Effect of Hypertonic Mannitol on Accumulation Rate and Distribution of Albumin in the Renal Papilla.* (28493)

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The circulation of the renal medulla, by virtue of its relation to the loops of Henle and collecting ducts in this region, is believed to play an important role in the concentration of urine(1,2). Berliner *et al.*(1) have suggested that the concentration of the final urine is inversely proportional to the square of the blood flow in the medulla.

The observation has been made that an infusion of an osmotic diuretic such as hypertonic mannitol, "washes out" the hyperosmolality of the interstitium of the renal medulla(3). The osmotic diuretic has been thought to increase the blood flow to the renal medulla. This concept has been supported by the observation of Thurau *et al*(4) that the mean transit time of Evans blue dye decreases during an osmotic diuresis.

Because no direct or definitive method of measuring blood flow in the renal medulla is available, it was thought reasonable to study the effects of an infusion of hypertonic mannitol on the accumulation rate of intra-arterially infused I^{131} albumin in the papilla, and on the size of the exchangeable albumin pool in this region, by methods previously reported from this laboratory(5,6).

Methods. In the first series, 5 mongrel dogs in the post-absorptive hydropenic state were anesthetized with intravenous sodium pentobarbital (25 mg/kg). Following tracheal intubation, polyethylene catheters were placed in a femoral artery and vein, and the abdominal cavity was entered through a midline abdominal incision. After a catheter was placed in the bladder for observation of urine flow, a 15 ml priming dose of 20% mannitol in distilled water was injected intravenously, and a continuous mannitol infusion was begun with the 20% solution. This infusion was continued throughout the procedure at a rate of 100 drops per minute. After a brisk diuresis was achieved, one millicurie of I^{131} albumin was injected intravenously. Fifteen minutes later the intestines were gently retracted and umbilical tapes were passed around both renal pedicles, taking care not to occlude renal inflow or outflow. Precisely 30 minutes after the albumin injection, both kidney pedicles were simultaneously ligated, and an arterial blood sample was obtained. The kidneys were removed and rapidly frozen in a mixture of dry ice and acetone. Subsequently, the frozen kidneys were sliced transversely with a band saw. Sections of papilla were removed, placed in vials, weighed, and assayed for radioactivity in a well-type scintillation counter. The arterial blood sample was also assayed in the counter.

The size of the exchangeable albumin pool in the renal papilla was expressed as the ratio of I^{131} activity in 100 g of tissue to that in one ml of plasma.

In another series of 9 dogs, after the same anesthesia, tracheal intubation, and insertion of femoral arterial and venous catheters, a third polyethylene catheter was introduced into the right carotid artery so that its tip lay in the ascending aorta. The abdominal cavity was entered and a catheter was placed in the bladder. The same mannitol priming dose and infusion were given intravenously and umbilical tapes were loosely placed around both renal pedicles. After waiting for a definite diuresis to take place, an infusion of I^{131} albumin solution in normal saline into the ascending aorta was begun at a rate of 0.6 ml/sec (5 μc/sec) employing a Harvard infusion pump. Samples of arterial blood were collected at one-second intervals into siliconized test tubes containing heparin. Exactly 15 seconds following the beginning of

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TABLE I. Size of Exchangeable Albumin Pool in Renal Papilla (ml plasma/100 g tissue) in Hydropenia and Hypertonic Mannitol Diuresis.

Hydropenia		Mannitol	
Kidney No.	Size of pool	Kidney No.	Size of pool
1017A1	33.5	0816A1	38.0
1017A2	43.1	0816A2	40.0
1017C1	51.9	0816B1	50.4
1017C2	40.8	0816B2	34.7
1022A1	38.4	0814A1	19.8
1022A2	36.6	0814A2	20.4
1022B1	50.6	0809A1	21.7
1022B2	57.5	0809A2	22.0
1022C1	49.5	0824A1	27.3
1022C2	41.2	0824A2	26.4
1024A1	24.3		
1024A2	27.5		
1024B1	21.5		
1024B2	21.7		
1024C1	34.9		
1024C2	31.5		
Mean	37.8	Mean	30.1
S.E.	± 2.8	S.E.	± 3.2
$t = 1.77$		$p: 0.1 > p > 0.05$	

the I^{131} albumin infusion, the ligatures around the renal pedicles were tightened and the kidneys were removed, rapidly frozen, and sliced on the band saw as before. Tissue samples from the papillae were placed in vials, weighed, and counted in the well-type scintillation counter. Aliquots of the arterial blood specimens were also analyzed in the scintillation counter.

