Accelerated Reabsorption in the Proximal Tubule Produced by Volume Depletion

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ABSTRACT The renal response to chronic depletion of extracellular volume was examined using the techniques of micropuncture. Depletion of salt and water was produced by administration of furosemide to rats maintained on a sodium-free diet. There was a marked fall in body weight, plasma volume, and glomerular filtration rate. The intrinsic reabsorptive capacity of the proximal tubule, measured by the split-droplet technique, was greatly enhanced. The acceleration of proximal fluid reabsorption could not be accounted for by changes in filtration rate, tubular geometry, or aldosterone secretion. The half-time of droplet reabsorption in the distal tubule was not altered by sodium depletion.

An increase in the reabsorption of fluid in the proximal tubule, as demonstrated directly in the present experiments, provides an explanation for a variety of clinical phenomena associated with volume depletion.

INTRODUCTION

It is now well established that expansion of the extracellular fluid leads to renal excretion of salt and water in large part through the mechanism of decreased reabsorption in the proximal tubule. It is also well-known that depletion of extracellular volume regularly induces retention of salt and water, but the mechanism is not so precisely established. There is reason to think that, in addition to a fall in glomerular filtration rate, there is an accelerated reabsorption of sodium in the proximal tubules resulting in decreased delivery of filtrate to the distal nephron. Thus far, however, the evidence has been largely indirect; for example, concentrating capacity is impaired (1-3). In addition, several studies have indicated that fractional reabsorption of glomerular filtrate is increased in the proximal tubule in volume depletion (4-6), but it is not clear that this is always the result of increased reabsorptive activity by the tubules. The present studies offer direct evidence that sodium depletion is associated with an increase in the intrinsic reabsorptive capacity of the proximal tubule, in a way that is independent of glomerular filtration rate, aldosterone secretion, and tubular geometry.

METHODS

Male Sprague Dawley rats weighing 150-400 g were used for all experiments. Control animals were fed Purina Chow and water ad lib. (sodium intake 1.8-2.5 mEq/day). Experimental rats were fed a diet containing no sodium (Nutritional Biochemicals Corp., Cleveland, Ohio) and were allowed free access to water. Volume depletion was produced by the intraperitoneal administration of 15 mg/kg furosemide, on 2 successive days. To assess the effects of this treatment, 12 animals were housed in metabolism cages (Acme Metal Products, Cincinnati, Ohio) in order to measure the changes in sodium balance and plasma volume. Food and water intake, as well as complete urine collections, was measured for 7 days while rats were maintained on the diet containing no sodium. Furosemide was administered on the 2nd and 3rd day. Samples of blood were obtained immediately before (from the orbital capillary plexus of the lightly anesthetized rat) and at the end of the collection period. Sodium and potassium content of plasma and urine was measured by flame photometry. Hematocrit of blood was measured with microhematocrit tubes. The biuret method was used to measure plasma protein concentration (7).

Micropuncture experiments were performed on rats, maintained on a sodium-free diet, 4-6 days after furosemide treatment, since at this time the action of the drug was no longer present and the fall in plasma volume was similar to the value found in the balance study. Surgical preparation for micropuncture was performed as previously described (8, 9).

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Stop flow studies. Stop flow studies were performed to measure intrinsic reabsorptive capacity of the proximal and distal tubules. The shrinking droplet method of Gertz was used to measure reabsorptive half-time ($t_1$) (8, 10). In order to improve the validity of the Gertz technique, all photographs were coded and measured in a random blind fashion. A regression line, passing through the origin, was calculated for each droplet sequence. The $t_1$ was derived from the mean slope of these regressions. Proximal and distal transit times were measured using lissamine green. The proximal transit time was estimated by the method as modified by Gertz (11) in which the end point is taken at the time of convergence of the columns of dye in proximal tubules before they descend beneath the surfaces to form pars recta. Distal transit time was measured from the initial blush of dye on the kidney surface to the earliest appearance of dye in distal tubules. The diameter and initial length of the isolated droplets was measured at the oil-saline interface as previously described (8, 9). Only droplets with an initial length of 60–90 µ were included in the analysis. Neither hydropenic control rats nor volume-depleted animals received intravenous infusions before or during these experiments. A second group of control rats, referred to as hydronated control rats, was given an initial infusion of 1% of body weight of isotonic saline intravenously to replace surgical losses; followed by a sustaining infusion of 1.2 ml/hr. A third group of normal animals received aldosterone in oil, 33 µg/kg of body weight, 16 hr and 2 hr before micropuncture. An additional 100 µg was infused intravenously in a volume of 0.1 ml of saline, 1 hr before micropuncture. This dose was chosen to provide supramaximal amounts of aldosterone (salt restriction provokes a secretion rate of aldosterone of approximately 60 µg/24 hr per rat (12)). No additional fluids were administered to this group.

