PART II
EDITORIALS
Editorial
The Natriuretic Hormone
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The suggestion that there exists a natriuretic hormone was first put forward by de Wardener et al (1961) who demonstrated that there must be factors other than changes in glomerular filtration rate and aldosterone which control sodium reabsorption. The experimental observations which gave rise to this hypothesis led to much fruitful work which has now established that many so-called physical factors have an effect on urinary sodium excretion. These include the peritubular capillary oncotic and hydrostatic pressures, the plasma sodium concentration, and the viscosity of the blood (Schrier & de Wardener, 1971). The suggestion that in addition there is a circulating substance other than aldosterone which controls sodium excretion was at first totally ignored and then vigorously denied. It was considered by some that the discovery of these physical factors automatically ruled out any other possibility. Nevertheless there is a continuing accumulation of evidence that demonstrates that some circulating substance, or substances other than aldosterone control sodium excretion. An outline of this evidence is given below.

To demonstrate that the changes in urinary sodium excretion that occur with variations in the volume of the body fluids are due in part to some humoral effect, the blood volume of an animal is expanded with blood that is in equilibrium with the animal's blood. The effect of this on the urinary sodium excretion of a denervated or isolated kidney constantly perfused at a controlled pressure with the animal's blood is then observed. In this way the increase in blood volume does not dilute any of the constituents of the animal's blood, and the physical factors known to influence sodium excretion are not disturbed. This experiment has now been performed by three groups (Bahlman et al, 1967; Kaloyanides & Azer, 1971; Bengele et al, 1972). In each, the blood of a dog is continuously exchanged with the contents of a reservoir which is initially primed with a solution of bovine albumin in Ringer Locke or Hartman's solution, or saline. After one to two hours of such an exchange the blood in the
reservoir is in equilibrium with the blood in the dog. The dog's blood volume is then expanded by about 30% in 15 min by lowering the fluid level in the reservoir. The dog's blood volume is therefore expanded without changing the composition of the blood (Figure 1). In all three sets of experiments the perfusion pressure of the kidneys was controlled and the kidney was denervated; in two the renal venous pressure was also kept constant. In one of the

![Diagram of an isolated kidney preparation.](image)

Figure 1. Diagram of an isolated kidney preparation. The isolated kidney (1) is placed in a constant temperature humidity chamber where it is perfused with blood from the femoral artery (2) of a second dog (10). Renal venous blood (3) flows by gravity into a reservoir (4) from which it is pumped (5) to the femoral vein of the perfusion animal. The perfusion animal (10) rests on an adjustable platform and by raising or lowering the platform with respect to the isolated kidney, renal arterial pressure in the isolated kidney can be regulated. Pressure in the femoral artery (9) and vein (8) and renal artery (7) and vein (6) are monitored with pressure transducers. Urine (11) is collected from a catheter secured in the ureter.


three reports the kidney was isolated in a warm saline bath, while in the other two the kidney remained in situ. In all three sets of experiments there was a 50 to 100% increase in urinary sodium excretion which lasted about 30 to 60 min. These three sets of results demonstrate unequivocally that the rise in urinary sodium excretion which occurs upon expanding the blood volume of an animal is in part due to the change in the concentration of some circulating substance. They do not distinguish whether there is a diminution of some antinatriuretic substance or an increase of some natriuretic substance, but in two of the reports it was possible to demonstrate that the natriuresis was due to a diminished tubular reabsorption of sodium (Kaloyanides & Azer, 1971; Bengel et al, 1972).

