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Quantitative Excretion of Water and Sodium Load by Isolated Dog Kidney: Autonomous Renal Response to Blood Dilution Factors

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Summary. Totally isolated dog kidneys of the same pairs are perfused with heparinized blood under identical conditions, one of the kidneys being submitted to a supplementary isotonic or hypotonic saline load. Excess sodium is excreted quantitatively as well as excess solute-free water. Autonomous renal response depends on blood dilution, changes in blood pressure being excluded as well as influence of volume expansion or of extrarenal hormonal factors. The experiments demonstrate kidney ability to control quantitatively fast changes in saline balance by autonomous mechanisms in the sense of intrarenal feed-back type relation to blood composition. Dilution factors (plasma sodium, potassium, proteins) control excretion primarily by adjustment of tubular reabsorption to filtration. Moreover, the absence of relation between basal control excretion and response to saline loading in the present experiments suggest that different mechanisms could insure long duration adjustment of the kidney to a definite blood composition and saline balance. It is demonstrated that many effects attributed to volume expansion can be caused by blood dilution; moreover, interference between dilution effects and specific hormonal control by eventual natriuretic factor should be avoided.

Key-Words: Sodium Excretion — Water Excretion — Isolated Kidney.

Schlüsselwörter: Na-Exkretion — Wasserexkretion — Isolierte Niere.

Two fundamental mechanisms are susceptible to control renal excretory response after saline and water loading, depending respectively on volume changes and on composition changes of blood and extracellular fluid. An increase of *blood volume* may result in a concomitant increase of water and sodium excretion by the way of reflex mechanisms involving receptors sensitive to volume variations and controlling the secretion of antidiuretic hormone and of a possible natriuretic factor (Lichardus [23]; Lichardus and Pearce [23]; Cort [6]; Pannier, Seroussi, Martineaud, Vassilikos, and Durand [35]). Modifications of *blood composition* caused by saline loading as, for example, decreased

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plasma protein concentration and lowered haematocrit may induce sodium and water elimination by the kidney: the importance of such physical factors has recently been emphasized again [25]. It is difficult to dissociate the role played by either volume changes or blood dilution in the response of kidneys in situ; isolation of these mechanisms and study of autonomous renal controls can be best performed on totally isolated kidneys; under such experimental conditions, blood pressure can be kept constant, extrarenal hormonal factors are ruled out, and renal response depends only on changes of blood composition related to water and sodium loading.

We have previously described a technique for the perfusion of isolated dog kidneys with heparinized blood [10,28]; this technique has been applied to the study of autonomous renal control of water and sodium excretion. The previous results showed an increase of sodium rejection by kidneys taken from dogs submitted to a high sodium diet for 3 weeks prior to isolation of kidneys [32]. Sodium rejection is at the highest during the first hour of artificial perfusion by heparinized blood; this observation is compatible with the presence of a natriuretic factor of short life. A large increase of sodium rejection is also observed after infusion of saline either to the animal 1/2 hour before isolation of the kidney [32] or during the artificial perfusion. In the last case, interference of extrarenal factors other than blood dilution is avoided [27,32]. In order to reduce individual differences of animals, further series of experiments have involved comparative and simultaneous perfusion of both kidneys of the same pair by two identical equipments with unilateral modification of the blood. It was demonstrated that sodium and water rejection by isolated kidney working at a constant blood pressure is not only related to changes in glomerular filtration, but primarily to reduced tubular reabsorption; decrease of haematocrit and increase of total renal blood flow are not the only factors involved [29]. Important parameters are plasma protein concentration and oncotic pressure: the addition of serumalbumin (or of dextran) reduces sodium and water excretion by isolated kidneys submitted to a saline load; the response is chiefly related to increased tubular reabsorption [31]. Other factors are ionic disequilibrium. Dilution of plasma by saline induces hypokalaemia which in turn reduces tubular reabsorption of sodium without changes in filtration [30]. Moreover, tubular sodium reabsorption is also controlled by plasma sodium concentration; autonomous rejection of hypertonic saline depends on reduced reabsorption as an immediate consequence of increased plasma sodium concentration [33].

It appears from these results that the kidney is able to eliminate a saline load by purely autonomous mechanisms; kidney response depends on cumulative factors bound to blood dilution [34].

The fundamental question is therefore raised to know *to what extent autonomous renal response to blood dilution is capable of insuring a quantitative excretion of the extra load* and, as a consequence, of keeping constant the composition of blood and extracellular fluid. The purpose of the experiments described in the present paper is to answer this question. Two kidneys of the same pair are perfused in identical basic conditions: one of the kidneys is submitted to an extra water and sodium load and the perfusion is prolonged until urine flow comes back to an identical

level on both sides. The differences in the total amounts of urine and sodium excreted during the whole experiment are respectively compared to the amounts of water and sodium added on one side.

Such a comparative type of experiment makes possible to investigate quantitatively the renal response to blood dilution by neutralizing individual differences between animals as well as the progressive functional changes that occur in the isolated perfused kidney [3, 32, 37]. These changes, involving a decrease of glomerular filtration, cannot be neglected in long duration experiments and should make impossible a quantitative determination of excess saline excretion. This technical difficulty is solved by using an identical kidney for simultaneous reference.

