Sodium Movement across Single Perfused Proximal Tubules of Rat Kidneys

GERHARD GIEBISCH, RUTH M. KLOSE, GERHARD MALNIC,
W. JAMES SULLIVAN, and ERICH E. WINDHAGER

From the Department of Physiology, Cornell University Medical College, New York.
Dr. Malnic's present address is Department of Physiology, Sao Paulo University Medical
School, Sao Paulo, Brazil. Dr. Sullivan's present address is Department of Physiology and
Biophysics, New York University School of Medicine, New York

ABSTRACT Using perfusion techniques in single proximal tubule segments of
rat kidney, the relationship between net sodium movement and active transport
of ions, as measured by the short-circuit method, has been studied. In addition,
the role of the colloid-osmotic pressure gradient in proximal transtubular fluid
and sodium movement has been considered. Furthermore, the limiting concen-
tration gradient against which sodium movement can occur and the relation-
ship between intratubular sodium concentration and fluid transfer have been
investigated. Comparison of the short-circuit current with the reabsorptive
movement of sodium ions indicates that this process is largely, perhaps ex-
clusively, active in nature. No measurable contribution of the normally existing
colloid-osmotic pressure gradient to transtubular water movement was de-
tected. On the other hand, fluid movement across the proximal tubular epi-
thelium is dependent upon the transtubular sodium gradient and is abolished
when a mean concentration difference of 50 mEq/liter is exceeded.

The major portion of renal salt and water reabsorption occurs within the
proximal tubule, amounting in mammalian kidneys to about 80 per cent of
the quantity present in the glomerular filtrate (1–4). Micropuncture work on
rats has demonstrated unequivocally the existence of active sodium transport
across proximal tubular epithelium (5–8) but has not defined its relative
contribution to the over-all movement of this ion species. The present work
attempts to clarify this point by correlating active transport of ions as measured
by the short-circuit method with net movement of fluid under comparable
conditions. Since all measurements of the osmotic pressure of tubular fluid
have shown proximal reabsorption to be isosmotic, i.e. it proceeds without
the establishment of an osmotic pressure difference (9, 10), net fluid movement
can be used as an index of the net transport of sodium. Thus, short-circuit
current can be correlated with net movement of sodium.

In addition, perfusion experiments on single proximal tubules were carried out using varying concentrations of sodium chloride but keeping the chemical activity of water constant by adding mannitol. In such studies the magnitude of the limiting concentration gradient against which sodium movement can occur and the relationship between intratubular sodium chloride concentration and fluid transfer were investigated. In an additional series of experiments the possible influence of the colloid-osmotic pressure difference between proximal fluid and peritubular blood on Na reabsorption was examined.

METHODS

All experiments were carried out on single proximal tubules of male rats (body weight 310 to 512 gm) *in vivo*. The methods of anesthesia, of preparation of the animals, and of micropuncture have been described previously (5). During perfusion experiments the kidney surface was covered with a layer of mineral oil whereas isotonic NaCl solution was used during all electrical measurements.

Measurement of Short-Circuit Current

Short-circuit current was measured either by the original short-circuit method of Ussing and Zerahn (11), using double-barrelled microelectrodes (12), or by a modification described by Karger *et al.* (13, 14).

Double-barrelled microelectrodes were made from 1.5 mm pyrex tubing. Two pieces of glass tubing were fused, twisted, and gently drawn over a small flame. In a second step, the pulling was completed in an apparatus described by Alexander and Nastuk (15). They were filled by the method of Caldwell and Downing (16) with 3 M KCl to which Na-fluoresceinate had been added to improve the visibility of the tip as suggested by Pillat and Heistracher (17). The tip potentials were less than 5 mv and the resistances of the individual barrels were between 5 and 30 megohms. The electrode holder was similar to that described by Whittembury and Windhager (18) but provision was made for a shielded polyethylene tubing filled with 3 M KCl agar to establish electrical contact between the second barrel and a current-generating device. As indifferent electrode a polyethylene tube filled with 0.9 per cent saline-agar bridged to a calomel electrode was placed into the saline pool in the vicinity of the kidney surface.

For measurements of potential differences the voltage barrel was connected to a Keithley model 200B electrometer and the output of the latter was displayed on a Grass model 5 polygraph. Tip potentials were compensated by the method of Adrian (19). Direct current was applied through the second barrel using a Grass stimulator as a source. This current was measured by a vibrating reed electrometer (Cary 31-31V) as the potential drop across a 10 megohms precision resistor and recorded on a second channel of the Grass polygraph.

It is well known that the application of a steady current through one barrel of a double-barrelled microelectrode causes a potential change to be recorded from the other barrel (12). This is due to the coupling resistance shared by the current-applying and the potential-recording electrode tips and requires correction. For this purpose

coupling resistances of individual microelectrodes were measured with the tips immersed in isotonic saline solution with currents of the magnitude and polarity needed for transtubular short-circuiting (50 to 200 $\times$ 10^{-10} amp). The potential drops across the coupling resistances ranged between 1 and 2 mv (mean 1.3). The transtubular current was adjusted for this value, which corresponds to the true short-circuit condition.

An alternative way of measuring short-circuit current across short segments of renal tubules is that of Karger (13, 14), requiring only the use of a single microelectrode within the tubule. The application of this technique for the measurement of short-circuit current in tubules of *Necturus* kidney has been described in detail by Eigler (20). In the present study a similar approach was used. The object is to reduce the transtubular potential difference to zero by passage of current through a microelectrode, the tip of which is in the tubule lumen. Under these conditions the effective transtubular resistance is reduced to zero, and virtually all the resistance in the external circuit is that contributed by the microelectrode. The situation is somewhat complicated by the fact that microelectrode resistance is a function of current flow through the electrode tip. Further, the resistance-current relation with the tip in the tubule differs from that obtained with the tip in the bathing solution. Operationally, therefore, one determines resistance *vs.* applied current with the tip in the bathing solution, and again with the tip in the tubule. The intersection of the two curves so obtained defines the current for which electrode resistance with the tip inside the tubule equals that with the tip in the bathing solution. As indicated above, this is the current required to short-circuit the tubule.