The plasma flow rate in ml plasma/0.25 min/100 g tissue, was obtained by dividing the radioactivity of the papillary tissue samples (in counts per minute per gram of tissue) by the average radioactivity of arterial plasma over the 15-second collection period (in counts per minute per ml of plasma).

In both series, systolic blood pressures of all animals were above 100 mm Hg at all times.

The results of these experiments were compared with previously reported studies in hydropenic dogs which were performed identically except for the mannitol infusion(5,6).

Results. The size of the exchangeable albumin pool in the series of dogs given mannitol was 30.1 ml plasma per 100 g tissue (Table I). The mean reported value of the hydropenic series was 37.5 ($0.1 > p > 0.05$).

The plasma flow rate in the renal papilla, as determined by the accumulation rate of I^{131} albumin in this region, was 5.1 ml plasma/0.25 min/100 g of tissue in the group receiving mannitol (Table II). The value for the control group was 4.32 ($0.6 > p > 0.5$).

Discussion. From the data there appears to be evidence for a large pool of albumin in the renal papilla of the dog. By means of micropuncture of hamster vasa recta, Gottschalk *et al.*(7) have found the albumin concentration of plasma in these vessels to be only 1.6 times that of vena cava plasma. This seems insufficient by itself to account for the size of the albumin pool in the papilla, although precise data on the magnitude of the vascular compartment in this area are not available. It seems likely, then, that some of the albumin in the papilla is located extravascularly.

Malvin and Wilde(3) have shown that an osmotic diuretic will decrease the hyperosmolality of the interstitium of the renal medulla below that found in hydropenia. As a consequence, there should be a decrease in the amount of water lost from the vasa recta to their environment during an osmotic diuresis. If most of the large amount of albumin in the papilla was in fact located intravascularly, the result of the diminished outward diffusion of water from the vasa recta would

TABLE II. Plasma Flow Rate in Renal Papilla (ml plasma/0.25 min/100 g tissue) in Hydropenia and Hypertonic Mannitol Diuresis.

Hydropenia		Mannitol	
Kidney No.	Flow rate	Kidney No.	Flow rate
16 R	2.1	0827A L	7.3
16 L	2.0	0827B R	4.5
17 R	6.5	0827B L	4.4
17 L	13.3	0905A R	3.4
18 R	4.2	0905A L	3.9
18 L	6.4	0907A R	4.8
19 R	3.0	0912 R	3.7
19 L	3.2	0912 L	4.4
20 R	2.2	0914 R	2.7
20 L	3.3	0914 L	1.9
21 R	2.6	0918 R	7.3
21 L	7.9	0919 R	2.3
22 R	5.3	0919 L	2.4
22 L	2.4	0911 R	16.4
23 R	1.4	0911 L	7.0
23 L	3.4		
Mean	4.3	Mean	5.1
S.E.	±.76	S.E.	±.99
$t = 0.633$		$p: 0.6 > p > 0.5$	

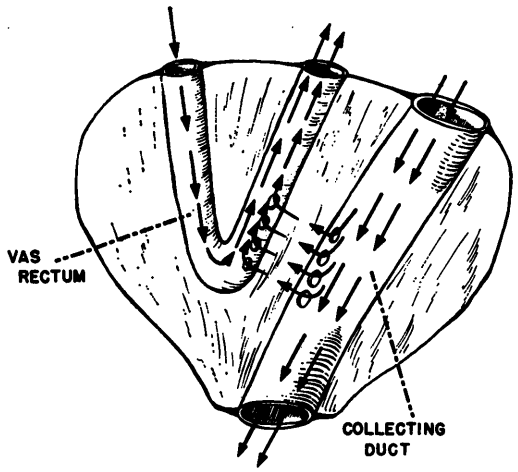

FIG. 1. Fluid flow in vasa recta and collecting ducts during osmotic diuresis. Direction of flow is indicated by arrows.

be a substantial decrease in intravascular concentration and a decrease in size of the exchangeable albumin pool in osmotic diuresis as compared to hydropenia. The results of the present study, however, indicate that under the influence of a mannitol diuresis, the exchangeable albumin pool decreases only slightly. Furthermore, a comparison of papillary wet and dry weight in hydropenia and osmotic diuresis in this laboratory revealed a 5% increase in water content in the papilla during osmotic diuresis. If this factor is taken into account, the observed difference of 7.4 ml of plasma per 100 g of tissue (30.1 *vs* 37.5) is reduced to 5.9 ml (31.6 *vs* 37.5). Thus, at best there is only a slight decrease in the size of the exchangeable albumin pool associated with a mannitol diuresis.

