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Received March 2, 1959. P.S.E.B.M., 1959, v101.

A Method for Investigating Net Water Fluxes Across Individual Proximal Tubules.* (24889)

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In attempting to evaluate possible mechanisms involved in transport of materials by the kidney, it would be useful to know the kinetics of the transport processes. Experiments estimating rates of water movement across the tubules of Necturus have been carried out by Schatzmann *et al.*(1), using a perfusion system. It is the purpose of this report to describe a simple method for determining net water fluxes across tubules of rat kidneys.

Methods. Micropipettes were drawn with a Brinkman Micro-puller. They were half filled with a test solution by gentle heating of the broad end and plunging the still hot pipette into a beaker containing the test solution. As the air within the pipette cooled and contracted, the solution was drawn into the pipette. The pipette was then mounted in the pipette holder of an Aloe microinjection apparatus. Oil was then forced in the open end of the pipette, expelling the remaining air through the micro-orifice, thereby filling the remainder of the pipette. The microinfusion apparatus is shown in Fig. 1. A one cubic centimeter syringe was mounted on a brass rack which held a threaded rod for adjusting delivery rate. A short length of rubber pressure tubing was wired to the syringe and the other end of the pressure tubing was wired to an adapter which was in turn connected to the pipette holder by means of plastic tubing. The whole system was filled with mineral oil. Upon pushing in the syringe plunger, the pressure tubing inflated and thereby acted as a pressure reservoir providing a relatively constant rate of infusion. Some pressure drop undoubtedly takes place as delivery proceeds but since the volume delivered is very small compared to total volume of the system (less than one part in 2000), pressure falls caused by fluid infusion can be neglected. To avoid leakage around the syringe plunger, tight fitting tuberculin syringes were selected, and no effect of leakage on infusion rate has been detected. A control experiment whereby delivery rate of solution was determined as a function of time is shown in Fig. 2. In this experiment, time required for the oil-solution meniscus to move between the major divisions of an ocular micrometer was determined as the infusion apparatus delivered solution into a pool of fluid held in a planchet. The volume delivered was estimated from measurements of the pipette dimensions. Rate of delivery was purposely made higher than that to be used in biological experiments since it was thought that at high delivery rates the chance of detecting inadequacies in the system would be enhanced. To determine infusion rate, movement of the oil-solution interface was monitored with a telescope mounted on a microscope. The fine adjustment knob on

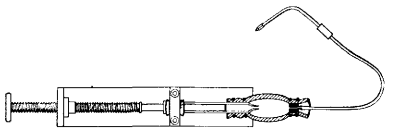
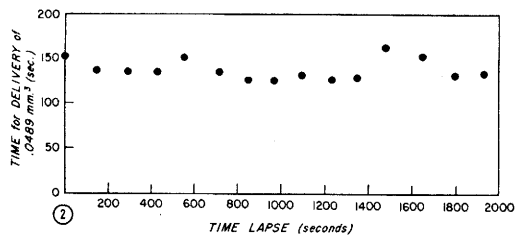


FIG. 1. Schematic drawing of infusion apparatus.

* Supported in part by N.I.H. Grant H-3676.



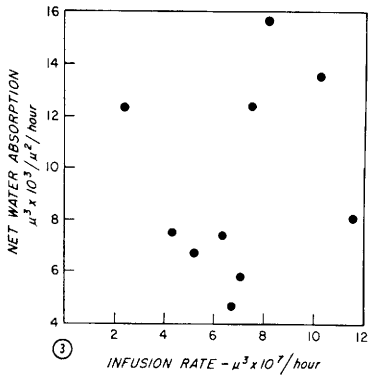


FIG. 2. Plot of delivery rate during continuous delivery showing relative constancy of flow.

FIG. 3. Plot of absorptive rate as function of infusion rate showing lack of correlation between these 2 factors.