All measurements of short-circuit current were performed on tubular fluid segments isolated from the rest of the tubular fluid by means of columns of mineral oil. This is accomplished by the injection of a small amount of mineral oil into a surface tubule and subsequent splitting of the oil droplet by the reinjection of a small amount of collected tubular fluid. When necessary, tubular fluid proximal to the site of measurement was collected to maintain a constant position of the injected fluid and oil.

Measurements of Net Movement of Fluid

The measurements of short-circuit current were compared with the net movement of sodium ions as calculated from fluid movement observed in perfusion experiments on single proximal tubules. In this technique, similar to that used in the *Necturus* kidney (21), colored mineral oil is injected into a surface tubule, and its movement is observed as it is carried away by the tubular fluid. This allows selection of a proximal tubule with an adequate number of surface loops. A small oil column is then injected and split by a small amount of perfusion fluid, usually of the order of 1 mμl. The position of the isolated column of perfusion fluid is then fixed by injecting mineral oil through a second pipette into the most distal surface loop of the tubule. The perfusion fluid is left within the tubular lumen for a short period and is then collected through one pipette while mineral oil is injected through a more proximally located micropipette. Tubules were perfused with three different perfusion fluids: (*a*) 145 mEq/liter NaCl, 25 mEq/liter NaHCO₃, 4 mEq/liter KCl; (*b*) 100 mEq/liter NaCl,

25 mEq/liter $NaHCO_3$, 4 mEq/liter KCl; and (c) 10 mEq/liter NaCl. All three contained glucose at a concentration of 5.5 mM. The chemical activity of water was kept constant by maintaining all perfusion fluids approximately isosmotic (300 mOsm/kg H_2O) with plasma by the addition of the appropriate amounts of mannitol. C^{14} carboxylic acid–labeled inulin (New England Nuclear Corp., Boston) at a specific activity in excess of 2.4 μc/mg, serving as a reference substance for fluid movement, was also added to the perfusion fluids. Samples of injected and collected perfusion fluid were counted in a Packard liquid scintillation counter as previously described (5). All samples contained activities of at least twice background and were counted to a total of 10,000 counts. The standard error of the mean as calculated from 10 replicate samples (volume ranging from 1.2 to 1.7 $m\mu l$, radioactivity 3 $\times$ background) was 4 per cent. In some experiments, analyses for mannitol concentrations have been made similarly, using C^{14} mannitol (California Corporation for Biochemical Research, Inc., Los Angeles). Sodium was measured in some samples of collected perfusate by ultramicroflame photometry (23).

Recently, an alternative technique for measuring transtubular fluid movement has become available (22) in which a fluid column is injected between colored oil droplets and its rate of disappearance *in vivo* is recorded photographically. From the half-time of disappearance the rate of transtubular fluid and sodium movement can be calculated. In the present study reabsorptive fluid movement was studied under control conditions using a solution of 145 mEq/liter NaCl as the injected fluid, and under conditions where the colloid-osmotic pressure gradient had been altered by addition of sufficient amounts of albumin to the perfusion fluid to reach concentrations of 2.5 or 5.0 gm per cent. Individual tubules were punctured with a double-barrelled injection pipette and filled with either silicone oil (Dow-Corning 702 fluid) or castor oil colored with Sudan black. This column of oil was subsequently split by the injection of the perfusion fluid, and the isolated fluid segment displaced distally by the injection of another small column of oil. The distance between the oil menisci bordering the fluid segment was photographed sequentially at either 2 or 5 second intervals with a Nikon F automatic reflex camera fitted with a Nikon electric motor drive (EM 10). These were mounted on the Nikon microflex attachment (NF 804) and fastened to one ocular of a Leitz dissecting microscope ($\times$ 100). As light source a Knisely quartz rod illuminator (24) was combined with a synchronized electronic flash light unit (Foto-Flash interval controller, Branch and Harrell, Grand Prairie, Texas). This unit includes a power supply for the strobe light, a source providing synchronizing pulses to the camera motor drive, and timers controlling both the frequency with which the strobe light fires and the total number of flashes in a single run. The enlarged image of the tubular segment was traced, and the area between the bordering oil droplets measured planimetrically and plotted as a function of time.

In agreement with the results obtained by Gertz (22), it was found that the residual fluid volume decreased logarithmically as a function of time. From the half-time and the radius, sodium transfer was calculated according to an expression given by Gertz:

$$0.521 \, \frac{r}{t_{1/2}} \, \frac{\mu Eq \, Na}{mm^2 \, sec.}$$

where r is the radius of the tubular lumen in millimeters, $t_{1/2}$ the half-time in seconds,

