192	0.93	days after righ
	35.3	30.0	25.0	33.1	4.0	24.3	36.1	220	212	0.94	nephrectomy
	35.0	50.0	23.5	31.9	3.8	20.8	35.3	230	237	0.91	After 0.5 mgm.
	36.3	38.0	25.0	29.1	4.2	23.8	35.8	226	190	0.86	per kgm. of ura-
	38.2	28.5	24.2	32.9	4.1	24.9	36.4	249	208	0.86	nium acetate
	32.9	46.0	27.0	25.5	4.8	24.4	29.7	174	149	0.78	After 1 mgm. per
	35.9	25.5	23.4	26.4	4.3	23.5	33.0	228	168	0.74	kgm. of ura-
	27.0	20.0	24.7	21.3	3.7	22.1	35.5	169	149	0.79	nium acetate

Summary. The explanation for the loss of concentrating power offered for uranium poisoning would seem to be applicable here also. Reduction in blood flow, however, may be a factor in the reduced clearances. Levy, Mason, Harrison, and Blalock (49) found reduced blood flow when the ureters were tied. The gradual reduction in the concentration of urea, increase in chloride, and appearance of sugar in the fluid obtained from a hydronephrotic sac, indicates the tubules have not only lost the capacity to concentrate urea, but to reabsorb chlorides and sugar to the normal extent.

Renal denervation

Claude Bernard (11) in 1859 found that division of the splanchnic nerves on one side led to an increased volume of urine from the ipsilateral kidney. This has been confirmed repeatedly.

Marshall and Kolls (54) cite the literature up to the time of their papers. An increase in renal blood flow is usually offered as the mechanism responsible for the

diuresis, although Bayliss and Fee (10) found that in the double heart-lung-kidney preparation, while splanchnic section increased blood flow, pituitrin decreased the urine volume without any change in blood flow. Rhoads *et al.* (70) did not find any increase in blood flow after splanchnic section. Denervated and normal kidney responded alike to ingestion of water, exercise, pituitary extract (44) and to afferent nerve stimulation (81). Apparently the response of an animal with denervated kidneys to a concentration test has not been studied.

Complete denervation of the kidney can only be secured, according to Quinby (65), by section and resuture of artery, vein, and ureter. Even under these conditions the diuresis disappears in about two weeks. Most authors have been content to divide all visible nerves entering the hilus, or to cut the splanchnic nerves.

After preliminary concentration tests, and measurement of water intake and urine excreted when water was allowed *ad lib,* the kidneys of two dogs (Numbers 47 and 59) were denervated. Under ether anesthesia, the kidney was exposed through a lumbar incision, delivered into the wound, the capsule stripped of all adherent fat, and artery, vein, and ureter carefully cleared and finally wiped rather vigorously with gauze. All other structures entering the hilus were divided.

TABLE VII

*Urine volumes and concentration tests before and after renal denervation**

Dog	Before denervation			After denervation			Specific gravity after pituitrin
	Mean urine volume	Concentration test		Mean urine volume	Concentration test		
		Mean urine volume	Mean specific gravity		Mean urine volume	Mean specific gravity	
47	102 (6)	74 (6)	1.035	278 (4)	93 (3)	1.020	1.035
59	198 (6)	86 (2)	1.048	583 (4)	260 (2)	1.019	1.028

* Figures in parentheses indicate the number of observations averaged.

After operation, the daily urine volume when water was allowed was increased, and the specific gravity on concentration test reduced (Table VII). In one dog pituitrin yielded a urine of specific gravity equal to that of the mean concentration test gravity before operation, in the other the increase after pituitrin was less marked. The mechanism of the polyuria and of the mode of action of pituitrin has not been studied. The experiments served only to show an experimentally produced loss of concentrating power without other impairment of kidney function.

