Intrarenal distribution of blood flow in diabetes insipidus: role of ADH

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ALTHOUGH OLIVER AND SCHAFER (23) had concluded, in 1895, that the pressor effect of pituitary gland extracts was the result of a direct effect on peripheral blood vessels, and subsequent investigators have verified the vascular action of the antidiuretic hormone (25), the role of ADH in the control of renal circulation has not been clarified. Verney (32), in his studies on the perfused isolated kidney, reported that the introduction of a normal dog head in the perfusion circuit reduced renal blood flow during the ensuing antidiuresis; Wakim et al. (34) noted that vasopressin decreased renal blood flow in the unanesthetized dog, but they used relatively large doses. From subsequent clearance measurements of renal blood flow and glomerular filtration rate, it was concluded that diuresis and antidiuresis were unrelated to changes in renal hemodynamics (24). Thus, emphasis was focused on the purely tubular effect of ADH. However, with the introduction of the concept of countercurrent exchange in the medulla, attention was drawn to the control of blood flow in this region for maintenance of the hyperosmotic interstitium (3), but recent studies suggest that ADH at physiological levels has no medullary vascular effect (1, 29). The role of ADH in regulation of cortical blood flow has not been studied. We have therefore investigated the distribution of renal blood flow in trained, unanesthetized dogs with diabetes insipidus (DI) using the $^{85}$Kr method (28). Our studies demonstrate that outer cortical flow is increased, whereas juxtamedullary cortical and outer medullary peritubular flow are reduced in DI. The major change in vascular resistance appears to be localized to the glomerular and postglomerular vessels. These observations suggest hormonal regulation of tone in this portion of the vascular tree.

MATERIALS AND METHODS

Mongrel dogs (20-30 kg) were trained to lie quietly for periods of several hours on a cushioned table in a quiet room. Training was easily accomplished and the effectiveness of the training was indicated by the docility of each animal. Resting heart rates never exceeded 55-60 beats/min during the observation periods and occasionally the animals would sleep during the course of an experiment. The animals were maintained on a constant diet of ground horsemeat supplemented with cereal pellets. Free access to water was allowed. Urine was collected in a pan placed beneath the metabolic cage. Each animal was exercised twice a day. A solution of diluted milk containing 10 mEq of sodium per liter of solution was given the animals when daily fluid intake was to be augmented. Chronic infusions were performed with a portable Sage pump (Sage Instruments Inc.) strapped to the back of unrestrained dogs.

Both chronic and short-term experiments were performed in the study. In six dogs chronic studies were made over an 8-month period, with serial determinations of renal blood flow and simultaneous measurements of fluid and electrolyte excretion. In seven additional animals, short-term experiments were conducted to obtain autoradiographs and silicon rubber (Microfil, Canton Bio-Medical Products, Swarthmore, Pa.) injection specimens of the kidneys as well as renal blood flow determinations.

Chronic experiments. After satisfactory training, either of two operations was performed as the initial procedure. In two dogs an hypothalamic-hypophysial tract section was performed as the first procedure and was followed in 7-13 days by placement of polyvinyl catheters in the renal artery and in the abdominal aorta. After vascular catheterization, four other animals were allowed to recover until distribution of renal blood flow was normal (usually 10-14 days) before hypothalamic-hypophysial tract section was per-
formed. An epistomy was also performed in some of the dogs. In one dog, implantation of renal catheters and tract section was followed by the urinary bladder splitting operation as described by Deautels (9).

Preoperatively, food was withheld for 18 hr and water for 8 hr. In the animals with established diuresis following the hypothalamic-hypophysial tract section, vasopresrin (Pitressin tannate in oil; Parke, Davis, 5 units) was given intramuscularly 24 hr prior to operation. Anesthesia was induced with pentobarbital (30 mg/kg intravenously) and maintained with additional small increments. The kidney was exposed through a flank incision with minimal dissection of the artery in order to maintain normal renal innervation. A polyvinyl catheter (od 0.030 inch and id 0.015 inch) was introduced into the renal artery as previously described by Herd and Barger (16). For catheterization of the infrarenal aorta, an incision through the left flank was made and a larger polyvinyl catheter (od 0.055 inch and id 0.025 inch) introduced into the aorta by the same technique.

