PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 765–773 (1975)

Effect of Blood Volume Expansion on Tubule Sodium Transport in the Isolated Dog Kidney[1] (38627)

GEORGE J. KALOYANIDES, GERALD F. DIBONA, AND ROBERT D. BASTRON

Renal-Hypertension-Electrolyte Division, Department of Internal Medicine and Department of Anesthesiology, University of Iowa College of Medicine and the Medicine and Research and Education Services, Veterans Administration Hospital, Iowa City, Iowa 52242

For the past several years we have been examining the effector mechanisms of volume expansion natriuresis in an isolated perfused dog kidney preparation in which it is possible to isolate the influence of various factors (neurogenic, hemodynamic, humoral and compositional) thought to be important determinants of the natriuretic response. In this model extracellular volume expansion with saline leads to natriuresis associated with significantly decreased proximal tubule fractional and absolute sodium reabsorption (1) similar to that described in the intact dog kidney (2–4). Preferential intravascular expansion with equilibrated blood also elicits a natriuresis in this model (5) similar in magnitude to that seen after saline loading (1). However, in contrast to saline loading, the natriuretic response to infusing equilibrated blood cannot be satisfactorily explained by changes in peritubular capillary forces as estimated from changes in plasma colloid osmotic pressure and renal hemodynamics. This observation raises the possibility that the resulting increase in urinary sodium excretion in the two types of volume expansion might involve different effector mechanisms, and, consequently, might reflect different patterns of segmental tubular sodium transport.

The present experiments were undertaken in an attempt to define where along the nephron sodium transport is altered in the isolated perfused dog kidney following volume expansion with equilibrated blood and to evaluate the possible mechanisms mediating the response.

Methods. Experiments were performed on mongrel dogs weighing 15–30 kg. One dog served as the kidney donor; the second dog was used to perfuse the isolated kidney. All animals were fed a standard kennel ration which was supplemented with 150 mEq of NaCl and 80 mEq of KCl a day for the perfusion animals. In addition, the perfusion dog was pretreated with 15 mg deoxycorticosterone acetate in oil administered intramuscularly each day for at least 14 days including the morning of the study. On the morning of the study the animals were anesthetized with sodium pentobarbital, 30 mg/kg, given intravenously with supplemental doses as required to maintain light anesthesia. An endotracheal tube was inserted and the animals were ventilated with a Harvard respirator adjusted to maintain the arterial pH between 7.35 and 7.45.

Preparation of the isolated kidney was similar to that previously described (5). The reservoir was filled with 5% albumin in 0.9% saline to a volume equal to 35 ml/kg plus 200 ml. The latter volume represents the basal volume maintained in the reservoir following volume expansion. After perfusion of the isolated kidney was established, the perfusion animal received a priming dose of inulin followed by a constant infusion of inulin in 0.9% saline calculated to achieve a plasma inulin concentration of 100 mg/100 ml. Aqueous Pitressin was added to the infusion to deliver 0.5 munits/kg per min. A minimum of 60 min was allowed for equilibration of the perfusion animal's blood with the fluid in the reservoir and for stabilization of renal function as evidenced by constant urine and blood flow.

Group I consisted of 13 experiments. Following the collection of two 15-min control urine samples, the dog was expanded with 35 ml/kg of equilibrated blood from the reser-

[1] This work was supported in part by USPHS research grant Nos. HL 13765, AM 15843 and Program Project HL 014388 and grants from the Iowa Heart Association.

voir over a 30-min period. The volume expansion stimulus was maintained by replacing urine losses with hypotonic saline (120 mEq/liter). Sixty min after initiating volume expansion, two 15-min experimental urine samples were collected. Renal arterial pressure (P_{RA}) was maintained constant throughout the study at approximately 105 mmHg; renal venous pressure was maintained at 0 mmHg.

Group II consisted of 10 experiments identical to group I except the perfusion dog was not volume expanded between the control and experimental periods.

