

# Studies on the Mechanism of Reduced Urinary Osmolality after Exposure of the Renal Papilla

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**ABSTRACT** Studies were performed in Munich-Wistar rats to determine whether changes in papillary plasma flow might be responsible for the concentrating defect which occurs after exposure of the extrarenal papilla. Papillary plasma flow was measured by  $^{125}\text{I}$ -albumin accumulation. Initial studies in hydropenic animals revealed that papillary plasma flow was 40% higher in the kidney with the exposed papilla, 41 vs. 29 ml/min per 100 g of papilla ( $P < 0.001$ ). This increase in papillary plasma flow was detectable 15 or 45 min after removing the ureter. Because it was unclear whether the rise in papillary plasma flow was a cause or the result of the fall in urine osmolality, similar studies were performed in animals undergoing a water diuresis. In this setting, papillary plasma flow still increased on the exposed side compared to the control side, 81 vs. 60 ml/min per 100 g, despite similarly low urine osmolalities of 155 and 174 mosmol/kg, respectively. This finding is compatible with the possibility that papillary exposure per se causes an increase in papillary plasma flow and that this hemodynamic alteration may lead to a reduction in urinary osmolality secondary to washout of the medullary interstitium. A final group of hydropenic rats was given either indomethacin or meclofenamate before removing the ureter. In these studies, there was no difference in either the papillary plasma flow or the urine osmolality between control and exposed kidneys. It is therefore suggested that opening the ureter induces an increase in papillary plasma flow by some mechanism which may involve an alteration in prostaglandin synthesis.

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## INTRODUCTION

Investigation of function in regions of the kidney inaccessible to conventional micropuncture has been made possible through the development of tubular microperfusion techniques and by micropuncture of papillary structures in certain species possessing an extrarenal papilla. This latter approach requires removal of the ureter in order to expose the papilla. Associated with this maneuver is the development of a defect in concentrating ability, first examined by Schütz and Schnermann (1) in 1972. These investigators demonstrated a time-dependent fall in urine osmolality which they sought to relate to the loss of pelvic urine recycling after removing the ureter. A correlation was discovered between the osmolality of solutions superfusing the papilla and of urine leaving the papilla. However, it was necessary to superfuse the exposed papilla at a rate much greater than hydropenic urine flow to stop or partially reverse the fall in urine osmolality which occurs after opening the ureter. Even with a 20-fold increase in the amount of fluid bathing the papilla, total correction of the concentrating defect was not possible. Thus, a decrease in the recycling of pelvic urine may not be the predominant cause of the fall in urinary osmolality with papillary exposure.

Other possible mechanisms for the observed fall in urine osmolality involve processes basic to the production of a concentrated urine. Since the maintenance of interstitial hypertonicity necessary for normal concentrating ability depends on the delivery of filtered solute to distal nephron sites as well as active reabsorption along the ascending limb of Henle's loop, alterations in these processes would affect urine osmolality. It is unlikely that distal delivery is affected by papillary exposure, since it has been shown that fractional solute excretion is not decreased in this model (2). Furthermore, exposure of the papilla does not interfere with normal diluting ability (3).

Since antidiuretic hormone activity is necessary for the formation of concentrated urine, resistance to endogenous antidiuretic hormone could diminish urine osmolality. Yet studies have demonstrated osmotic equilibration between collecting duct fluid and adjacent vasa recta plasma, making this possibility unlikely (4).

Finally, alterations in papillary plasma flow could explain this concentrating defect. Because no data are available concerning this possibility and because other explanations are seemingly inadequate, we chose to examine this interrelationship.

## METHODS

Munich-Wistar rats weighing 125–190 g and from which food was withheld the night before study were used. Water was not withheld since we wished to simulate the procedures utilized in our previous micropuncture studies (2). The animals were anesthetized with i.p. Inactin (100 mg/kg body weight), and normal body temperature was maintained on a heated platform. Tracheal cannulation and bladder catheterization were performed. Two polyethylene catheters were placed in a single jugular vein for infusions, and a carotid arterial line was maintained for blood pressure monitoring and blood withdrawal. For the purpose of arterial label visualization (further explained below) at the time of papillary plasma flow measurement, a femoral artery was exposed.

