Redistribution of Renal Blood Flow Produced by Furosemide and Ethacrynic Acid

By A. G. Birtch, M.D., R. M. Zakheim, M.D., L. G. Jones, M.D., and A. C. Barger, M.D.

ABSTRACT

Outer medullary blood flow (85Kr method) is markedly reduced by furosemide and ethacrynic acid injected into the renal artery of unanesthetized dogs; juxtamedullary cortical flow is also decreased. Anatomic localization of the redistribution was verified by autoradiography and by silastic injection studies. The injected specimens demonstrated a relative increase in the resistance of the peritubular capillaries of the juxtamedullary cortex and outer medulla, the blood supply of the juxtamedullary tubules, Henle’s loops and collecting ducts; the vasa recta were dilated. Total renal blood flow (Doppler flowmeter) decreased 10% 1 min after injection, and then gradually began to increase at 2 to 3 min, reaching levels 25 to 30% above control as cortical blood flow increased. The vasodilatation in the cortex is probably localized in the pars radiata. The onset of the diuresis coincided with the decrease in renal blood flow, and persisted after total renal blood flow returned to control value. Thus, the time course of the diuresis correlated more closely with the sustained decrease in juxtamedullary and outer medullary blood flow.

ADDITIONAL KEY WORDS

diuretics diuresis vasa recta
renal medulla unanesthetized dogs

In an attempt to determine whether redistribution of renal blood flow may alter sodium and water excretion, we have studied the effects of the new potent diuretic agents, furosemide (4-chloro-N-[2-furyl methyl]-5-sulfamyl-anthranilic acid) and ethacrynic acid (2,3-dichloro-4-[2 methylenebutyl]-phenoxyacetic acid) on renal blood flow using the 85Kr technique (1). These drugs abolish the concentrating ability of the kidney, and impair the diluting capacity; these effects suggest that a major site of action is in the ascending portion of the loop of Henle in the outer medulla (2). Evidence for a more proximal site of action is conflicting (3, 4) as are the data concerning glomerular filtration rate and renal plasma flow (5, 6). The more recent observations (7) indicate that renal plasma flow is elevated if plasma volume is maintained during the diuresis. Although total renal blood flow is increased, the data to be presented in this paper demonstrate that juxtamedullary cortical and outer medullary blood flow are reduced during the diuresis.

Methods

ACUTE EXPERIMENTS

Four mongrel dogs (20 to 30 kg), on ad libitum fluid intake, were carefully and rapidly anesthetized with pentobarbital (30 mg/kg) to avoid excitement, which alters the distribution of renal blood flow. Through a midline incision the ureters were catheterized after local injection of procaine, and one renal artery was catheterized by technique previously described (8). After a 30- to 60-min recovery period a control measurement of renal blood flow was obtained by the 85Kr method (1). Then, 5 mg of furosemide or ethacrynic acid, dissolved in 5% dextrose in water, was injected slowly (30 sec) through the renal artery catheter. Urine flow increased within
1 to 2 min of the beginning of the injection of the diuretic. The urinary excretion was balanced by intravenous infusion of Ringer's lactate solution (composition in mEq/liter: Na⁺, 130; K⁺, 4; Ca²⁺, 2.7; Cl⁻, 109; and lactate, 28). The sustaining infusion contained, in addition, approximately 25 mg of the diuretic agent. When the diuresis was at a peak (5 to 8 min after first injection) we measured renal flow using the ⁸⁵Kr technique. To localize the alterations in distribution of renal blood flow, similar acute experiments were performed for autoradiography (1) and silastic injections. The silicon rubber¹ was injected through the renal artery at a pressure of 150 mm Hg immediately after the removal of the kidney, and allowed to polymerize and harden for 12 hr. The kidney was cut into thick sections, dehydrated in increasing concentrations of alcohol, and finally cleared in methyl salicylate for examination and photographing.

