On the Biology of Sodium Excretion: The Search for a Natriuretic Hormone

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One of the foremost functional attributes of the mammalian kidney in health is its ability to regulate the excretion of sodium with sufficient precision to allow for the maintenance of a constant content of sodium in the extracellular fluid (ECF). In essence, whatever quantity of sodium enters the ECF from dietary intake must be excreted by the kidney, and this commitment must be upheld regardless of the range over which sodium intake varies. The capability of the kidney to maintain the equality between excretion rates and acquisition rates of sodium must also be preserved in the face of advancing nephron loss if either salt retention or salt depletion is to be prevented. Thus when the nephron population is reduced progressively in the course of advancing renal disease, the residual nephrons must increase their individual excretion rates of sodium in inverse proportion to the glomerular filtration rate.

The complexity of regulating the excretion rate of sodium in health and the increasing complexity of this regulation in advancing renal disease make it extremely unlikely that the mechanisms of control reside entirely within the nephron. Rather, it seems essential that a biologic control system exists that has the general design shown in Fig. 1. A detector element is shown that has the capability of monitoring the rate of sodium entry into the ECF. The evidence is clear that the detector element does not perceive changes in the concentration of the sodium ion (i.e., it does not operate as a "sodium electrode"). Rather, it appears that what is detected is a translocation from the steady state of some property of the ECF volume.

A widely held view is that the derivative of ECF that is monitored is the "effective arterial blood volume" and that receptors, possibly sensitive to pressure or volume, are distributed diffusely throughout small subdivisions of the vascular tree. If detector elements do exist throughout the body, then it becomes essential that there be an integrator element, presumably located in the central nervous system and having the ability to collate the messages arising from the multiple detector components. The basis for this becomes evident when one examines the potential impact on a sodium control system imposed by the sequestration of extracellular fluid in a localized area of the body. If, for example, there is localized edema formation in one limb, the detector elements in that limb would see an expanded ECF volume, which would necessitate an increase in the rate of sodium excretion by the kidney. However, the effective ECF volume in the rest of the body would be diminished by virtue of the loss of fluid into the affected limb, and detector elements in the nonaffected areas would see a decrease in ECF volume, which would be interpreted as a need to decrease the rate of sodium excretion. An integrator element would analyze the various stimuli arising from all of the detector elements throughout the
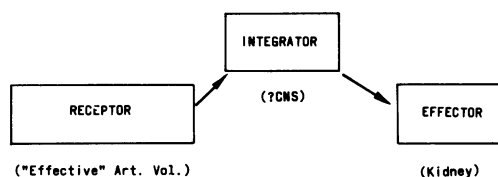
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FIG. 1. Biologic control system for the regulation of external sodium balance.

body and would then establish the nature of the information to be transmitted to the kidney, the end-organ in the system.

In Fig. 1, an arrow is shown leading from the integrator to the effector element. The nature of this arrow is the subject of intense interest, scrutiny, and investigation throughout the world. It is clear that aldosterone exercises an important influence over the capacity of the nephron to transport sodium. It also is clear that the "physical factors" within the renal parenchyma, including the peritubular capillary oncotic pressure and peritubular capillary hydrostatic pressure, influence net sodium reabsorption in the proximal tubule. An increase in the former will serve to increase net sodium reabsorption; an increase in the latter will serve to decrease net sodium reabsorption. Both forces appear to work through influencing the "back leak" of fluid from the peritubular capillary surface of the epithelial cells through intercellular channels into the tubular fluid. It appears, however, that even marked changes in proximal tubular sodium reabsorption have little influence on the ultimate delivery of sodium into the urine (1–6). Thus, the influence of physical factors on the day-to-day regulation of sodium excretion remains to be determined. Finally, changes in glomerular filtration rate do not provide an adequate explanation for the modulation of sodium excretion.