Two other groups have performed almost the same experiment, but instead of using equilibrated blood, the blood volume was expanded, in one instance, with blood from another dog (Lichardus & Nizet, 1972), and in the other by 'artificial' blood consisting of washed canine erythrocytes resuspended in 6%
bovine albumin in Ringer Locke solution (Lichardus & Pearce, 1966). Lichardus and Nizet (1972) transplanted a kidney into the neck. It was then perfused at constant arterial pressure and venous pressure while the blood volume was expanded by about 33% in 15 min with blood from donor dogs whose plasma protein concentration and haematocrit matched those of the experimental animals. Blood volume expansion caused a 50% rise in urinary sodium excretion though there were no significant changes in the haematocrit or in the plasma concentrations of protein, sodium and potassium. In Lichardus and Pearce’s (1966) experiment the blood from a donor dog perfused an isolated aortic sac of another dog in situ. The sac was created by tying the aorta above and below the left renal artery. The right renal artery was ligated. The renal venous blood of the left kidney emptied into a reservoir before being returned to the donor dog. The aortic sac, and thus the left kidney, was then perfused at constant arterial and venous pressure. Upon infusing a quantity of 'artificial' blood (see above) into the donor dog (approximately 30% of its estimated blood volume) there was a sustained rise in urinary sodium excretion from the isolated left kidney in the recipient animal. The natriuresis persisted even when the perfusion pressure and glomerular filtration rate were lowered substantially by an aortic clamp.

Tobian et al (1967) performed yet another variation on this same theme in that they placed an isolated rat kidney between two reservoirs into one of which arterial blood flowed from a donor rat and into the other venous blood flowed from the kidney. The isolated kidney was perfused at constant pressure. When a mixture of 2/3 blood and 1/3 Ringer's solution was placed into the venous reservoir without expanding the donor rat's blood volume, there was no increase in sodium excretion by the isolated kidney. But when the same amount of blood and Ringer was infused intravenously into the rat there was usually a pronounced rise in urinary sodium excretion from the isolated kidney. The donor rat supplying the blood to the isolated kidney had been given dibenzylamine, and had had both adrenals and kidneys removed. It was concluded that expansion of the blood volume changed the circulating level of some humoral substance which increased urinary sodium excretion. The protocol of the experiment also allowed some conclusions about the possible site of production of this substance.

If the rise in urinary sodium excretion that occurs when the blood volume is expanded is due in part to a change in the concentration of a humoral substance it is reasonable to suppose that the hormone should also be demonstrable in an experiment between two animals, one of which is expanded with equilibrated blood. In this instance the expanded donor animal is perfusing a whole recipient animal, as opposed to an isolated kidney. At first, cross circulation experiments in which one animal was expanded with blood did not cause a rise in urinary sodium excretion in the other animal (Pearce et al,
1970; Nizet, 1970; Bonjour & Peters, 1970). Nevertheless Pearce et al (1969) performed a fascinating modification of the classical cross circulation experiment which, in a rather round about way, certainly demonstrated the presence of a humoral phenomenon. They first demonstrated that upon expanding the blood volume of rats that had been deprived of sodium there was only a small rise in urinary sodium excretion. They then showed that the same blood volume expansion in rats that had been given large amounts of DOCA and sodium, produced a much greater rise in urinary sodium excretion. The definitive experiment consisted in cross circulating sodium depleted rats with sodium loaded DOCA rats for one hour via a common reservoir. At the end of the hour each animal was transfused with equilibrated blood from the reservoir. There was a large rise in sodium excretion from both the DOCA rats and the sodium depleted rats. Pearce et al (1969) concluded that the altered responsiveness of the salt deprived rat to blood volume expansion, after it had been cross circulated with the salt loaded DOCA rats, was due to a circulating factor in the blood of the DOCA rats.

Lichardus and Ponec (1970) were the first to demonstrate the presence of a humoral factor in a straightforward cross circulation experiment between two rats. One rat (the donor) was expanded with equilibrated blood but both had a rise in sodium excretion. Lichardus and Ponec (1970) succeeded because they re-infused the donor rat’s urine into its own jugular vein throughout the experiment. They had noticed in previous cross circulation experiments that when the blood volume of one of the animals is expanded, the ensuing natriuresis and diuresis from this animal causes a significant increase in its plasma protein concentration. In a cross circulation experiment the plasma is common to both animals, therefore the plasma protein concentrations of the other animal (the recipient) must also rise. They argued that, in the previous unsuccessful cross circulation experiment, it was perhaps this increase in plasma protein concentration which had prevented a rise in urinary excretion in the recipient animals.