**CHRONIC EXPERIMENTS**

Repeated experiments were performed on 3 trained, unanesthetized mongrel dogs (30 to 40 kg). The animals were allowed to recover for 3 to 4 weeks after surgical implantation of a renal artery catheter before experiments were started. After a control ⁸⁵Kr curve was obtained, 5 mg of furosemide or ethacrynic acid in 5% dextrose in water were injected through the renal artery catheter, followed by a constant infusion of 0.5 mg/min. The experimental measurement of renal flow was started 5 to 8 min after the first injection. To enable the dogs to lie quietly without catheterization for the 70-min period, intravenous fluid replacement was not given. In 1 dog a Doppler flowmeter was implanted proximal to the renal artery catheter to determine changes in total renal blood flow simultaneously with the ⁸⁵Kr curves. The transducer was calibrated for accuracy, linearity and reproducibility of absolute flow before implantation. However, since occlusion of the renal artery, even though momentary, may produce persistent alterations in distribution of flow, zero flows were not obtained in vivo. Therefore, only relative flow rates are reported in this paper.

¹Microfil (M. V. 112), Canton Bio-Medical Products.
INTRARENAL BLOOD FLOW

Results

Although the redistribution of renal blood flow produced by furosemide and ethacrynic acid was first noted in the unanesthetized dog, the significance of the alterations in the $^{85}$Kr curves was not evident until a series of renal autoradiographs had been prepared. Thorburn et al. (1) had demonstrated in the normal dog that component 1 of the $^{85}$Kr curve represented cortical flow, and component 2 outer medullary flow (Fig. 1). However, following the intrarenal injection of the diuretics, the autoradiographs indicated that components 1 and 2 were both localized to the cortex (Fig. 1, Cortex A & B); very little of the radioactivity entered the outer medulla. The changes induced in the kidneys are illustrated in Figure 2, and compared with control observations. When the kidney was removed immediately after $^{85}$Kr injection (0 sec), the density in the cortex was essentially uniform in the control and the kidney that received the diuretic. However, little activity was present in the outer medulla of the kidney that had received the diuretic (i.e. below the arcuate vessels). Even at 15 and 30 sec after the $^{85}$Kr injection no significant amount of $^{85}$Kr was present in the outer medulla of the experimental kidneys, indicating a slow rate of blood flow in this region. In addition, the 30-sec ethacrynic acid specimen and the 60-sec furosemide autoradiograph show most clearly two distinct rates of flow in the cortex. The more rapid outer cortical flow had removed much of the $^{85}$Kr originally deposited in this region, showing a more dense juxtamedullary

![Figure 2](image-url)

 Autoradiographs of control and experimental kidneys removed at stated times after the intrarenal injection of $^{85}$Kr (0 sec, 15 sec, 30 sec, and 60 sec), and 5 to 8 min after the injection of 5 mg of furosemide (Furo) or ethacrynic acid (Etha) into the renal artery. The exposure time for the later autoradiographs was progressively increased to obtain equivalent density. (COR = cortex, OM = outer medulla, IM = inner medulla, AV = arcuate vessels.)
Silicon rubber injection specimens of control kidney (A), and kidney removed after intrarenal infusion of 5 mg ethacrynic acid (B). (JC = juxtamedullary peritubular capillaries; MC = outer medullary peritubular capillaries; VR = vasa recta.) The dilated, juxtamedullary efferent arterioles are indicated by the upper arrows in B. The coalescing terminal branches of the vasa recta, at the junction of the outer and inner medulla, are shown between the lower arrows in B. Bracket indicates 100 μ.
area with slower "washout." At 60 sec the greatest density in the control kidney was in the outer medulla; no similar concentration of radioactivity was observed in these kidneys. The outer and inner medulla of the experimental kidneys appeared to have the same slow blood flow rate. Thus, the autoradiographs of kidneys that had received the diuretic demonstrated two blood flow rates in the cortex (Cortex A & B, Fig. 1) and a much reduced outer medullary flow rate comparable to, and indistinguishable from, the slow rate of the inner medulla.