A mid-abdominal incision was made in all animals. After both kidneys were freed from perirenal fat and the adrenal glands, a loose tie was placed around each kidney at its hilus. Removal of fat and freeing of the ureter was done on the left side in both control and experimental animals to facilitate removal of the ureter in the latter group. The papilla was exposed by momentarily displacing it into the renal pelvis and severing the ureter at its origin so that the papilla returned to its normal position maximally exposed.

The method used to measure papillary plasma flow (PPF)<sup>1</sup>

<sup>1</sup> Abbreviations used in this paper: PPF, papillary plasma flow;  $U_{osm}$  urine osmolality.

is a modification of the albumin accumulation technique originally described by Lilienfield and colleagues (5). Using this technique, Solez et al. (6) have shown the circulation time for radiolabeled albumin through the renal papilla of normal hydropenic rats to be approximately 40 s. A similar determination of papillary circulation time is illustrated in Fig. 1. It can be seen that if the ratio ( $V_t$ ) of radioactivity in papillary tissue to systemic blood radioactivity is examined at various perfusion periods (here, at 8, 16, 24, 30, and 60 s), a linear relationship holds to an extrapolated value of approximately 40 s. It is at this time that, at any given papillary blood flow rate, significant radioactive label begins to leave the papilla, thereby altering the previous relationship between the accumulation of albumin in systemic blood and papillary tissue. Thus, for any period of perfusion less than the circulation time, the radioactivity of systemic arterial blood should reflect that of blood traversing the papillary vasa recta.

At the time of PPF measurement, an infusion of <sup>125</sup>I-albumin (approximately 15  $\mu$ Ci/ml, Mallinckrodt Inc., St. Louis, Mo.) and 7% FDC green dye was begun at a rate of 0.45 ml/min via the jugular vein. When the arterial label reached the kidney, as approximated by the appearance of dye at the femoral artery, arterial blood collection was started at the same rate as the infusion. Matching of infusion and withdrawal rates prevented significant change in arterial blood pressure and was made possible through the use of a two-syringe reciprocal action Harvard pump (Harvard Apparatus Co., Inc., Millis, Mass.). After a perfusion and collection period of 12 s, the kidneys were ligated and the blood collection simultaneously stopped. After placing the kidneys at  $-10^\circ\text{C}$  for 20 min, the papillas were removed, weighed, and counted along with aliquots of the collected plasma. It should be pointed out that the PPF measured by this method is entering flow as opposed to exiting flow. PPF can be calculated by relating counts accumulated in the papilla to counts in arterial blood with the formula:

$$\text{PPF (ml/min per 100 g)} = \frac{\text{cpm/100 g papilla}}{\text{cpm/ml plasma}} \times \frac{60 \text{ s/min}}{\text{Perfusion time (s)}}$$

Five groups of rats were studied.

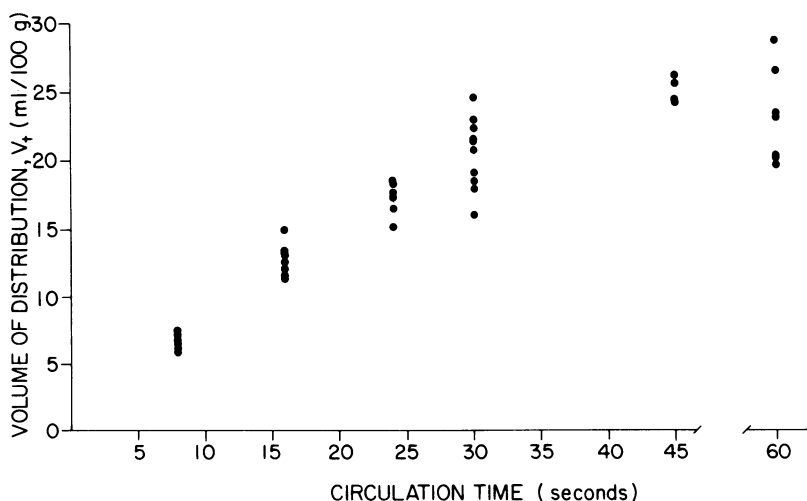


FIGURE 1 Relationship ( $V_t$ ) between radioactivity of renal papilla and arterial blood over time during constant intravenous infusion of <sup>125</sup>I-albumin. Each point represents a single kidney.