Free flow studies. Free flow studies were performed in a separate series of animals. Glomerular filtration rate (GFR), and tubular-fluid-to-plasma ratios (TF/P) of inulin were measured using inulin-methoxy-^1^H (New England Nuclear Corp., Boston, Mass.). After a priming dose of 100 µCi in 0.25 ml of isotonic saline given intravenously, 100 µCi in 1.2 ml of saline was infused per hr. Samples of plasma, urine, and tubular fluid were counted in Scintisol Complete (Isolab Inc., Akron, Ohio). No quenching effect was observed with this method, using an H^3^ internal standard. The site of tubular puncture was identified by injection of latex (General Biological Supply House Inc., Chicago, Illinos) and subsequent microdissection (13). Tubular diameters during free flow were measured from direct photographs of the surface of the kidney from histological sections of kidneys subjected to instant freezing (8).

Glomerular filtration rate and renal plasma flow were measured in control- and volume-depleted animals by clearances of inulin-^3^H and H^3^ aminohippuric-acid (H^3^PAH). A priming dose of 50 µCi of H^3^ PAH and 50 µCi of inulin-^3^H were given intravenously in 0.4 ml saline. 50 µCi of each in 1.2 ml saline was then infused per hr. Filtration fraction (FF) was calculated by the equation: FF = Crin/CpaH. All data have been expressed as the mean plus or minus the standard error. Fisher's test has been used to determine significance.

Abbreviations used in this paper: C, reabsorptive rate constant; FF, filtration fraction; GFR, glomerular filtration rate; TF/P, tubular-fluid-to-plasma ratios.

RESULTS

General effects of chronic volume depletion (Fig. 1). Diuretic treatment and continued sodium restriction produced a marked reduction in body fluid. In balance studies performed on 12 rats, average body weight fell from 313.5 ±10.0 g to 263.2 ±8.0 g, resulting in a mean loss of 15.4 ±0.9%. The mean net sodium loss was 2.15 ±0.14 mEq while the mean potassium balance was +0.76 ±0.44 mEq per rat.

Associated with this contraction of body fluid, the glomerular filtration rate, measured in a separate group of eight control- and nine volume-depleted animals, fell 24% from 531 ±35.0 μl/kidney/min per 100 g body weight to 403 ±47.0 (P < 0.001). Urine sodium concentration decreased from a control value of 40.0 ±12.0 mEq/liter to 1.5 ±0.3 mEq/liter. The serum sodium did not change.

The gross appearance of the kidneys of volume-depleted rats was markedly altered compared to the kidneys of normal animals. The kidney frequently appeared shrunken and dark in color. The surface was irregular because the tubes were narrowed and often collapsed. After injection of lissamine green, the appearance of

Figure 1 Consequences of chronic volume depletion. Body weight, urine-sodium concentration, hematocrit, and glomerular filtration rate, before and after volume depletion. Values represent mean ±SE. All changes are significant (P < 0.001).
dye across the renal surface was uneven because of an abnormally great heterogeneity of nephron transit time.

During volume depletion the hematocrit rose 21% from 42.9 ±0.52% to 52.3 ±2.6% (P < 0.001), presumably owing to contraction of the extracellular fluid about a relatively fixed red cell mass. Plasma volume, estimated from the change in hematocrit, decreased by 16%.