In order to investigate the relations between autonomous excretion of sodium and of water, two series of experiments are performed, involving respectively the addition of isotonic and of hypotonic saline.

Material and Methods

For the detailed description of perfusion device and of basic experimental conditions, we refer to previous papers [10, 28]. Two identical machines are used for simultaneous perfusion of both kidneys of the same pair. The dogs are not submitted to a special dietary preparation. Blood is taken from the same donor different from kidney donor; the animals are under pentobarbital anaesthesia (26 ml/kg I.V.) and receive 25,000 I.U. heparin intravenously. The same volume of blood (450 ml) is used in all experiments, with a perfusion pressure of 110 mm Hg and a temperature of 37.5° C. Initial vasoconstriction is minimized by addition of 25 mg promethazine (Phenergan Specia) to the blood in the machine before starting perfusion. Saline load (75 ml isotonic or hypotonic NaCl) is added on one side at the beginning of perfusion. The duration of each double experiment depends on the time required for equalization of urine flow on both sides (150 to 330 min). Urine samples are collected after each 30 min period. In each machine a continuous injection of a solution containing 1 g glucose, 1 g urea and 2,650 g potassium chloride per 100 ml Ringer's solution is made at a rate of 6 ml/hour. Glomerular filtration is measured from creatinine clearance. A priming dose of 20 mg creatinine per 100 ml blood is added at the beginning and is followed by a continuous infusion at 6 ml/hour of a 2 p. 100 creatinine solution in Ringer. In both machines, and at intervals of 10 to 30 min, Ringer's solution diluted to one third is added in volumes corresponding to the volume of urine excreted by the control kidney minus the amount of fluid added by continuous infusion, i.e. 12 ml/hour. In order to avoid the possible interference of blood changes that might occur during bleeding of donor dog, blood is introduced alternately in both machines in separated and successive fractions. Blood introduction starts on control side in half of the experiments and on saline loading side in the other half. Moreover, left and right kidneys are respectively used in half of controls and half of saline loading experiments.

Wherever comparison of datas involves successive values concerning the same kidney at various times or kidneys of the same pair at corresponding time intervals, statistical significance of differences has been calculated by the method of pair comparison, differences being taken for significant for $2P \leq 0.05$.

Moreover, limits of confidence are indicated in the tables between brackets after each average values: they are calculated from Student's distribution for

$2P = 0.05$. Comparison of average values of independent series between themselves or with a given value are made by calculation of the statistical significance on the same distribution basis.

Experimental Results

1. Isotonic Sodium Chloride Loading

A technical difficulty of the experiments involving addition of isotonic sodium chloride lies in the decrease of concentrating ability of isolated kidney which cannot be entirely counteracted by vasopressin supplementation. It is known that hypotonic urine can be excreted by the dog after saline loading even in the presence of vasopressin (CLAPP and ROBINSON [5]). The same phenomenon is observed in the isolated kidney [28] and is aggravated by at least two mechanisms: development of medullary oedema and changes in medullary blood flow [32]; antagonism between vasopressin and bradykinin liberated in the machine (such an antagonism has been demonstrated by FURTADO [14]). The consequence of hypotonic urine secretion is a progressive increase of plasma osmolality and sodium excretion [29,33] which becomes troublesome in long duration experiments. In the present series, the difficulty has been solved by limitating the saline load to 75 ml for 450 ml blood and by replacing urine by Ringer's solution diluted to one third; moreover, vasopressin (lysin-8-vasopressin, Sandoz) has been added at a rate of 0.5 I.U. every 30 min in each machine.

The saline load corresponds to 75 ml water and 10,875 μEq sodium in all experiments of the series.

Table 1 gives the average results of 12 identical experiments.

Duration of experiments ranges between 150 and 330 min. *Kidney weight* shows no significant difference between both sides ($2P = 0.07$). *Total renal blood flow* is moderately increased by saline supplementation ($2P < 0.005$) it increases on both sides and finally reaches an identical level ($2P = 0.35$). *Haematocrit and plasma protein content* do not change on control side; after saline loading they are lower than controls ($2P < 0.001$) but they increase steadily to values higher than controls ($2P < 0.005$). *Plasma osmolality and plasma sodium* are identical on both sides at the beginning and remain steady on control side; at the end of perfusion they become higher on saline side ($2P < 0.001$). *Plasma potassium* values cannot be interpreted because of wide individual differences ($2P > 0.07$). Urine flow is considerably increased by saline addition ($2P < 0.005$) but becomes identical on both sides at the end of perfusion. *Urine osmolality* is slightly decreased after saline loading ($2P = 0.04$). Urine becomes hypotonic; osmolality is the same at the end ($2P = 0.25$). *Sodium excretion* at the beginning is considerably higher after saline addition ($2P < 0.005$). On control side, sodium ex-

Table 1. *Excretion of water and sodium after isotonic saline loading*
The Table gives the values at the beginning and at the end of perfusion
Limits of confidence are given between brackets for $2P = 0.05$