The operative exposure of the hypothalamic region was a modification of the transoral approach of McLean (20). Anesthesia was induced with a minimal amount of pentobarbital. A specially designed operating table and gag maintained the jaws widely separated and provided excellent visualization of the roof of the mouth. The head of the operating table was elevated to a 45° angle to lower cerebral venous pressure. The tongue was withdrawn and affixed to the ventral surface of the lower jaw; a cuffed tube was inserted into the trachea. A binocular operating microscope was centered over the open mouth of the animal. Oculars of X12.5 magnification and a 300-mm objective lens were found to supply appropriate magnification. The oropharynx was washed with benzalkonium chloride (1%) and the area draped with sterile towels. A 4-cm incision was made through the soft palate in the midline starting at the edge of the hard palate. Silk traction sutures placed through the edge of the wound exposed the nasopharynx. The hamulate process of the os palatinum was identified on the lateral wall of the nasopharynx. The processes usually mark the level of the interseptal suture, the important landmark for placement of the burr hole. The midline incision in the mucoperiosteum extended from the tympanic bullae to the level of the hard palate. The mucoperiosteum was reflected from the base of the skull and retracted laterally with silk sutures. Care was taken to avoid shredding of the mucoperiosteum which subsequently was used to close the defect in the skull. A dental drill with a 4-mm burr was used to drill through the outer table and diploc of the inferior surface of the skull, starting 5 mm anterior to the interseptal suture, and extending 8 mm posterior to the suture. The width of the hole in the outer table was approximately 6 mm. Care was exercised with the burr when approaching the inner table since the dural venous sinuses lie beneath the thin inner table; bone curettes were used to remove the inner table. The inner table was penetrated anteriorly; the glistening dura lying between the optic chiasm and the anterior hypophysis was identified. The dura was then opened in the midline with a hook knife and the incision was extended over the anterior one-third of the pituitary gland. A second set of sterile instruments was utilized for the intracranial procedure. The tip of a double-edged knife blade was inserted for a depth of 1–2 mm into the hypothalamic area anterior to the adenohypophysis. The incision was extended around the lateral margins of the hypophysis for 180°. No material was placed in the cleft and no attempt was made to close the dural incision. The opening in the skull was packed with hemostatic gelatin pads. The mucoperiosteum and palate were closed with interrupted gut sutures. The animals were offered fluids 8 hr after the procedure. Prophylactic penicillin and chloramphenicol were administered for 3 days. The ensuing renal response of all the animals followed the same characteristic pattern, i.e., initial transient diuresis lasting 4–7 days, an antidiuretic interphase of 2–6 days, and an intermediate level of diuresis which persisted for the life of the animals (11).

For the measurement of the distribution of renal blood flow, the 85Kr method was used as previously described (28). The washout curves were analyzed graphically into four components. The regional flow rate (k) in milliliters per gram per min of perfused region, the relative volume of each region, the percentage of radioactivity entering each region, and the flow in milliliters per 100 g per min of kidney were calculated as described in previous reports (7). The total renal blood flow (in ml/100 g per min of kidney) was estimated as the sum of the flow in the first two components; this method has been validated by direct measurement of total renal venous effluent (7, 26). To verify that the elevated cortical flow in DI was not the result of the high urine flow per se, in three normal dogs, prior to tract section, water diuresis (urine flow above 3 ml/min) was induced by acute oral water load (2–4% body wt); flow rate in component I and calculated total renal blood flow were unchanged during water diuresis when compared to values obtained during basal conditions (urine flow below 1 ml/min).

Daily urines were collected for determinations of sodium and potassium excretion and osmolarity; more frequent collections were made when necessary. Serum sodium and potassium concentrations were determined by flame photometry (Instrumentation Laboratory, Inc. no. 407) and plasma osmolarity was measured with a Fiske osmometer (Advanced Instruments, Inc.).

**Short-term experiments.** In order to localize the sites of vascular changes in the kidneys of dogs with diabetes insipidus, seven dogs were prepared for short-term experiments. Each animal underwent transoral hypothalamic-hypophysial tract section as described above and after recovery was sacrificed for autoradiography or Microfil-injection studies.