In volume-depleted rats studied by micropuncture, the changes in plasma volume and hematocrit were similar. The hematocrit was 43.0 ±0.41 and 43.5 ±0.39% in the hydrated and hydropenic groups, respectively, and increased 24% to 53.5 ±0.55% in animals with chronic volume depletion.

**Stopped flow studies of the proximal tubule** (Table I, Fig. 2). The reabsorptive half-time (t₁) of the hydrated control group was 10.4 ±0.8 sec. The t₁ of the hydropenic controls was slightly lowered to 9.0 ±0.8 (P not significant). There was a marked and statistically significant reduction to 6.4 ±0.4 sec in the volume-depleted animals (P < 0.001, compared to both hydrated and hydropenic control groups). These measurements are especially interesting because they demonstrate for the first time an accelerated reabsorption of fluid from the proximal tubule during stopped-flow. Aldosterone given in doses above physiological amounts failed to alter the reabsorptive half-time (t₁) in an additional group of rats which were not volume depleted.

The diameter of the isolated split droplet measured at the droplet-oil interface is considerably greater than the diameter measured during free flow, probably because of expansion of the tubule by the oil. The diameters during stopped-flow in the proximal tubule were 32.8 ±0.6 μ for the hydrated controls, 32.9 ±0.6 μ for the hydropenic controls, 30.8 ±0.5 μ for the volume-depleted animals, and 32. ±0.9 μ for the aldosterone-treated rats.

Since the diameter of the oil-filled tubule was similar in each group of rats, the reabsorptive half-time (t₁) becomes directly proportional to the reabsorptive rate constant (C)† (10, 11), expressed as mm³/mm sec. The value of C was calculated from the mean t₁ and radius of each of the three groups of rats. The reabsorptive rate constant (C), estimated in this way, was 5.7 X 10⁻⁴ and 6.5 X 10⁻⁴ mm³/mm sec. in the hydrated and hydropenic control groups, respectively. In volume-depleted rats in which the reabsorptive half-time was markedly reduced, C was increased 24% to 8.1 X 10⁻⁴.

Volume depletion induced a marked prolongation and irregularity of the lissamine green transit times. Proximal transit time rose by 50%, from 12.0 ±0.7 sec in the hydropenic control group to 18.8 ±2.5 sec in the hypovolemic animals. Aldosterone administration did not alter the transit time of control animals. Fractional reabsorption estimated from t₁ and transit time by the equation of Gertz ² (11) was 60% in the control group and 87% in the sodium-depleted animals. The relationship between the variables t₁ and transit time are shown graphically in Fig. 2. The slope of the shaded areas represent the rate of reabsorption in the hydropenic control and volume-depleted groups and transit time (sec)

### Table I

<table>
<thead>
<tr>
<th>Reabsorptive half-time (t₁)</th>
<th>Transit time</th>
<th>Diameter</th>
<th>Reabsorptive rate constant (C)†</th>
<th>Fractional reabsorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>sec</td>
<td>sec</td>
<td>μ</td>
<td>sec</td>
<td>%</td>
</tr>
<tr>
<td>Hydrated controls (6 animals)</td>
<td>10.4 ±0.8</td>
<td>11.5 ±0.9</td>
<td>32.8 ±0.60</td>
<td>5.69 X 10⁻⁴</td>
</tr>
<tr>
<td>(n = 36)</td>
<td>(n = 15)</td>
<td>(n = 35)</td>
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<tr>
<td>Hydropenic controls (8 animals)</td>
<td>9.0 ±0.8</td>
<td>12.0 ±0.7</td>
<td>32.9 ±0.55</td>
<td>6.54 X 10⁻⁴</td>
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<tr>
<td>(n = 71)</td>
<td>(n = 20)</td>
<td>(n = 71)</td>
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<tr>
<td>Volume depleted (12 animals)</td>
<td>6.4 ±0.4*</td>
<td>18.8 ±2.5*</td>
<td>30.8 ±0.51</td>
<td>8.06 X 10⁻⁴</td>
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<td>(n = 110)</td>
<td>(n = 75)</td>
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<tr>
<td>Aldosterone (4 animals)</td>
<td>9.8 ±1.0</td>
<td>12.0 ±1.0</td>
<td>32.8 ±0.91</td>
<td>6.00 X 10⁻⁴</td>
</tr>
<tr>
<td>(n = 18)</td>
<td>(n = 10)</td>
<td>(n = 18)</td>
<td></td>
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</table>

Values represent mean ±SE; n, number of observations.

