Effect of Variation in Dietary NaCl Intake on the Intrarenal Distribution of Plasma Flow in the Rat

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Abstract: The effect of dietary variation in sodium chloride intake on the intrarenal distribution of plasma flow was investigated in rats using the antiglomerular basement membrane antibody technique. Rats were placed on a liquid diet containing either 9.86 (n = 9) or 0 (n = 9) mEq NaCl daily portion for 2 wk. Labeled antibody was injected and the diets were reversed. After an additional 2 wk period, antibody labeled with a different radionuclide was injected and the animals were sacrificed. Fractional plasma flow distribution was then calculated for each dietary period. No change in flow to any cortical region could be detected. In six additional awake rats on identical dietary regimen, total plasma flow was estimated by the clearance of hippuran-3H. No change in this parameter occurred with changes in NaCl intake. We conclude, therefore, that no change in either total renal plasma flow or intracortical distribution of plasma flow occurs with wide variations in dietary sodium chloride intake in the rat. The implications of this constancy of regional plasma flow are discussed with reference to presumed concomitant alterations in the intrarenal distribution of nephron filtration rate.

Introduction: It has been proposed that a redistribution of glomerular filtrate and renal blood flow from deep nephrons, which are assumed to have a high reabsorptive capacity, to superficial nephrons with a lower capacity, might account for a portion of the natriuresis associated with the administration of sodium chloride (1). Most of the studies to test this thesis have involved the rapid administration of large intravenous saline loads (2–9). Results from these experiments may not be directly applicable to the more important question of the daily regulation of sodium chloride excretion as a function of dietary intake.

Only a limited number of studies have been performed to determine the distribution of filtrate and blood flow during variations in dietary sodium chloride intake. Horster and Thurau (10) using micropuncture techniques, and de Rouffignac and Bonvalet (11), employing the Hanssen technique, have demonstrated a disproportionately greater superficial filtration rate in rats on a high sodium chloride diet as compared with low sodium chloride diet. Also, Hollenberg et al. (12) and Blafox and coworkers (13) have demonstrated superficial redistribution of blood flow in human subjects on high salt diets using the krypton washout technique.

These findings of a redistribution of blood flow and glomerular filtrate to the superficial cortex during periods of high salt intake have led these investigators to propose that redistribution is a major regulatory factor in the renal maintenance of sodium balance. We have recently developed a new technique for the measurement of the intrarenal distribution of plasma flow (14) using a radionuclide-labeled antibody to glomerular basement membrane. This technique allows the measurement of plasma flow distribution in the awake animal, without surgical manipulation, with each animal acting as his own control. Applying this technique, we could demonstrate no significant change in the distribution of plasma flow with wide variations in sodium chloride intake and excretion.
METHODS

Protocol. Studies were performed on male Sprague-Dawley rats weighing initially 150-210 g. They were divided into three groups: Animals (group I, n = 9) were placed on low sodium diet for a period of 14 days, at the end of which 125I-labeled antibody to glomerular basement membrane (Ab 125) was injected via tail vein in the awake animal. The diet was then changed to high salt, and at the conclusion of 14 more days, 1.5 µCi of 131I-labeled antibody was injected (Ab 131); in group II (n = 9) the order of diets was reversed. In group III (n = 6) normal salt diets were given for two consecutive 14-day periods with antibody injections as above. Fig. 1 graphically displays the experimental protocol. A basic electrolyte-deficient liquid diet of the following composition was used. The diet contained approximately 2 g of protein, carbohydrate, fat, amino acid supplements, trace elements, and full vitamin requirements per daily portion. To this was added 29 g NaCl/2 liters for the high salt diets, 0 g NaCl for the low salt diets, and 11.6 g NaCl/2 liters for the normal salt diets. This concentration delivered 9.86 mEq NaCl/ml to the high salt group, 0 mEq/ml to the low salt group. All animals received approximately 4 mEq KCl/20 ml per day. Animals were placed in individual metabolic cages; 24-hr urine volumes were collected on alternate days under oil and total electrolyte excretion determined. The animals were weighed on alternate days, and estimates of total dietary intake made daily.