During the control and experimental periods single nephron glomerular filtration rate (SNGFR), fractional and absolute reabsorption rate of superficial end-proximal tubules were measured using micropuncture techniques previously reported and validated for the isolated dog kidney (1). Because of technical problems related to performing recollection micropuncture in a heparinized kidney, in each period tubular fluid was collected from 5 or 6 previously unpunctured nephrons and the individual values were averaged to give a mean value for that period.

Distribution of cortical blood flow was determined using the radiolabeled microsphere technique. Microspheres of approximately 15-mμ diameter and labeled with either ^{85}Sr or ^{141}Ce were injected into the renal arterial catheter at the end of the control and experimental periods. The techniques used for slicing the kidney, assaying the radioactivity and calculating the data were identical to those previously reported (6).

Blood pressure monitoring and recording, urine collection and blood sampling were carried out as previously described (5). All blood and urine samples were analyzed for sodium and inulin; packed cell volume (PCV) and plasma protein concentration were determined on all blood samples. Sodium was measured with an Instrumentation Laboratories flame photometer. Inulin was measured by the method of Fuhr, Kaczmarczyk and Kruttgen (7) and plasma protein concentration by refractometry. PCV was determined using a microhematocrit centrifuge. RBF was measured directly by timing the flow from the renal vein into a graduated cylinder. Filtration fraction (FF) was determined from the formula $FF = C_{IN}/RPF$ where C_{IN} is the inulin clearance and RPF is the renal plasma flow calculated according to the formula RPF = RBF (1–0.95 PCV). Tubule fluid sample volume was measured with a calibrated micropipette; tubular fluid inulin concentration was measured in duplicate by means of a fluorometric method (8). SNGFR was calculated according to the formula $SNGFR = V_{TF} \times (TF/P)_{IN}$ where V_{TF} is the tubular fluid flow rate in nl/min and $(TF/P)_{IN}$ is the tubular fluid to plasma inulin ratio. Proximal tubule fractional reabsorption (FR) was determined according to the formula $FR = 1\text{-}(P/TF) \times 100$; proximal tubule absolute reabsorption (AR) was determined according to the formula $AR = SNGFR\text{-}V_{TF}$.

The data in the text are expressed as the mean ± 1 SE. Student's t test was used for statistical analysis of paired data within each group (9).

Results. Figure 1 summarizes the sodium excretion data from the volume expansion and control experiments. Similar to our previous study (5) we observed a significant rise in absolute and fractional sodium excretion in the isolated kidney following volume expansion of the perfusion dog with equilibrated blood whereas in the absence of volume expansion (control) these variables did not change. The increase in sodium excretion occurred in the face of a significant decrease in C_{IN} and RBF (Fig. 2) which tended to be proportional as indicated by the fact that the slight rise in filtration fraction (0.19 ± 0.01–0.23) was not statistically significant ($P > 0.2$). In the control group only C_{IN} decreased significantly. P_{RA} remained constant in both groups. Plasma protein concentration and PCV remained constant in the volume expansion experiments. Plasma protein concentration measured 4.9 ± 0.2 g/100 ml and PCV measured 31 ± 1 during both periods. In the control group plasma protein concentration decreased from 4.3 ± 0.1 to 4.1 ± 0.1 g/100 ml ($P < 0.025$) and PCV from 31 ± 1 to 30 ± 1 ($P < 0.05$).