The precision with which sodium excretion is regulated in health has been responsible for the view that at least one additional factor (over and above changes in aldosterone activity, physical factors, and GFR) must be involved in the effector end of the control system. Current attention is being directed to the possibility that this factor is hormonal in nature, and that its principal effect is to inhibit tubular sodium reabsorption, thereby increasing the rate of sodium excretion. Most investigators have chosen to focus their search for the so-called "natriuretic hormone" on normal animals or man subjected to varying degrees of acute and chronic ECF volume expansion.

The work in our laboratories in search of the same hormone was initiated several years ago using uremic animals and man, and the rationale for this approach will be sketched briefly. The change in the biology of sodium excretion imposed by any renal disease that destroys nephrons is predictable and progressive. With normal renal function and a given intake of salt, excretion of sodium is shared by approximately two million nephrons. After the onset of a progressive form of chronic renal disease, if salt intake remains unchanged, the rate of excretion of sodium must fall below the rate of acquisition each time GFR falls, thus, transient sodium retention will ensue and will persist until the excretory contribution of the surviving nephrons is readjusted upward. In Fig. 2, the change in the rate of excretion of sodium per nephron (expressed as fractional sodium excretion or FE_{Na}) throughout the natural history of chronic renal disease is shown schematically. FE_{Na} rises each time GFR falls. Also shown in the figure is the fact that the increment in FE_{Na} must relate to a concomitant and parallel rise in "natriuretic activity." If the natriuretic forces include a natriuretic hormone, then the concentration of this hormone in the
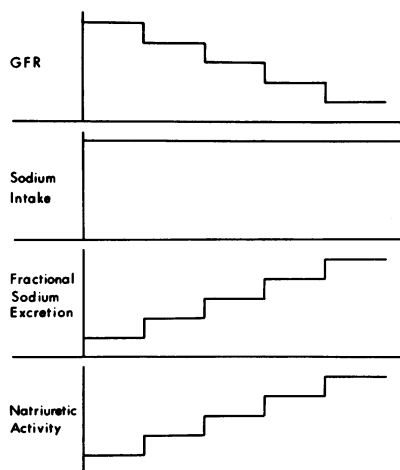
FIG. 2. Effects of reduction of glomerular filtration rate (GFR) at constant sodium intake on fractional sodium excretion and natriuretic activity. (Courtesy of *Excerpta Med.*, Amsterdam.)

blood may well increase with each permanent reduction in GFR, and in the advanced stages of chronic renal disease, the levels of the hormone in the circulation could exceed greatly those achieved in normal subjects or animals subjected even to heroic degrees of volume expansion. It is this line of reasoning that provided the theoretical basis for the use of patients and animals with chronic uremia in the search for a natriuretic hormone. Implicit in the approach is the view that if a natriuretic hormone can be identified in biologic fluids of uremic subjects, it must also exist, albeit in considerably lower concentrations, in normal subjects.

The first bioassay system that we employed was an indirect one, namely, the uptake of para-aminohippurate (PAH) by rabbit and rat kidney cortical slides (7). There was previous evidence that uremic serum inhibited the uptake of PAH by kidney slices (8,9), and because the transport of PAH *in vitro* appears to be linked to sodium transport, we attempted to isolate the fraction of uremic serum that contained the PAH inhibitor. In Fig. 3, an elution pattern obtained by filtering
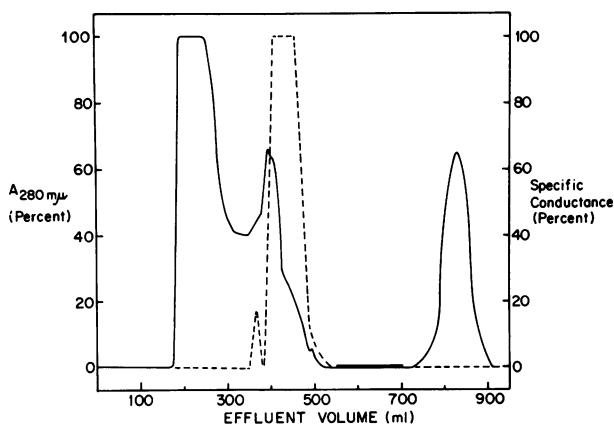