	Control				75 ml water + 10,875 μEq Na			
	Begin		End		Begin		End	
Renal blood flow (ml/g/min)	3	(0.44)	4.8	(0.44)	3.7	(0.66)	4.5	(0.88)
Haematocrit (p. 100)	54	(4.4)	52	(4.4)	49	(4.4)	55	(4.4)
Plasma Na (mEq/l)	152	(4.4)	155	(4.4)	152	(6.6)	162	(4.4)
Plasma K (mEq/l)	4.3	(0.66)	5.5	(1.3)	3.9	(0.66)	4.9	(0.88)
Plasma proteins (g/l)	65	(4.4)	65	(4.4)	57	(4.4)	72	(6.6)
Plasma osmolality (mOsm)	312	(4.4)	317	(6.6)	311	(4.4)	336	(8.8)
Urine flow (ml/100 g/min)	0.86	(0.59)	2.6	(0.88)	2.52	(0.95)	2.6	(0.88)
Urine osmolality (mOsm)	438	(70)	208	(31)	355	(62	216	(31)
Na excretion (μEq/100 g/min)	108	(81)	189	(70)	322	(123)	221	(75)
K excretion (μEq/100 g/min)	38	(14.3)	49	(14.3)	61	(14.3)	44	(14.3)
Glomerular filtrationrate (ml/100 g/min)	26	(4.4)	25	(4.4)	38	(6.6)	24	(4.4)
Na Load (μEq/100 g/min)	4,066	(924)	3,873	(1,382)	5,790	(1,245)	4134	(823)
Na rejection (p. 100 of load)	2,8	(2)	5.8	(1.76)	6	(2)	6.3	(1.54)
Total Na output (μEq)		9,864	(3,190)			20,933	(3,335)	
Total water output (ml)		134	(39.6)			232	(42)	
Kidney weight (g)		44	(6.6)			42	(6.6)	
Differential total Na output (μEq)				11,069	(2,143)			
Differential total water output (ml)				98	(12)			

cretion increases significantly during the experiments ($2P = 0.025$); on the other side sodium excretion drops ($2P = 0.001$); at the end of perfusion, there is no significant difference between both sides ($2P = 0.04$). *Potassium excretion* is moderately increased by saline loading ($2P < 0.001$); there is no significant difference at the end ($2P = 0.25$). *Glomerular filtration rate, sodium load and percentage of sodium rejection* behave similarly; they are increased initially by saline loading ($2P < 0.05$), reach an average maximum of 36 ml/100 g/min on control side and 47 ml under load, and exhibit no significant difference at the end of experiments ($2P > 0.5$).

Total sodium output is increased by saline supplementation; the *difference from control side, after equalization of urine flow, corresponds to the amount* of sodium added ($2P = 0.85$). Total water output is also increased by saline addition; *the difference in total is moderately but significantly higher than the volume of water added* ($2P < 0.005$).

Essential results of typical experiments are given in Fig. 1 and 2.

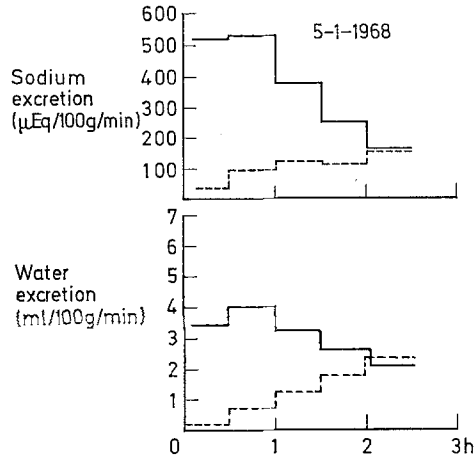

Fig. 1. Convergence of sodium and water excretion by two kidneys of the same pair, one of them being submitted to an isotonic saline load

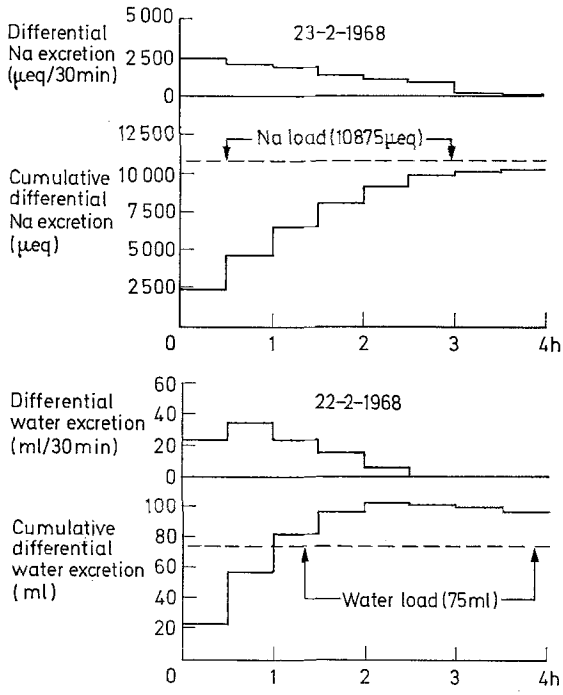

Fig. 2. Isotonic sodium chloride loading. The difference in the amounts of sodium excreted during each 30 min period decreases progressively while total difference excreted comes nearer to the amount of excess sodium added. Water excretion tends also to equalization; total excess volume excreted exceeds moderately extra water load

2. Hypotonic Sodium Chloride Loading

An identical experimental setup is used. 75 ml of sodium chloride solution containing 3625 μEq of sodium are added on one side. Solute-free water addition corresponds to 50 ml. No measurable haemolysis is produced because of quick mixing. The opportunity of vasopressin supplementation requires consideration: addition of vasopressin in conditions of marked plasma hypoosmolality would be in contradiction with physiological conditions; while vasopressin could reduce water excretion on control side, it is not obvious that it should modify differential water excretion in the coupled experiments; finally an influence of vasopressin on either basic or differential sodium excretion is even more questionable.