Following volume expansion a slight decrease in end-proximal $(TF/P)_{IN}$ was detected (Fig. 3) and indicates that proximal

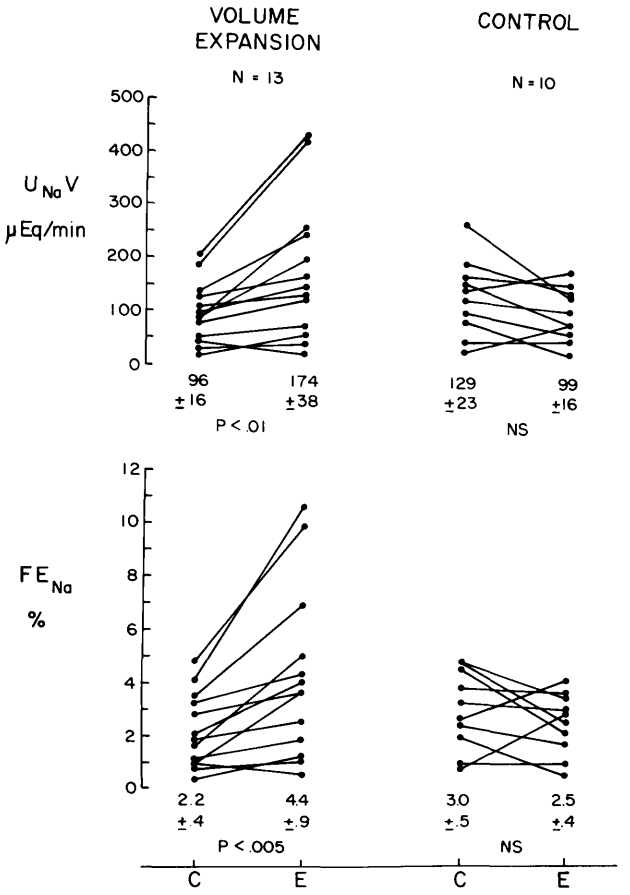

FIG. 1. Plot of absolute ($U_{Na}V$) and fractional sodium excretion (FE_{Na}) of individual experiments for the control (C) and experimental (E) periods of the volume expansion (Group I) and control groups (Group II). Numbers are mean ± SE for each period.

tubule fractional reabsorption declined from 38.5 ± 1.3 to 34.2 ± 1.7%, $P < 0.025$. No significant change occurred in the absence of volume expansion. SNGFR changed in a variable manner in both groups but there was no significant change in the mean in either group. No consistent change in the distribution of glomerular filtrate, as estimated from the ratio of $SNGFR/C_{IN} \times 10^{-6}$, was detected in either group. $SNGFR/C_{IN} \times 10^{-6}$ was 3.1 ± 0.2 and 3.3 ± 0.2 for the control and experimental periods of the volume expansion group ($P > 0.2$) and 2.9 ± 0.1 and 2.9 ± 0.2 for the same periods of the control group.

Proximal tubule absolute reabsorption ($SNGFR-V_{TF}$) decreased significantly in the volume expansion group but did not change in the control group (Fig. 4). End-proximal tubular fluid flow rate (V_{TF}) changed in a variable manner in individual experiments of both groups, but there was no change in the mean value of either group. Thus, although proximal tubule fractional and absolute reabsorption rates decreased significantly during volume expansion, these changes were balanced by the slight decrease in SNGFR so that there was no change in the delivery of filtrate out of superficial end-proximal tubules.

Measurement of fractional distribution of renal cortical blood flow using the radiolabeled microsphere technique revealed no significant differences between the control and experimental periods of either group (Table I) indicating that the decrease in ab-