FIG. 3. Elution pattern of human serum obtained with a 10-mM ammonium acetate solution on a column packed with Sephadex G-25. The absorbance at 280 mμ (left abscissa) and the specific conductance (right abscissa) are indicated by solid and broken lines, respectively. The fraction routinely used for assay is depicted by the heavy line. (Courtesy of *J. Clin. Invest.*)

uremic serum through Sephadex G-25 and using 0.01 M ammonium acetate as the buffer is shown. The effluent solution was characterized by monitoring both the UV absorption at 280 nm and the specific conductance of the successive fractions. The inhibitor of PAH uptake was contained in a fraction of eluate that appeared immediately after the major peak of sodium, urea, and creatinine. The same gel filtration fraction from the serum of normal individuals failed to inhibit PAH uptake (10).

For many reasons, the PAH system was deemed too imprecise to serve as a definitive bioassay, and thus, efforts were made to develop a more specific bioassay system. Two such systems were developed. The first employs the isolated frog skin or urinary bladder of the toad. Both of these anuran membranes transport sodium transepithelially, and the net rate of sodium transport can be quantitated simply by measuring the short-circuit current. The second bioassay system involves the measurement of sodium excretion by the rat kidney. In the anuran membrane, the effects on short-circuit current of the addition of a test fraction to the Ringer's solution bathing the blood side of the membrane are measured. In the rat, the effects of the infusion of the test fraction intravenously on the rate of sodium excretion are determined.

In Fig. 4, a representative experiment is shown in which the gel filtration fraction from a uremic patient is added to the inside (i.e., blood) surface of an isolated frog skin. During the equilibration period, both the short-circuit current (SCC) and the transepithelial potential difference (P.D.) were stable. After addition of the fraction, the SCC and the P.D. began to fall, and the decrements continued until the Ringer's solution containing the plasma fraction was removed and replaced by fresh Ringer's. With the removal of the inhibitory fraction, both SCC and P.D. increased towards their control levels. The same gel filtration fraction from normal serum had no significant inhibitory effect on sodium transport by either the toad bladder or the frog skin (11).

A representative experiment using the unanesthetized rat subjected to a reduction of renal mass of approximately 75% is shown in Fig. 5. After several control clearance periods, during which GFR and absolute and fractional sodium excretion were measured, 1 ml of the gel filtration fraction shown in Fig. 3 and obtained from the serum of a uremic patient was infused intravenously over a 10-min interval. This quantity of fraction is the equivalent of the amount of natriuretic material contained
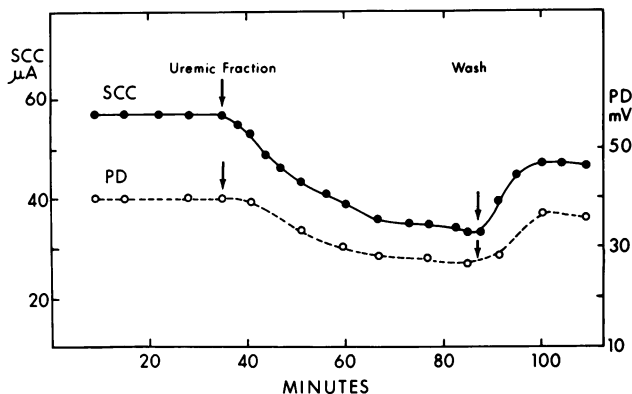