**Group I. Time course studies (n = 8).** In these initial studies, urine samples were collected before opening the ureter and from the exposed left papillary tip at intervals of 5, 15, 30, and 45 min after removing the ureter. These animals and subsequent hydropenic groups (II, III, V) received a Ringer infusion at a rate of 0.02 ml/min via the jugular vein during study. The initial control sample was obtained by puncturing the ureter with a 7 to 10- $\mu$ m oil-filled micropipette attached to a Leitz micromanipulator. After opening the ureter, papillary urine samples in this and the remaining groups were collected in a cleaned capillary glass between droplets of mineral oil. This was accomplished by manually touching the papillary tip momentarily with the oil-filled end of the capillary glass and immediately isolating the sample with additional oil. Contralateral urine samples were obtained in identical fashion from an abbreviated bladder catheter.

**Group II. 45-min exposure studies (n = 11).** After initial surgery, this group was prepared for PPF measurement as described above. 45 min after removing the ureter, urine samples were collected from the left papilla and bladder catheter and PPF was measured.

**Group III. Control and 15- and 45-min papillary exposure studies (n = 20).** Rats were prepared in the same manner as the group II animals. 15 or 45 min after removal of the ureter, PPF was measured. Control animals were studied in the same fashion except that the ureter was not removed.

**Group IV. Water diuresis (n = 6).** These animals were prepared for water diuresis in the following manner. Rats were given 5% sucrose drinking water for 14–28 days. During surgical preparation, each received a hypotonic infusion containing 0.3% NaCl through the jugular vein until  $U_{osm}$  was less than 250 mosmol/kg. At this point, the left ureter was removed. 45 min later, urine samples were obtained from the left papilla and bladder catheter and PPF was measured.

**Group V. Indomethacin and meclofenamate (n = 10).** Indomethacin (n = 13) (Merck Sharp & Dohme, West Point, Pa.) or meclofenamate (n = 6) (Parke, Davis & Co., Detroit, Mich.) dissolved in 0.05 M sodium phosphate buffer (pH 8.0) was given intravenously to a final group of hydropenic rats. A dose of 5 mg/kg of either drug was given 15 min before and 15 min after papillary exposure.  $U_{osm}$  and PPF were then measured 45 min after the ureter had been removed.

Osmolalities were measured with a Clifton osmometer utilizing a modification of the technique of Ramsay and Brown (7). Plasma and papillary tissue samples were counted simultaneously in a Packard model 5912 auto-gamma counting system (Packard Instrument Co., Inc., Downers Grove, Ill.). All values were expressed as the mean  $\pm$  1 SEM with either a paired or nonpaired *t* test.

## RESULTS

**Group I. Time course studies.** Fig. 2 demonstrates the time course of the change in  $U_{osm}$  after exposure of the renal papilla. Immediately before opening the ureter,  $U_{osm}$  averaged 1,660 mosmol/kg. Values obtained 15, 30, and 45 min after opening the ureter were significantly less than control values at time zero ( $P < 0.005$  or less). In three of the studies, urine was collected from the contralateral kidney and did not significantly change over the course of the study.

**Group II. 45-min exposure studies.** In each of these studies,  $U_{osm}$  was significantly lower in the experimental kidney ( $P < 0.001$ ) when compared to the untouched contralateral kidney, values averaging 854 vs.