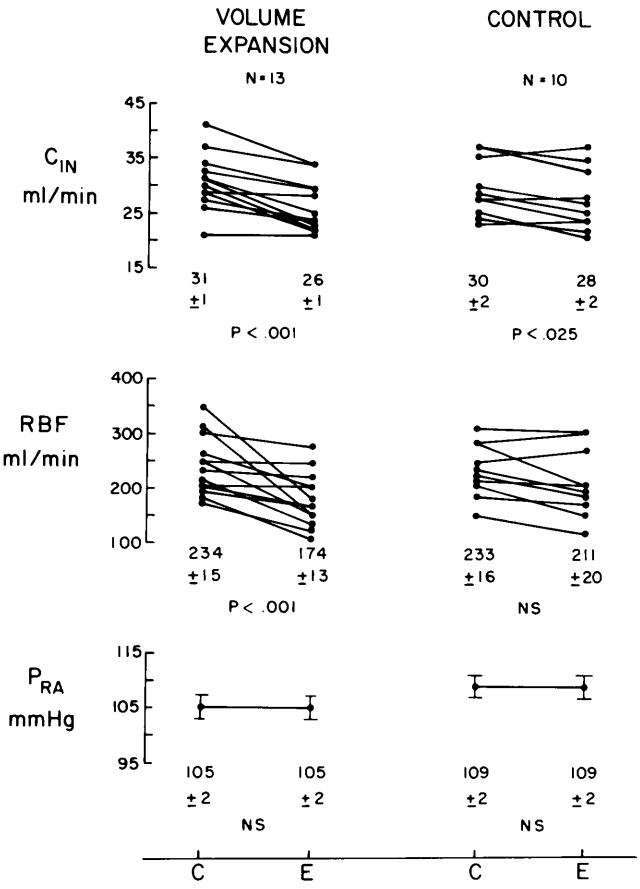

FIG. 2. Plot of inulin clearance (C_{IN}), renal blood flow (RBF) and renal arterial pressure (P_{RA}) for the control (C) and experimental (E) periods of the volume expansion (Group I) and control groups (Group II). Individual experiments are plotted for C_{IN} and RBF. Mean ± SE is plotted for P_{RA}. Numbers represent mean ± SE for each period.

solute RBF was shared proportionately by the four cortical zones.

Discussion. The clearance data from these experiments confirm the results of our previous study (5), namely that volume expansion of a DOCA-loaded dog with equilibrated blood results in a significant natriuresis in an isolated kidney perfused with blood from the femoral artery of the expanded animal. Similar to our previous experiments the natriuresis occurred in the presence of a significant decrease in GFR and RBF but in the absence of change in renal arterial pressure, PCV or plasma protein concentration.

Micropuncture techniques were applied in an attempt to define the tubular sites where sodium transport was depressed. A significant fall in proximal tubule $(TF/P)_{IN}$ was observed following volume expansion with equilibrated blood (Group I) whereas no change was observed in group II experiments in the absence of volume expansion. This finding is in agreement with that of Knox *et al.* (10) who also reported a fall in proximal tubule fractional sodium reabsorption in the dog following infusion of equilibrated blood. In addition to the fall in fractional reabsorption, we detected a slight but significant decrease in absolute sodium reabsorption along the proximal tubule.

It is of interest that the depression of proximal tubule fractional and absolute sodium reabsorption observed following volume expansion with equilibrated blood was significantly less than that seen following volume expansion with saline in this prepara-