In three dogs, 48 hr after tract section acute experiments were performed during the period of maximal diuresis. Anesthesia was induced by intravenuous chloralose (50 mg/kg) after preliminary injection of phencyclidine hydrochloride (0.4 mg/kg iv). A sustaining solution of 3–5% dextrose and water was administered at the average rate of urine flow during the previous 12 hr. The right renal artery was catheterized with a small polyvinyl catheter as described above. After the measurement of renal blood flow by the 85Kr method, the kidneys were removed for autoradiographs at predetermined times after the injection into the renal artery of a standardized amount of 85Kr. Auto-
radiographs were made from cross sections of the kidneys by the method previously described (28).

At the peak of the initial diuretic phase following hypothalamic-hypophysial tract section, two dogs were quickly sacrificed by the rapid administration of an intravenous bolus of concentrated pentobarbital (500 mg/kg). Two additional animals were sacrificed in a similar manner 2 and 3 weeks after tractotomy during the permanent diuretic phase. As controls for the Microfil injections, kidneys were obtained from normal dogs with free access to water. Immediately after the death of the animals, the kidneys were removed and quickly prepared for silicon rubber injection (4). One kidney was filled through the renal artery at a pressure of 150 mm Hg while the opposite kidney was filled through the renal vein at 25 or 50 mm Hg. The silicon rubber was allowed to polymerize and harden for 12 hr. The kidney was then cut into thick sections, dehydrated in increasing concentrations of alcohol, and finally cleared in methyl salicylate for examination and photographing.

RESULTS

The urinary excretion pattern of the dogs following hypothalamic-hypophysial tract section was similar to that described for cats by Fisher et al. (11). As illustrated in Fig. 1, the diuresis began immediately after operation, reached a peak of 4–10 liters/day, and persisted for 4–7 days. Urine flow then returned to normal levels for 2–6 days, reportedly the result of liberation of ADH during the degeneration of the hypothalamic-hypophysial fiber tracts (22). The second, or permanent, diuretic phase became apparent 7–10 days after tract section, and urine flow remained relatively constant thereafter for the duration of the experiment (up to 6 months). With the dogs on a constant diet and with free access to water, the daily urine volume stabilized at a level below the initial peak diuresis and at a value characteristic for each dog. The magnitude of the diuresis, however, differed from dog to dog, varying from 2 to 8 liters/day.

The urine from each dog was hypotonic to plasma during both diuretic phases. Measurements of renal blood flow were made in each of the three phases: transient phase, normal interphase, and permanent phase.

Transient phase. At the peak of the diuresis following hypothalamic-hypophysial tract section, renal blood flow was markedly elevated. The augmented perfusion of the kidney in the absence of ADH is illustrated in the data from three dogs (Table 1) showing a rapid flow rate in component I with a large percentage of total blood flow in this region and a high total renal blood flow; the flow rate in component II did not change significantly. Similarly, in dogs with DI maintained with a constant infusion of physiological dose of vasopressin, total renal blood flow increased markedly when the infusion was stopped, as shown in Fig. 2 (dogs 1 and 2). These experiments were performed 3 weeks to 5 months after tract section, when the animals were fully recovered from the surgical procedure. Vasopressin was infused systemically for at least 18 hr at a rate of 0.5–1.0 μg/min; then the infusion was suddenly stopped. The rate of infusion of vasopressin was in the physiological range, for the urine flow was not reduced below 600 ml/day with the dog allowed free access to water, and the urine osmolarity was 1,000–1,900 mOsm/liter. Random urine osmolarity in normal dogs, under similar conditions in this laboratory, may be 2,000 mOsm/liter or more. Blood pressure, during vasopressin infusion and after its cessation, was unchanged.

In order to establish that the increase of renal blood flow after tract section was not related to the operative procedure per se but to the suppression of ADH release, one dog (dog 3, Fig. 2) was maintained on intravenous vasopressin for 2 weeks after tract section until the time of permanent phase of DI was attained, as predicted from our experience with
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TABLE 1. Distribution of renal blood flow at peak of initial diuresis in three dogs with diabetes insipidus

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Component I</th>
<th>Component II</th>
<th>Total Renal Blood Flow, ml/100 g per min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flow Rate, ml/g per min</td>
<td>Flow Rate, ml/g per min</td>
<td>% 85Kr I of 85Kr Total flow</td>
</tr>
<tr>
<td>1</td>
<td>8.4</td>
<td>2.9</td>
<td>72%</td>
</tr>
<tr>
<td>2</td>
<td>9.3</td>
<td>2.3</td>
<td>83%</td>
</tr>
<tr>
<td>3</td>
<td>8.3 R</td>
<td>2.5</td>
<td>72%</td>
</tr>
<tr>
<td></td>
<td>9.4 L</td>
<td>2.6</td>
<td>60%</td>
</tr>
</tbody>
</table>