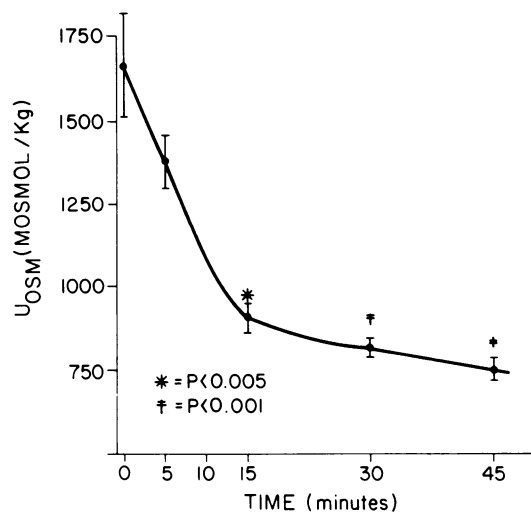


FIGURE 2 Effect of papillary exposure on urine osmolality ( $U_{osm}$ ).

1,676 mosmol/kg, respectively (Fig. 3). The fall in  $U_{osm}$  after opening the ureter was accompanied by a greater PPF when compared with the contralateral control kidney. As can readily be appreciated, PPF was significantly higher in the kidney with the exposed papilla, 41 ml/min per 100 g of papilla as opposed to 29 ml/min per 100 g on the control side ( $P < 0.001$ ).

**Group III. Control and 15-, and 45-min papillary exposure studies.** The effect of removing the ureter on PPF after 15 and 45 min compared with control values is shown in Fig. 4. In control animals in which the ureter was not removed, but the experimental kidney otherwise handled similarly, there was no significant difference in PPF between the two sides, 37 vs. 35 ml/min per 100 g. In contrast, flow was significantly greater in the experimental kidney 15 and 45 min after removing the ureter. At 15 min, the values were 38 vs. 45 ml/min per 100 g ( $P < 0.005$ ), while at 45 min the mean blood flows were 32 vs. 46 ml/min per 100 g in the control and experimental kidney, respectively ( $P < 0.001$ ).

**Group IV. Water diuresis studies.** To clarify this observed relationship between PPF and  $U_{osm}$ , the effect of papillary exposure was evaluated in a model in which basal  $U_{osm}$  was already markedly reduced. In the water diuresis studies shown in Fig. 5, very low urine osmolalities were obtained which were not significantly different between the two sides, 171 vs. 155 mosmol/kg in control and experimental kidney, respectively. Removal of the ureter still led to a significant elevation in PPF on that side. These values averaged 81 ml/min per 100 g in the kidney with the exposed papilla compared with a control mean value of 60 ml/min per 100 g ( $P < 0.005$ ). It should also be noted that the absolute values for PPF are definitely elevated in

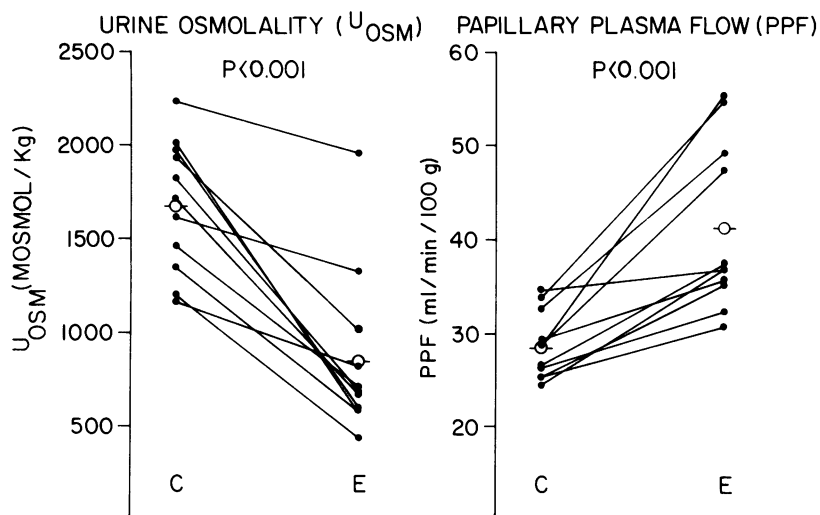


FIGURE 3 Comparison of urine osmolality ( $U_{osm}$ ) and papillary plasma flow (PPF) in control (C) and experimental (E) kidneys after 45-min papillary exposure. The open circles represent the mean values.

water diuresis when compared with hydropenia (Fig. 6) in both the control kidney and the kidney with the exposed papilla ( $P < 0.001$  for both). Yet in both models, papillary exposure leads to a rise in PPF in comparison with the contralateral control side.

