Effect of Protein in Tubular Fluid upon Proximal Tubular Absorption.* (29716)

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Estimates of water permeability in the proximal tubule of the rat have suggested that hydrostatic or colloid-osmotic pressure gradients can have only minor effects upon the movement of fluid across the proximal tubule(1). In the proximal tubule of Necturus reabsorption continues despite a reversal in the usual oncotic pressure gradient(2), and in the rat kidney, perfusion of single proximal tubules with fluid initially containing 2.5 and 5.0 g% albumin is said to have no noticeable effect upon fluid movement(3). The continued reabsorption of fluid in kidneys with severe tubular necrosis or in the presence of metabolic poisons, on the other hand, has been attributed to the colloid-osmotic pressure of the serum proteins(4).

In the present study the reabsorption of an isolated droplet of isotonic saline in the proximal tubule was compared to the reabsorption of diluted serum in order to determine the effect of colloid osmotic pressure on reabsorption.

Methods. Male white rats weighing 150-250 g fed a standard laboratory diet and permitted to drink water *ad lib* were anesthetized with Inactin. The left kidney was isolated through a left upper quadrant transverse incision, and the capsule and perinephric fat removed. The kidney was immobilized in a plexiglas boat using the method described by Wirz(5) and the rat's body temperature was maintained using a heated operating table. As previously described by Gertz(6), a double-lumen capillary pipette with a beveled tip was used for the micropuncture. One lumen was filled with castor oil colored with Sudan Black and the other lumen with the fluid being studied. Visualizing the kidney with a dissecting microscope, the tip of the pipette was inserted into a proximal tubule and the oil-Sudan Black mixture injected into the tubule. The oil column was then broken by injection of the test solution from the other barrel of the pipette. A second injection of oil to seal off the tip of the pipette was then made and photographs were taken through the microscope using a Robot Star II automatic camera with an intervalometer which allowed sequential photographs at the rate of one every 5 seconds. The time lapse between injection of fluid and the first picture was measured with a stop watch.

The reabsorption of 2 different solutions from the proximal tubule was studied. The first was a solution of isotonic saline containing 152 mEq/l of sodium chloride; the second a 50% dilution of rat serum with isotonic saline. From enlarged photomicrographs, the linear decrease between the 2 columns of colored oil was measured, and the percentage decrease was plotted against time on semi-log paper taking into consideration the time lapse before the first picture. When time elapsed from the injection of fluid to the first picture was less than 5 seconds, the first picture was taken as the initial volume. When this time interval was greater than 5 seconds, the initial volume was obtained by extrapolation.

Results. The average half-time for the resorption of a series of 15 saline perfusions (152 mEq/l) was 10.8 $\pm$ 1.8 seconds (mean $\pm$ standard error). Reabsorption of the saline solution was complete. Plotting the volume at time t over the volume at t_0 (V/V_0) against time on semi-log paper (Fig.

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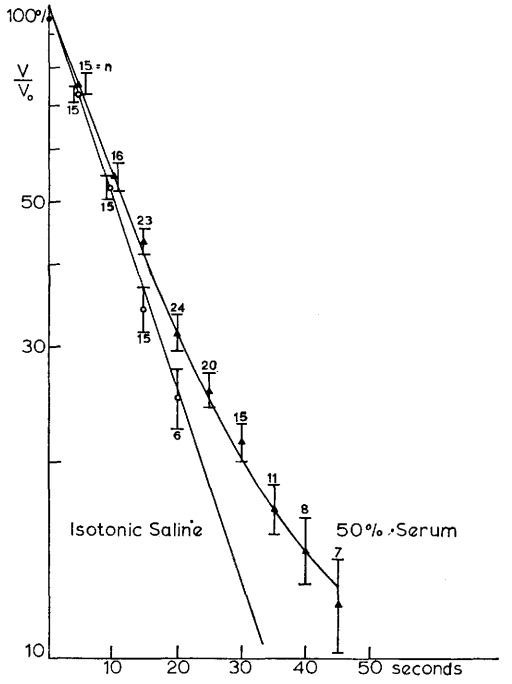

FIG. 1. Reabsorption of proximal tubular perfusate; relative volume change plotted as a function of time.

1), the curve approximated that of a straight line. This indicates that the saline solution was reabsorbed at a constant rate per unit area of tubular lumen. Using the formula $\phi = .347\ r/t_{1/2}(6)$, where ϕ equals volume resorbed per unit area of tubular surface per second, $t_{1/2}$ equals the half-time for resorption and r equals the radius of the tubule (average 15 μ), ϕ is found to equal 4.91 $\pm$ 0.96 10^{-4}mm^3/mm^2/sec for isotonic saline.

Using 50% serum, the average half-time for reabsorption was 12.0 $\pm$ 1.4 seconds. Curves comparing the reabsorption of the test solutions are shown in Fig. 1. When 50% serum was studied, the droplet was not completely reabsorbed during the time of observation.