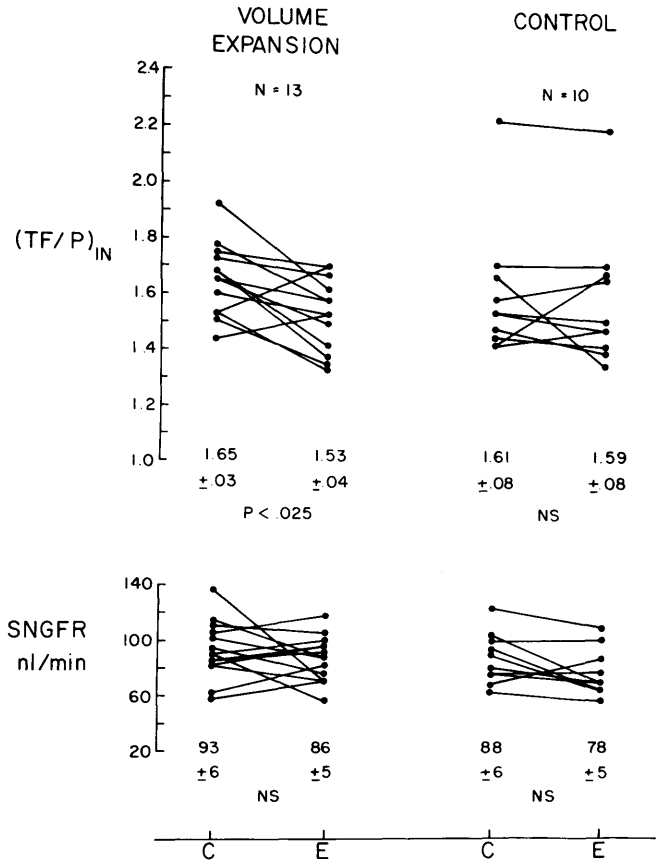

FIG. 3. Plot of tubular fluid to plasma inulin ratio ($(TF/P)_{IN}$) and single nephron GFR (SNGFR) of superficial end-proximal tubules of individual experiments for the control (C) and experimental (E) periods of the volume expansion (Group I) and control groups (Group II). Numbers represent mean ± SE for each period.

tion (1). In response to saline loading proximal tubule fractional sodium reabsorption declined from 51 ± 3 to 39 ± 3% ($P < 0.001$) and absolute reabsorption from 50.9 ± 3.4 to 40.4 ± 4.0 nl/min ($P < 0.005$). These changes were accompanied by an increase in filtrate delivered out of superficial end proximal tubules of 17 ± 4 nl/min ($P < 0.005$). Since urinary sodium excretion rose only 102 ± 20 μEq/min ($P < 0.01$) it is evident that a large fraction of the increment in distal sodium delivery must have been reabsorbed by distal nephron segments. In contrast to the saline loading experiments, volume expansion with equilibrated blood caused only a slight depression of fractional and absolute proximal tubule sodium reabsorption which, due to the slight fall in SNGFR, was not associated with a detectable increase in distal sodium delivery. Nevertheless, the increase in urinary sodium excretion following volume expansion with equilibrated blood was not significantly different from that observed in response to saline loading ($\Delta U_{Na}V$ equilibrated blood = 79 ± 24 μEq/min, $\Delta U_{Na}V$ saline = 102 ± 20 μEq/min, $P > 0.4$). These data suggest that volume expansion with equilibrated blood also depressed sodium reabsorption at some site distal to the proximal convoluted tubule.

Before accepting this interpretation, alternative explanations require consideration. Firstly, the observed changes in sodium transport of superficial proximal tubules may not be representative of changes occurring in deeper nephrons; thus, the increase in sodium excretion might reflect a

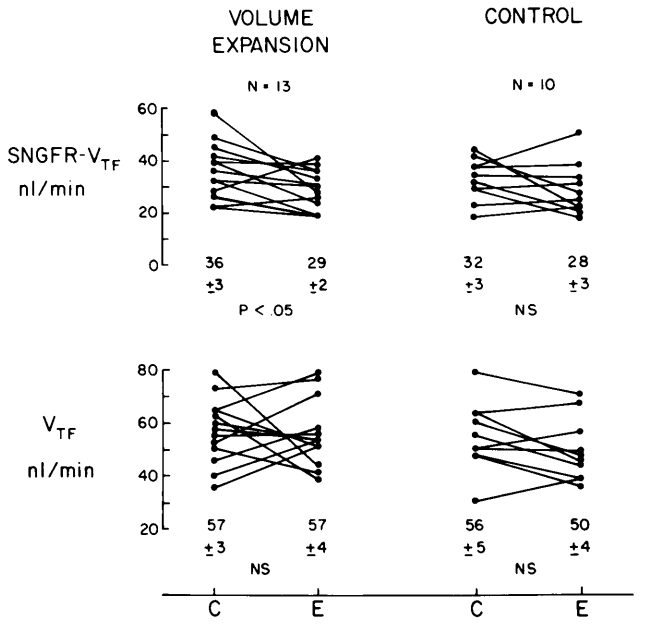

FIG. 4. Plot of proximal tubule absolute fluid reabsorption (SNGFR-V_{TF}) and tubular fluid flow rate (V_{TF}) of superficial end-proximal tubules of individual experiments for the control (C) and experimental (E) periods of the volume expansion (Group I) and control groups (Group II). Numbers represent mean ± SE for each period.