Analysis of tissue. At the conclusion of the experiment, the animal was anesthetized with Inactin (Promonta, Germany) 100 mg/kg body wt and intubated, and a left femoral catheter PE 50 was placed. The abdomen was opened, the aorta clamped at the level of the diaphragm, and an infusion of iso-oncotic albumin Ringer's solution begun through the arterial cannula. The renal veins were then cut, and the kidneys perfused with approximately 100 ml of solution. The purpose of this perfusion was to remove nonspecific labeled globulin from the renal circulation. The kidney was then sliced as previously described (7). The slices were counted in a three window gamma spectrometer (Packard 3003; Packard Instrument Co., Downers Grove, Ill.), and fractional plasma flow (f)1 calculated from the equation f = q/W, where q = the counts in a weighed slice, W = the total radioactivity of the kidney, and W was the weight of the slice. Total renal plasma flow was measured in six awake animals in the following fashion. At least 1 wk before the first dietary period, a right carotid artery catheter (PE 50) filled with heparinized saline was placed. The catheter was run subcutaneously down the back and the tip sealed with wax near the base of the rat's tail. Baroreceptor adaptation has been demonstrated in the rat to be complete within 4-6 days (15). The animals were first placed on either a low or high NaCl diet for a period of 14 days. At the end of this period, the animal was lightly anesthetized with ether and placed in a rat restraining device and allowed to awaken. The tip of the carotid catheter was then unsealed and connected to an infusion pump and syringe filled

1 Abbreviations used in this paper: C-1, C-2, cortical slices 1 and 2; CH, clearance of hippuran; f, fractional plasma flow; GFR, glomerular filtration rate; HS, high salt; IV, measured count rate infused per minute; JMC, juxtaglomerular apparatus; K, number of counts per milliliter at equilibrium; q, counts in weighed slice; Q, total radioactivity of the kidney; SNGFR, single nephron GFR; W, weight of slice.

FIGURE 1 The experimental protocol of this study is graphically displayed. After the first period of 2 wk, a specific 125I-labeled antibody to glomerular basement membrane was injected intravenously while the animal was awake. 131I-labeled antibody was similarly administered after the second 2 wk period on the diets listed. The animal was then sacrificed after the kidneys were perfused with 125I-labeled hippuran (Hippuptope, Squibb Laboratories, New Brunswick, N. J.) in isotonic NaCl-NaHCO₃ solution (approximately 20 µCi/ml). Infusion was maintained at a rate of 0.02 ml/min for a period of 3 hr. Blood samples were obtained from tail vein until blood levels of radioactivity reached a constant level. Blood samples were spun and plasma proteins precipitated in equal 10-ml volumes of trichloroacetic acid (10%). The capillary tube was then centrifuged and 10-ml samples of the supernate were counted on a Packard three window gamma counter. No urine was collected or analyzed by this method. The two kidney renal plasma flow was determined by the formula: C₉ = IV/P where IV equals the measured count rate infused per minute and P is the measured plasma counts per ml at equilibrium of indicator. In repeated preliminary studies, the steady-state plasma count rate of 131I was found to be uniformly constant at 3 hr or less. The catheter was sealed, the rat was returned to its cage, and the procedure repeated after 2 wk on the alternate diet.

Electrolytes were determined on a flame photometer (Instrumentation Laboratory, Inc., Lexington, Mass.) and plasma proteins were determined by a modification of the Lowry protein method (16) utilizing 2-µl samples of plasma.

RESULTS

Fig. 2 displays the metabolic balance data for all 18 experimental animals. While the animals were on low salt

FIGURE 2 Metabolic balance. Sodium chloride dietary intake and urinary excretion are depicted in the first and second panels, and urine flow while on high and low salt diets in the third panel. High salt (n = 18) refers to values for groups I and II while on high NaCl intake and low salt similarly refers to values for both group I and II animals.

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diet, sodium chloride intake was zero and output averaged 0.03±0.001 SEM mEq/24 hr. While on high salt diet, intake averaged 8.73±0.4 mEq/24 hr and output averaged 7.95±0.4 mEq/24 hr. There was no significant difference between group I and II rats while on either diet. Urine flow averaged 17.1 ml/24 hr during low sodium intake and 20.3 ml/24 hr during the high salt diet. This high rate of urine flow probably represents the forced water diuresis imposed by the liquid diets. Serum proteins averaged 6.8±0.2 g/100 ml while on high salt diet and 7.6±0.3 g/100 ml while on low salt diet (P < 0.05). Hematocrits averaged 53.7±2 vol/100 ml on low sodium intake and 53.1±0.8 vol/100 ml on high sodium intake (P > 0.9).