**Group V. Indomethacin and meclofenamate studies.** The administration of either of the prostaglandin synthetase inhibitors, indomethacin or meclofenamate, abolished the differences seen in both  $U_{osm}$  and PPF with papillary exposure. The results were qualitatively similar with the two agents and have been analyzed together. As shown in Fig. 7,  $U_{osm}$  from the control and experimental sides averaged 1,652 and 1,563 mosmol/

kg, respectively, while PPF averaged 35 and 36 ml/min per 100 g, respectively. No significant difference exists in either  $U_{osm}$  or PPF between the two kidneys.

## DISCUSSION

The possibility that altered PPF might be involved in the concentrating defect seen after removal of the ureter was addressed in the studies described here. Plasma flow in the papilla was measured with the albumin accumulation technique originally described by Lilienfield et al. (5) and subsequently modified by Solez et al. (6) and Ganguli and Tobian (8). The

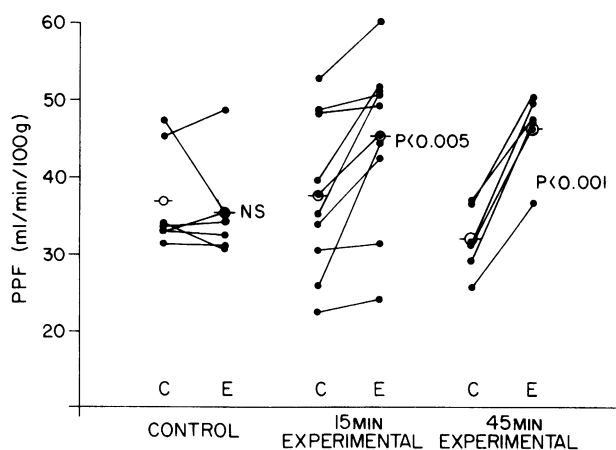


FIGURE 4 Summary of papillary plasma flow (PPF) data. PPF values are shown for control rats as well as rats after 15- or 45-min papillary exposure. The open circles represent the mean values.

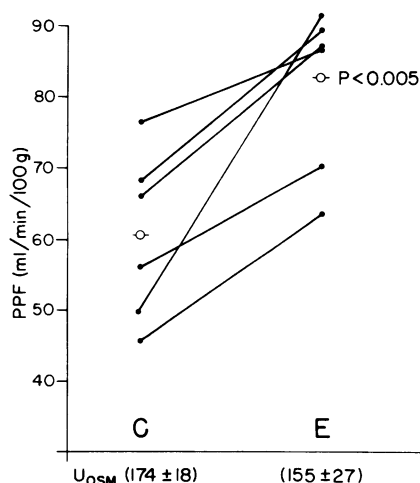


FIGURE 5 Comparison of papillary plasma flow (PPF) in control and experimental kidneys during water diuresis. The open circles represent the mean values.

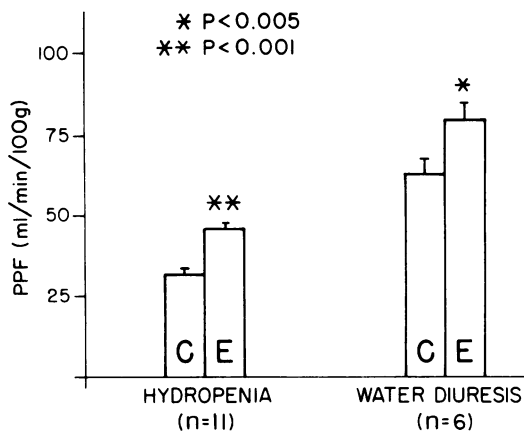


FIGURE 6 Comparison of papillary plasma flow (PPF) in control and experimental kidneys during hydropenia and water diuresis.

theoretical and technical aspects of this method have been discussed in detail by each of these investigators.