Sonnenberg (1971) used the technique of re-infusion of urine during volume expansion in single animals. He noted the much greater rise in urinary sodium excretion that occurs. Thus subsequently, Sonnenberg et al (1972) re-infused the donor animal’s urine, or equivalent amounts of saline or Ringer, into the donor animal’s femoral vein in a beautiful set of cross circulation experiments (Figure 2). These workers obtained a brisk rise in urinary sodium excretion in the recipient rat when the blood volume of the donor rat was expanded with homologous blood. In the first hour after expansion of the donor rat, the rise in urinary sodium excretion in the recipient rat was considerably less than in the donor animal, but in the second hour the rise was about the same in both animals. Furthermore though the rise in sodium excretion in the recipient animal was a little less when the donor
had had its kidneys and adrenals removed, the natriuresis was still substantial. Sonnenberg et al (1972) concluded that in cross circulation experiments a humoral component of the natriuretic affecter mechanism apparently can only be revealed with sustained blood volume expansion, i.e. the loss of sodium and water in the urine must be replaced intravenously. They suggested that without re-infusion of urine into the donor animal the rapid correction of the blood volume expansion by the natriuresis prevents the humoral component from reaching a level sufficient to manifest a significant effect in a recipient animal.

The results of some micropuncture experiments have also suggested that expansion of the blood volume gives rise to a natriuretic hormone. These experiments are not so clear cut as those described above, but they do not support the hypothesis that the rise in urinary sodium excretion which accompanies volume expansion is due entirely to 'physical factors'. Micropuncture techniques have been used to measure sodium reabsorption in the proximal tubule before and after the administration of saline. Brenner et al (1971) confirmed that as the plasma oncotic pressure fell there was a fall in tubular fluid reabsorption. They then microperfused the peritubular capillaries with a solution of albumin which was of slightly higher oncotic pressure than that of the peritubular plasma which had been in the peritubular capillaries during the control period. This produced a return of the proximal tubule fluid reabsorption towards normal but reabsorption was still depressed. Knox et al (1972) in a technically more advanced study measured proximal tubule reabsorption during saline infusion in the dog. They measured the peritubular capillary oncotic and hydrostatic pressure directly. The fall in peritubular capillary oncotic pressure caused by the saline was then corrected by an infusion of albumin into the renal artery. In one set of experiments, even though the capillary oncotic pressure rose to above control values tubular sodium reabsorption remained lower than during the control periods. In
another set of animals the plasma volume was expanded with 25% hyperoncotic albumin. The peritubular capillary protein oncotic and hydrostatic pressures after the expansion were in the same range as they had been before expansion, but after expansion the reabsorption of sodium in the proximal tubule was still significantly lower than before (Figure 3). These experiments confirm and

![Figure 3. The effect of expansion of the plasma volume with 25% hyperoncotic albumin solution on proximal reabsorption. Absolute reabsorption was measured with free flow micropuncture techniques. Starling forces in the peritubular microcirculation were measured directly with a servo-nulling device and sampling of efferent arteriolar blood for protein analysis. Absolute reabsorption was significantly reduced in the absence of changes in Starling forces in peritubular capillaries. (To be reproduced in the Proceedings of the 9th International Congress of Nephrology 1972) Knox, F C, Schneider, E G, Strandhoy, J W, Willis, L R, Warm, P C and Davis, B B. Microperfusion evidence for a natriuretic hormone: Effect of volume expansion on intrarenal sodium reabsorption in the presence and absence of Starling forces.)

extend Brenner et al's (1971) previous observation. Holzgreve and Schrier (1972) microperfused the peritubular capillary of the same proximal tubule in the rat before and after the administration of saline. After the administration of saline there was a fall in sodium reabsorption of the proximal tubule though its peritubular environment was unchanged. The results of these four studies demonstrated that the fall in proximal tubule sodium reabsorption which takes place as a result of a saline or hyperoncotic albumin infusion is due in part to factors other than Starling forces. Or in other words they reinforce the results obtained with the isolated kidney and cross circulation experiments described earlier that the increase in urinary sodium excretion produced by expansion of body fluids is due to a change in the concentration of some circulating substance.