Three experiments have been performed with vasopressin supplementation at the same doses as in the first series (0.5 I.U. every 30 min) and 5 experiments without vasopressin supplementation. Comparative average results are figured in Table 2.

Table 2. *Vasopressin and excretion of hypotonic saline load (3,627 μEq sodium, 75 ml water)*

	without vasopressin	with vasopressin	
Total sodium output on control side (μEq)	7,037	8,883	$2P = 0.55$
Excess sodium output after loading (μEq)	3,697	5,141	$2P = 0.27$
Total water output on control side (ml)	158	141	$2P = 0.48$
Excess water output after loading (ml)	76	75	$2P = 0.75$

The first series of experiments has shown that vasopressin is unable to insure hypertonicity of urine after isotonic loading; its only effect is to reduce the degree of hypotonicity. This finding is even more marked after hypotonic loading; vasopressin exhibits no significant influence on basic as well as differential sodium and water excretion within the range required for the interpretation of present investigation. All experiments involving hypotonic load have therefore been treated as one series.

Average results are quoted in Table 3.

Duration of experiments is comprized between 150 and 300 min. *Kidney weight* is identical ($2P = 0.2$). *Total renal blood flow* is not significantly different at the beginning ($2P = 0.1$); it increases on both sides and becomes moderately higher on control side ($2P = 0.02$). *Haematocrit*

Table 3. *Excretion of water and sodium after hypotonic saline loading*
The table gives the values at the beginning and at the end of perfusion
Limits of confidence are given between brackets for $2P = 0.05$

	Control				75 ml water +3,625 μEq Na			
	Begin		End		Begin		End	
Renal blood flow (ml/g/min)	4	(0.94)	6.2	(1.4)	4.5	(0.94)	5.4	(1.2)
Haematocrit (p. 100)	50.1	(5.2)	48.8	(5)	46.3	(3.78)	48.1	(4)
Plasma Na (mEq/l)	150.6	(5.2)	159.2	(10.4)	135.2	(6.1)	160.2	(12.5)
Plasma K (mEq/l)	4.9	(0.7)	6	(1.65)	4.4	(0.94)	5.4	(1.4)
Plasma proteins (g/l)	67.8	(4.2)	72.5	(7.6)	56.2	(3.78)	75.1	(9)
Plasma osmolality (mOsm)	327	(9.9)	339	(23.6)	289	(6.4)	336	(28.3)
Urine flow (ml/100 g/min)	1.6	(0.94)	2.5	(1.4)	2.6	(1.2)	2.7	(1.2)
Urine osmolality (mOsm)	410	(85)	228	(80)	292	(66)	217	(80)
Na excretion (μEq/100 g/min)	157	(120)	128	(54)	212	(151)	160	(94)
K excretion (μEq/100 g/min)	60	(22)	74	(9.4)	70	(28)	72	(16.5)
Glomerular filtration rate (ml/100 g/min)	35.8	(10.6)	23.8	(7.1)	37.2	(13)	22.6	(5.9)
Na load (μEq/100 g/min)	5,333	(1,402)	3,733	(1,029)	4,961	(1,645)	3,535	(850)
Na rejection (p. 100 of load)	3.3	(2.8)	3.5	(0.71)	4.5	(3.3)	4.8	(2.1)
Total Na output (μEq)		7,729	(3,108)			11,967	(3,759)	
Total water output (ml)		151.8	(57)			227	(59)	
Kidney weight (g)		37.2	(5.9)			37.7	(6.4)	
Differential total Na output (μEq)				4,238	(1,709)			
Differential total water output (ml)				75.4	(15.8)			
Differential solute-free water output (ml)				45	(11.3)			

does not change significantly on control side ($2P = 0.09$); initially decreased by dilution it comes back to control value ($2P = 0.35$). *Plasma osmolality* is initially decreased by hypotonic saline addition; it increases moderately on control side ($2P = 0.05$), more on saline side ($2P = 0.05$), and reaches the same final value ($2P = 0.25$). *Plasma proteins* are also diluted by extra water load. They increase on both sides ($2P < 0.005$); at the end of perfusion they are moderately higher on saline side than on control side ($2P = 0.02$). *Plasma sodium* is initially decreased by water dilution; it increases later on control side ($2P = 0.02$) and more on saline side ($2P = 0.004$). Final concentration becomes identical ($2P = 0.35$). *Plasma potassium* exhibits wide individual differences; it increases progressively on saline side ($2P = 0.005$); difference between final levels is barely significant ($2P = 0.07$). *Urine flow* initially higher after saline addition comes to final identical values on both sides ($2P = 0.25$). *Urine osmolality* is