Anemia

Christian (20) and Mosenthal (58) have noted low gravity in patients suffering from pernicious anemia, with improvement during remission. Fouts and Helmer (26) reported low urea clearances with improvement on liver therapy. If this were due simply to anoxemia of the tubule cells, it seemed that it should be reproduced in animals if hemoglobin was maintained at a low level by repeated bleedings. Since, in the dog, hemoglobin regeneration is very rapid on the stock diet, the animals were given either a bread and milk or Whipple's bread and salmon diets. On these diets alone, concentration test specific gravities are lower than on the stock diet. After bleeding, the plasma was separated and reinjected in order to maintain plasma proteins at a normal level. If necessary, additional plasma was supplied from normal dogs. Dog 33 showed no impairment of concentrating power or decrease in creatinine clearance after her hemoglobin had been reduced from 16 grams per 100 cc. to approximately 3.9 grams and maintained at that level for a month. Dog 42 was maintained at a level of about 7.8 grams per 100 cc. for two and a half months, and then at from 4.6 to 5.9 grams per 100 cc. for an additional month. While during this time some concentration tests showed gravities as low as 1.020, others were well within the range of those obtained during the control period. The same was true of Dog 49 whose hemoglobin was maintained at 2.5 to 3.9 grams for a month. These dogs did not show any significant change in creatinine or urea clearances or in blood pressure during the period of anemia.

While the number of experiments is small, they indicate that the mechanism of the diminished renal function in pernicious anemia may not be due to the low hemoglobin alone, or if it is, the anemia must be present for a longer time than it has been maintained in these animals.

Through the kindness of Dr. Goldblatt, an opportunity was offered to study the concentrating ability of some of his dogs with experimental hypertension produced by renal ischemia. In Table VIII are shown the maximum specific gravities on concentration tests and the mean blood pressure before and after constriction of

the renal arteries or of the aorta above the renals. It is evident that hypertension can be produced by these means without any reduction in the ability of the kidney to excrete a concentrated urine. The renal blood flow and intrarenal blood pressure are probably reduced in these animals. As long as the reduction in flow is not sufficient to interfere with the nutrition of the tubule cells, a concentrated urine is to be expected. Animals with the "malignant" type of hypertension have not been studied.

TABLE VIII

Mean blood pressure and maximum specific gravity of urine on concentration test in Dr. Goldblatt's dogs before and after the production of renal ischemia

Dog	Control period		After production of renal ischemia	
	Mean blood pressure	Maximum specific gravity	Mean blood pressure	Maximum specific gravity
235	140	1.042	170	1.038
240	135	1.047	190	1.045
330	120	1.050	192	1.053
340	125–140	1.037	177	1.046
344	120	1.035	195	1.040
368	130	1.027	210	1.040

Dr. T. Birch generously allowed observations on two of his animals during development of black tongue on a vitamin B_6 deficient diet and its cure by nicotinic acid. No change in the ability to excrete a urine of high specific gravity on a concentration test during these nutritional changes was noted.

Two dogs happened to be pregnant at the time observations were started. One failed to attain a normally high specific gravity during the last month of pregnancy, and both failed for a month after whelping. Subsequently, both excreted urines of normal concentration.

The lower test specific gravities on a low protein diet are not necessarily due to the same mechanism as the reduction in clearances. Pitts (64) and Herrin, Rabin, and Feinstein (35) have suggested that the latter is related to the level of protein metabolism, and have shown that ingestion of salts or urea are without effect. Dog 50 had a mean specific gravity of 1.026 for three tests after a month on the cracker meal diet. She was then given 10 grams of urea and 5 grams of NaCl and put in a metabolism cage. The urine volume for the following twelve hours was 210 cc. and its specific gravity 1.028; for the next twelve hours the volume was 130 cc. and the specific gravity 1.046. After 40 units of pituitrin she excreted a urine having a specific gravity of 1.051. Dog 52, which showed a mean specific gravity of 1.037 for six tests on a bread and salmon diet, excreted a urine of 1.050 after 12 grams of urea and 5 grams of NaCl. Her blood urea rose from 12.0 to 19.5 mgm. per cent. After pituitrin, however, the highest gravity obtained during the succeeding twelve hours was 1.041. Dog 53, which had shown a decrease from 1.061 on stock diet to 1.026 on cracker meal during the winter, still had a mean of 1.047 for six tests with one as high as 1.060 after a month of cracker meal diet during the summer.