and the concentration of sodium in the tubular reabsorbate is taken as 150 mEq/liter. This calculation rests upon the assumption that a full cylinder of fluid is exposed to the reabsorbing tubular area. Since, however, a convex meniscus of oil of inconstant shape extends into the fluid segment an error is introduced leading to overestimation
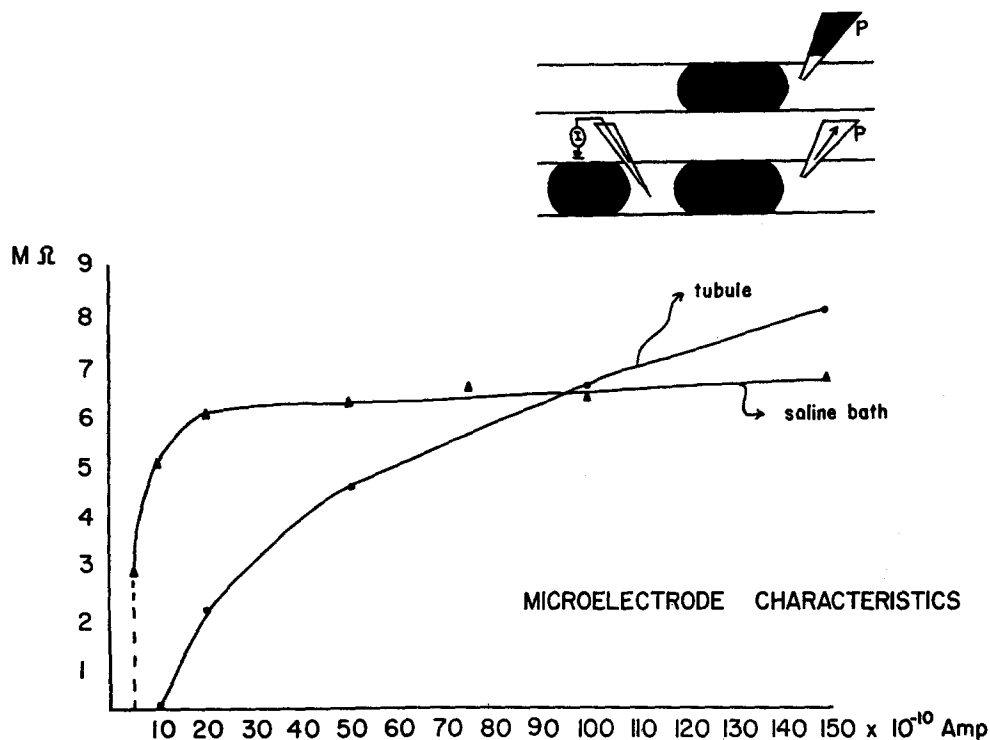

FIGURE 1.   Representative measurements of short-circuit current using single intra tubular microelectrode. Insert shows method of impalement and isolation of small fluid sample. Colored mineral oil was injected through one micropipette inserted into the lumen of a superficial proximal tubule. Subsequently, a small volume of tubular fluid was aspirated and reinjected, being isolated from the rest of the tubular contents by further oil injection. The position of the oil-fluid-oil assembly can be stabilized by suitable upstream aspiration of tubular fluid through the right-hand pipette (*P*).

of the net flux of fluid and sodium. This error becomes relatively more important the shorter the distance between the separating oil droplets. No attempt was made to correct for this error. Gertz (22) had drawn attention to the fact that fluid and sodium reabsorption calculated in this way may be too high by as much as 20 per cent. On the other hand, volume changes estimated by analysis of C^{14} inulin, as used in some of our experiments, are devoid of this error.

RESULTS

Measurements of Short-Circuit Current

A representative experiment of a short-circuit measurement using the technique of Karger (13, 14, 20) is shown in Fig. 1. Microelectrode resistance is

a function of current flow through the electrode tip (20, 25). Accordingly, resistance was determined at several values of electrode current with the tip outside the tubule lumen. These measurements were repeated with the tip inside the lumen. The intersection of the two characteristic lines so obtained indicates the particular current which renders the electrical resistance of the probing microelectrode within the tubular lumen identical to that of the same microelectrode when it is outside the nephron. In the specific instance shown in Fig. 1 a short-circuit current of 97×10^{-10} amp is indicated.
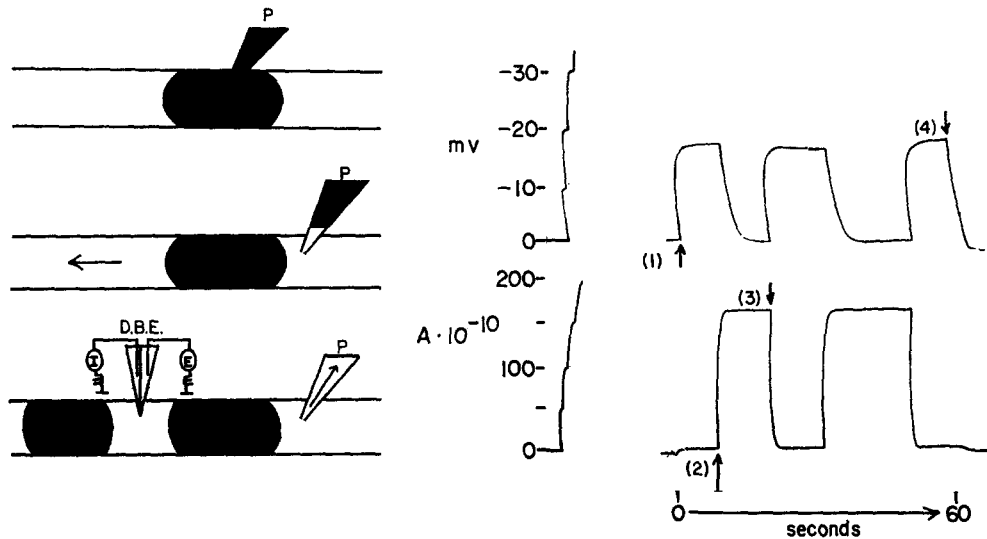

FIGURE 2. Representative measurement of short-circuit current using double-barrelled intratubular microelectrode (D.B.E.). Insert shows method of impalement and isolation of small fluid sample. Details of technique are illustrated in the insert and are essentially similar to those described in Fig. 1. The numbered arrows indicate: (1) Tubular impalement by double-barrelled microelectrode; (2) Short-circuit current on; (3) Short-circuit current off; (4) Withdrawal of double-barrelled microelectrode.