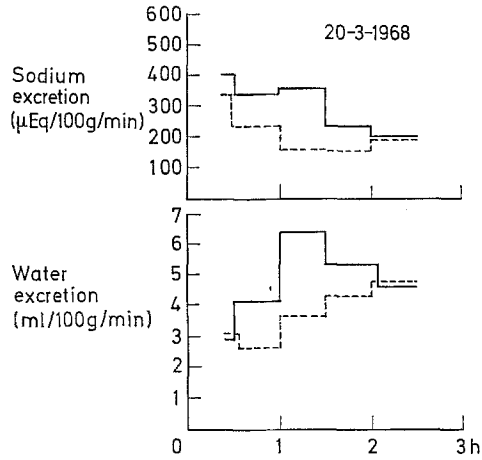

Fig. 3. Convergence of sodium and water excretion by two kidneys of the same pair, one of them being submitted to a hypotonic saline load

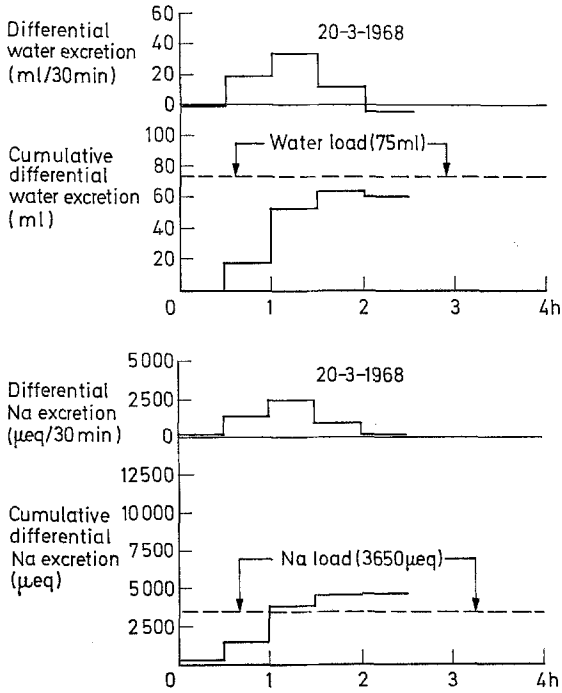

Fig. 4. Hypotonic sodium chloride loading. Sodium and water excretion become equal in both kidneys and differences in excretion disappear when excess water and sodium supplements have been quantitatively excreted

decreased at the beginning by hypotonic loading. Urine becomes hypotonic on both sides; at the end of perfusion there is no significant difference ($2P = 0.1$). *Sodium excretion* is increased moderately by loading ($2P = 0.04$). It does not change significantly on control side ($2P = 0.35$) but diminishes on saline side ($2P < 0.001$). At the end the difference is small and not clearly significant ($2P = 0.1$). Glomerular filtration rate changes during perfusion are parallel, without any significant difference between both sides ($2P < 0.08$), with an average maximum of 42.7 ml/100 g/min for controls and 45.3 ml on loaded side. *Sodium load* at the beginning is moderately reduced by dilution of plasma sodium ($2P = 0.05$); it becomes identical at the end of experiments. *Percentage of sodium rejection* exhibits relatively considerable individual variations.

Differences in total sodium and water output correspond respectively to extra water ($2P = 0.05$) *and sodium load* ($2P = 0.45$). *Solute-free water excretion corresponds also to free water load* ($2P = 0.35$).

Essentials of one experiment are reproduced in Fig. 3 and 4.

Discussion

Appreciable individual differences in water and sodium excretion as well as in blood composition are observed on control side. On the contrary there is a good concordance in the comparative behaviour of the same kidney in the course of perfusion experiment as well as in the differences observed between kidneys of the same pairs submitted to perfusion with or without water and sodium load.

The fundamental point is the demonstration that the extra water and sodium loads are quantitatively eliminated.

After isotonic saline supplementation, the volume of water excreted exceeds moderately the volume added to the blood. An explanation can be offered on the basis of the experiments quoted in the introduction: the hypotonicity of excreted urine results in a higher increase of plasma sodium and osmolality on the side submitted to saline load. By itself, increased plasma sodium maintains prolonged and excessive sodium loss because of reduced tubular reabsorption [33]; this effect is compensated by the increase in plasma protein concentration which in turn increases tubular reabsorption [31]. The final result is that a new equilibrium is reached at the time extra sodium load has been excreted. It is therefore obvious that the kidney eliminates quantitatively the extra saline load by autonomous mechanisms; in the absence of arterial pressure changes and extrarenal hormones, kidney response can be monitored only by blood dilution: decrease in plasma protein concentration [31], plasma potassium [30], perhaps haematocrit. Although glomerular filtration is increased in the present experiments, we know from previous results that this factor is not the only one; changes in reabsorption play a major role [29].