There has been considerable interest into the precise site in the nephron
where the natriuretic hormone has its action. It is difficult, however, to disentangle this information for many of the experiments that have been performed to investigate this point have not been designed to exclude physical factors. For instance, there are no micropuncture studies of tubular function in the isolated kidney perfused by an animal whose blood volume is expanded with equilibrated blood. Nevertheless some interesting points have emerged. It is well established that with the administration of saline, solutions of bovine albumin, and blood there is a fall in sodium reabsorption in the proximal tubule (Dirks et al, 1965; Cortney et al, 1965; Hayslett et al, 1967; Landwehr et al, 1967; Knox et al, 1968; Howards et al, 1968; Wright et al, 1969; Brenner & Berliner, 1969; Sonnenberg, 1971). An important point that was stressed by many of these workers was that the depression of proximal tubule sodium reabsorption produced by the various infusions were of the same order, but that the accompanying increase in urinary sodium excretion varied widely, being higher with saline and lowest with whole blood infusions. In some experiments the conditions were such that though there was a considerable fall in proximal tubule sodium reabsorption there was no increase in urinary sodium excretion (Howards et al, 1968; Sonnenberg, 1971). These results have inevitably led to the conclusion that the principal reason why volume expansion produces a rise in urinary sodium excretion is by inhibiting sodium reabsorption in the distal tubule and collecting ducts.

Recently further evidence has been produced to support this conclusion. Sonnenberg (1972) infused whole blood into DOCA loaded rats and into chronically sodium deprived rats. In the DOCA loaded animals the amount of sodium excreted in the urine, during the natriuresis caused by volume expansion, was greater than the amount of sodium delivered from the distal tubule into the collecting duct. The inevitable conclusion is that during volume expansion sodium either diffuses or is actively transported into the lumen of the collecting duct from the peri-collecting duct environment. Sonnenberg (1972) points out that sodium can diffuse into the rat collecting duct (Uhlich et al, 1969) and that aldosterone decreases the collecting duct's permeability for sodium. The latter phenomenon, however, is unlikely to be relevant to the natriuresis of acute volume expansion for the rise in sodium excretion is the same if the animal is given large amounts of aldosterone (Sonnenberg, 1972). It is also uninfluenced by the simultaneous administration of vasopressin (Sonnenberg, 1972). The hypothesis that the main site in the nephron responsible for the natriuresis of volume expansion is probably the collecting duct has received increasing support from Knox and his co-workers. They have demonstrated in a variety of ways that changes in sodium excretion produced by acute and sustained volume expansion are unrelated to the rate of delivery of sodium from the proximal tubule into the distal tubule (Knox et al, 1970; Schneider et al, 1971; Willis et al, 1972; Knox, 1973; Knox et al, 1973).
insulin (Nizet et al, 1971); calcitonin (Keeler et al, 1970); bradykinin and kallikrein (Adetuyibi & Mills, 1972; Marin-Grez et al, 1972); and a substance from the liver (Milies, 1960). It is most unlikely to be any substance made in either the kidneys or adrenals for two of the most conclusive cross circulation experiments which have demonstrated the presence of the natriuretic hormone were performed in donor animals that had been nephrectomised and adrenalectomised (Tobian et al, 1967; Sonnenberg et al, 1972). And in Tobian et al's 1967 experiment the animals had, in addition, been given dibenzylxine. The natriuretic hormone does not appear to be one of the prostaglandins for though prostaglandin A and E are natriuretic their plasma concentrations either remain unchanged, or fall, with volume expansion (Zusman et al, 1973). Schrier et al (1968) have demonstrated that oxytocin is not an important factor in the natriuresis accompanying isotonic saline infusion for this produces no significant change in the concentration of plasma oxytocin. And conversely the administration of oxytocin during the administration of saline does not influence the natriuresis. In addition these authors found that the circulating concentration of arginine vasopressin was unchanged during isotonic saline administration. The possibility of vasopressin being the natriuretic hormone is also excluded on the basis that many of the blood volume expansion experiments have been performed during the administration of large amounts of pitressin or arginine vasopressin (Bahlman et al, 1967; Kaloyanides & Azer, 1971; Lichardus & Nizet, 1972; Sonnenberg et al, 1972).

