WATER METABOLISM IN HYPERTENSIVE RATS*

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In studies of the influence of adrenochrome upon experimental hypertension in rats (Oster and Sobotka, 1943), a striking increase in the water consumption and the urinary excretion was observed in the majority of hypertensive animals, as compared with normal ones. This finding suggested to us an investigation of the influence exerted by renal ischemia on the water metabolism, and of the possible interrelation of the latter with experimentally produced hypertension in rats.

Material and Methods

Animals.—White rats, weighing between 250 and 300 gm. were used, from the stock of Rockland Farm. They were placed in individual metabolism cages and they appeared lively, ate well, and kept their weight during the course of the experiment.

Diet.—The diet consisted of Rockland Farm dried rat pellets *ad libitum*. This ration is composed as follows:—

	per cent
Carbohydrates	47.3
Protein	27.0
Fat	6.0
Ash	9.8
Moisture	9.9
	100.0

The moisture content was neglected in the computation of the water intake. Vitamin D was supplied in the form of codliver oil.

The water intake of the animals was controlled daily. The water bottles held more than 1 day's requirement; their nipples were so constructed as to prevent spilling. The main part of the water was drunk at night and the animals were found to consume rather constant amounts of fluid during a test period lasting from 1 to 4 weeks. The daily deviation from the average volume rarely exceeded 8 ml. The influence of the outside temperature was negligible.

Production of Renal Ischemia.—In order to produce perinephric scar, the kidney was wrapped in cellophane (Page, 1939). Constriction of the left renal artery was accomplished by placing a mandrin alongside the blood vessel, tying with a double loop of silk, and subsequent removal of the mandrin (Drury, 1931).

The blood pressure of the normal animals, as well as of those which had been operated upon, was measured twice weekly according to the plethysmographic method of Williams, Harrison, and Grollman (1939). The type of operation performed, the degree of hypertension, the body temperature, and the procedure of measuring the blood pressure did not influence the water intake.

The occurrence of otitis, peritonitis, and nasal discharge never raised the water intake but in some instances decreased it. Operative procedures, for example, laparotomy and the draining of abscesses, were followed by an immediate drop in water intake and a return to normal levels within 3 to 5 days. Unilateral nephrectomy diminished the animal's thirst from 1 week to 10 days with subsequent return to normal. The warming of the rats which was necessary prior to blood pressure measurements left their drinking rate unaffected. Vascular crisis, as described by Wilson and Byrom (1941) in hypertensive rats, was followed by a drop in blood pressure, but was not always accompanied by a reduction of water consumption. Severe convulsions due to hypertension were followed by a drop of both blood pressure and water intake.

When normal saline was substituted for water the fluid intake of the normal rat increased 55 per cent (average of 6 animals). In hypertensive rats the same substitution doubled fluid consumption (average of 6 animals) and the rats developed generalized edema with weight increases up to 40 gm. One unit of pitressin, injected intramuscularly twice daily, caused a drop of about 20 per cent in the water intake of normal rats, which became ill as a result of this high dosage. Injection of the same amount of pitressin into hypertensive rats caused a pronounced reaction and consequent drop in blood pressure, but did not result in a similar lessening of the water intake.

RESULTS

The daily water intake of normal rats under our experimental conditions averaged 46 ml. in a group of 34 animals observed over a period of 2 to 3 months. An equal number of rats survived one of the following two procedures designed to produce hypertension: production of perinephric scar by wrapping the left kidney in cellophane and production of ischemia by clamping the left renal artery. Of 24 animals, which survived the former procedure, 11 developed arterial blood pressures of 160 mm. or higher within 1 month. Of 10 animals, that survived the clamping operation, 9 became hypertensive within the 1st week. Eight animals of the latter group and 9 of the 11 hypertensive rats with perinephric scar showed significant increases of water intake over prolonged periods of observation. The average increase for these 20 hypertensive animals was 32 ml. per day, which represents a 70 per cent increase over the normal average value, and a 76 per cent increase over the preoperative average of these 20 individuals. Amongst the group of 14 animals, in which we failed to raise the blood pressure to 160 mm., 6 increased their water requirements following operation.