The second series of experiments, involving hypotonic saline load, brings a confirmation of above-mentioned interpretations; moreover, it demonstrates that dilution factors can work differentially and insure quantitative excretion of excess solute-free water independently from sodium excretion. The reduced differential sodium excretion under hypotonic saline loading could possibly be related to the fact that a significant increase of filtration is observed in the present experiments under isotonic saline and not under hypotonic saline; however, the minor role of moderate changes of total filtration rate should be emphasized again [29]. Blood dilution is identical in both series of experiments with the only obvious difference that plasma sodium is diluted and plasma osmolality decreased in the second series and not in the first one. An important factor is the decrease of plasma sodium concentration whose direct influence on sodium reabsorption has been previously demonstrated in the isolated kidney [33] as well as in the whole animal (Goldsmith, Rector, and Seldin [15]; Kamm and Levinsky [19]; Bálint and Forgács [1]; Eknoyan, Suki, Rector, and Seldin [13]). The important point in the present experiments is that the control of sodium excretion is so accurately adjusted that excess sodium is quantitatively excreted at the time plasma sodium, protein and osmolality as well as haematocrit have come back to equal values on both sides, what implies quantitative excretion of water load within the same time.

Quantitative excretion not only of excess sodium but also of excess solute-free water after hypotonic loading is insured as a consequence of kidney integration of the dilution factors, in spite of the absence of the osmoreceptor—ADH control mechanism.

While sodium rejection by isolated kidney is easily controlled by dietary sodium intake when both kidney and blood are taken from the same animal [32], basic sodium rejection differs widely when kidney and blood are provided by different animals as evidenced by the present experiments. Initial percentage of sodium rejection in control kidneys is comprized between 0.06 and 9.9 p. 100. However, there is no significant correlation between control and differential sodium and water excretion.

The coefficient of correlation of Bravais-Pearson calculated from individual results gives the following values.

Isotonic saline loading:		
Sodium excretion	$r = 0.23$	$2P = 0.45$
Water excretion	$r = 0.04$	$2P = 0.90$
Hypotonic saline loading:		
Sodium excretion	$r = 0.15$	$2P = 0.70$
Water excretion	$r = 0.11$	$2P = 0.75$

It appears that each kidney pair is initially adjusted to a definite level of excretion for the blood composition of the kidney donor. When perfusion is performed with the blood of a different animal, initial equilibrium is disrupted. Differences in basal excretion correspond to the same phenomenon as differential excretion of the extra saline load.

Control of blood sodium and water by autonomous renal effect works in the sense of a stable equilibrium by adjustment of sodium and water reabsorption to filtration under monitoring of blood composition. The level of the stable equilibrium corresponds to the above-mentioned basal adjustment of the kidney. The question is raised to know whether the response of kidney to fast water and sodium loading changes and the more permanent adjustment to definite extracellular fluid and plasma composition depend or not on identical mechanisms.

It is worth while to discuss these two problems with reference to some intrarenal and extra-renal factors susceptible to be involved.

a) The Fast Variations of Water and Sodium Excretion

Apart from control of ADH secretion by volume receptors and osmoreceptors [35] which will not be considered here, a considerable amount of work supports the idea that sodium excretion following saline loading is related to extracellular volume expansion [13,16], and that the response to extracellular or intravascular volume expansion might involve a specific natriuretic factor [18,23,24,40] whose action could be controlled by the way of volume-reflex mechanism [6,7,22]. Natriuresis is the consequence of decreased tubular reabsorption [18,21,36,41].

The present experiments demonstrate that some of the effects attributed to volume expansion with or without interference of natriuretic hormone are explainable by immediate renal response to blood dilution that occurs together with volume expansion after saline loading. Decreased fractional tubular reabsorption is observed as a consequence of blood dilution without any possible interference of extrarenal hormonal factors or volume changes [29,34].

While careful reevaluation of some interpretations seems advisable, there is, however, an impressive amount of experimental material in which dilution factors seem to be ruled out. In LICHARDUS' experiments [22], volume expansion has been induced by "reconstituted" blood without dilution. A natriuretic factor has been detected in urine by KRÜCK [20]. Preliminary characterization of a hormonal inhibitor of proximal tubular reabsorption has been performed by MARTINEZ-MALDONADO, KURTZMAN, RECTOR, and SELDIN [26]. Origin, nature and mechanism of action of natriuretic hormonal material is investigated by CORT, LICHARDUS and coll. [6,7,8]. A hepatic role in the control of sodium

excretion following saline infusion has been evidenced by Daly, Roe, and Horrocks [11].

There is no contradiction in the possible simultaneous action of both dilution effects and natriuretic hormone. It is conceivable that the correction of dilution changes in the blood is a purely autonomous renal mechanism, which controls primarily blood composition, but is unable to control volumes. The stability of intravascular and extracellular volumes could be monitored primarily by volume-sensitive receptors, Gauer-Henry reflexes, antidiuretic and natriuretic hormones [22, 35], other mechanisms being not excluded.

It is also conceivable that the autonomous and quantitative renal excretion of excess water after hypotonic loading should combine its effect with the system of osmoreceptors, voloreceptors and ADH secretion.