TABLE I. FRACTIONAL DISTRIBUTION OF RENAL CORTICAL BLOOD FLOW[a]

	C_1 %		C_2 %		C_3 %		C_4 %	
	C	E	C	E	C	E	C	E
Group I	30.0	29.3	27.4	27.4	24.7	25.2	17.9	18.1
N = 12	±1.5	±1.7	±0.8	±1.3	±1.3	±1.6	±1.7	±2.4
P	NS		NS		NS		NS	
Group II	31.6	29.1	26.0	24.6	23.1	24.4	19.3	21.9
N = 7	±2.0	±1.4	±0.9	±0.7	±1.6	±1.4	±2.4	±2.2
P	NS		NS		NS		NS	

[a] C_1, outermost cortex; C_4, innermost cortex; C, control period; E, experimental period. Group I, volume expansion experiments; Group II, control experiments.

depression of proximal tubule sodium reabsorption of deeper nephrons. This possibility is not subject to direct experimental testing by presently available techniques and, therefore, cannot be excluded in our experiments.

Secondly, we considered the possibility that redistribution of filtrate to deep nephrons might have augmented the delivery of sodium out of the proximal tubules of these nephrons. However, no consistent change in glomerular filtrate distribution, as estimated from the SNGFR/C_{IN} ratio, was evident in these studies. Moreover, the constancy of fractional distribution of renal cortical blood flow also argues against redistribution of filtrate as a factor contributing to the natriuresis.

Finally, it is possible that the technique of puncturing random end-proximal tubules is not sufficiently sensitive to detect small changes in proximal tubule reabsorptive and tubular fluid flow rates. Admittedly, recollection micropuncture is a more sensitive technique than puncturing random end-

proximal tubules. Nevertheless, using the latter technique we were able to detect changes in $(TF/P)_{IN}$ and tubular fluid flow rate in the isolated kidney following saline loading (1) that were similar to those observed by other investigators employing recollection micropuncture in the intact dog kidney (2–4). Moreover, since the micropuncture techniques employed in the equilibrated blood experiments were identical to those used in the saline experiments, the limitations of these methods apply to data from both groups of experiments and, consequently, do not invalidate conclusions drawn from a comparison of these data. Thus, in saline loading the natriuresis appeared to derive from a decrease in proximal tubule fractional and absolute sodium reabsorption resulting in a large increase in distal sodium load most of which was reclaimed by distal nephron segments. In the equilibrated blood expansion studies increased sodium excretion occurred in the absence of a consistent increase in distal sodium load which suggests that the natriuresis derived from a decrease in sodium reabsorption along some site distal to the proximal convoluted tubule. Given the variability of end-proximal tubule fluid flow rate measured in the present experiments, it is possible that a change of 4 nl might have escaped detection. An increase in distal delivery of this magnitude would be more than sufficient to account for the observed natriuresis. However, even under these circumstances it is evident that the capacity of the distal nephron to augment sodium reabsorption in response to a modest increase in sodium load must have been significantly impaired and that distal tubule fractional sodium reabsorption must have decreased.

These experiments emphasize that distal tubule sodium reabsorption is an important determinant of natriuresis in the isolated perfused dog kidney following volume expansion with equilibrated blood. In this respect our findings are in agreement with previous studies which implicate a regulatory role for the distal tubule in determining urinary sodium excretion of the intact kidney during volume expansion (11–17). In addition, however, our experiments suggest that a large depression of proximal tubule sodium reabsorption is not essential for natriuresis to occur with preferential intravascular volume expansion. This finding is similar to our previous observation that depression of proximal tubule sodium reabsorption is not essential for natriuresis to occur following a rise in renal arterial pressure in this model (18).