Some workers are exploring the possibility that the natriuretic hormone comes from the hypothalamus and posterior pituitary. Gitelman and Blythe (1972) have prepared a natriuretic substance from the posterior pituitary which they consider is not vasopressin or oxytocin. Nevertheless they are having considerable difficulties making this differentiation. Similarly Clarkson (1972) and Clarkson et al (1973) have prepared much cruder extracts from the hypothalamus which they claim are natriuretic. Again the main difficulty has been to exclude the natriuretic effect of the extracts being due to vasopressin. They have tried to exclude this possibility by treating the extracts with thioglycollate and only assaying for natriuretic activity those samples which subsequently no longer had any antidiuretic activity when assayed formally on alcohol anaesthetised rats. The results show that extracts treated in this way when obtained from the hypothalamus are natriuretic whereas similar extracts obtained from the cerebral cortex are not.

The suggestion that the hypothalamus in man might secrete a substance which controls urinary sodium excretion directly was first put forward by Homer Smith in 1957, but he proposed that the substance was antinatriuretic. It does seem odd that the hormonal control of water with ADH should be so deeply embedded in the oldest part of the brain whereas the only known hormonal control of sodium comes from a gland that is anatomically distant and
functionally unrelated to water control. In mammals vasopressin and oxytocin may, in an unpredictable way, increase urinary sodium excretion in the rat, dog, camel and sheep but not in man (Bentley, 1971). It is doubtful if this action of vasopressin and oxytocin is of any relevance to the control of sodium balance in these animals for they are both secreted in response to a reduction of fluid volume. But it is interesting to find natriuretic properties in the known neurohypophyseal hormones, however inappropriate these may be. The properties of the various known neurohypophyseal hormones tend to overlap which suggest that perhaps there may be another, as yet unknown neurohypophyseal substance which is mainly natriuretic. This possibility gains some support from the work of Jard and Morel (1963), Chan and du Vigneaud (1970), Cort et al (1973), Gillessen et al (1973) and Piška et al (1973) who have made natriuretic in animals in which the original substance had either little effect on sodium excretion or had a marked antinatriuretic effect.

Some lower animals secrete a natriuretic substance from the central nervous system. In certain frogs, toads and snakes vasotocin from the neurohypophyseal system increases tubular reabsorption of sodium (Bentley, 1971). This is particularly well defined in Bufo Marinus (Chester Jones et al, 1971). Teleosts on the other hand have a specialised system of glandular neurones situated in the caudal spinal cord called the urophysis (Enami, 1958). Its ultrastructure is similar to that of the neurohypophysis and its venous effluent drains into the caudal vein which irrigates the renal portal system (Bern, 1967). Extracts from the urophysis stimulate sodium transport across the gill, and reduce urinary sodium excretion (Maetz et al, 1964). The final example of a neurosecretory substance that influences sodium transport is from the Triatamidae or blood sucking insects sometimes called Assassin bugs (Wigglesworth, 1931; Maddrell, 1964; 1966). Rhodnius prolixus sucks up ten times its own weight of blood. The consequent abdominal distension causes afferent impulses to be transmitted from the abdominal wall to a fused neuroganglionic mass situated in the mesothorax. Within this neuroganglionic structure there are neurosecretory cells. When stimulated these cells release a substance into the haemolymph which rapidly increases the transport of sodium from the haemolymph into the blind ended malpighian tubules which are floating in the haemolymph. This results in a massive increase in urinary sodium and water excretion and the insect rapidly returns towards its normal size and weight.

In each of these three examples of a substance secreted by the central nervous system which has a direct effect on sodium transport the substance appears to stimulate sodium transport. This is an action opposite to that proposed for the action of the natriuretic hormone in mammals which, until recently, has been considered to be an inhibitor of sodium transport. Nevertheless if Sonnenberg’s (1972) findings in the rat are confirmed, that volume
expansion causes sodium to be transferred from the peritubular environment into the lumen of the collecting tubule, it is just possible that the properties of the mammalian natriuretic hormone may be more like those of other species than was originally suspected.