A representative experiment is displayed in Fig. 3. Fractional flow on the vertical axis is plotted as a function of increasing depth of slice into cortex on the horizontal axis. On the low salt diet (solid lines and circles) flow in the most superficial cortical slice (C-1) and the second cortical slice (C-2) were relatively greater per gram than fractional flow to juxtedudillary cortex (slice 5—JMC). This greater flow per gram has been demonstrated to be a function of greater glomerular density in the superficial cortex (5, 14, 17). Fractional flow determined in the same slices of tissue while on high salt diet (open circles-broken lines) closely approximates the curve obtained while on low salt diet.

Table I depicts the values for fractional plasma flow in groups I and II. In this table, the change in fractional plasma flow to three layers of cortex (C-1, C-2, and JMC) are expressed as the ratio of fractional plasma flow on high salt diet to fractional plasma flow on low salt diet (HS/LS). In group I animals, high salt diet was given after a period of low salt diet. In group II animals the high salt diet was given first. The order of the diets was deliberately reversed in the two groups (Table I) so as to eliminate the possibility that the sequence of administration influenced the distribution of fractional plasma flow independent of dietary intake. HS/LS ratio for C-1 was 1.00±0.02, for C-2 was 1.01±0.03 and for JMC was 1.01±0.01. Comparison of the arisin of the square root of these ratios to 1.00 revealed P values of 0.95 for C-1, 0.90 for C-2, and 0.3 for JMC. It is evident that there is no influence of variations in sodium chloride intake on plasma flow distribution to any analyzed layer of cortex. When group I and group II were analyzed separately no different results were obtained (P > 0.2). Fractional plasma flow was compared in the same animal as the ratio of superficial (C-1) to juxtedudillary fractional flow (C-1/JMC). This ratio during low salt diet was 1.36 and during high salt diet was 1.34. Comparisons of the arisin of the square root of these ratios for the 18 animals by paired t test revealed no significant difference (P > 0.6) (18).

As a control study, six animals were maintained on 4 mEq/24 hr sodium chloride intake for the entire 4 wk period of the study, and fractional flow was determined

![Figure 3](http://www.jci.org) Fractional flow on high and low salt diets: a representative experiment is shown. Fractional plasma flow expressed as g/0.4W × 100, on the vertical axis is plotted as a function of increasing depth of slice into the rat kidney cortex. Fractional plasma flow on the low NaCl diet is described by solid lines and circles. Fractional plasma flow on high NaCl diet is depicted by dashed lines and open circles. No significant difference in the distribution of plasma flow is noted between diets. Slice 5 is the juxtedudillary cortex (JMC).
TABLE II
Fractional Flow in Control Animals

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>2/1*</th>
<th>Superficial/deep</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-1</td>
<td>C-2</td>
</tr>
<tr>
<td>19</td>
<td>1.18</td>
<td>0.89</td>
</tr>
<tr>
<td>20</td>
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<td>0.85</td>
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</tr>
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<td>0.91</td>
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<td>0.95</td>
</tr>
<tr>
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<td>0.08</td>
</tr>
<tr>
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<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>P</td>
<td>&gt;0.20</td>
<td>&gt;0.30</td>
</tr>
</tbody>
</table>

* Fractional flow—period 2/fractional flow—period 1.
† P value for the difference from one by paired t analysis of arcsin √x.
‡ P value for the difference between paired samples.

at 2 and 4 wk. Table II displays the ratio of fractional flow in the second period to fractional flow in the first period for C-1, C-2, and JMC. None of these ratios was significantly different from 1.0. Superficial to juxta-
medullary ratios for the first and second periods were not different. These results indicate that lapse of time
and growth did not influence the distribution of plasma flow.

Since the distribution of fractional plasma flow was not altered by changing sodium chloride intake, in-
crease in plasma flow to any area of the cortex could only be accomplished through an increase in total
renal plasma flow. Fig. 2 graphically displays total renal plasma flow expressed as the clearance of hippuran-
131I as measured in a similar group of awake animals on high and low salt diets. Renal plasma flow was 7.33±0.7
ml/min on low sodium intake and 7.30±1.02 ml/min on high salt diet (P > 0.9). Therefore, no significant al-
teration in renal plasma flow to any area of the cortex could be detected.