TABLE I. Net Efflux of Sodium Chloride from Proximal Tubule.
(10^{-4} mm^3/mm^2 sec^{-1})

Time interval (sec)	Isotonic saline	50% Serum in saline
0-10	4.91 $\pm$ .25* (N = 15)	4.71 $\pm$ .37 (N = 14)
10-20		4.99 $\pm$ .83 (N = 8)
20-30		3.42 $\pm$ .40 (N = 10)
30-40		3.25 $\pm$ .41 (N = 6)

* Values are mean $\pm$ S.E.

The value $\phi = 4.91 \pm 0.96\ 10^{-4}$mm^3/mm^2/sec obtains at any time during the resorption of isotonic saline from t = 0 to complete resorption. However, ϕ was constant only up to 20 seconds when the protein solution was resorbed. From that time on the reabsorption rate gradually decreased. The formula $\phi = \frac{r}{2} - \frac{\ln V_2/V_1}{t}(6)$ allows us to calculate the average rate of resorption of a solution during a small interval of time. ϕ for 50% serum has been calculated over various time intervals where V_1 equals the volume at the beginning and V_2 the volume at the end of each interval, and r is tubular radius (15 μ). Table I gives the results of such calculations and shows that the rate of reabsorption of 50% serum does not differ significantly during the first 20 seconds from the rate calculated for saline. By 20-30 seconds (when the volume of the droplet is 1/4 to 1/8 its initial volume), with 50% serum-saline as the initial perfusate, the intratubular concentration of protein is probably greater than that of plasma. After this time the reabsorptive rate is significantly less than with saline alone.

If no protein is reabsorbed from the tubular lumen and if the further simplifying assumption is made that the only force retarding outward flux of water, when 50% serum is placed in the tubule, is the colloid osmotic pressure of the protein in the droplet, which opposes the colloid osmotic pressure of the protein in serum, then, $\phi_t = \phi_{Na} + \phi_{OP} - \phi_{OP}\frac{V_o}{2V_t}$, where ϕ_t equals net outward flux at time t, ϕ_{Na} equals net outward flux due to active reabsorption of sodium, and ϕ_{OP} equals net outward flux due to serum oncotic pressure. When $\phi_t = 0$, ϕ_{OP} can be estimated as $\frac{\phi_{Na} \cdot 2V_t}{V_o - 2V_t}$. By plotting ϕ_t against the observed volume at each time interval and extrapolat-

ing to $\phi_t = 0$, an estimate of V_t of 1/16 V_o is achieved. This corresponds to a protein concentration of eight times plasma when flux is zero. Under these circumstances, $\phi_{OP} = \phi_{Na}/7$. If, therefore, the oncotic effect of plasma proteins has any influence upon the reabsorption of glomerular filtrate in the proximal tubule, it is calculated to be approximately 1/7 or 14% of that exerted by active sodium transport. Since the possibility of a change in potential due to the Donnan effect and the deviation from ideality of concentrated protein solutions have been ignored in this treatment, the calculated limiting value is likely to be an overestimate of the effect of oncotic pressure.

Discussion. The validity of the method used here has been discussed in detail elsewhere(6). The calculated net fluxes are only estimates of the true flux since they are based on a measured tubular diameter which ignores the contribution of the cellular brush border to surface area. Gertz(7) has used this method to determine the effect of various substances on tubular reabsorption. He found 30% inhibition of net efflux of isotonic saline with 2-4 DNP and hygroton, demonstrating that tubular reabsorption was diminished by inhibitors of active sodium transport. Complete stoppage of tubular reabsorption has not been shown using metabolic inhibitors.

The present study has demonstrated that tubular reabsorption continues at a rate very close to normal when the transtubular oncotic gradient has been reduced by an initial intraluminal protein concentration equal to that of 50% plasma. At a time when luminal protein concentration was probably much greater than plasma, inhibition of tubular reabsorption was observed to the point where efflux appeared to stop. Using the assumption that protein was not reabsorbed at all, protein concentration at this point was calculated to be roughly 8 × plasma concentration. It seems likely that the late inhibition of tubular reabsorption with 50% serum is related to a substantial increase of intraluminal colloid osmotic pressure and its related Donnan effect. A direct effect of protein on active outward transport of sodium or cellular permeability cannot, however, be ruled out. The effect of intratubular oncotic pressure on reabsorption in animals whose tubular epithelium has been damaged is yet to be studied.

Summary. Evidence has been presented which demonstrates that protein placed in proximal tubular fluid in a concentration similar to that of serum exerts little influence on tubular reabsorption in the normal rat. The results are consistent with the thesis that active sodium transport and its concomitant reabsorption of water are the main forces in proximal tubular reabsorption.

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