In order to relate the short-circuit current to the area of tubular wall involved, measurements were performed on a segment isolated from the rest of tubular fluid by means of colored mineral oil as indicated schematically in the upper right-hand corner of Fig. 1. This was accomplished by splitting an oil column by the injection of previously collected tubular fluid. The same approach was used when double-barrelled microelectrodes were employed for the measurement of short-circuit current. A representative experiment is shown in Fig. 2. Current is applied through one barrel and the transtubular potential difference recorded by means of the second. Since the current is applied from a point source and the tubular wall constitutes a structure of high ionic conductance, it is unlikely that a constant density of the applied

current obtains over an extended length of tubular segment. Therefore, short-circuit current measurements were made on fluid segments of different length to relate the current to the area involved in the measurements. Tubular area was estimated from optical measurements of the individual tubular diameters and the extent of the exposed tubular wall. Since fluid reabsorption continues after deposition of the fluid sample, special care was taken to coordinate the time of the current measurement exactly with that of the estimation of the tubular area involved. This is accomplished more easily with double-barrelled electrodes. Only one measurement is needed with this method in contrast to the several individual resistance determinations necessary to establish the electrode characteristics in Karger's method.
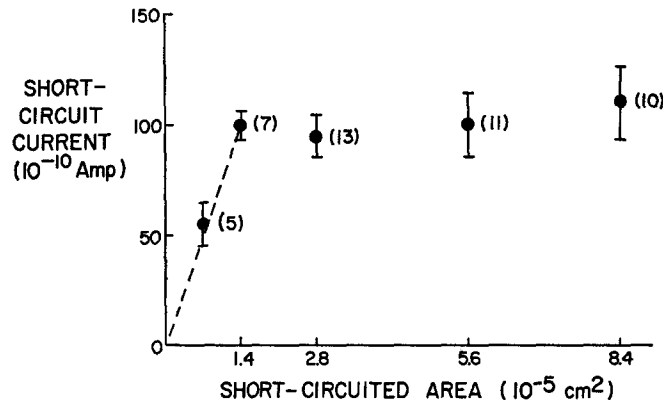

FIGURE 3. Short-circuit current as function of tubular surface area. Means and standard errors are presented. Numbers in parentheses indicate number of observations.

Fig. 3 shows the relationship between short-circuit current and tubular area. Short-circuit current per unit area was found to be constant only when tubular segments of less than $1.4 \text{ cm}^2 \times 10^{-5}$ were isolated. This is indicated by the fact that the first two current values lie on a straight line passing through the origin. This was found to be true in spite of the fact that, when the oil droplets are in close proximity, the measurement of tubular area presents some difficulties. Quantitatively similar results have been obtained in amphibian nephrons by Eigler (20). The transtubular potential differences averaged -18 ± 1.0 mv (mean $\pm$SE, 49 observations) and agree well with the results obtained by others (8, 26, 27). The average short-circuit current ($\pm$SE) as measured in a series of experiments by means of both Karger's method and that of Ussing and Zerahn using double-barrelled electrodes was $73 \pm 6.9 \times 10^{-5}$ amp/cm². This value corresponds to 75×10^{-10} Eq/cm² sec. of monovalent, positively charged ions moving out of the tubular lumen into the peritubular fluid or to an equivalent number of anions moving in the

opposite direction. Since sodium constitutes the bulk of the cations reabsorbed, it is obvious that the short-circuit current largely reflects the outward movement of this ion.

Microperfusion Experiments on Single Proximal Tubules

A series of 19 microperfusion experiments was carried out in which tubules were initially filled with solutions containing various concentrations of sodium, mannitol being added to maintain isosmolarity with plasma. In such a way the relationship between the transtubular sodium concentration gradient and net movement of tubular fluid and sodium can be obtained. All samples were collected 30 seconds after intratubular deposition of the perfusion fluid. At a sodium concentration of 145 mEq/liter, the C^{14} inulin concentration ratio of collected perfusate over injected perfusion fluid averaged 4.4. When the initial intratubular sodium concentration was 100 mEq/liter a ratio of 1.43 was obtained, whereas net entry of fluid into the tubular lumen was indicated by the mean C^{14} inulin concentration ratio of 0.54 when the initial sodium concentration was only 10 mEq/liter. Two additional pieces of information are necessary to correlate the fluid movement associated with sodium transfer to the concentration gradient of this ion. First, at the low initial concentration of sodium (10 mEq/liter) it is to be expected that the sodium concentration of the injected perfusion fluid will change as a function of time as additional fluid enters the lumen. Thus, it appears desirable to correlate the volume changes as a function of the mean, instead of the initial, intratubular sodium concentration, since this is a more appropriate parameter of the chemical driving force for sodium entry in determining the transtubular concentration gradient. Secondly, since mannitol was also present in the perfusion fluid, the increase in inulin concentration of the perfusate cannot solely be ascribed to the effect of intratubular sodium concentration, and must be corrected for water movement secondary to mannitol efflux. The contribution of other osmotically active solutes such as urea and glucose is negligible since proximal fluid transfer proceeds without the establishment of osmotic pressure differences. The fractional contribution of these solutes to the total osmolarity of the reabsorbate does not exceed 3 per cent.

In order to obtain a value for mean sodium concentration when the initial sodium concentration of the perfusion fluid was 10 mEq/liter, a series of 13 perfusions, summarized in Fig. 4, was done in which samples were collected after varying time intervals and the sodium concentration was measured by ultramicroflame photometry. It can be seen that the concentration of sodium rises as a function of time, and after 30 seconds approaches a value of 80 mEq/liter. A mean effective concentration of 60 mEq/liter of sodium is obtained by graphical methods. In other perfusions where time was prolonged, the sodium concentration in the perfusate appeared to approach a steady-state value of 95 mEq/liter.