Preoperative values (10) 6.1 ± 1.2 2.1 ± 0.1 65% ± 9.3 95 ± 59

R = right kidney. I = left kidney. Preoperative values are means ± SD with number of observations given in parentheses.

ml/100 g per min with a normal distribution; 3 hr after stopping vasopressin infusion, the renal blood flow was 640 ml/100 g per min, with a flow rate of 9.3 ml/g per min in the first component, and 84% of 85Kr initially present in this component.

To localize the components of the 85Kr curves, autoradiographs were prepared at selected times (from 15 to 70 sec) after injection of the isotope into the renal artery. The animals with DI were sacrificed either in the transient diuretic phase or during the diuresis which ensued following the withdrawal of vasopressin. The autoradiographs were then compared with similar preparations from kidneys of normal dogs. The autoradiographs shown in Fig. 3 were obtained 45 sec after 85Kr injection. In the normal dog (A) the "washout" of the entire cortex is essentially uniform and a large amount of radioactivity has entered the outer medullary region. In contrast in the dog sacrificed during the initial diuretic phase after withdrawing vasopressin (B), autoradiographs demonstrate two flow rates in the cortex, a very rapid one in the outer two-thirds of the cortex (k1) and a slower juxtamedullary cortical flow (k2). Moreover, little activity appears in the outer medullary region despite the high urine flow which might be expected to carry 85Kr into this region, in addition to that transported by the blood. Further evidence of the slow flow in the juxta-

![Flow rate component I](image1)

![Flow rate component II](image2)

![Total renal blood flow](image3)

**FIG. 2.** Changes in distribution of renal blood flow in 3 dogs with diabetes insipidus following cessation of systemic vasopressin administration. For dogs 1 and 2 vasopressin was infused for at least 18 hr at a rate of 0.5-1.0 μg/min, and final flow determinations were made 3-6 hr after the infusion was stopped. Increases in total renal blood flow (bottom) and percentage of radioactivity initially present in component I (outer cortex) is clearly illustrated. Dog 3 was maintained on vasopressin for 15 days after tract section. Mean and range of flow determinations during this period are presented (sec text).
FIG. 3. Autoradiographs of kidneys obtained from a normal dog (A) and from a dog with diabetes insipidus (B) during initial diuretic phase. Both kidneys were removed 45 sec after injection of a bolus of $^{32}$K. Exposure times were adjusted to obtain comparable densities. During diuresis (B) density is decreased in outer cortex with activity remaining in juxtamedullary cortex; little activity is noted in outer medullary region. In contrast, in normal dog (A) density of entire cortex is essentially uniform and a large amount of radioactivity has entered outer medullary region.

FIG. 4. Autoradiograph of a kidney obtained from a dog with diabetes insipidus during initial diuretic phase. Kidney was removed 70 sec after injection of $^{32}$K. An abnormal amount of radioactivity is noted in juxtamedullary cortex; band of radioactivity in outer medulla is narrow.

To define more precisely the sites of altered vascular resistance in the absence of ADH, silicone rubber microvascular casts of the kidneys were prepared. Four dogs with diabetes insipidus were sacrificed, two during the transient diuretic phase, one at the onset of the permanent phase, and one during the permanent phase. Kidneys were also obtained from normal animals with free access to water and prepared in a similar manner. In the four animals in which ADH secretion had been suppressed, the arterial injection specimens (Fig. 5) demonstrated poor filling of the juxtamedullary cortex and peritubular capillaries of the outer medulla, indicating a high arterial and/or arteriolar resistance in these regions; the outer cortex was uniformly well filled. Conversely, the striking feature of the venous silicone rubber injection specimens from the dog with DI was the dense filling of the venous structures throughout the kidney, even in the region of poor arterial filling (Fig. 6, B and D) and the retrograde injection of numerous glomeruli. In some regions of the kidney all glomeruli were filled by this retrograde venous injection as shown in Fig. 7B. Such prominent filling of venous system and glomeruli indicates a lowered venous vascular resistance and diminished efferent arteriolar and glomerular vascular tone when ADH is suppressed for a sufficient interval. In contrast, in normal animals with free access to water, retrograde injection of the venous system resulted in incomplete venous filling (Fig. 6, A and C); glomerular tufts were not filled with Microfil (Fig. 7A) even with injection pressures exceeding 150 mm Hg.