This method, which essentially measures plasma flow entering the papilla, requires that the albumin reach the papilla totally by way of the medullary circulation and that the albumin accumulation rate be measured over some period within the initial papillary transit time of the marker. In regard to the first point, there is no evidence that quantitatively significant amounts of albumin reach the papilla of normal rats by way of the glomerular filtrate or other routes. Furthermore, as is shown in Fig. 1, the volume of distribution of albumin increases linearly in at least the initial 30-s period after the start of the albumin infusion. The extrapolated peak of this line was approximately 40 s, a value quite similar to that obtained by Solez et al. (6). In any case, we chose to measure PPF at 12 s after initiation of the infusion, a time period well within the linear portion of the albumin accumulation curve shown in Fig. 1. In addition, we designed the majority of studies in such a way that the primary technical problem of the method would be obviated. It is apparent from the previous studies in both dog and rat that there is a significant variation in the absolute plasma flow measurement in a given experimental model (5, 6, 8). This presumably relates to both methodologic considerations and animal variation. It therefore seemed advantageous to utilize the untouched contralateral kidney for comparison to the kidney with the exposed papilla.

Thus, from the considerations discussed above, this method would seem to be a reasonable index of plasma flow entering the papilla. Furthermore, comparison of PPF between the kidney with the exposed papilla and the contralateral side may be a more sensitive technique than that utilized in previous studies where only group comparisons were possible.

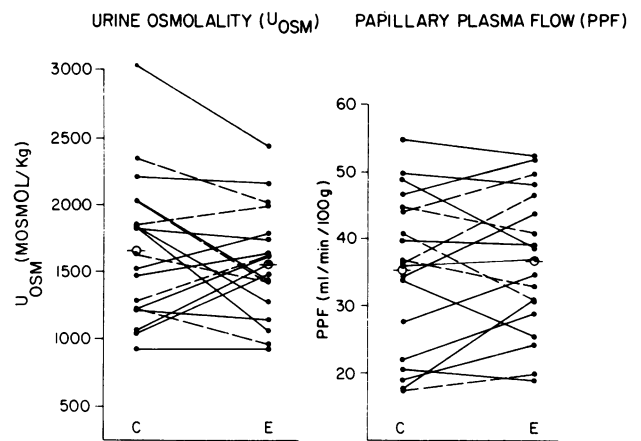


FIGURE 7 Comparison of urine osmolality ( $U_{osm}$ ) and papillary plasma flow (PPF) in control and experimental kidneys of indomethacin-treated (solid lines) and meclofenamate-treated (broken lines) animals. The open circles represent the mean values.

As is shown in Fig. 3, PPF was consistently higher and  $U_{osm}$  markedly lower in the kidney with the exposed papilla 45 min after opening the ureter. PPF was also consistently elevated 15 min after papillary exposure, the earliest time at which  $U_{osm}$  was found to be decreased after opening the ureter (Fig. 2). In contrast, there was no change in PPF in kidneys handled in an identical manner, with the exception that papillary exposure was not performed. Thus, these studies would suggest that there was a relationship between the fall in  $U_{osm}$  and the rise in PPF. Yet, the order of occurrence of these events could not be established from these studies. An increase in papillary plasma flow could lead to a fall in urine osmolality by removing more solute from the medullary interstitium than is added to it (9, 10). On the other hand, Schmid-Schonbein and colleagues have proposed that changes in interstitial tonicity can alter blood flow by affecting the viscosity and other physical properties of blood as it traverses the vasa recta (11).

Thus, it is possible that an increase in PPF led to a decrease in  $U_{osm}$  after papillary exposure or that the converse occurred. In an attempt to resolve this issue, the water diuresis experiments were performed. In these studies,  $U_{osm}$  was markedly reduced in the control kidney and the kidney with papillary exposure. This reduction in  $U_{osm}$  was also presumably associated with a marked fall in interstitial tonicity. Yet, papillary exposure still led to an increase in PPF when compared with the control kidney (Fig. 5).