The rise of blood pressure following operation may occur simultaneously with the onset of polydipsia or it may precede or succeed it. Once both symptoms

are established, their fluctuations in degree tend to run parallel to each other. Injections of 20 mg. iodo-adrenochrome each on 3 successive days lowered the water intake *pari passu* with the blood pressure in all of 3 animals so treated.

The observed polydipsia was found to be associated in all rats with polyuria. The urinary output was studied in a number of animals and compared with their water intake throughout the same period. The individual animals were placed in cages in which the urine could be collected without loss by evaporation. The urinary volumes were noted daily for 2 to 3 weeks. The daily excretion was 19 ± 1.5 ml. in the average of 6 normal rats. The specific gravity varied between 1024 and 1060 with an average of 1044. There was no correlation between water intake on the one hand, and body temperature or sex on the other. The urine always contained traces of albumin. The ratio "water intake/urinary output" was 2.5.

The daily urinary excretion was more than doubled in the average of 6 hypertensive rats (Table I). In these instances the specific gravity varied from 1010 to 1036 with an average of 1027; this is a significant drop in density, but the figure is definitely above the isosthenuric level. The albumin in the urine was not significantly increased over that in normal animals. With the augmentation of water metabolism the ratio "water intake/urinary output" dropped to an average of 1.9, since a smaller relative portion of the water was diverted to the feces and insensible perspiration.

Bilateral Renal Ischemia.—In 6 rats which had developed neither hypertension nor polydipsia after wrapping of the left kidney, perinephric scar of the right kidney was subsequently produced by the same procedure. Four of these animals now developed hypertension and polydipsia, whereas the remaining 2 stayed normal in both respects (Table II).

Removal of Ischemic Kidney.—In order to test whether both hypertension and polyuria were due to the presence of the ischemic kidney, the latter was removed in 5 rats which had developed the described combination of both symptoms. In all but 1 rat, the blood pressure dropped after removal of the wrapped or clamped kidney, although not always to the initial level. This drop occurred either abruptly or gradually, seemingly depending upon the duration of the established hypertension. There was a corresponding drop in water intake in 3 out of 5 animals so treated.

Removal of Normal Kidney.—It was noticed in some instances that water consumption dropped again almost to the normal level in hypertensive rats after the initial rise evoked by wrapping or clamping of the left kidney. In the case of rat 17 (Fig. 1, Table III, (a)), polydipsia reached its height 10 days after operation and within 18 days after this peak had been reached the water intake fell to normal levels. This may be ascribed to a compensating mechanism involving the right normal kidney. When this kidney was removed, the blood pressure rose further and with it the water intake increased to a plateau
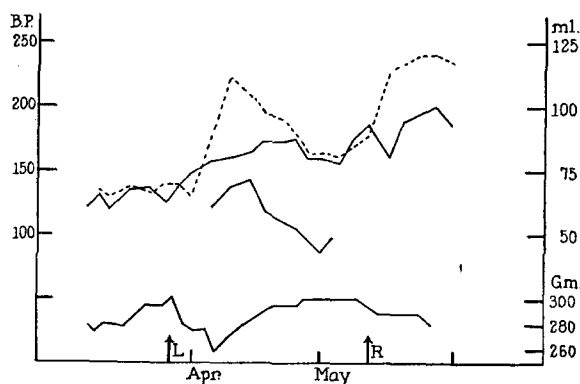
Fig. 1. Blood pressure and water intake of rat 17. Solid line on top shows blood pressure according to scale on the left, broken line shows daily water intake according to upper scale on the right, solid line on bottom shows body weight according to scale at lower right, short graph during April indicates urinary output on the same scale as water intake. March 27: left kidney clamped (L), causing rise of blood pressure, polydypsia, and polyuria. May 11: right kidney removed (R), causing exacerbation of symptoms.
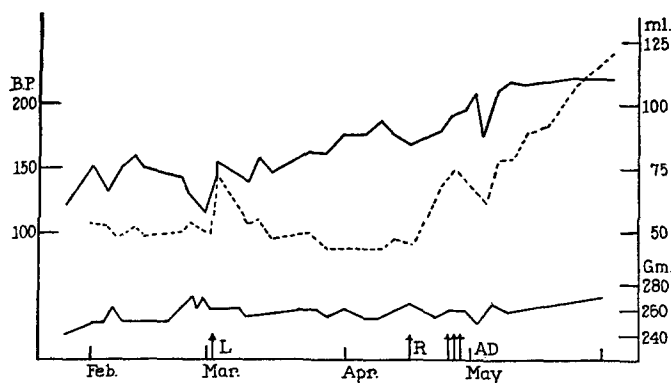