FIG. 4. Effects of the Serum fraction from a patient with chronic uremia on short-circuit current (SCC) and potential difference (PD) across the isolated frog skin (Courtesy of *J. Clin. Invest.*).
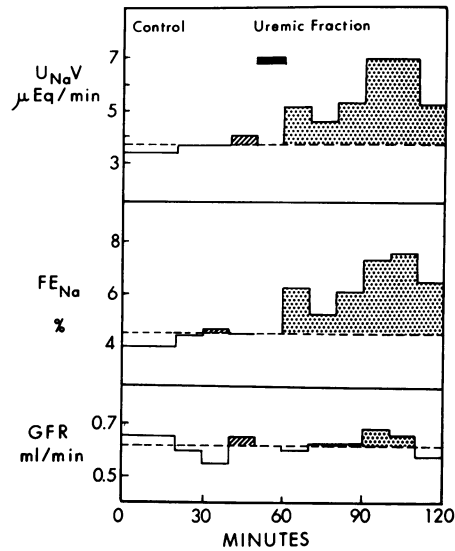
FIG. 5. Effects of the serum fraction from a patient with chronic uremia on absolute sodium excretion ($U_{Na}V$), fractional sodium excretion (FE_{Na}), and glomerular filtration rate (GFR) in the assay rat. The dotted line indicates the mean base-line control values observed before infusion of the fraction. The solid lines depict the absolute values for each clearance period before and after infusion of the fraction.

in 10 ml of original serum, assuming no loss in any of the preparative procedures. GFR and sodium excretion then were followed over the ensuing 60-min interval. There was no change in GFR, nor have any consistent changes been observed in renal plasma flow, filtration fraction, or blood pressure. However, both absolute and fractional sodium excretion increased, peaking approximately 40 min after injection of the fraction. Values then fell back toward the control level. With the identical protocol, the same gel filtration fraction from normal individuals on an average salt intake produced no significant change in either absolute or fractional sodium excretion rates (12).

The demonstration of a natriuretic factor in the serum fraction of chronically uremic patients, and subsequently of chronically uremic dogs (13), coupled with the inability to detect the presence of the factor in normal subjects and dogs is consistent with the thesis that the active material could play a physiologic role in modulating sodium excretion in uremia. However, the possibility that the factor is a nonphysiologic "toxin" retained in the blood due to failure of excretion could not be excluded. Consequently, studies were undertaken using urine of chronically uremic patients and dogs and employing the same gel filtration technique employed for serum. Fig. 6 compares the effects of the fraction from uremic patients and normal subjects on sodium transport by the toad bladder. With the normal fractions there was no inhibition of short-circuit current; indeed, slight stimulation was observed. In contrast, the uremic fractions produced inhibition of SCC, which was apparent within 10 min of addition of the fraction and continued over a 60-min period of observation (14). Similar data have recently been obtained by using the fraction derived from the urine of chronically uremic dogs (15). The urine fraction from uremic patients and dogs was also found to be natriuretic in the rat (15, 16). Thus, not only is there increased activity of the natriuretic factor in uremic serum, but the factor also is excreted in increased quantity in the urine of patients and dogs with low GFRs. The increased excretion rate precludes the possibility that the retention
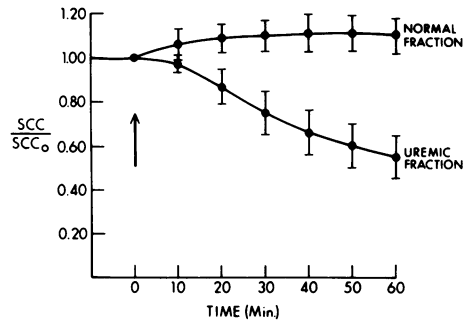
FIG. 6. Mean changes, ± SE, in short-circuit current (SCC) across the isolated toad bladder during exposure to urine fractions from normal subjects and patients with chronic uremia.

of the factor in the blood is due to failure of its excretion. Rather, the data require either that the factor is produced in increased quantity in uremia and/or that its biologic half-life is prolonged.

The degree of natriuresis produced by the factor in the rat was next shown to be a function of the amount of material administered. Fig. 7 depicts a dose–response curve. The initial natriuresis occurred in response to the administration of the quantity of natriuretic factor harvested from a 2-hr sample of urine. Sodium excretion increased by approximately 2 μequiv/min. After 1 hr, a tenfold concentrate of the same material was given intravenously. As can be seen in Fig. 7, the natriuretic response was striking. Similar changes occur in fractional sodium excretion (16); moreover, dose–response relationships also have been observed using serum fractions.