* P < 0.001 compared with hydropenic controls.

† C was estimated from mean values for t₁ and radius of the droplet.

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C = \frac{0.693πr^2}{t₁}

% Fractional reabsorption = \left[1 - \frac{1}{\text{antilog}(0.301 \cdot TT)}\right] \cdot \frac{100}{T₁}

TT, transit time.
Fig. 3 demonstrates that the TF/\( \text{P}_{\text{IN}} \) was elevated above 24 observations in control animals throughout the entire length of the proximal nephron in the volume-depleted rats. Mean nephron GFR was \( 22.9 \pm 1.4 \) ml/min/nephron per g kidney weight in the control group and \( 19.8 \pm 2.9 \) in the volume-depleted rats \( (P \) not significant). Nephron GFR in the experimental group is probably an overestimate of the average value for surface nephrons since only fluid-filled tubules were punctured. Fractional reabsorption can be calculated directly from the TF/\( \text{P}_{\text{IN}} \) \( (\text{fractional reabsorption} = (1-P/\text{TF}_{\text{IN}}) \times 100) \). Control fractional reabsorption during free flow was 58.3%. Volume depletion induced a rise to 83.6%.

The diameters of proximal tubules during free flow were measured by direct photographs and by instant freezing. Although there is considerable difference between these methods of measurements, both techniques demonstrate a diminution of tubular diameter in volume depletion. The snap-freezing technique showed a decrease of about 20% from \( 25.3 \pm 0.4 \) to \( 20.6 \pm 0.4 \mu \). Measurement of direct photographs indicated a decrease of approximately 15% from \( 20.7 \pm 0.4 \) to \( 17.9 \pm 0.3 \mu \).

Serum protein concentration and filtration fraction in chronic volume depletion. Measurements of \( t_1 \), plasma protein, filtration fraction, and hematocrit were made in 10 normal hypodermic control rats and in 11 rats subjected diuretic treatment and sodium restriction. While

![Graph showing reabsorptive half-times, transit times, and predicted fraction reabsorption in the proximal tubule. Shaded area represents mean ±SE.](image)

The shaded area indicates the predicted fraction reabsorption in both groups of rats.
volume depletion was associated with a 12.3 ±3.6% reduction in body weight and an 16% increase in hematocrit to 49.6 ±0.5% compared with 42.8 ±51% in control rats, there was no demonstrable change in plasma protein concentration. The plasma protein concentration was 5.68 ±0.26% g per 100 ml before and 5.32 ±0.16 g per 100 ml after volume depletion. The plasma protein of the control animals was 5.47 ±0.19 g per 100 ml. Filtration fraction (Cfu/Cfum) was 0.32 ±0.03 (11 observations) in volume-depleted rats, similar to the control value of 0.35 ±0.04 (21 observations, P not significant). A significant decrease in the t(f) from 10.3 ±0.5 sec (n = 38) for the hydropenic controls to 7.6 ±0.3 sec (n = 38, P < 0.001) in volume depletion confirmed the previous findings.

Since the plasma protein concentration, and therefore the oncotic pressure, in the postglomerular microcirculation is proportional to the peripheral plasma protein concentration and filtration fraction, there was no evidence that enhanced renal reabsorption of sodium was associated with increased oncotic pressure in chronic volume depletion. The filtration fraction of the whole kidney, however, might not have mirrored changes which occurred in superficial nephrons, where an increased capacity for sodium reabsorption in the proximal tubules was observed. Whether changes in proximal tubular function in volume depletion occur independently of alterations in peritubular capillary oncotic pressure can only be settled by direct measurement of protein concentration in such animals.