A single exponential curve was fitted to the data of Fig. 4 in the following manner: For each of the data depicted in Fig. 4, $(Na)_\infty - (Na)_t$ was calculated, where $(Na)_\infty$ is the steady-state value of 95 mEq/liter, and $(Na)_t$ is the concentration determined at any time t. The logarithm of $[(Na)_\infty - (Na)_t]$ was then plotted against time, and a straight line fitted by eye to these data. By a retransformation from this straight line, an exponential curve was fitted to the experimental points of Fig. 4 from 5 seconds onward (solid curve) and extrapolated back to zero time (dotted curve). The zero-time intercept on the vertical axis is 23 mEq/liter, whereas the Na concentration actually established at zero time was 10 mEq/liter. This discrepancy suggests that the
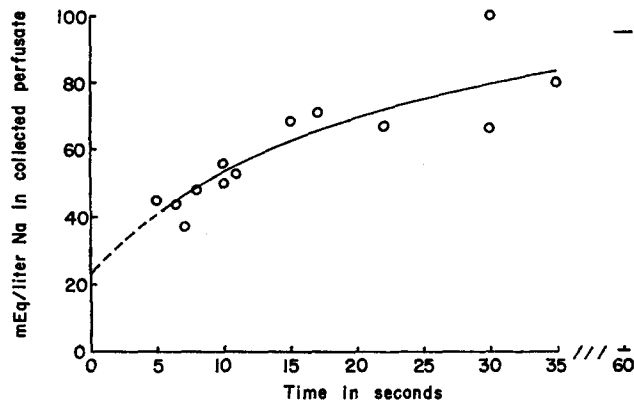

Figure 4.   Sodium concentration in collected perfusate plotted against duration of tubular perfusion. The exponential curve has been fitted to the points by the method described in the text.

over-all time course of the change in sodium concentration in the perfusate cannot be fitted by a single exponential, at least not during the first 5 seconds, and hence apparently does not obey simple first order kinetics throughout. The absence of simple first order kinetics may be related to the fact that the inward diffusion of sodium chloride is counteracted by active transport of sodium ions out of the lumen. It is possible that during the initial period the relative contribution of the sodium transport mechanism is small, and increases as the intratubular concentration of sodium rises. An exact quantitation of these processes appears to be difficult in the absence of information on such parameters as the transtubular electrical profile, intracellular sodium concentration, the sodium permeability of the luminal cell boundary as a function of intraluminal concentration, and possible active movement of sodium across this cell border. In contrast to the increase in concentration of sodium observed when the initial concentration was 10 mEq/liter, no significant change was observed in experiments in which a solution with 100 mEq/liter of sodium was placed into the tubular lumen (6 perfusions, time: 6 to 30 seconds, mean: 102 mEq/liter, range: 92 to 110 mEq/liter).

Earlier studies on proximal tubules of *Necturus* kidneys have demonstrated that the only significant driving force for net water flux arises from the net transport of NaCl. Such dependence of water upon sodium movement was found over a range of perfusion fluid concentration from 15 to 100 mEq/liter sodium (plasma sodium concentration 100 mEq/liter) (28). In such studies, the osmotic pressure of the collected perfusate was found to be equal to that of plasma.

That a similar situation obtains in the rat proximal tubule is indicated by the isosmotic character of fluid reabsorption during strong osmotic diuresis in the rat (3, 10). In this situation, the intratubular sodium concentration falls to values as low as 90 mEq/liter (5). Although osmotic pressure was not measured in the present study, the data cited above strongly suggest that isosmotic transtubular movement also occurs at initial Na concentrations in perfusion fluids below 90 mEq/liter.

To correct the data on inulin concentration for water movement secondary to mannitol flux, a separate series of 9 experiments was performed under the same experimental conditions (collection periods lasting 30 seconds), except that C^{14} mannitol was measured rather than C^{14} inulin. The ratios of concentrations of C^{14} mannitol between collected perfusate and injected perfusion fluid averaged 0.31 (4 observations, range: 0.17 to 0.49) at 10 mEq/liter intratubular sodium, and 0.84 (5 observations, range: 0.63 to 0.94) at a sodium concentration of 100 mEq/liter. Calculation of the net water movement which may be ascribed to transfer of sodium chloride, after correction for mannitol-induced transfer (28), shows that the net movement of sodium chloride out of the tubule can take place against a considerable concentration gradient. Fig. 5 shows that absorptive net transfer ceases between a solution containing 95 mEq/liter sodium and one containing sodium at a concentration of 145 mEq/liter (*i.e.* plasma). When the concentration gradient exceeds 50 mEq/liter, the direction of water movement, ascribed to sodium transfer, is reversed and water enters the tubule. At an intratubular sodium concentration of 145 mEq/liter, fluid movement amounted to 77 per cent of the injected volume. Since proximal tubular fluid movement is isosmotic in nature (*i.e.* proceeds without establishment of an osmotic pressure difference), this value corresponds to a mean net sodium reabsorption of 52×10^{-10} Eq/cm^2 sec.

Microperfusion experiments in which the rate of volume reduction of injected fluid was recorded by time-sequence photography (22) were designed to test specifically the effect of alterations in the normal colloid-osmotic pressure gradient across the proximal tubular wall. Fig. 6 shows the results of a representative series of experiments in which the volume change of isosmotic NaCl solutions without, and with 2.5 and 5 per cent serum albumin present was plotted semilogarithmically as a function of time elapsed after injection of the perfusion fluid. It is evident that the half-time of disappearance is