Quite recently, studies have been performed using the isolated perfused cortical collecting tubule of the rabbit. When the natriuretic factor was added to the solution bathing the peritubular capillary surface of the isolated tubule, there occurred a highly significant decrease in intraluminal negativity. When the factor was added to the perfusate so as to expose the luminal surface of the tubule to its effects, no change in P.D. was observed. The same fraction from normal subjects produced no effects when added to either the luminal or contraluminal solution. Finally, the decrease in intraluminal negativity has been associated with inhibition of net sodium
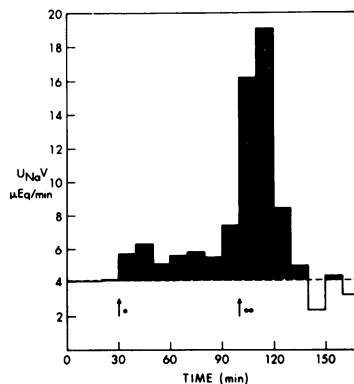

FIG. 7. Dose-response experiment in the assay rat with the urine fraction from a uremic patient. The first arrow (*) represents the injection of the vector harvested from a 2-hr urine sample, the second arrow (**) represents the injection of a tenfold concentrate of the same material.

reabsorption due to a decrease in efflux (i.e., transport from lumen to bath), with no effect on the leak of sodium back into the tubular fluid from the peritubular surface (17).

The data thus far indicate that a natriuretic factor exists in the serum and urine of chronically uremic patients and dogs with high rates of fractional sodium excretion, that the same factor cannot be detected in nonuremic patients and dogs with low rates of fractional sodium excretion with the available bioassay techniques, and that the rate of production of the factor and/or its biologic half-life is increased in uremia.

The next step in seeking the answer as to whether or not this factor is a component part of the sodium control system involved examining its relationship to the concurrent patterns of sodium excretion. Still restricting the search to chronic uremia, two sets of data were obtained in support of such a relationship. The first was made possible by the availability of a group of patients with far advanced chronic uremia (mean GFR approximately 4 ml/min) who did not exhibit the high rates of sodium excretion per nephron seen in the typical patient with chronic uremia. These patients were not only uremic but nephrotic. Thus, despite their low GFRs, they had high rates of protein excretion, hypoalbuminemia, and marked edema formation. Indeed several of the patients had virtually no sodium in their urine. Gel filtration fractions of serum and/or urine were examined in the subjects, and the results are shown in Fig. 8. The typical response of the natriuretic uremic patient is included for comparison. Despite all of the chemical stigmata of chronic uremia, the "natriuretic" fraction of serum or urine from the nephrotic patients produced no increase in either absolute or fractional sodium excretion in the rat (16).

The second experiment designed to test the relationship between the natriuretic factor and sodium excretion was performed in two groups of dogs in which renal mass was reduced in sequential steps so as to decrease GFR from normal values to values of approximately 5–10 ml/min (13). One group of animals was maintained on a constant sodium intake of 120 mequiv/day. In the other group, sodium intake was initially set at 120 mequiv/day; but each time renal mass was reduced, sodium intake was decreased in exact proportion to the measured fall in GFR. By this technique, which we have termed "proportional reduction of solute," or PRS_{Na}, the rise in fractional sodium excretion that typically attends the fall in GFR in advancing renal disease is obviated, and even at very low levels of GFR, values for FE_{Na} remain at less than 0.5%. The comparison of the bioassay results using the serum fractions
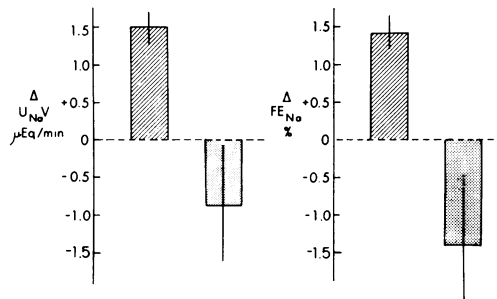