one side of the microscope was removed and a 360° protractor mounted in its place. The fine adjustment of the microscope was calibrated by determining number of degrees turn necessary to move the telescope cross hair one mm. In the apparatus used in these experiments, one mm was equal to 2160 degrees of turn. Repeat measurements were reproducible to within 30°. Rats were prepared for the experiments according to methods previously described(2) with the modification that mineral oil was layered over the peritoneal Ringer bath to improve visualization of the kidney. Tubules were first impaled with an oil filled pipette in order to fill the whole nephron with heavy mineral oil. Before filling, however, a first test was done to localize the proximal loop. A small droplet of oil was injected into the lumen, and watched as it was swept through the tubules. Time required for the droplet to pass through a proximal surface loop is less (less than a second usually) than that required to pass through a distal loop (more than 1.5 seconds usually). A second evaluation of localization of the proximal portion was made during the filling procedure. Most surface nephrons do not have any distal representation. These were most often found and identified by observing that filling was rapid and continuous and that the oil filled loops formed a relatively compact mass of tubules. When the tubule had a surface distal representation, filling of the loops occurred in 2 phases, a rapid filling of the tubules around the puncture site followed at an appreciable time later by a filling of a second group of tubules, usually somewhat displaced from the first group. In the experiments to be described, the original puncture was always made in a proximal loop. A mineral oil filled loop other than that by which the filling was accomplished was selected for study and impaled with a micropipette containing the test solution. Test solution was then infused at a slow rate (.01 to .1 cmm per hour). A bubble of test solution formed in the tubular lumen which grew somewhat in size over the first few minutes, then stopped growing. When the "steady state" size was reached, net rate of water removal across the walls of the tubule in contact with the solution bubble was equal to rate of infusion. Dimensions of the intraluminal droplet were measured with a Spencer ocular micrometer, and knowing the infusion rate, it was possible to calculate the net flux per square micron of luminar area.

Results. In a series of 10 determinations, using Ringer solution as the test solution, net rate of water absorption ranged from 5-15 $\times$ 10^3 μ^3/μ^2/hr. The mean was 9.3 with a standard error of 1.3 $\times$ 10^3 μ^3/μ^2/hr.

The possibility should be considered that the water which leaves the tubule does so because of filtration rather than by tubular activity. One would then expect that infusion rate would be related to pressure, and that if the water showed bulk movement through the tubular wall because of high hydrostatic pressure, the derived net flux rate would be proportional to rate of infusion. Fig. 4 shows a plot of absorption rate as a function of infusion rate, and it can be seen that net flux values are scattered about an average value instead of being positively correlated with infusion rate.

It may be argued that in formation of the

solution droplet, the oil is not moved from the tubular walls, and that the area over which absorption takes place is less than that which is measured because of the presence of oil microdroplets. One of the more striking features of the solution droplet is the fact that it is sharply curved inward. This shows that the tubule walls are strongly hydrophilic. It does not seem reasonable therefore, that interference by residual oil is likely. Tubule hydrophilia may, however, be responsible for introducing an error into the results. Because the contact angle is small, the effective area over which water movement is taking place may actually be greater than that which is measured, thereby causing overestimation of the reabsorption rate.

Two kinds of control experiments have been done. In animals which have died during preparation for an experiment, a steady state size has not been observed to be attained within a surface loop at a time which is at least 10 minutes after time of death. This shows that water movement had been depressed. When infusion solution was lightly colored with trypan blue, it was seen that color intensity of the intratubular droplet increased as time passed. These experiments showed that removal of fluid did not result from infusion solution slipping past the oil column. It has now become standard practice to color slightly the infusion solution with dye to make the solution droplet more apparent. Furthermore, if excessive damage is done in the puncturing process, intense local staining of the cells around the pipette tip is usually observed. As might be expected, certain proximal cells show some cellular staining(3).

Summary. A method has been described for measuring net rate of water movement across single tubules of rat kidney *in situ*. Water is reabsorbed at a rate of 9.3 $\pm$ 1.3 μ^3/μ^2 proximal tubular surface/hr. Possible criticisms of the method have been discussed.

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Received April 8, 1959. P.S.E.B.M., 1959, v101.

Secretion of Cholesterol by Intestinal Mucosa in Patients with Complete Common Bile Duct Obstruction.* (24890)

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A mixture of endogenous and exogenous cholesterol is absorbed from the lumen of intestine. The endogenous portion (total secretion) normally consists of one fraction secreted by liver in bile and a second secreted by the intestine. It has been determined in normal man under standard conditions of food intake that there is a total secretion of approximately 2 g/day of cholesterol into the gut(1,2, unpublished). The method by which these results were obtained is summarized below. Two other groups of investigators, one using a special balance method(3), the other by determining dilution of fed C^{14}-cholesterol in chyle collected from urinary fistula(4), have obtained similar results. Such determinations indicate the *sum* of quantities secreted in bile and by intestinal mucosa since in normal man contributions from the 2 sources can not be separated. This paper reports results of such studies in 3 patients with complete obstruction of common bile duct due to carcinoma of the head of the pancreas. In these patients the fraction usually gaining access to gut *via* the bile had been excluded so that all of endogenous cholesterol in the lu-

* Aided by grants from Nat. Inst. of Arthritis and Metabolic Dis., N.I.H., P.H.S.