Fig. 2. Blood pressure and water intake of rat 8. Symbols and scales as in Fig. 1. March 2: left kidney wrapped in cellophane (L), gradual increase in blood pressure, but unchanged water intake. April 17: right kidney removed (R), with subsequent steep rise of blood pressure and water intake, except for temporary drop upon administration of 20 mg. adrenochrome on April 26, 27, and 28, respectively, (AD). Body weight unchanged throughout observation.

above the previous peak. In another animal, the condition was not exacerbated by removal of the right kidney (Table III, (a)).

Seven rats were selected which showed the following combinations of states after operation on the left kidney: 2 rats in which neither water metabolism nor arterial pressure had been affected, 4 rats with normal blood pressure but

increased water consumption, and 1 rat with hypertension but normal water metabolism. Since the presence of the normal kidney might mask potential
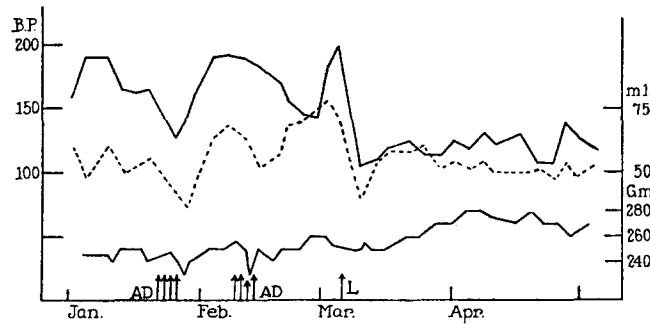

FIG. 3. Blood pressure and water intake of rat 6. Preoperative average daily water intake 39 ml. November 11: left kidney wrapped. 20 mg. adrenochrome injected daily January 24 to 27, and February 10 to 13 (AD), causing temporary drop in blood pressure and water intake. March 6: left kidney removed (L), blood pressure and water intake fell, slight rise in body weight.
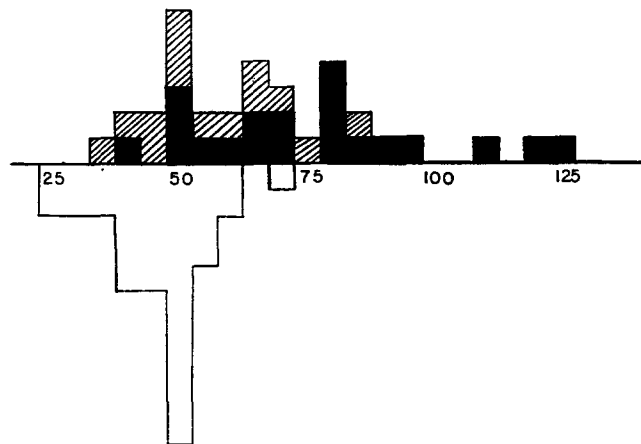