FIG. 8. Mean changes, ± SE, in absolute sodium excretion ($\triangle U_{Na}V$) and fractional sodium excretion ($\triangle FE_{Na}$) produced by serum fractions from patients with chronic uremia in the absence (left bars) and in the presence (right bars) of the nephrotic syndrome (Courtesy of J. Clin. Invest.).
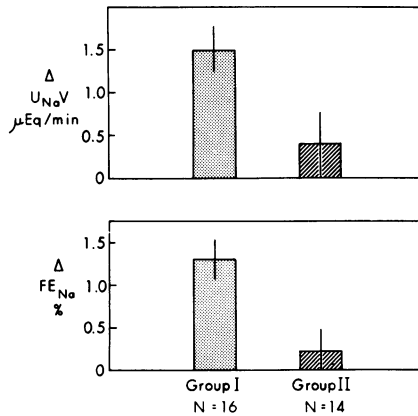
FIG. 9. Mean changes, $\pm$ SE, in sodium excretion produced by the serum fraction from two groups of uremic dogs with different salt intakes. Group I was maintained on a constant salt intake of 120 mequiv/day. Group II was subjected to the PRS $_{Na}$ regimen (see text).

from the two groups of dogs is shown in Fig. 9. In the animals maintained on the constant salt intake, a significant natriuretic response was induced in the bioassay rats. In the animals maintained on the PRS $_{Na}$ regimen, no significant natriuresis was observed; and the difference between the constant salt intake group and the PRS $_{Na}$ group was statistically significant.

The foregoing experiments added one additional link to the chain of evidence supporting a physiologic role for the natriuretic factor. However, the onus of working with uremic serum and urine fractions remained, and the possibility still could not rigorously be excluded that the material was either unique to the uremic state or a "toxin" with some fortuitous relationship to the dictates of sodium excretion. The next essential step, therefore, was to demonstrate the presence of the natriuretic factor in normal dogs. Efforts have been made to accomplish this goal.

The studies had the following design. Four groups of animals were studied. All received a synthetic semiliquid diet that was essentially salt-free. To this diet a predetermined amount of sodium was added each day. The first group received a total of 3 mequiv/day of Na, the second 91 mequiv/day, and the third and fourth 258 mequiv/day. In the first and fourth groups, a supermaximal dose of a potent mineralocorticoid hormone (0.2 mg of fludrocortisone a day) was added to the food. The animals were maintained in metabolic cages on their respective regimens for 4 days, and on the morning of the fifth day, clearance studies were performed and urine was collected for a 5-hr period for purposes of assaying for natriuretic factor. The results of sodium balance data on these groups are shown in Fig. 10. In Group I, negative sodium balance ensued over the 4-day interval, averaging −51 mequiv. In Group II, there was a modest degree of sodium retention over the 4 days. In Group III, positive sodium balance averaged 148 mequiv. Finally, in Group IV, positive sodium balance averaged 246 mequiv. It is of interest that all of the dogs in the latter group "escaped" from the effects of the mineralocorticoid hormone prior to collection of urine for bioassay. The results of the bioassays in the rat are shown in Fig. 11. In the animals on the low salt diet and in the animals on the 91-mequiv/day sodium intake, no significant natriuresis was observed in the bioassay tests. In the groups receiving 258 mequiv/day of sodium without or with mineralocorticoid hormone, a significant natriuresis was induced. Similar results were obtained using the toad bladder assay (18).
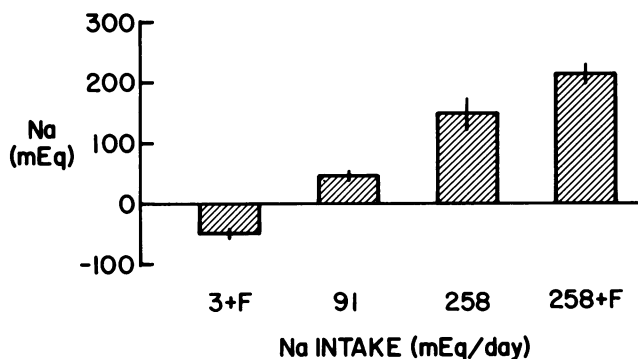
FIG. 10. Four-day cumulative sodium balance on ordinate in four groups of dogs with normal GFR, maintained on 3, 91, or 258 mequiv/day of sodium. Animals from Groups I and IV received 0.2 mg/day of fludrocortisone (F). The balance data represent the difference between oral intake and urinary excretion.