Stein *et al.* (17) recently reported that volume expansion with saline caused a greater depression in collecting duct sodium reabsorption than did volume expansion with hyperoncotic albumin. Other investigators have also reported a differential effect between extracellular volume expansion with saline and preferential intravascular volume expansion with albumin or dextran on distal tubule sodium reabsorption (12, 13, 15). The fact that we observed a greater depression of distal tubule sodium reabsorption with equilibrated blood than with saline might appear to be at variance with the above reports. However, it should be noted that the equilibrated blood experiments were performed in chronically sodium and DOCA-loaded animals in contrast to the saline experiments in which the perfusion dog received DOCA only on the morning of the study. The study of Knox *et al.* (14) also indicates that distal tubule sodium reabsorption is depressed in dogs which have escaped from the salt retaining effects of DOCA. In their experiments DOCA-escaped dogs excreted more sodium than non-DOCA-escaped animals although distal sodium delivery was not different in the two groups.

The mechanisms by which volume expansion may alter tubular sodium reabsorption have been summarized in a recent review (19). Brenner and colleagues (20) have marshalled convincing evidence that the depression of proximal tubule fractional and absolute sodium reabsorption accompanying volume expansion with colloid free solution is related to and causally mediated by the accompanying dilution of postglomerular plasma protein concentration. These authors demonstrated that raising peritubular capillary plasma colloid osmotic pressure (PCOP) largely restored proximal tubule fractional and absolute sodium reabsorption to control levels indicating that alteration in PCOP was the major if not the sole determinant of proximal tubule sodium transport. Thus, the decline in proximal tubule fractional and

absolute sodium reabsorption in our saline experiments (1) may be readily explained as a consequence of the associated fall in systemic and therefore peritubular capillary plasma protein concentration. This mechanism, however, does not satisfactorily explain the depression of proximal tubule fractional and absolute sodium reabsorption that occurred following volume expansion with equilibrated blood. In the latter experiments arterial plasma protein concentration remained constant whereas filtration fraction increased slightly. If filtration fraction of superficial nephrons changed in a manner proprotional to whole kidney filtration fraction (the fact that $SNGFR/C_{IN}$ and fractional distribution of renal blood flow remained constant suggests such was the case) then peritubular capillary plasma protein concentration would have changed in a direction favoring proximal tubule sodium reabsorption. Moreover, in view of the fact that P_{RA} was held constant and RBF fell, it seems likely that peritubular capillary hydrostatic pressure would have decreased thereby also favoring reabsorption of sodium by the proximal tubule.

As noted in our previous study (5), the fact that sodium excretion increased in the isolated kidney following volume expansion with equilibrated blood despite decreases in renal blood flow and glomerular filtration rate, in the absence of renal nerves and in the absence of change in plasma protein concentration, packed cell volume and renal perfusion pressure constitutes phenomenological evidence that a humoral mechanism may have mediated the decrease in tubular sodium reabsorption. Since $SNGFR/C_{IN}$ and fractional distribution of RBF remained constant, it is unlikely that the factor(s) promoted a natriuresis via alterations in intrarenal hemodynamics. The micropuncture data suggest that this factor may depress sodium reabsorption along the proximal tubule as well as some as yet unidentified segment of the distal nephron.