DISCUSSION

These experiments confirm many in the literature in demonstrating that there are several ways in which loss of concentrating power may be brought about. These include: reduction in the number of nephrons; tubular poisoning; tubular degeneration resulting from back pressure, or back pressure plus ischemia; (temporarily at least) interference with the nerve supply to the kidney; at times low protein diet; and possibly pregnancy. Lesions in the mid-brain, producing diabetes insipidus should be included. Three of these are associated with other evidence of renal impairment, diminished clearances and elevation of blood urea and creatinine. Only one exhibits any tendency to be associated with hypertension (reduction in kidney mass). Some are reversible processes (diet, pregnancy, denervation) from which restoration to normal regularly occurs. Others (tubular damage) may recover if the injury has not been too severe. Reduction in kidney mass is irreversible, and while hypertrophy of the remaining tissue may be accompanied by some improvement in function, this is not apparent when renal mass has been reduced beyond a certain point.

The response of animals with these different types of hyposthenuria to various attempts to obtain a concentrated urine differs. Pituitary extract leads to a urine of normal specific gravity after renal denervation, low protein diet, and (from the literature) after lesions of the mid-brain; it is without significant effect in tubular damage and with reduction in kidney mass. In the latter, increase in the concentration of plasma colloids and reduction of blood pressure to near the critical level lead to the excretion of a more concentrated urine. In the presence of tubular damage these are without effect.

The urine volume in all tends to be above normal except in advanced tubular degeneration, when it is diminished, or even suppressed. In uranium poisoning, the presence of a normal blood flow with a diminished extraction ratio, which is lower for creatinine than for inulin, indicates that not only has the ability of the tubules to reabsorb

sugar and water been impaired, but also the ability to prevent such substances as creatinine, concentrated to some extent by reabsorption of water, from re-entering the blood stream. The oliguria or anuria in extreme damage seems explicable on the assumption that these severely damaged cells act like a dead membrane, and that the glomerular filtrate may be completely reabsorbed, the absorbing force being the osmotic pressure of the plasma colloids in the peritubular capillaries. This occurrence was demonstrated by Richards in frogs anuric from mercuric chloride poisoning (71).

Hyposthenuria results from lesser degrees of tubular damage because of the loss of capacity of the damaged cells to reabsorb water to the normal degree against the increasing osmotic pressure of the fluid in the lumen of the tubules. Accompanying this, there is loss of the ability to resist back diffusion of substances concentrated by the reabsorption of water. This back diffusion is greater for urea than for creatinine, and greater for creatinine than for inulin. Evidence is not available to decide the relative importance of the two factors, nor the parts of the tubule involved. When the tubules are severely damaged, most or all of the glomerular filtrate is reabsorbed, the little urine that is excreted approaching an ultrafiltrate of plasma in composition. With lesser degrees of tubular damage there is an increased volume of dilute urine. As the degree of damage increases, the urine volume diminishes, but remains dilute. No means have been found which will permit the excretion of a concentrated urine from such kidneys.

The polyuria and hyposthenuria resulting from decreased kidney mass is adequately explained by increased blood flow and greater volume of filtrate per remaining glomerulus. This results in a more rapid flow of fluid down the tubule, lack of time for reabsorption accounting for the hyposthenuria. Under suitable conditions a small kidney remnant excretes a urine of high specific gravity. There is no evidence in the experiments presented here that continued polyuria exhausts or damages the tubular cells. It seems unnecessary, therefore, to assume tubular damage in addition to the circulatory changes in order to explain this polyuria.

Other mechanisms for hyposthenuria, such as disturbances in circulating hormones, undoubtedly exist, but sufficient evidence is not at hand to make discussion profitable.

Hyposthenuria might be classified as renal and extrarenal, or parenchymal and extraparenchymal, or tubular and non-tubular. In the former category belong the definite tubular degenerations; in the latter, reduction in renal mass. Sufficient evidence is not yet available to know where to place the hyposthenuria of denervation, low protein diet, and of pregnancy.

CONCLUSIONS

Hyposthenuria, or loss of ability to excrete a concentrated urine under usual conditions, may be produced experimentally in dogs in a number of ways. These include reduction in kidney mass, uranium poisoning, ureteral obstruction, denervation, and a low protein diet.

Dogs subjected to subtotal nephrectomy will excrete a concentrated urine under certain conditions, including increased concentration of plasma colloids, low blood pressure, and injections of sodium sulphate after water deprivation. A urine of high specific gravity has not been obtained from dogs with tubular damage. Pituitrin leads to the excretion of a concentrated urine after renal denervation, but is without significant effect in the other groups.

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