In conjunction with these attempts to find the source of the natriuretic hormone several assay techniques have been developed to try and detect its presence in blood and urine. Extracts have been prepared by a variety of simple methods consisting mainly of dialysis, gel filtration, and ultrafiltration. They have been assayed for their effect on the urinary sodium excretion of the dehydrated or hydrated rat, or the sodium transport of the isolated renal tubule, frog skin or toad bladder. These experiments have demonstrated that both plasma and urine contain a substance or substances which are natriuretic and which inhibit sodium transport in 'in vitro' systems.

Attempts to repeat some of these experiments, have not been successful. In some others there are no published accounts that the experiments have been repeated. And finally a few have been repeated and the original claims have been confirmed.

Schrier, Verroust and de Wardener working in one laboratory (Schrier & de Wardener, 1971) and Keimowitz, Brenner and Berliner working in another were unable to repeat the findings of Cort et al (1968) and Lichardus et al (1968). The latter had claimed that extracts of plasma prepared with trichloracetic acid diethyl ether from animals which are expanded are natriuretic in the rat. Wright, Brenner, Bennett, Keimowitz, Berliner, Schrier, Verroust, de Wardener and Holzgreve (1969) working in three different laboratories were unable to confirm the findings of Rector et al (1968) that plasma dialysates from volume expanded animals inhibit sodium reabsorption in the proximal tubule as measured either by micropuncture or by the rate of free water excretion in the hereditary diabetes insipidus rat.

Buckalew and his associates (Buckalew et al, 1970; Buckalew & Lancaster, 1971; Buckalew, 1972a; Buckalew, 1972b) have claimed that dialysates, ultrafiltrates and extracts obtained with Sephadex G10 chromatography from the plasma of acutely saline loaded dogs, or dogs which have escaped from the prolonged administration of DOCA, inhibit sodium transport in the toad bladder. There are no published accounts that these experiments have been repeated though the experimental protocol appears simple and undemanding.

Neither has Viskoper et al's (1971) experimental technique been repeated. These workers used an extraction procedure previously described by Krück (1967): 200ml urine samples from salt loaded individuals are dialysed and then ultrafiltered through a 5μ filter. The residue on the ultrafiltering membrane is then taken up in a barbital buffer and the barbital soluble fraction dialysed against distilled water. Lyophilized extracts of the residual volume are injected into rats that have been maintained on a high sodium diet and
which are given DOCA and saline at the beginning of the test procedure. The urine is collected for five hours and each sample is assayed on 12 to 15 rats simultaneously, half of which act as controls. The results of two such assays on a sample carried out on 2 consecutive days are pooled for the calculation and evaluation of the results. Thus each individual sample is assayed on 12-15 rats with an equal number of simultaneous controls. Viskoper et al (1971) found that 6 to 12 μg of urine extracts obtained from normotensive subjects given a salt load causes a significant rise in urinary sodium excretion in the rats. Similar results were obtained with 3 μg of urine extracts obtained from salt loaded hypertensive subjects. The results of assays with urine extracts obtained before the administration of saline were not given but Viskoper et al (1971) stated that such extracts consistently fail to show natriuretic properties. This experiment has not been repeated. Krück (1969) had previously assayed urine extracts prepared in the same way, but from normal individuals given a moderate but persistent water load orally. The extracts were tested

Figure 4. The circuits used in the determination of the effect of expanding the blood volume of a dog on the short-circuit current across a frog skin incorporated in the circulation of the dog. AP and VP, arterial and venous pressure-manometers, respectively; U, urine collection from bladder catheter; F, Watson-Marlow MHRE Flow-Inducer pump; R₁, transfusion reservoir; R₂, top reservoir for the frog skin; R₃, funnel-reservoir; G, damp gauze; M, Perspex cell for frog skin; C₁ and C₂, coils for cooling the blood; C₃, coils for reheating the blood; Q, overflow chimney; mV, Vibron Electrometer; H₁ and H₂, KCl-agar leads; I, calomel half-cell; J, saturated KCl solution; V, voltmeter; μA, micro-ammeter; K, Ag-AgCl wires; L, saturated AgCl in saturated KCl.