Studies of the distal tubule (Table II). The split-droplet method of Gertz was also employed to study fluid reabsorption in the distal convolution. In contrast to the proximal tubule, reabsorption of the isolated droplet in the distal convolution was unchanged by volume depletion. Distal t(f) was 38.8 ±2.5 sec in the control and 37.2 ±2.0 sec in the volume-depleted group.

The distal transit-time is an indication of the rate of flow of tubular fluid through the proximal tubule and the loop of Henle. This was almost doubled from 43.5 ±3.5 sec in the control animals to 77.7 ±8.1 sec in the volume-depleted rats.

As in the proximal tubule, the free flow diameters of the distal tubule, measured by the snap-freezing technique, were significantly diminished. Control distal diameter was 18.3 ±0.4 μ. The diameter of the distal tubules of the volume-depleted rats was 13.7 ±0.6 μ.

**DISCUSSION**

Contraction of the volume of body fluids usually produces prompt renal retention of sodium (14). Since a depression of the glomerular filtration rate is usually associated with hypovolemia, it was thought earlier that this was responsible for the concomitant urinary

| TABLE II |
| Distal Tubular Function in Volume Depletion |
| --- | --- | --- |
| Hydrogenic controls (4 animals) | Reabsorptive half-time (t(f)) | Transit time |
| 38.8 ±2.5 | 43.5 ±3.5 |
| n = 28 | n = 20 |
| Volume depleted (4 animals) | 37.2 ±2.0 | 77.7 ±8.1* |
| n = 28 | n = 13 |

All values represent mean ±se; n, number of observations. * P < 0.001 compared with control. All other values not significantly different from controls.

Changes (15). More recent investigations have suggested that alterations in reabsorption of fluid by the tubules are also important in determining the final composition of the urine.

What portion of the nephron is chiefly responsible for the conservation of sodium? Indirect studies have suggested that volume depletion induces an increase in sodium reabsorption by the nephron at a site proximal to the diluting and concentrating segments. Patients with congestive heart failure, decompensated cirrhosis or severe salt depletion have been shown to have an inability to excrete a water load, due to an impairment in urinary diluting ability (14, 16, 17). Concentrating ability is similarly impaired by acute or chronic sodium depletion in a way which cannot be explained by a decrease in filtered load (1-3).

Direct evidence for an increase in fractional reabsorption in the proximal tubule was presented by Dirks, Cirkensia, and Berliner (4) who showed by micropuncture techniques that diuretic treatment of hydropenic dogs provoked a rise in the proximal TF/Puin. These experiments were complicated by the fact that salt depletion produced by diuresis lowered the glomerular filtration rate. Several studies have demonstrated that a drop in filtration rate per se, induced for example by constricting the renal artery or aorta, is associated with a rise in fractional reabsorption of glomerular filtration in the proximal tubule (5, 6). Brenner and Berliner (18) demonstrated with a recollection technique that acute volume depletion, produced by instillation of polyethylene glycol into the peritoneal cavity of rats, also induced a significant rise in the proximal TF/Pxin. Recognizing that a reduction of filtration rate might be in part responsible for these findings, additional experiments were performed in three rats in which carotid artery ligation produced hypertension, thus minimizing or preventing a fall in the individual surface nephrons that were punctured. TF/Pxin increased with volume depletion nevertheless. The validity of these experiments
is subject to the errors involved in the accuracy of the measurement of GFR in individual nephrons.

The split-droplet method was employed in the present experiments because it has the inherent advantage of measuring the intrinsic reabsorptive capacity of the tubular surface in a way that is independent of glomerular filtration rate or passage time. In order to reduce observer bias and increase the validity and reliability of the technique, all photographs were coded and measured in a random blind fashion, and the measurements evaluated by statistical methods. Differences in the length of the saline droplet or oil column, noted by others (19) and ourselves to influence the rate of shrinkage, cannot account for the changes in ti which we observed, since these factors were held constant.