FIG. 4. Comparison of water intake of normal and hypertensive rats. Average daily water intake of 34 normal rats = white field below horizontal axis; 20 hypertensive animals = black areas; 14 animals, operated on left kidney without production of hypertension = cross-hatched areas.

hypertension in unilateral renal ischemia, and might compensate for potential polyuria, the right kidney was removed in this group of 7 animals. Two of them succumbed because the remaining left kidney had become largely necrotic. Of the 5 surviving animals, 2 had not developed either condition, but upon removal of the normal kidney, one of these 2 developed both hypertension and polydipsia whereas the other did not. (Table III, (d)). It was found at

TABLE I

Hypertension and Polydypsia in Rats with Unilateral Ischemia

Ischemia of left kidney by	No. of rats with blood pressure		With polyuria	Without polyuria	Average increase of postoperative water intake	
					Rats with polyuria	All rats
Cellophane wrapping: 24 rats	Above 160 mm: 11 rats	B.P.........	220 210 210 200 200 190 190 180 175 170	170 160	42	33
		Δw.........	80 45 40 25 65 20 35 30* 40	5 0‡§		
		U	54 34 76			
	Below 160 mm: 13 rats	B.P.........	150 140 130 130 125	150 140 140 135 130 125 120 120 120	25	10
		Δw.........	30‡ 25 20 10 40	0‖ 0‖ 0‡ 0‡ 0‡ 0‖ 0‖ 0‖ 0‖		
		U	28	24 21		
Clamping of artery 10 rats	Above 160 mm: 9 rats	B.P.........	200 180 175 175 170 170 170 170	160	29	26
		Δw.........	70 35 25 20 25‡ 20‡** 20 15	0		
		U	18 58 26			
	Below 160 mm: 1 rat	B.P.........	140‡		10	10
		Δw.........	10			

B.P. = blood pressure plateau in mm.; Δw = difference of postoperative minus preoperative averages of daily water intake in ml.; U = average daily postoperative urinary volume.

* Cf. Fig. 3.
‡ Cf. Table III.
§ Cf. Fig. 2.
‖ Cf. Table II.
** Cf. Fig. 1.

autopsy that the left kidney of this latter rat had hypertrophied and had ripped the cellophane capsule, for which reason presumably there had been no development of ischemia. A third rat (Fig. 2, Table III, (b)) had shown hypertension, but a normal water intake. After dextro-nephrectomy, it developed polydipsia and polyuria, while hypertension persisted. Finally 2 normotensive rats (Table III, (c)) with increased water consumption developed high blood pressure after removal of the normal kidney, while polydipsia per-

TABLE II

Effect of Bilateral Renal Ischemia on Blood Pressure and Water Intake

After wrapping of left kidney	B.P............	150	140	130	125	120	120
	Δw............	0	0	0	0	0	0
Time between operations	Days..........	37	28	39	37	37	35
After wrapping of right kidney	B.P............	160	145	135	205	200	180
	Δw............	45	5	0	30	55	45

For significance of B.P. and Δw see Table I.

TABLE III

Removal of Normal Kidney in Rats with Unilateral Renal Ischemia

Type of operation		Cl. (a) Cl.	(b) Wr.	Wr. (c) Cl.	Wr. (d) Wr.
After operation on left kidney	B.P........	170 170	160	150 140	140 135
	Δw........	25 20	0	30 10	0 0
Time between operations	Days.......	45 45	46	32 38	85 41
After removal of right kidney	B.P........	200 155	200	175 170	190 140
	Δw........	50 10	20	45 35	30 0

For significance of B.P. and Δw see Table I.
Cl., clamped. Wr., wrapped.

sisted. Hence, removal of the healthy kidney brought out both changes in most cases in which the ischemia of one kidney had not sufficed to give the characteristic results.