To provide more definitive evidence of the direct effect of ADH on cortical vessels, vasopressin was infused into the renal artery of dogs with DI during the permanent phase at rates of 0.03–0.1 μl/min for 18–40 hr. These rates are approximately $\frac{1}{40}$ of those used in the systemic infusions. As indicated below renal blood flow was low and variable during the permanent phase; therefore values of renal blood flow during DI presented in Fig. 8 are below the normal range. Thus the intrarenal infusion of vasopressin did not...
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Figure 6. Silicone rubber (Microfil)-injection specimens of venous vasculature of kidneys removed from a normal dog (A) and from a dog with diabetes insipidus (B) during transient diuretic phase (50 mm Hg filling pressure). Poor filling of venules in pars radiata of normal dog is seen more clearly at higher magnification (C).

consistently change the total renal blood flow, but did reduce the amount of $^{99}$Kr delivered to the first compartment (Fig. 8); the flow rate in component I did not change. After the abrupt withdrawal of vasopressin a rapid renal blood flow was consistently observed within the first 3–6 hr. The results obtained during intrarenal infusion and after its withdrawal further demonstrate that the antidiuretic hormone has a direct effect on the renal vasculature when given in small physiological doses.

Interphase: Renal blood flow began to decrease in the latter part of the transient phase. In two dogs flows were also measured during the interphase. During this 5- to 7-day interval the renal blood flow was stable, with a relatively normal rate and distribution. In the experiment illustrated in Fig. 9, during the early transient diuretic phase a rapid renal blood flow (715 ml/100 g per min) with 85% of the flow in the rapid component ($k_1 = 9.3$ ml/g per min) was found; however, 5 days later during the normal interphase, renal blood flow had returned to normal range. Similar results were obtained in the second dog.
FIG. 7. Silicone rubber-injection specimens of venous vasculature of kidneys removed from a normal dog (A) and from a dog with diabetes insipidus (B) during transient diuretic phase. Low glomerular and postglomerular resistance in absence of ADH is illustrated by abundant venous and glomerular filling (B). Glomeruli do not fill with retrograde injection in normal animal even at much higher pressures than used in these specimens (50 mm Hg). Brackets indicate 100 microns.

Permanent phase. For the pilot experiments in this investigation, dogs were studied in the permanent diuretic phase of diabetes insipidus. During this period, as others have described (6, 15, 21, 36), a low and unstable renal blood flow was found. Blood flows varied as much as 200 ml/100 g per min from day to day. As shown in Fig. 10, renal blood flow in this animal was widely variable in rate and distribution during the permanent diuretic phase.

The low and variable renal blood flow in the permanent phase made it difficult to assess the role of vasopressin in the regulation of renal vascular resistance in this stage. Therefore attempts were made to elevate and stabilize renal blood flow by administration of ACTH (H. P. Acthar gel; Armour Pharmaceutical Co.) or hydrocortisone, 30 or 20 mg/day (although no direct evidence for pituitary insufficiency was present), or by expansion of extracellular fluid volume by increased water intake. During replacement hydrocortisone therapy, the level of diuresis was modified in only one animal and in this dog there was a 45% increase in urine output. Hydrocortisone clearly augmented renal blood flow during the permanent diuretic phase as shown for two animals in Table 2; the mean values are obtained from determinations made during 4- to 18-day periods. However, renal blood flow was stable in only one animal. In the remaining animal wide fluctuations in renal blood flow persisted despite hydrocortisone therapy.