Thus, exposure of the extrarenal papilla, even in a situation in which papillary tonicity is already markedly reduced, will cause a rise in PPF. From this finding, it does not seem unreasonable to suggest that this hemodynamic alteration occurs as a primary event

in hydropenic animals after papillary exposure. This rise in PPF in hydropenia would be expected to increase the removal of solutes from the papillary interstitium and reduce papillary tonicity and thus  $U_{osm}$ . In water diuresis, however,  $U_{osm}$  would not be expected to fall to any significant extent as PPF rose, since the former parameter was already markedly reduced. It should also be emphasized that the reduction in papillary tonicity which occurs in hydropenic animals may further perpetrate the hemodynamic changes in the medullary circulation which occur as a consequence of papillary exposure. Thus, although these studies do not prove that the rise in PPF caused a reduction in  $U_{osm}$ , the findings are seemingly compatible with this view.

The studies utilizing the prostaglandin inhibitors also lend further doubt to the suggestion that the concentrating defect in this model is the consequence of the interruption of pelvic urea recycling. As noted previously, experiments performed to test this hypothesis utilized a superfusate flow rate far in excess of the normal hydropenic urine flow (1). Even with an excessive superfusate rate, restoration of  $U_{osm}$  to normal levels was usually not possible (1). In similar studies, systemic hypertonicity also occurred with superfusion even if measures were taken to prevent absorption of the solution into the circulation (12; unpublished observations). It also seems inconceivable that either indomethacin or meclofenamate prevented the fall in  $U_{osm}$  after papillary exposure by altering the recycling of pelvic urine.

Fig. 6 demonstrates that PPF was consistently higher in animals undergoing a water diuresis than in hydropenic rats. This finding is in agreement with previous studies by Thureau et al. (13) and Fourman and Kennedy (14). Whether this increase in flow was related to suppression of antidiuretic hormone release or action, volume expansion, local changes in the rheology of the medullary circulation, or other factors was not evaluated in this study.

An attempt was made to identify a mechanism whereby exposure of the papilla leads to an increase in PPF. There are obviously multiple possibilities. Since prostaglandins are produced in the renal medulla and have been suggested to regulate blood flow in this area (15), studies were performed to evaluate the effect of administration of inhibitors of prostaglandin synthesis on the changes which occur after papillary exposure. The administration of indomethacin or meclofenamate in doses shown, to inhibit prostaglandin synthesis in the rat kidney, at least for the former compound, (16–18) prevented both the rise in PPF and the fall in  $U_{osm}$  (Fig. 7), suggesting the possibility that exposure of the papilla may lead to an increased PPF by a prostaglandin-mediated pathway.

This possible relationship between  $U_{osm}$  and prostaglandin synthesis and release deserves further comment. There are a number of in vivo (16, 19, 20) and in vitro (21–23) studies which seem to demonstrate that prostaglandin E antagonizes the hydro-osmotic effect of vasopressin. The in vivo studies have been performed on a background of water diuresis, a situation in which papillary tonicity is low (24). In this setting, an increase in PPF would probably have little effect on  $U_{osm}$ . In hydropenic animals like those utilized in the present studies, however, an increase in PPF mediated by enhanced prostaglandin synthesis could cause a profound decrease in  $U_{osm}$ . This does not exclude the possibility that a prostaglandin E-mediated vasopressin antagonism may also be operative after papillary exposure. It is possible that the fall in  $U_{osm}$  in this model may be due to both an increase in PPF and the antagonistic effect of prostaglandin E on the hydro-osmotic action of vasopressin. Yet, a significant effect of prostaglandin E on the action of vasopressin should lead to osmotic disequilibrium between the collecting duct and surrounding medullary structures. In two studies, however, this alteration has not been noted (3, 4).

Lastly, it should be emphasized that indomethacin and meclofenamate have multiple actions (17) and that their role in preventing the change in  $U_{osm}$  and PPF after papillary exposure may have occurred by a mechanism independent of prostaglandin synthesis and release. Further studies are needed to investigate this possibility.

In summary, the exposed rat papilla is a model which has made in vivo study of medullary function possible. For some time, a poorly understood defect in concentrating ability has been recognized to develop after removal of the ureter. An increase in PPF was found to occur after exposure of the papilla and may be responsible, at least in part, for the fall in  $U_{osm}$ .

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