Table I is a synopsis of the results of operation in 34 animals. The arterial pressure given for each animal represents the average of all measurements performed on the animal, beginning with a date 4 weeks after the cellophane wrapping or 2 weeks after the clamping operation. Fig. 4 illustrates the variations in water intake of 34 normal rats which averaged 46 ± 9 ml. In order to take into account the individual preoperative drinking level of the polydipsic animals, we have listed in Tables I to III the differences between

average postoperative and preoperative water levels instead of the postoperative water levels themselves. The absolute amounts of the latter are given in Fig. 4, with 3 hypertensive individuals averaging more than 100 ml. *per diem*. In several instances, the water consumption rose above 160 ml. on individual days, an amount more than one-half of the rats' body weight. It appears from Table I that the clamping operation produces hypertension more uniformly than cellophane wrapping does. If one considers those groups of animals only in which hypertension had been produced there appears to be a slight difference in the degree of the polydipsia-polyuria syndrome in favor of the wrapping procedure; this difference, however, is hardly of statistical significance.

Table II illustrates the effect of two-stage bilateral cellophane wrapping, which produced hypertension and polyuria in 4 out of 6 animals.

The effect of removal of the normal kidney on animals with various combinations of conditions, is summarized in Table III, which gives both levels of hypertension and of water intake after the original operation on the left kidney as well as after the removal of the right one.

DISCUSSION

The disturbance of water metabolism observed in the foregoing experiments has not been previously described as a characteristic happening when hypertension has been experimentally induced by renal ischemia. The occurrence of this disturbance provides an approach to the study of the excretory function of the ischemic kidney. The deviation from the clinical picture, and also from the state of affairs in dogs with renal ischemia, may help to elucidate differences in the mechanism of essential and experimental hypertension, as well as differences in the renal mechanism from species to species.

Chanutin and Ferris (1932) in experiments with rats, in which hypertension had been induced by progressive reduction of kidney tissue, found a marked polyuria in those rats which had only one-half of one kidney left. This experience is in agreement with the clinical observation of polyuria in various diseases of the kidney associated with progressive atrophy of the renal parenchyma. Eichelberger (1943) describes a change in the distribution of intra- and extracellular fluid in the tissues of hypertensive dogs; however, no change in the water intake has been observed in such animals. The degree, to which the increased water intake may be taken up by the body in the form of edema, is rather limited in rats since their normal water consumption, and even more so that in polydipsia, is exceedingly high compared to man and other species. Thus, the amount of fluid in transudates and ascites could account but for a minute fraction of the total intake. Moreover, frank edema was not observed in our hypertensive animals except during periods of substitution of saline for water, when typical edema became evident; then the animals dragged their abdomens on the ground and their body weight increased 10 per cent or more.

The body weight during the usual water regime was quite constant (Figs. 1 to 3) and there was no evidence that a weight increase due to a generalized state of edema were possibly concealed by a concomitant wasting of tissue. The occasional observation of puffed eyes and, at autopsy, of localized edema of the lungs and mild hydrothorax was certainly in the nature of a secondary effect and could not account for prolonged polydipsia.

It is reasonable to suppose that the polydipsia observed in the present studies on experimentally hypertensive rats was a sequel of an induced polyuria. The increased water intake served in fact as a useful indicator for the polyuria, but the latter was also directly confirmed and measured in individual cases (cf. Table I). The cause of polyuria may be either increased filtration in the glomeruli, or decreased reabsorption from the tubuli, or a combination of both effects. The experiment of Toth (1937), who produced polyuria in normal dogs by slow infusion of epinephrine, speaks in favor of the first assumption. He suggested that the constriction of the efferent capillaries increases the pressure in the glomeruli, thereby increasing filtration. The same hypothesis might hold true in operatively induced hypertension, which, according to Smith, Goldring, Chasis, and Ranges (1938), is also partly due to constriction of the efferent blood vessels in the kidney.[1]