Intensive efforts are currently in progress to determine the chemical nature of the natriuretic factor. Isolation procedures have been employed on the gel filtration fraction containing natriuretic activity from both normal and uremic urine. The techniques include ion exchange chromatography, descending paper chromatography, and amino acid analysis. When the active Sephadex G-25 fractions have been subjected to ion exchange chromatography on Dowex 50, biologic activity consistently has been recovered in a single fraction that contains no free amino acids. However, after acid hydrolysis, seven amino acids, asp, thr, ser, glu, gly, ala, and leu or val, have been identified repeatedly. No basic amino acids have been found. Paper chromatography analysis of the Dowex 50 biologically active fraction has revealed several UV absorbing spots and chlorine-0-tolidine staining bands but no ninhydrin positive bands. The results to date suggest that the natriuretic factor obtained from both uremic and normal urine is the same substance and that it appears to be acidic,
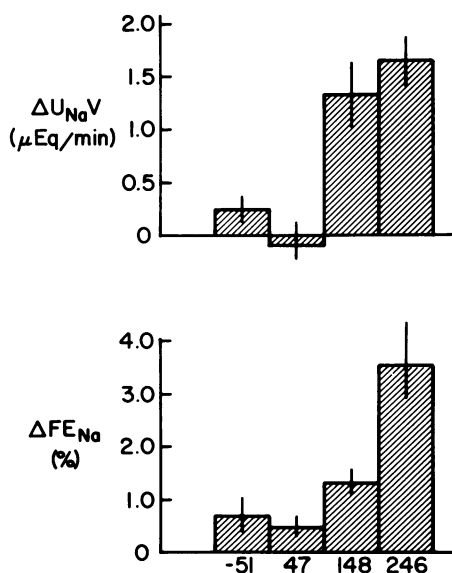

FIG. 11. Mean changes, ± SE, in sodium excretion in rats produced by the urine fraction from dogs with normal GFRs. (See text).

nonvolatile, of low molecular weight, and possibly peptidic in nature. The biologic activity appears at a nitrogen concentration of 2 μg/ml of the active Dowex fraction (19).

In summary, studies have been undertaken to determine whether a natriuretic hormone exists in nature and, if so, what its role is in the modulation of sodium excretion. The search to date indicates that there is a low molecular-weight substance in serum and urine of uremic patients exhibiting a high rate of fractional sodium excretion and that a natriuretic substance, presumably the same as that found in uremia, is present in dogs with substantial ECF volume expansion induced by the chronic administration of a high salt diet and a potent mineralocorticoid hormone. The presence of the factor thus appears to correlate with the dictates of sodium excretion and/or the concurrent patterns of sodium excretion. Attempts to isolate and characterize the nature of the active molecule are in progress, and available evidence suggests that it is a peptide that is unrelated to vasopressin, oxytocin, angiotensin, and parathyroid hormone.

Future studies will determine whether this natriuretic factor is a key modulator of the rate of sodium excretion in health and in diseases associated with increased rates of sodium excretion per nephron. Future studies also will determine whether a decrease in the activity of this substance is a concomitant of certain sodium-retaining states.