The outstanding finding in the present paper is that volume depletion markedly accelerated the rate of droplet shrinkage in the proximal tubule. The reabsorptive rate constant, which expresses absolute reabsorption in terms of volume of fluid absorbed per unit of tubular surface area, was consequently increased. The implication is that severe volume depletion greatly enhances the capacity for active reabsorption of sodium by the proximal tubule, regardless of the rate of flow through the tubule, or the rate of glomerular filtration. This finding cannot be duplicated by the administration of large doses of aldosterone, in spite of the evidence that aldosterone has an action in the proximal tubule (20). There was likewise no evidence that a rise in the oncotic pressure within the postglomerular microcirculation of the kidney during volume depletion, although oncotic pressure has been shown to influence proximal reabsorption (21, 22). Since the peritubular capillary oncotic pressure of surface vessels was not directly measured, a final conclusion about the importance of this factor in producing the acceleration of proximal tubular absorption that we observed must await further study.

The rise in hematocrit that accompanied volume depletion might also have affected proximal tubular function by changing the viscosity of the blood and the flow and pressure in peritubular capillaries (23, 24). Neither hydrostatic pressure in peritubular capillaries nor systemic blood pressure was measured in these experiments; it is conceivable that these "physical factors" might have influenced reabsorption from the proximal tubule (25).

The fraction of the glomerular filtrate reabsorbed in the proximal convolution may be estimated from ti and transit time according to the equation of Gertz. Fractional reabsorption calculated in this way was 86.9% in volume depletion compared with 60% in the control animals. Direct confirmation of this elevated fractional reabsorption was sought by collecting fluid from the tubules during free flow. In these experiments great care was taken to avoid an artifactually high tubular fluid concentration of inulin due to retrograde flow (6). A large oil block was placed distal to the collection site, negative pressure was not applied to the collecting pipette, and the sample was discarded if the oil block moved during the collection period. The mean end proximal TF/Pmean was 6.1. This represents a fractional reabsorption of 83.6%, remarkably similar to that estimated from the stopped-flow data. The volume of fluid delivered to the distal tubule (total GFR-per cent fractional reabsorption × GFR) was therefore markedly diminished in hypovolemic rats to one third of control. This reduction offers a direct explanation for the curtailment of urinary concentrating and diluting ability in hypovolemic states. It should be noted that fractional reabsorption during free flow in the proximal tubule was increased, even though the measured diameter of the tubular lumen was decreased by volume depletion.

In the present studies, an attempt was made to assess function in the distal convolution with the shrinking droplet technique. The half-time of reabsorption is longer here than in the proximal tubule and sodium transport cannot automatically be equated with droplet shrinkage because the concentration of sodium in the drop quickly falls below that of isotonic saline (26). To allow the sodium concentration in the droplet to approach its equilibrium levels, photographic measurements were delayed at least 15 sec after the introduction of the droplet of saline. In contrast to the proximal tubule, reabsorption of an isolated droplet in the distal convolution was unchanged by volume depletion. It is interesting that, by contrast, the half-time of reabsorption in the same segment is actually shortened during rapid saline infusion (8).

It is appropriate to compare the alterations in nephron function induced by chronic sodium depletion with changes in the opposite direction, associated with acute volume expansion. Infusions of isotonic saline or Ringer's solution produce a decrease in reabsorption in the proximal tubule with a prolongation of the split-droplet half-time (8, 27, 28). The present experiments establish that the reabsorptive capacity of the proximal tubule is powerfully influenced by the volume of the extracellular fluid over the entire spectrum of bodily hydration (8).

These findings provide an explanation for several clinical observations associated with depletion or dislocation of body fluids. Salt deprivation, congestive heart failure, and decompensated cirrhosis are often accompanied by a dilutional hyponatremia secondary to a decrease in the ability of the kidneys to excrete free water and dilute the urine (14, 16, 17). The urinary excretion of calcium and urate, which are chiefly reabsorbed in the proximal tubule, is curtailed by sodium deprivation (29, 30). The polyuria of diabetes insipidus is improved by depletion of salt (31). The mechanism responsible for these phenomena lies in the accelerated proximal reabsorption of tubular fluid that characterizes the renal re-
response to depletion of the volume of the extracellular fluid.

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