similar in the 3 solutions, in this instance about 9 seconds. Since tubular protein reabsorption is too small to be of significance in the concentration ranges used (29, 30), it should be emphasized that, due to continuous fluid reabsorption, the intratubular albumin concentration increases as a function of time. This will actually lead to a reversal of the direction of the normally prevailing colloid-osmotic gradient. In a number of experiments in which protein was
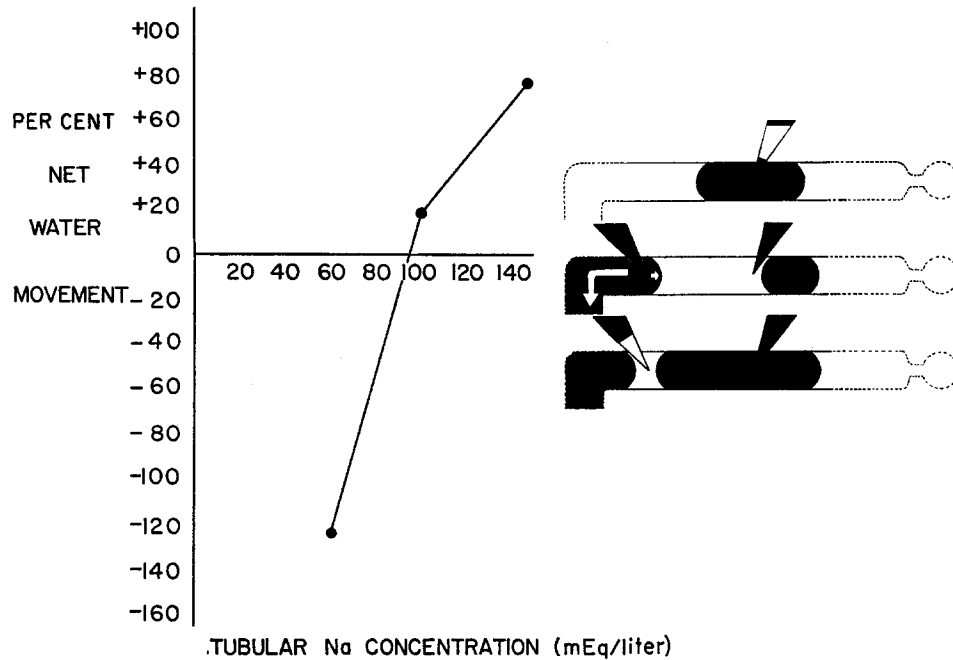

Figure 5. Fractional net water movement as a function of mean intratubular Na concentration. Perfusion time: 30 seconds. Insert shows method of isolation of fluid column and of collection of perfusate. Colored mineral oil is injected into a proximal tubule through a pipette and split by injection of perfusion fluid. The position of the oil-fluid-oil assembly is stabilized by downstream injection of mineral oil. Collection is accomplished by injection of additional oil through the proximally located micropipette and by simultaneous aspiration of perfusion fluid through the more distally located micropipette: +, out of tubular lumen; −, into tubular lumen.

present we observed a retardation of transtubular fluid absorption when 2 to 3 half-times had elapsed. Since under these conditions the separating oil droplets had reached close proximity, it was felt that a quantitative estimate of fluid reabsorption had become unreliable. Also, albumin must have been concentrated to such a degree that transtubular fluid movement was further influenced by the high viscosity of the perfusate. A total of 109 perfusion experiments was performed and a frequency distribution of the initial half-times is presented in Fig. 7. The mean half-time of 57 perfusion experiments with

isosmotic sodium chloride solution was 8.7 seconds, a value quite similar to that of 9.8 seconds reported by Gertz (22).

The observed half-time corresponds to a mean net flux of sodium of 90 × 10^{-10} Eq/cm² sec., a value in agreement with that of 82 × 10^{-10} Eq Na/cm² sec. calculated by Gertz (22) using the same methods for assessing transtubular sodium movement. It is also quite similar to the value arrived at by Thurau
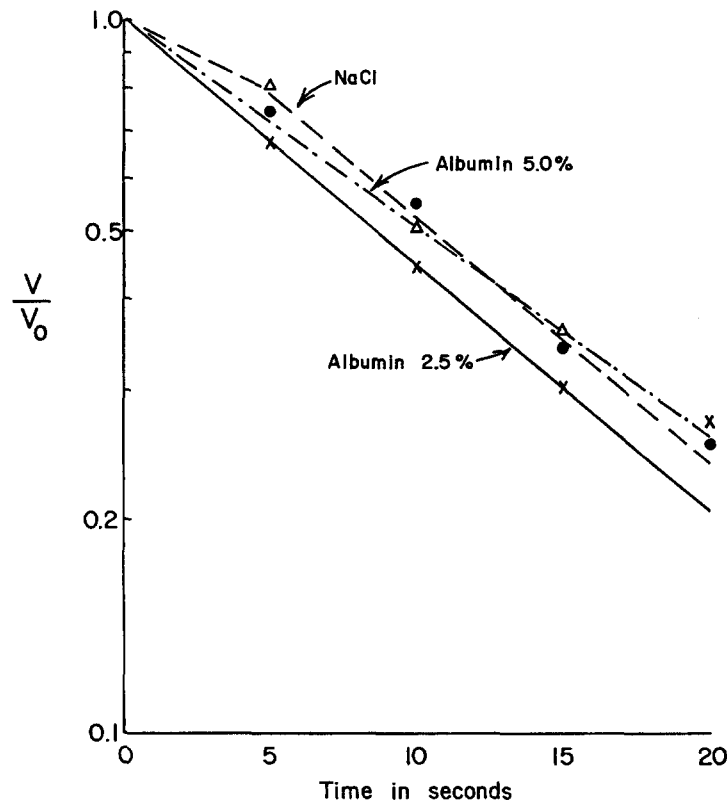

FIGURE 6. Time course of volume changes of fluid drop injected into proximal tubules of rat. Initial albumin concentration in drop from 0 to 5 gm/100 ml.