Summary. Nonrecollection end-proximal tubule micropuncture technique and the microsphere method for estimating fractional distribution of renal cortical blood flow were applied to further define the mechanism of the natriuresis in the isolated dog kidney in response to volume expansion with equilibrated blood. Following volume expansion sodium excretion increased $+79 \pm 24$ μEq/min ($P < 0.01$) in the face of significant decreases in inulin clearance (C_{IN}) and renal blood flow (RBF) and in the absence of changes in renal perfusion pressure, plasma protein concentration or packed cell volume. $(TF/P)_{IN}$ of end-proximal tubular fluid decreased from 1.65 ± 0.03 to 1.53 ± 0.04, $P < 0.025$, and proximal tubule absolute reabsorption decreased from 36 ± 3 to 29 ± 2 nl/min, $P < 0.05$. The decrease in absolute reabsorption, however, was balanced by a decrease in single nephron GFR (SNGFR) so that no increase in distal delivery of fluid (V_{TF}) out of the proximal tubule was detected. $SNGFR/C_{IN}$ remained constant. No change in fractional distribution of RBF was detected. The data indicate that volume expansion with equilibrated blood depresses proximal tubule fractional and absolute reabsorptive rates in the isolated kidney but since V_{TF} did not increase, they imply that the natriuresis derives from a decrease in sodium transport along more distal nephron segments.

1. DiBona, G. F., Kaloyanides, G. J., and Bastron, R. D., Proc. Soc. Exp. Biol. Med. **140,** 1405 (1972).
2. Dirks, J. H., Cirksena, W. J., and Berliner, R. W., J. Clin. Invest. **44,** 1160 (1965).
3. Mandin, H., Israelit, A. H., Rector, F. C. Jr., and Seldin, D. W., J. Clin. Invest. **50,** 514 (1971).
4. Stein, J. H., Osgood, R. W., and Ferris, T. F., Amer. J. Physiol. **223,** 984 (1972).
5. Kaloyanides, G. J., and Azer, M., J. Clin. Invest. **50,** 1603 (1971).
6. Kaloyanides, G. J., Bastron, R. D., and DiBona, G. F., Circ. Res. **35,** 400 (1974).
7. Fuhr, J., Kaczmarczyk, J., and Kruttgen, C. D., Klin. Wochschr. **33,** 729 (1955).
8. Vurek, G. and Pegram, S., Anal. Biochem. **16,** 409 (1966).
9. Huntsberger, D. V. and Leaverton, P. E., *in* "Statistical Inference in the Biomedical Sciences" (Allyn and Bacon, eds.) Boston (1970).
10. Knox, F. G., Howards, S. S., Wright, F. S., Davis, B. B., and Berliner, R. W., Amer. J. Physiol. **215,** 1041 (1968).
11. Auld, R. B., Alexander, E. A., and Levinsky, N. G., J. Clin. Invest. **50,** 2150 (1971).

12. Davidman, M., Alexander, E., LaLone, R., and Levinsky, N., Amer. J. Physiol. **223,** 188 (1972).
13. Howards, S. S., Davis, B. B., Knox, F. C., Wright, F. S., and Berliner, R. W., J. Clin. Invest. **47,** 1561 (1968).
14. Knox, F. G., Schneider, E. G., Dresser, T. P., and Lynch, R. E., Amer. J. Physiol. **219,** 904 (1970).
15. Knox, F. G., Schneider, E. G., Willis, L. R., Strandhoy, J. W., and Ott, C. E., J. Clin. Invest. **52,** 1642 (1973).
16. Levy, M., J. Clin. Invest. **51,** 922 (1972).
17. Stein, J. H., Osgood, R. W., Boonjarern, S., and Ferris, T. F., J. Clin. Invest. **52,** 2313 (1973).
18. DiBona, G. F., Kaloyanides, G. J., and Bastron, R. D., Proc. Soc. Exp. Biol. Med. **143,** 830 (1973)
19. Schrier, R. W. and DeWardener, H. E., New Eng. J. Med. **285,** 1231 and 1292 (1971).
20. Brenner, B. M., Troy, J. L., and Daugharty, T. M., J. Clin. Invest. **50,** 1596 (1971).

Received September 6, 1974. P.S.E.B.M., 1975, Vol. 148.