To localize more precisely the changed vascular resistance in the juxtamedullary cortex and outer medulla produced by furosemide and ethacrynic acid, the microvasculature was examined after arterial injection of silicon rubber and clearing of the tissue. Figure 3 is an illustrative example of the arterial filling in control and experimental kidneys. In the normal kidney (Fig. 3A) the peritubular capillaries of the juxtamedullary cortex (JC) and outer medulla (MC) were well filled, as were the vasa recta (VR). Five to eight minutes after the injection of furosemide or ethacrynic acid (B) into the renal artery, the relative resistance to filling was markedly higher in

### Table 1

Effect of Ethacrynic Acid and Furosemide on the Distribution of Renal Blood Flow in 4 Anesthetized Dogs

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Location of compartment</th>
<th>Flow rate (ml/min per g of compartment)</th>
<th>Fraction of kidney volume</th>
<th>Flow rate (ml/min per g of kidney)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>5.4 × .58 = 3.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cortex</td>
<td>1.3 × .42 = .6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Outer medulla</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ethacrynic acid</td>
<td>A 7.0 × .73 = 5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cortex</td>
<td>B 1.3 × .27 = .8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Outer medulla</td>
<td>.2 × ? = ?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>5.9 × .65 = 3.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cortex</td>
<td>2.4 × .35 = .8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Outer medulla</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ethacrynic acid</td>
<td>A 7.9 × .72 = 6.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cortex</td>
<td>B 1.3 × .28 = .8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Outer medulla</td>
<td>.2 × ? = ?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>4.9 × .41 = 2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cortex</td>
<td>1.2 × .59 = .7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Outer medulla</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Furosemide</td>
<td>A 10.0 × .63 = 7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cortex</td>
<td>B 1.9 × .37 = .7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Outer medulla</td>
<td>.2 × ? = ?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>6.2 × .49 = 3.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cortex</td>
<td>1.3 × .51 = .8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Furosemide</td>
<td>A 9.3 × .66 = 6.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cortex</td>
<td>B 1.6 × .34 = .7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Outer medulla</td>
<td>.2 × ? = ?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

See text for explanation of A, B and calculation of total flow rate.

_Circulation Research, Vol. XXI, December 1967_
these peritubular vessels in the juxtamedullary cortex (JC) and outer medulla (MC); the vasa recta appeared dilated. Hence, the silastic injection specimens corroborated the autoradiographic evidence for decreased rate of blood flow in the juxtamedullary cortex and the peritubular capillaries of the outer medulla.

The quantitative data derived from the $^{85}$Kr curves in the anesthetized and chronic, unanesthetized dogs are summarized in Tables 1 and 2. Since inner medullary flow did not change significantly, only cortical and outer medullary data are presented. Flow rate in each compartment (i.e. cortex, outer medulla) was determined from the slope of the component ($k_i$), and the fraction of kidney volume ($f'$) as suggested by Carriere et al. (9).

$$f' = \frac{A_0/k'}{A_0/k' + A'_0/k''}$$

where $A_0$ is the zero time intercept. In the normal dog, the flow rate per gram of kidney (i.e. in contrast to flow per gram of renal compartments) was obtained by multiplying the flow rate (ml/min per g) by the fraction of kidney volume for each compartment. Since the inner medulla contains little activity for the first several minutes, the inner medullary component can be neglected with only a small error in total flow. In addition, in the kidney that received the diuretic, 95% or more of the radioactivity entered the cortex; therefore, outer medullary flow can also be neglected in calculating total flow without significant error in these experiments. Thus, with furosemide and ethacrynic acid, flow rate in Cortex A (Fig. 1) was multiplied by fraction of Cortex A, and added to the flow calculated for Cortex B. For example, in experiment 1 of Table 1, cortical flow rate was 5.4 ml/min per g, and outer medullary 1.3, with an average flow rate of the whole kidney of 3.7 ml/min per g. Following the administration of ethacrynic acid, outer cortical flow (Cortex A) increased to 7.0, while inner cortical flow decreased to 1.3 ml/min per g; total kidney flow increased approximately 50% (to 5.5 ml/min per g). Outer medullary flow rate decreased from 1.3 to 0.2 ml/min per g. The data for the chronic dogs (Table 2) were similar to that of the acute experiments, with an increase in outer cortical flow, and a decrease in juxtamedullary cortical and outer medullary flow.