The name of "third factor" given sometimes to natriuretic hormone seems quite inadequate. In such a conception the two other factors should be glomerular filtration and mineralocorticoids [4]. There is now ample evidence that glomerular filtration and mineralocorticoids do not play the only major role in fast changes of sodium excretion and that factors other than natriuretic hormone play a major role.

b) The Basic Adjustment of Kidney to Blood Composition and Sodium-Water Balance

The distinction between fast and slow control of extracellular volume and sodium balance is strongly suggested by the autonomous renal effects demonstrated by the experiments described here: feed-back-type interactions between blood dilution factors and glomerular-tubular balance in the sense of a stable equilibrium at a definite level of blood composition. What are the determinants of that level? Various control factors can be taken into consideration, as, for example, basal arterial pressure, renin-angiotensin system, mineralocorticoids and sodium content of tubular cells. According to Thurau and Schnermann [39], glomerulotubular balance could be controlled intrarenally by renin and angiotensin. Stumpe and Ochwadt [38] have demonstrated that in rats chronically loaded with salt the inhibition of fractional proximal reabsorption promoting sodium rejection is related to a decreased secretion of mineralocorticoids and is corrected by aldosterone supplementation. According to Behrenbeck, Dörge, and Reinhardt [2], intracellular sodium concentration might be one factor influencing renal regulation of sodium homeostasis.

Several results suggest that the mechanism of natriuretic response to chronic sodium feeding is not identical to the mechanism of response to acute sodium loading as induced by saline infusion. While a decrease in

proximal tubular reabsorption of sodium after saline infusion has been observed by many investigators [9,12,21,36,41] this response is not influenced by prior dietary salt loading or salt restriction (HAYSLETT, KASHGARIAN, and EPSTEIN [16]). Moreover, HORSTER and THURAU [17] dit not observe any change in proximal reabsorption in rats chronically submitted to either high or low sodium diets; according to these authors the kidney in these conditions adjusts sodium excretion by altering the respective contribution of superficial and juxtamedullary nephrons to total kidney glomerular filtration.

It might be reasonable to admit that fast response and prolonged adjustment of sodium excretion correspond to different mechanisms which combine their effects but are, to some extent, independent, as evidenced in our experiments by the absence of relation between basal control excretion and acute response to saline loading.

References

1. BÁLINT, P., u. I. FORGÁCS: Natriumreabsorption und Sauerstoffverbrauch der Niere bei osmotischer Belastung. Pflügers Arch. ges. Physiol. **288**, 332 (1966).
2. BEHRENBECK, D. W., A. DÖRGE u. H. W. REINHARDT: Untersuchungen an wachen Hunden über die Einstellung der Natriumbilanz. III. Electrolytbilanzen und Natriumrejektion nach akutem Natriumentzug durch Peritonealdialyse oder wiederholter Mannitolinfusion. Pflügers Arch. ges. Physiol. **300**, 226 (1968).
3. BERKOWITZ, H. D., L. D. MILLER, and H. D. ITSKOVITZ: Renal Function and the renin-angiotensin system in the isolated perfused kidney. Amer. J. Physiol. **213**, 928 (1967).
4. BRICKER, N. S.: The control of sodium excretion with normal and reduced nephron population. The pre-eminence of third factor. Amer. J. Med. **43**, 313 (1967).
5. CLAPP, J. R., and R. R. ROBINSON: Osmolality of distal tubular fluid in the dog. J. clin. Invest. **45**, 1847 (1966).
6. CORT, J. H.: The source and chemical nature of the natriuretic activity of plasma evoked by saluretic "volume reflexes". Canad. J. Physiol. Pharmacol. **46**, 325 (1968).
7. — B. LICHARDUS, V. PLIŠKA, V. UHRÍN, T. BARTH, and J. RUDINGER: The origin, nature and mechanism of action of natriuretic hormone. In: protein and polypeptide hormones. Proceedings of the International Symposium Liège, 1968, p. 523. International Congress Series n° 161, Excerpta Medica Foundation, Amsterdam 1968.
8. — V. PLIŠKA, and T. DOUŠA: The chemical nature and tissue source of natriuretic hormone. Lancet **1968I**, 230.
9. CORTNEY, M. A., M. MYLLE, W. E. LASSITER, and C. W. GOTTSCHALK: Renal tubular transport of water, solute, and PAH in rats loaded with isotonic saline. Amer. J. Physiol. **209**, 1199 (1965).
10. CUYPERS, Y., A. NIZET, and A. BAERTEN: Technique pour la perfusion de reins isolés de chien avec du sang hépariné. Arch. intern. Physiol. **72**, 245 (1964).
11. DALY, J. J., J. W. ROE, and P. HORROCKS: A comparison of sodium excretion following the infusion of saline into systemic and portal veins in the dog:

Evidence for a hepatic role in the control of sodium excretion. Clin. Sci. **33**, 481 (1967).