DISCUSSION

The present study clearly indicates that variations in the salt content of the diet did not change plasma flow
to any region of the cortex. Animals were studied at wide extremes of dietary NaCl intake, and total plasma
flow and plasma flow distribution were determined after a steady state was attained following a period of 14 days
on each diet.

Two control measures were instituted in this study. In the first type of control, the order of the high and low
salt diets was reversed, so as to prevent either the lapse of time or growth of the animal from obscuring the ef-
f ect of diet alone upon the results. Neither over-all nor regional plasma flow was altered by the order in which
the diets were administered. In a second control, the constancy of plasma flow was examined by studying
animals during two consecutive periods on the same normal NaCl diet. Once again, no change could be de-
tected. The error of the blood flow method with regard to measurements made 2 wk apart was small, with a stan-
dard deviation of approximately 10%. The method should, therefore, detect even a modest change in the distribu-
tion of plasma flow. We conclude that wide variations in oral NaCl intake, within the physiologic range, pro-
duced no alteration in total renal plasma flow or its distribution.

The only previous experiments on blood flow distribution during dietary variations in salt load have been those of Hollenberg and coworkers (12) and Blafox et al. (13), both of whom demonstrated a superficial re-
distribution of blood flow on high salt as compared with low salt diets. These studies were performed in human
subjects using inert gas washout techniques. Perhaps species difference as well as differences in analytic tech-
niques (19) account for the disparate results.

The failure to demonstrate redistribution in the present
study applies only to plasma flow, and does not neces-
sarily imply a similar constancy in the distribution of
glomerular filtrate. The micropuncture studies of Horster
and Thurau (10) on rats on low and high salt diets have
stimulated much of the interest in redistribution of fil-
trate as a mechanism controlling sodium excretion. They
demonstrated that in the transition from low sodium to
high sodium diet, superficial nephron filtration rate rises
while juxtamедullary filtration rate falls. This finding
was subsequently confirmed by de Rouffignac and Bon-
valet (11) using the Hanssen technique, although the
absolute changes in nephron filtration rate were smaller
than those observed by Horster and Thurau. In addition
to these studies on chronic dietary sodium effects, acute
saline infusion has been shown by some investigators

![Figure 4](http://www.jci.org)
(2, 3) to be associated with disproportionate increases in the superficial nephron filtration rate as compared with total filtration rate. It has been assumed that this redistribution of glomerular filtrate in both acute and chronic studies occurs as a result of a similar redistribution of plasma flow. However, in the present study we have failed to demonstrate any change in the distribution of plasma flow after manipulations of dietary sodium chloride intake similar to those utilized by Horster and Thurai (10).

This difference between the constancy of plasma flow in the present study and the increase in superficial nephron filtration rate in the studies on the distribution of nephron GFR can be explained in two ways. First, the hypothesis may be ventured that nephron filtration rate changes in strict proportion to changes in plasma flow. If this is the case, then the constancy of plasma flow in our studies and the rise in superficial nephron GFR in the studies of others must be attributable to differences in experimental design and technique. No comparison may be made between our results in a chronic study to the acute studies of filtrate distribution after saline infusion (2, 3, 9) since different mechanisms may be operative in the natriuresis induced by massive saline infusion which are not directly applicable to the daily regulation of changes in dietary NaCl intake. Also, the chronic studies of Horster and Thurai and de Rouffignac were group comparisons, and the micropuncture studies required surgical preparation and anesthesia. The present studies were purposely designed to avoid surgical intervention and anesthesia, since the exact influence of these factors upon the distribution of plasma flow is not known. In our studies, distribution of plasma flow was measured in awake, unanesthetized animals; no surgery was necessary; the sodium chloride load was given orally and chronically rather than intravenously, and each animal acted as his own control. The antibody technique is readily adaptable to this type of experiment in that it may be given intravenously and has been demonstrated by our control studies to be distributed in a reasonably reproducible fashion when injections are given 2 wk apart (Table II).