and Deetjen, using a cinematographic method (31). On the other hand, it is significantly higher ($p < 0.001$) than our value for sodium transfer as calculated from the increase in C^{14} inulin concentration (52 × 10^{-10} Eq Na/cm² sec.). A number of technical considerations, characteristic for each method, are pertinent. As mentioned earlier, the time-sequence photography technique tends to overestimate the flux of fluid and of sodium due to the assumed constancy of the surface over volume ratio. On the other hand, two factors may tend to reduce the apparent fluxes when using C^{14} inulin as an index for fluid transfer. With the relatively larger fluid segments used, the decrease in volume
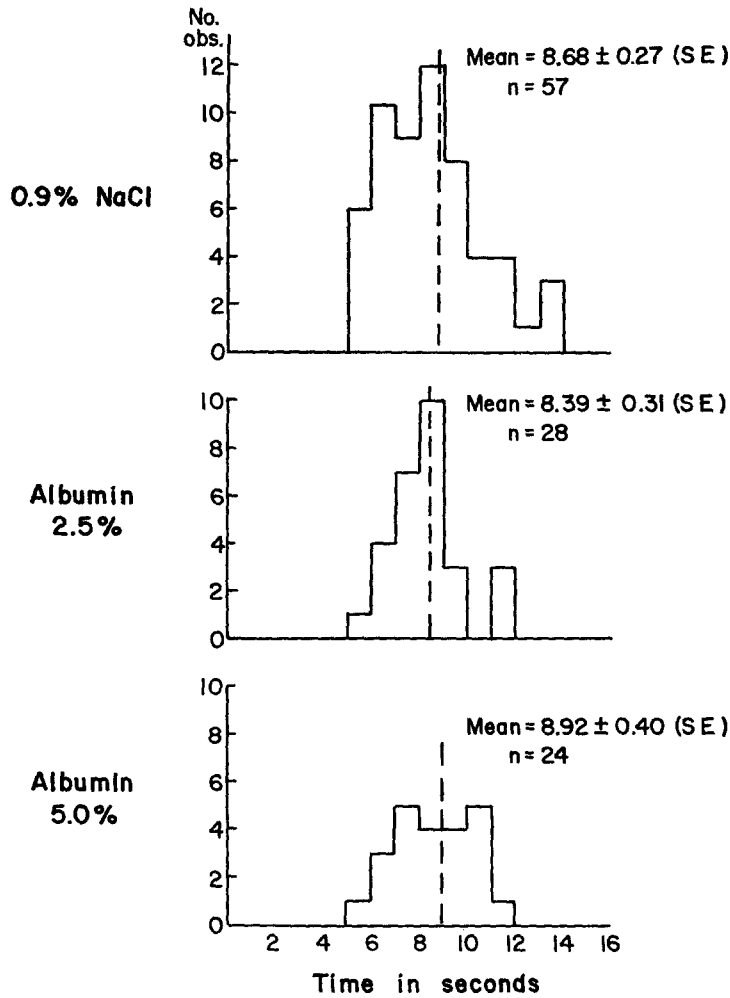
FIGURE 7.   Frequency distribution of initial half-times of volume changes.

may be retarded to a greater degree by the friction between oil columns and tubular wall. Furthermore, the volume reduction under these conditions not only may take place in the longitudinal axis of the fluid segment but also may involve a decrease in the tubular diameter. Both these factors would tend to reduce the calculated net flux of sodium and water.

Mean values of the half-time of disappearance for the protein-containing solutions were: 8.4 seconds (initial albumin concentration 2.5 gm/100 ml), and 8.9 seconds (initial albumin concentration 5.0 gm/100 ml). These values are not significantly different from the half-time of disappareance of fluid in the control experiments in which isotonic sodium chloride alone was used (2.5 gm/100 ml albumin: $0.5 > p > 0.4$; 5.0 gm/100 ml albumin: $0.9 > p > 0.8$).

DISCUSSION

Comparison of the net movement of charged particles as estimated from short-circuit current with the calculated reabsorptive transfer of sodium ions indicates that this latter process is largely active in nature. This conclusion follows from the well established facts that the proximal tubular reabsorbate is isotonic (9, 10), that the sodium ion constitutes the bulk of the osmotically active cationic solutes, and that the direction of the short-circuit current corresponds to resorptive movement of sodium. It should be noted that the short-circuit current is a non-specific measure of the active transfer of ions under conditions where tubular and peritubular solutions are the same, and the contribution of charged particles other than sodium must be considered. A contribution to the short-circuit current could be made either by the resorptive movement of cations or by movement of anions in the opposite direction. As far as other intratubular cations are concerned, recent evidence indicates that K ions are actively transported across the tubular wall (23, 32, 33). Considering the relative concentrations of sodium and potassium in the luminal fluid, it appears that potassium transfer cannot amount to more than about 3 per cent of that of sodium. The contribution of other ion species whose transfer is active, such as possibly hydrogen ions, calcium (34), and phosphate (35) must be of a similarly small magnitude. It is more difficult to assess any possible contribution of the movement of chloride ions to the measured short-circuit current. Two considerations are relevant in this respect. First, it has recently been suggested by Ullrich and Marsh (32) on the basis of measurements of the electrochemical potential difference of chloride made by Kashgarian et al. (8) that the reabsorption of chloride is accomplished against a component of active movement of this ion into the lumen. Such active entry of chloride would increase the short-circuit current above that to be expected from active transport of sodium ions alone. Secondly, it has been observed that the chloride concentration in proximal tubular fluid exceeds that in plasma (1, 3, 8, 36). Thus, a chemical driving force for chloride movement out of the tubular lumen might still be present under short-circuit conditions. Such movement of Cl would reduce the measured net transfer of charge. Presently, there is no information in mammalian nephrons permitting a quantitative assessment of these two opposing factors. On the other hand, in perfused proximal tubules of Necturus no change in the short-circuit current was observed when chloride was replaced by sulfate (20). Therefore, it appears unlikely that in Necturus the chloride ion movement contributes to the measured current.