The large urinary water losses of the dog with diabetes insipidus may produce a state of dehydration if the animal fails to ingest sufficient water. The altered fluid balance could result in wide fluctuations of extracellular fluid producing secondary or reflex changes in renal blood flow. A 48-hr systemic infusion of vasopressin at a physiological rate in two dogs with diabetes insipidus resulted in an increase in weight of 1–2 kg, further evidence that these animals do not remain in stable water balance during the permanent diuretic phase. Moreover, we found that we could induce the animals to increase fluid intake by providing dilute milk as well as water. As shown in Fig. 11, renal blood flow increased simultaneously with the increase in total body water; dehydration secondary to an episode of diarrhea temporarily reduced renal blood flow. These observations indicate that the reduction and instability of renal blood flow during the permanent phase can be explained, in part, by the unstable water balance of dogs with...
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TRANSIENT DIURETIC PHASE

RADIO-ACTIVITY

\(^{85}K\!r\)

\(^{85}K\!r\) DISAPPEARANCE CURVE

COMPONENT I

COMPONENT II

CALCULATED TOTAL RENAL BLOOD FLOW

375 ml/100g-min

550 ml/100g-min

675 ml/100g-min

450 ml/100g-min

FIG. 9. Comparison of first 3 min of \(^{85}K\!r\) disappearance curves (heavy lines) during transient diuretic phase (left panel) and during antidiuretic interphase (right panel). Components I and II (lighter lines) have been obtained by graphical analysis of curves. During transient diabetes insipidus, they also emphasize the difficulty of demonstrating a renal vascular effect of ADH during this phase. Studies during the early transient phase would provide better opportunity to document renal vascular effects of vasopressin, but this phase is short for detailed studies in each animal.

DISCUSSION

The increased renal blood flow observed in the unanesthetized dog during the transient diuretic phase following hypothalamic-hypophysial tract section is evidence for the physiological role of ADH in the regulation of renal vascular tone. If the dog with tract section is maintained in the non-diuretic state with vasopressin infusion for several weeks until the animal is fully recovered, renal blood flow is not increased until the cessation of the ADH infusion. Thus, the observed renal vasodilatation is not the result of the operative procedure per se, but the suppression of ADH release. Further evidence for the vascular role of ADH is the marked elevation of renal blood flow noted in the hours after stopping vasopressin which was infused in physiological doses during the permanent diuretic phase. Still more direct proof for local renal vascular action was obtained by infusion of minute doses of vasopressin into the renal artery of unanesthetized dogs with diabetes insipidus.

Since the renal blood flow changes during the early transient phase are so striking, it is surprising that the observation has not been previously reported. However, Pickford (24) noted in 1966 that she had been unable to find any flow measurements performed during the transient phase, and we have seen no reports in the ensuing 3 years. Yet renal blood flow determinations during this short phase are of particular significance since changes in vascular tone at this stage result primarily from the absence of ADH, without the complicating secondary changes induced by chronic dehydration. The chronic water imbalance during the permanent diuretic phase has been documented in man and animals, with elevation or fluctuations in serum electrolytes (2, 31, 35). Thus during the permanent phase of diabetes insipidus, dehydration may lead to reflex alterations in renal function, with low or variable flows as reported by previous investigators (6, 15, 21, 36) and as illustrated in Fig. 10. Since renal blood flow is depressed and unstable in the permanent diuretic phase, it is difficult under such abnormal conditions to demonstrate a physiological role of ADH in regulating renal vascular tone.

The increase in total renal blood flow we observed during the initial diuretic phase was the result of markedly increased outer cortical flow and was localized to this region by auto-
radiography; inner cortical flow and outer medullary flow were decreased. The Microfil-injection specimens demonstrated that the major site of vasodilatation was in the glomerular and postglomerular vessels. In the normal animal, glomeruli did not fill with retrograde venous injection of silicone rubber even at pressures higher than that required to fill glomeruli during arterial injection. Bowman (5), in 1842, probably was the first investigator to call attention to this paradox of glomerular filling in arteriolar specimens and the surprising absence of glomerular filling in kidneys prepared by retrograde venous injection. These observations have been confirmed by von Kugelgen et al. (33). The effect of ADH on renal efferent arterioles has previously been suggested by Corcoran and Page (8); evidence for the action of the hormone on venous tone has recently been described by Kramer and associates (29) could not demonstrate an effect on medullary vessels of ADH in physiologic concentrations. Similarly, Aukland (1) was unable to show any effect of vasopressin on hydrogen clearance in the outer medulla in dogs with water diuresis. However, Fourman and Kennedy (12), using fluorescent dye, found that staining of the vasa recta was greater than that of the peritubular capillaries in rats with diabetes insipidus. The arterial Microfil-injection specimens are in agreement with Fourman and Kennedy’s observations showing good filling of the vasa recta, but little filling, and therefore higher resistance, of the peritubular capillaries of the outer medulla in dogs with diabetes insipidus. The slow flow in the outer medulla is also apparent from the autoradiographs. Thus, whereas in the normal dog component I