ACKNOWLEDGMENTS

REFERENCES

1. Howards, S. S., Davis, B. B., Knox, G. F., Wright, F. S., and Berliner R. W., Depression of fractional sodium reabsorption by the proximal tubule of the dog without sodium diuresis. *J. Clin. Invest.* **47**; 1561, 1968.
2. Davidman, M., Alexander, E., Lalone, R., and Levinsky, N., Nephron function during volume expansion in the rat. *Amer. J. Physiol.* **223**; 188, 1972.
3. Sonnenberg, H., Proximal and distal tubular function in salt-deprived and in salt-loaded de-oxycorticosterone acetate-escaped rats. *J. Clin. Invest.* **52**; 263, 1973.
4. Stein, J. H., Osgood, R. W., Boonjarern, S., and Ferris, T. F., A comparison of the segmental analysis of sodium reabsorption during Ringer's and hyperoncotic albumin infusion in the rat. *J. Clin. Invest.* **52**; 2513, 1973.
5. Knox, F. G., Role of the proximal tubule in the regulation of urinary sodium excretion. *Mayo Clin. Proc.* **48**; 565, 1973.
6. Stein, J. H., Osgood, R. W., Boonjarern, S., Cox, J. W., and Ferris, T. F., Segmental sodium reabsorption in rats with mild and severe volume depletion. *Amer. J. Physiol.* **227**; 351, 1974.
7. Bricker, N. S., Klahr, S., Purkerson, M. L., and Schultze, R. G., An *in vitro* assay system for a humoral substance present in plasma and serum during extracellular fluid volume expansion and uremia. *Nature* **218**; 1058, 1968.
8. Preuss, H. G., Massry, S. E., Maher, J. F., Gilliece, M., and Schreiner, G. E., Effects of uremic sera on renal tubular p-aminohippurate transport. *Nephron* **3**; 265, 1966.
9. Hook, J. B., and Munro, J. R., Specificity of the inhibitory effect of "uremic" serum on p-aminohippurate transport. *Proc. Soc. Exp. Biol. Med.* **127**; 289, 1968.
10. Klahr, S., Bourgoignie, J., Miller, C. L., Lubowitz, H., and Bricker, N. S., Studies in search of a natriuretic hormone in uremic patients. *In* "Proceedings of the 4th International Congress of Nephrology, Stockholm, 1969," Vol. 2, p. 88, Karger; Basel, Munchen, New York.
11. Bourgoignie, J., Klahr, S., and Bricker, N. S., Inhibition of transepithelial sodium transport in the frog skin by a low molecular weight factor of uremic serum. *J. Clin. Invest.* **50**; 303, 1971.

12. Bourgoignie, J. J., Hwang, K. H., Espinel, C., Klahr, S., and Bricker, N. S., A natriuretic factor in the serum of patients with chronic uremia. *J. Clin. Invest.* **51**; 1514, 1972.

13. Schmidt, R. W., Bourgoignie, J. J., and Bricker, N. S., On the adaptation in sodium excretion in chronic uremia: The effects of "Proportional Reduction" of sodium intake. *J. Clin. Invest.* **53**; 1736, 1974.

14. Kaplan, M. A., Bourgoignie, J. J., Rosecan, J., and Bricker, N. S., The effects of the natriuretic factor from uremic urine on sodium transport, water and electrolyte content, and pyruvate oxidation by the isolated toad bladder. *J. Clin. Invest.* **53**; 1568, 1974.

15. Bourgoignie, J., Unpublished observations.

16. Bourgoignie, J. J., Hwang, K. H., Ipakchi, E., and Bricker, N. S., The presence of a natriuretic factor in urine of patients with chronic uremia: The absence of the factor in nephrotic uremic patients. *J. Clin. Invest.* **53**; 1559, 1974.

17. Fine, L., Bourgoignie, J. J., and Bricker, N. S., The influence of the natriuretic factor from uremic patients on bioelectric properties and sodium transport of the isolated mammalian collecting tubule. *Clin. Res.* **23**; 430A, 1975.

18. Favre, H., Hwang, H. K., Schmidt, R. W., Bricker, N. S., and Bourgoignie, J. J. An inhibitor of sodium transport in the urine of dogs with normal renal function. *J. Clin. Invest.*, in press.

19. Bourgoignie, J. J., Kaplan, M., Eun, C., Favre, H., Hwang, K., Blumenfeld, O., and Bricker, N. S., On the characterization of natriuretic factor. *Clin. Res.* **23**; 429A, 1975.