If, on the other hand, polyuria in experimentally hypertensive rats is to be ascribed to impairment of tubular excretion, it would appear that the functioning of the tubules under hypoxic conditions is diminished without the occurrence of any demonstrable anatomical changes. Because tubular reabsorption requires the local expenditure of energy for the selective secretory processes, it seems reasonable to suppose that it is specifically impeded by the withholding of oxygen from the kidney. Lack of oxygen has also been adduced as an explanation for the occurrence of hypertension; the significance of oxidative processes for the destruction and control of pressor substances has been discussed elsewhere (Oster, 1942; Oster and Sorkin, 1942; Oster and Sobotka, 1943). According to this conception the occurrence of hypertension and that of polyuria are simultaneous sequels of renal ischemia, but independent of each other. This view is favored by the incidence of hypertension without polyuria and vice versa in some animals.

Pickering and Prinzmetal (1940) found that renal extracts containing renin when injected intravenously into anesthetized or hydrated unanesthetized rabbits raised the blood pressure and, if the doses were not too small, accelerated the flow of urine conspicuously for 1 hour. This diuresis was accompanied by a large increase in the excretion of sodium and chloride, whereas creatinine

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[1] The vicinity of the suprarenal gland to the kidney may lead to the surmise that the hormonal regulation of water metabolism was affected by the operative procedures instituted. However the anatomical position of the suprarenal glands in the rat and the separate blood supply of the left one speak against this assumption.

clearance was not affected.   They ascribe this diuretic action to "an inhibition of tubular reabsorption of water, sodium, and chloride.   This may represent the effects of a pressure diuresis in which relatively few nephrons are involved, but it is more probably due in part to a direct action of renin on the activity of the renal tubule cells."   On the other hand, they ascribe the antidiuretic action produced by renin in unanesthetized rabbits during the ascending limb of water diuresis to the action of renin "on the glomerular vessels reducing the rate of glomerular filtration."   The observation of Pickering and Prinzmetal on acute diuresis in rabbits, produced by injection of renin, parallels our experiences in chronic polyuria in renal ischemia in rats.   It seems reasonable to explain both observations on the same basis.

The abolishment of both changes in the early stages of unilateral renal ischemia by removal of the ischemic kidney requires no comment.   When, on the other hand, the operative procedure on the left kidney had failed to produce the anticipated changes three explanations may be offered: (1) No sufficient ischemia had been established, and hence no effect could be achieved even by removal of the right kidney since the remaining left kidney parenchyma continued to function normally.   (2) The left kidney had become moderately ischemic without manifest symptoms and removal of the right kidney brought out the existence of a hitherto compensated dysfunction of the left one.   (3) The operative procedure eliminated the left kidney altogether with necrosis, and removal of the right kidney provoked the most severe uremic changes and the animal perished.

Whether polyuria is caused directly by ischemia in the rat kidney, or is, according to the alternative view, secondary to the intra- and extrarenal effects of high blood pressure, will have to be decided by clearance tests and electrolyte studies which we are unable to carry out at present owing to extraneous circumstances.   Our preference for the former hypothesis is based on the facility with which it explains the observed phenomena.

SUMMARY

The water intake in hypertensive rats was investigated.   Rats made hypertensive by renal ischemia increased their water consumption by 75 per cent over the preoperative level.   Polyuria was associated with this polydipsia and the independence of these occurrences from a number of other factors was demonstrated.

It was found that the presence of a normal kidney exerted a compensatory influence which may mask either hypertension or polyuria or both.   The appearance or exacerbation of the changes upon removal of the normal kidney, on the one hand, and the elimination or mitigation of the symptoms upon removal of the ischemic kidney on the other support the view that the changes observed cannot have been due to passive elimination of the kidney tissue by

ischemia, but to active malfunction of the renal, and especially the tubular, mechanism upon withdrawal of oxygen. The view is put forward that polyuria is a primary sequel of ischemia rather than secondary to the intra- and extra-renal effects of hypertension. A number of concomitant observations are in harmony with this hypothesis.

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