Finally, the rate of net sodium transport would be expected to be greater in the presence of the zero electrical potential gradient obtaining during short-circuit conditions than when a finite gradient has to be overcome. It has been shown in a number of epithelial structures that the rate of active transport

of sodium ions depends on the size of the sodium pool in the cells (37). This in turn will be affected by the electrical driving force for passive movement of sodium ions from luminal fluid into the cells. The magnitude of the difference between short-circuit current and net movement of sodium under these two conditions must depend upon the true electromotive force of the sodium pump and the lumped conductance of all passively transferred ions. These data are at present not available with regard to the proximal tubule of the rat kidney.

Values for the mean net movement of sodium as calculated from the three series of experiments, 52×10^{-10} (Eq/cm²)/sec. (C^{14} inulin method), 92×10^{-10} (Eq/cm²)/sec. (time-sequence photography), and 75×10^{-10} (Eq/cm²)/sec. (short-circuit current method), show reasonable agreement. These values are higher than those previously reported by us on the basis of a smaller group of experiments (7). However, in view of some of the uncertainties just discussed this agreement must not be taken as absolute proof that all of the sodium movement out of the tubular lumen is active in nature. Some passive reabsorptive movement of sodium chloride could conceivably be attributed to the colloid-osmotic pressure difference which normally exists across the proximal tubular wall. To investigate this possibility directly the colloid-osmotic pressure gradient was diminished or even reversed. The absence of a noticeable effect upon net fluid and sodium movement demonstrates that fluid and sodium reabsorption must be attributed to other forces than the protein osmotic pressure.

A similar conclusion has been reached by F. C. Rector, Jr. (personal communication) who observed that the initial slope of the disappearance of an isotonic NaCl solution was quite similar after addition of 5 gm per cent of albumin. Our conclusion is also in agreement with that of Ullrich and Rumrich (38), who, from calculated data of proximal tubular water permeability of rat kidneys, assigned no important role to the colloid-osmotic pressure gradient with respect to fluid reabsorption. Finally, the same general conclusion has been reached by Whittembury *et al.* (39) and by Eigler (20) from experiments carried out on proximal tubules of *Necturus*.

Perfusion experiments in which the sodium concentration was varied but the chemical activity of water kept constant by the addition of mannitol provide evidence for the close coupling between proximal sodium and water movement. They have also aided in assessing the limiting concentration difference against which net reabsorption of sodium chloride can take place. Our results indicate that the net flux of sodium approaches zero when a concentration ratio of 0.66 in perfusate over plasma has been reached. They also show an approximately linear relationship between net fluid movement and intratubular sodium concentration, a situation closely similar to that found in *Necturus* kidneys (28). These results also agree with those of Kashgarian *et al.* (8), who reported a limiting sodium concentration ratio of 0.7 under condi-

tions when net transtubular sodium fluxes had been reduced almost to zero by the intratubular injection of raffinose. Since the luminal fluid remains negative in the presence of lowered intratubular sodium concentrations (8, 28), the net absorption of sodium takes place against an electrochemical gradient and thus qualifies as an active process.

Experiments in which the transtubular sodium concentration gradient was measured during strong mannitol diuresis have shown that a limiting gradient of similar magnitude can be reached even under free flow conditions (5). This observation confirms the previously expressed thesis that little further sodium and water movement out of the lumen occurs in the pars recta of the proximal tubule during strong mannitol diuresis. The similarity of these two gradients also minimizes the possibility that the increased flow rate of tubular fluid during osmotic diuresis may contribute to the reduction in proximal tubular water reabsorption.

Within this context it might be of interest to compare the rate of proximal fluid reabsorption under free flow conditions with that under conditions of stopped flow in the absence of non-reabsorbable solutes. Assuming normal values for glomerular filtration rate in rats (6 ml/minute per kg body weight (40)), and number of glomeruli (61,600 in a 240 gm rat (41)), a filtration rate per glomerulus per second of 4×10^{-7} cm^3 is obtained (42). Taking a fractional proximal tubular reabsorption of 80 per cent, a tubular radius of 20 μ under free flow conditions (43), and a mean length of proximal tubules in rat kidneys of 10 mm, one arrives at a mean rate of sodium reabsorption of 76×10^{-10} Eq/cm^2 sec. This is in reasonable agreement with the values obtained under stopped flow conditions in the present study.

Concerning the details of sodium movement across the individual boundaries of the proximal tubule cell a number of comments seem pertinent. First, it appears most likely that the electrical profile across the proximal tubular epithelium is qualitatively similar to that of the Necturus tubule (44, 45). Thus, the cell interior would be asymmetrically negative with respect to the luminal and peritubular fluid, and would also contain sodium at a concentration significantly below that of either the tubular or interstitial fluid. Net movement of sodium from the cell interior to the peritubular fluid would therefore take place against an electrochemical potential gradient. Thus, the sodium extrusion mechanism must be located within the peritubular cell boundary (44, 45). The situation appears to be more complex with respect to sodium entry at the luminal cell boundary where the direction of the electrochemical potential gradient would favor diffusion of Na into the cell. Exchange diffusion or active extrusion of Na into the tubular lumen had to be invoked at this site to account for the relationship between the unidirectional flux ratio and the estimated electrochemical potential gradient (46, 47). Similar conclusions have been reached by Marsh et al. (32). In this context attention

should be drawn to the demonstration of a mechanism for sodium entry on the mucosal boundary of the toad bladder which, although passive, involves some kind of interaction of sodium ions with the mucosal membrane. This thesis is based upon the observation that beyond a given concentration in the medium neither sodium transport nor the sodium content of the tissue increased (37). Since it is difficult to demonstrate saturation kinetics for sodium transport in the proximal tubule of the rat during hypertonic NaCl loading (48, 49), it is doubtful whether any interaction of the luminal membrane ever becomes rate-limiting for transepithelial net movement of sodium.

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