12. Dirks, J. H., W. J. Cirksena, and R. W. Berliner: The effect of saline infusion on sodium reabsorption by the proximal tubule of the dog. J. clin. Invest. **44**, 1160 (1965).
13. Eknoyan, G., W. N. Suki, E. C. Rector, Jr., and D. W. Seldin: Functional characteristics of the diluting segment of the dog nephron and the effect of extracellular volume expansion on its reabsorptive capacity. J. clin. Invest. **46**, 1178 (1967).
14. Furtado, M. R. F.: Bradykinin inhibition of vasopressin and oxytocin. Effects on the permeability to water of the toad bladder. Proceedings of the International Symposium on vasoactive polypeptides: bradykinin and related kinins, 277. Sao Paulo: Riceirao Prêto 1967.
15. Goldsmith, C., F. C. Rector, Jr., and D. W. Seldin: Evidence for a direct effect of serum sodium concentration on sodium reabsorption. J. clin. Invest. **41**, 850 (1962).
16. Hayslett, J. P., M. Kashgarian, and F. H. Epstein: Changes in proximal and distal tubular reabsorption produced by rapid expansion of extracellular fluid. J. clin. Invest. **46**, 1254 (1967).
17. Horster, M., and K. Thurau: Micropuncture studies on the filtration rate of single superficial and juxtamedullary glomeruli in the rat kidney. Pflügers Arch. ges. Physiol. **301**, 162 (1968).
18. Johnston, C. I., and J. O. Davis: Evidence from cross circulation studies for a humoral mechanism in the natriuresis of saline loading. Proc. Soc. exp. Biol. (N. Y.) **121**, 1058 (1966).
19. Kamm, D. E., and N. G. Levinsky: Inhibition of renal tubular sodium reabsorption by hypernatraemia. J. clin. Invest. **44**, 1144 (1965).
20. Krück, F.: Biologischer Nachweis eines humoralen natriuretischen Prinzips im Urin gesunder Menschen. Klin. Wschr. **45**, 30 (1967).
21. Landwehr, D. M., R. M. Klose, and G. Giebisch: Micropuncture study of sodium reabsorption in saline-loaded rats. Amer. J. Physiol. **212**, 1327 (1967).
22. Lichardus, B.: Volume reflex. The experimental model to evidence a probable existence of a natriuretic hormone. Endocr. Exp. **1**, 181 (1967).
23. —, and W. Pearce: Haemodynamic and humoral factors in natriuresis following blood volume expansion. Fed. Proc. **24**, 404 (1965).
24. — — Evidence for a natriuretic factor released by blood volume expansion. Nature (Lond.) **209**, 407 (1966).
25. Martino, J. A., and L. E. Earley: Demonstration of a role of physical factors as determinants of the natriuretic response to volume expansion. J. clin. Invest. **46**, 1963 (1967).
26. Martinez-Maldonado, M., N. A. Kurtzman, F. C. Rector, Jr., and D. W. Seldin: Evidence for a hormonal inhibitor of proximal tubular reabsorption. J. clin. Invest. **46**, 1091 (1967).
27. Mills, I. H., G. W. Osbaldiston, G. M. Craig, and B. L. Wise: The perfused isolated kidney: control of sodium and water excretion by integration of blood pressure and haematocrit. Congress of Nephrology. Abstracts. Washington: Excerpta Medica Foundation 1966.
28. Nizet, A.: Recherches sur le rein isolé perfusé par un système artificiel. Bull. Acad. roy. Méd. Belg. 7è serie **111**, 283 (1963).
29. — Excretion of water and sodium loading by isolated perfused dog kidney. Pflügers Arch. ges. Physiol. **287**, 156 (1967).

30. Nizet, A.: Control by plasma potassium concentration of sodium excretion by isolated perfused dog kidney. Pflügers Arch. ges. Physiol. **297**, 162 (1967).
31. — Influence of serumalbumin and dextran on sodium and water excretion by isolated dog kidney. Pflügers Arch. ges. Physiol. **301**, 7 (1968).
32. — Y. Cuypers, P. Deetjen, and K. Kramer: Functional capacity of the isolated perfused dog kidney. Pflügers Arch. ges. Physiol. **296**, 179 (1967).
33. — J. P. Godon, and P. Mahieu: Comparative excretion of isotonic and hypertonic sodium chloride by isolated dog kidney. Arch. intern. Physiol. Biochem. **76**, 311 (1968).
34. — — Nouvelles recherches sur l'excrétion de léau et du sodium par le rein. Bull. Acad. roy. Méd. Belg. 7e série **7**, 95 (1968).
35. Pannier, C., S. Seroussi, J. P. Martineaud, D. Vassilikos, and J. Durand: Les volumes sanguins circulants chez l'homme. II. Répartition, rôle et régulation du volume sanguin circulant. Rev. franç. Etud. clin. biol. **13**, 124 (1968).
36. Rector, F. C., Jr., J. D. Sellman, M. Martinez-Maldonado, and D. W. Seldin: The mechanism of suppression of proximal tubular reabsorption by saline infusion. J. clin. Invest. **46**, 47 (1967).
37. Schröder, E., B. Ochwadt, and H. Bethge: Herstellung und Funktion eines isolierten Nierenpräparates vom Hund. Pflügers Arch. ges. Physiol. **286**, 189 (1965).
38. Stumpe, K. O., u. B. Ochwadt: Wirkung von Aldosteron auf die Natrium und Wasserresorption im proximalen Tubulus bei chronischer Kochsalzbelastung. Pflügers Arch. ges. Physiol. **300**, 148 (1968).
39. Thurau, K., u. J. Schnermann: Die Natriumkonzentration an den Macula densa Zellen als regulierender Faktor für das Glomerulumfiltrat. (Mikropunktions-Versuch.) Klin. Wschr. **43**, 410 (1965).
40. Wardener, H. E. de, I. H. Mills, W. F. Clapham, and C. J. Hayter: Studies on the efferent mechanism of sodium diuresis which follows the administration of intravenous saline in the dog. Clin. Sci. **21**, 249 (1961).
41. Watson, J. F.: Effect of saline loading on sodium reabsorption in the dog proximal tubules. Amer. J. Physiol. **210**, 781 (1966).

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