However, the assumption of a strict proportionality between nephron filtration rate and plasma flow in all circumstances is highly questionable. It is more reasonable to assume that nephron GFR and plasma flow need not change proportionately in all conditions or even in the same direction (7, 20, 21). If this is correct, then the technical variations in the experimental designs may not fully explain the different results. Instead, a more reasonable conclusion would be that both sets of data (plasma flow and glomerular filtrate) may well be correct. Were this the case, it would mean that in the face of a constant over-all and regional plasma flow, superficial nephron filtration rate increases and juxtamedullary filtration rate falls in rats changed from a low to high salt intake.

Redistribution of glomerular filtrate in the presence of constant plasma flow requires a disparity in filtration fraction between superficial and juxtamedullary nephrons. On a high salt intake, the superficial nephron filtration fraction would be significantly higher than that of juxtamedullary nephrons, while on low salt intake, the filtration fractions may be nearly identical in both nephron populations (17). In fact, such a postulate does not constitute a unique precedent in renal physiology. In acute progressive volume expansion with saline, a disproportionate increase in superficial nephron filtration rate has been observed (2), and with an identical protocol a disproportionate increase in juxtamedullary nephron plasma flow has been demonstrated (7). Barratt, Wallin, Rector, and Seldin (20) have provided evidence that in the same animal, the filtration fraction falls in juxtamedullary nephrons after saline infusion. Similar results were also reported by Blantz et al. with hyperoncotic albumin infusion (21). This fall in juxtamedullary filtration fraction was due both to a disproportionate increase in superficial nephron filtration rate and a disproportionate increase in deeper nephron plasma flow.

From this analysis, the change in superficial and deep filtration fraction emerges as a critical determinant of sodium excretion with variation in dietary salt intake. Unfortunately, data are not available for superficial and deep filtration rates in unanesthetized, unoperated rats. Nevertheless, the micropuncture data of Horster and Thurai (10) may be used for illustrative purposes with the assumption that the changes in single nephron filtration rate in rats changed from a low to high salt diet and subjected to surgery are qualitatively similar to what would be observed in the undisturbed animal. In their studies, over-all GFR changed from 0.94±0.16 ml/min on a low salt diet to 1.01±0.24 ml/min on a high salt diet, a change that does not appear to be significant. (In a study on undisturbed rats, Kleinman, Radford, and Torelli (22) demonstrated no difference in over-all GFR in rats on a low or high salt diet.) Comparison of data for single nephron glomerular filtration rate (SNGFR) in low salt and high salt groups (10) discloses a rise in superficial SNGFR from 23.5 to 38.1 ml/min per glomerulus, while deep SNGFR fell from 5.82 to 16.5 nl/min per glomerulus. The constancy of TF/P inulin ratios indicated that fractional reabsorption remained unchanged. When these data (10) are combined with the present findings of a constancy of total and fractional plasma flow, the transition from a low to a high salt diet can be estimated to produce a 60% rise in superficial nephron filtration fraction and a fall of 70% in juxtamedullary filtration fraction while over-all filtration fraction remains relatively unchanged. Since these estimates are derived from a combination of plasma flow data from
awake rats and data for nephron filtration rate and fractional reabsorption from rats subjected to deep anesthesia and surgery, it is clear that the calculated changes in filtration fraction are not quantitative expressions of what transpires in the normal animal. Nevertheless, they probably do reflect directional changes.

The constancy of fractional reabsorption, on a high salt diet, in the face of an elevation of superficial SNGFR means that absolute reabsorption has increased. The rise in filtration fraction, by increasing peritubular protein concentration, could account for the increase in absolute reabsorption. Nevertheless, increased fluid issues out of the proximal tubule because the increased absolute reabsorption is not commensurate with the rise in SNGFR (TF/P inulin does not rise). The deep SNGFR, on the high salt diet, is sharply reduced (10), but it is not clear whether the anticipated reduction in reabsorption, owing to a fall in the filtration fraction, is sufficient to promote increased sodium delivery out of the proximal tubule.

We conclude from the present study that wide variations in NaCl intake within the physiologic range of the rat do not result in changes in over-all or intrarenal distribution of plasma flow. If classic redistribution of glomerular filtrate (1) exists and is a factor in the daily control of NaCl excretion and volume homeostasis, then superficial and juxtamedullary nephron filtration fractions must change reciprocally in response to a high oral NaCl intake, and may contribute to the natriuresis associated with a high salt intake.

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