on rats but the assay procedure is not described in detail. The results appear to be similar to those of Viskoper et al. (1971) in that these extracts from water loaded man caused a natriuresis in the rat. Nevertheless, it is difficult to understand why they should for an oral water load does not increase the urinary excretion of salt (Miles and de Wardener, 1953). The fourth set of unconfirmed observations are those of Nutbourne et al. (1970). They used an 'on line' technique with a frog skin to detect the presence of a natriuretic substance. The blood from a dog flows in and out of a membrane cell that contains a frog skin (Figure 4). The dog's blood also flows into and out of a reservoir that initially contains artificial plasma but which after 30 min contains blood in equilibrium with the blood of the dog (Bahlman et al., 1967).

Four to eight hours later when the frog skin settles down, the blood volume of the dog is expanded by transfusing some of the blood from the reservoir into the dog. In this way, the dog's blood volume is expanded without changing the composition of the blood. After the blood volume is expanded the sodium transport across the skin diminishes as the urinary sodium excretion rises.

In contrast to these unconfirmed reports, Clarkson et al. (1970) found that the plasma from blood volume expanded dogs impairs PAH and sodium transport of renal tubule fragments suspended 'in vitro'. This confirmed and extended the findings of Bricker et al. (1968) who had previously demonstrated that such plasma inhibits PAH transport of kidney slices. The relevance of this observation to a circulating natriuretic substance is that volume expansion diminishes $T_m$ PAH. Brown et al. (1972) have confirmed an original observation of Sealey et al. (1969, 1971) that urine extracts obtained from salt loaded individuals and assayed on the water loaded rat cause a natriuresis (Figure 5). In support of this finding, Clarkson and de Wardener (1972) have found that such extracts also inhibit sodium transport of suspended fragments of renal tubules 'in vitro'.

In conclusion it is necessary to mention the increasing evidence of Bricker and his group that there is a natriuretic substance in the blood and urine of patients with chronic renal failure. They were the first to propose that the remarkable adaptation of the failing kidney to maintain sodium balance by gradually reducing the proportion of the filtered sodium that is reabsorbed from the gradually diminishing number of nephrons might be due to the presence of a circulating natriuretic substance (Schultze et al., 1966; Bricker, 1967). They pointed out that a normal person with a glomerular filtration rate of 120ml/min who is ingesting 7g of sodium chloride per day reabsorbs about 99.5% of the filtered sodium in order to excrete about 7g of salt and stay in balance. But that a patient with a glomerular filtration of 4ml/min on the same salt intake only remains in sodium balance if he reduces sodium reabsorption to 84% of the filtered sodium. Bricker and his colleagues have prepared extracts of serum and urine on Sephadex G25 with a
very dilute solution of ammonium acetate eluant. They have found a substance which comes out of the gel, after the electrolytes, which inhibits sodium transport of the frog skin, and increases sodium excretion of the rat (Bourgoignie et al., 1970; Bourgoignie et al., 1971). For some time there was a widespread feeling that this substance might be a toxic product related to the uraemic state. Recently, however, Bricker has devised an experiment which makes this unlikely. Dogs are gradually made increasingly uraemic by unilateral nephrectomy and ligation of segmental branches of the renal artery of the remaining kidney. As the glomerular filtration rate falls the content of sodium in the diet is lowered so that the dog can remain in sodium balance without reducing tubular sodium reabsorption. Extracts of serum or urine from these uraemic dogs are not natriuretic when injected into the rat (Bricker, 1972). It appears therefore that the presence of the natriuretic substance in the urine of an uraemic animal is dependent on the need to maintain sodium balance and not on the retention, or manufacture, of some toxic end product of protein metabolism. It is possible therefore that the natriuretic hormone which appears with volume expansion in normal animals may be the same as the substance responsible for the fall in tubular reabsorption of sodium in uraemic animals.
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