![Image of table]

**TABLE 2. Total renal blood flow during permanent phase of diabetes insipidus in two dogs**

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Without Hydrocortisone</th>
<th>With Hydrocortisone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>230 ± 70 (11)</td>
<td>355 ± 30 (6)</td>
</tr>
<tr>
<td>2</td>
<td>220 ± 55 (4)</td>
<td>380 ± 70 (3)</td>
</tr>
</tbody>
</table>

Values are means ± SD with number of observations given in parentheses. Total renal blood flow is given in ml/100 g per min.

The nature of the control of glomerular and postglomerular resistance has been puzzling since nerve fibers have rarely been described in this region. Even the most recent histochemical studies of adrenergic innervation indicate that cortical nerve fibers terminate at the junction of the afferent arteriole and the glomerulus (19). The absence of innervation of the glomerulus and efferent arteriole suggests that the tone of these vessels is controlled by other mechanisms; our results suggest that ADH plays an important role in regulating vascular tone in this region. In contrast, the adrenergic innervation could mediate the changes inafferent arteriolar tone noted during the permanent phase of DI, which result from the marked variations in fluid balance. Thus it would appear that the control of the afferent arteriolar tone may be primarily neurogenic, whereas control of glomerular and postglomerular resistances may be predominantly hormonal. It has been suggested that angiotensin, another polypeptide, may also serve to regulate efferent tone (18, 27).

Berlins et al. (3) emphasized the regulatory role of medullary flow for the maintenance of the hyperosmotic interstitium, but the subsequent hemodynamic studies during urinary dilution and concentration have been conflicting. Thurau et al. (30) reported that inner medullary flow was increased during water diuresis and restored to normal levels by ADH. However, as noted by Thurau, in subsequent studies Kramer and associates did not demonstrate an effect on medullary vessels of ADH in physiologic concentrations. Similarly, Aukland (1) was unable to show any effect of vasopressin on hydrogen clearance in the outer medulla in dogs with water diuresis. However, Fourman and Kennedy (12), using fluorescent dye, found that staining of the vasa recta was greater than that of the peritubular capillaries in rats with diabetes insipidus. The arterial Microfil-injection specimens are in agreement with Fourman and Kennedy’s observations showing good filling of the vasa recta, but little filling, and therefore higher resistance, of the peritubular capillaries of the outer medulla in dogs with diabetes insipidus. The slow flow in the outer medulla is also apparent from the autoradiographs.
of the $^{85}$Kr disappearance curve represents cortical flow and component II largely outer medullary flow, in the dog with diabetes insipidus component I represents outer cortical flow and component II primarily juxtamedullary cortical flow. This observation of two flow rates in the cortex during diuretic states is similar to the findings of Birtch et al. (4) during diuresis induced by ethacrynic acid and furosemide. Component III would then represent blood flow through the peritubular capillaries of the outer medulla, the vasa recta, and papilla. Little or no change in the slope of component III was noted, but the countercurrent exchange in the vasa recta makes it difficult to assess the significance of this finding.

The inability of previous investigators to demonstrate the role of ADH in the regulation of renal vascular resistance may have been due to the experimental design of the studies. There is considerable question whether water diuresis, particularly under general anesthesia, is an adequate model for the suppression of ADH. Since anesthesia and operative trauma lead to the release of ADH (15), excessive water must be administered to provide a moderate water diuresis. Under such conditions extracellular fluid volume may be markedly increased with reflex renal changes. The infusion of vasopressin leads to further distortion of fluid balance. Thus, the true physiological role of ADH on renal vascular resistance may be masked.

ADDENDUM

Since this paper was submitted, Wunderlich and Scherman reported that the hydrostatic pressure in the vasa recta of the papilla of diabetes insipidus rats on a water diuresis was $13.0 \pm 4.2$; the pressure fell to $8.3 \pm 2.3$ with ADH infusion. The elevated pressure in the vasa recta of the DI animal, with the low venous tone, provides additional evidence for rapid blood flow within the bundles. (Wunderlich, P., and Scherman. Fortlaufende Registrierung des hydrostatischen Drucks im Nieerestdiuni und Blutcapillaren. Arch. Ges. Physiol. 312: R77-8, 1960.)

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