Because of the failure of the osmotic diuresis to effect a substantial decrease in albumin content of the papilla, it appears likely that much of the albumin in this region is in an extravascular location, and that the walls of the vasa recta are permeable to albumin.

The plasma flow rate, as determined by rate of uptake of the I^{131} albumin, did not change appreciably during the mannitol infusion. This means that albumin accumulates in the renal papilla at substantially the same rate during osmotic diuresis as during hydropenia. The absence of a change in the accumulation rate during osmotic diuresis is interpreted to mean that there is no change in the inflow rate of plasma to the papilla. However, this does not necessarily mean that blood flow in this region does not increase.

Fig. 1 shows schematically a vas rectum and a collecting duct in the renal papilla. There are two sources of vasa recta outflow: the inflowing blood, and that amount of fluid absorbed from the interstitial space, coming from the collecting ducts and Henle's loops.

During osmotic diuresis, rate of inflow, as measured by the albumin accumulation rate, may not change. However, the volume flow of fluid and the hydrostatic pressure in the collecting ducts and Henle's loops are increased during osmotic diuresis. Since negative free water clearance is increased during osmotic diuresis(8,9) it is likely that there is an increase in the volume of water diffusing into the interstitium and thence into the vasa recta. This, combined with a decrease in the diffusion of water out of the vasa recta, would result in an increase in the flow of blood through the ascending limbs of the vasa recta if the out-flow resistance were to decrease. In this situation there would be an increase in the velocity of blood flow through the ascending vessels but no change in inflow rate. This increased rate of outflow during osmotic diuresis would wash out solute and reduce the interstitial osmolality of this region.

This model would also explain the decreased albumin mean transit time in this region during osmotic diuresis as described by Thurau *et al*(4) employing a photocell recorder placed near the papilla. The increased outflow of blood could result in increased velocity through the ascending limb, thus decreasing mean transit time through the papilla when taken as a whole.

It would appear, then, that the reported washout of the osmolality gradient and the increased average velocity of plasma flow in this region which occur under the influence of an osmotic diuretic, are probably due to increased reabsorption of fluid into the vasa recta rather than to an increase in inflow of blood to the medulla. The mecha-

nism whereby the medullary blood outflow resistance falls during mannitol diuresis remains to be elucidated.

Summary. Measurements were made of the size of the exchangeable albumin pool and the plasma flow rate in the papilla of dogs during hypertonic mannitol diuresis. The former, in ml plasma/100 g tissue, was determined by comparison of labeled albumin in the papilla with that in peripheral plasma. The latter was computed by determining the I^{131} albumin accumulation rate in ml plasma/0.25 min/100 g tissue. The results were compared with similar studies made in hydropenic dogs.

The data are interpreted to support the concept of an extravascular location of albumin in the renal papilla. In addition, it appears that the reported washout of the osmolality gradient and the increased average velocity of plasma flow in this region are probably due to increased reabsorption of fluid into the vasa recta rather than to an increase in inflow of blood to the medulla.

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Serine-Ethanolamine-Phosphate, Taurine and Free Amino Acids of Muscle in Hereditary Muscular Dystrophy of the Chicken.* (28494)

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Muscular dystrophy occurs in New Hampshire chickens homozygous for an autosomal recessive gene(1,2). Birds affected with this disorder cannot raise their wings or right themselves normally when placed on their backs. Marked alterations are found in the large superior pectoral muscle. Muscular degeneration with increasing deposition of fat occurs as the birds grow older(3). Catabolic processes in this, as in other dystrophies, appear to be associated with increased levels of lysosomal enzymes including the cathepsins(4). Possibly related to this enhanced proteolytic activity is a highly characteristic pattern of free amino acids in the dystrophic pectoral muscle. Unusually high levels of taurine and reduced levels of anserine and carnosine have been reported(5). The present study was carried out to define the free amino acid pattern and to characterize a previously unidentified ninhydrin positive component which occurs in considerable amounts in dystrophic pectoral muscle but is present only in traces in normal pectoral muscle. This substance has now been identified as serine-ethanolamine-phosphate.

Methods. Superior pectoral muscles were taken after sacrifice from adult New Hampshire chickens ranging in age from 53 to 137 weeks of age. Females and males with muscular dystrophy were compared with normal females and heterozygous normal male phenotypes.

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