The implantation of a Doppler flowmeter in 1 chronic dog enabled us to follow the time course of the effect of the diuretic agents on

### TABLE 2

**Effect of Ethacrynic Acid and Furosemide on the Distribution of Renal Blood Flow in 3 Chronic Dogs**

<table>
<thead>
<tr>
<th>Location of compartment</th>
<th>Flow rate (ml/min per g of compartment)</th>
<th>Fraction of kidney volume</th>
<th>Flow rate (ml/min per g of kidney)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Cortex, N = 10</td>
<td>6.0 $\times$ .52 = 3.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N = 5 Cortex,</td>
<td>1.9 $\times$ .48 = .9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outer medulla</td>
<td></td>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td>Ethacrynic acid Cortex</td>
<td>A 9.7 $\times$ .39 = 5.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N = 5 Outer medulla</td>
<td>B 2.7 $\times$ .61 = ?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furosemide Cortex</td>
<td>A 9.9 $\times$ .49 = 6.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N = 5 Outer medulla</td>
<td>B 2.6 $\times$ .51 = ?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

See text for explanation of A, B and calculation of total flow rate.
total renal blood flow (Fig. 4). Surprisingly, in each experiment, a 10 to 15% decrease in blood flow was observed immediately after the intrarenal administration of furosemide or ethacrynic acid, followed by a slower increase in blood flow of 25 to 30%. No change in renal blood flow was noted with injection of diluent alone. Blood flow returned to control levels in 30 min in the dog not given fluid replacement.

Discussion

The potency of furosemide and ethacrynic acid, which may induce the loss in the urine of a third of the filtered sodium and water, supposedly by blocking tubular reabsorption, suggests that these drugs should be ideal agents for the study of inhibition of sodium transport in isolated systems. It is surprising then to learn that the evidence in the literature for inhibition of sodium transport in the toad bladder or frog skin has been conflicting. Although Lipson and Hays (10) reported that ethacrynic acid and furosemide decreased the short-circuit current in the toad bladder, Herms and Hofmann (11) observed an increase in the current in the frog skin. Ferguson (12) noted no effect of furosemide on the resting short-circuit current of the toad bladder, but found that the drug antagonized the stimulation of sodium transport produced by arginine vasopressin, cyclic AMP and caffeine. In addition, Daniel (13) reported that ethacrynic acid in low doses increased the extrusion of sodium from sodium-rich smooth muscle cells from rabbits, and blocked the extrusion only with very high levels of the drug. Perhaps the relatively minor effects noted above may be further examples of species specificity of the drugs, e.g. ethacrynic acid, which produces a marked diuresis and natriuresis in man and dog, is ineffective in the rat. It is of interest, however, that Hook and Williamson (14) have shown comparable inhibition of renal Na-K-activated ATPase of the rat by ethacrynic acid and furosemide.

The conclusion that ethacrynic acid and furosemide acted primarily on the ascending limb of Henle was based originally on the decrease in free water clearance induced by these drugs, and by the obliteration of the medullary concentration gradient (2); confirmatory evidence for this action of furosemide was obtained by micropuncture studies.
in the rat (3). Recently, Clapp and Robinson (15) have presented more direct evidence for this site of action in micropuncture studies of the distal tubule of the dog. They found that furosemide approximately doubled the distal tubule osmolal TF-P ratio, which suggests decreased sodium transport by the ascending limb. Although current concepts would suggest that the diuretics act by direct inhibition of the transport process in the ascending limb, the same end result would occur if the tubular metabolism were depressed by reduction of oxygen supply. Although the exact relationship between metabolism and transport is still not clear, there is general agreement that a direct association exists between the amount of sodium reabsorbed by the kidney and oxygen utilized. Moreover, the ions transported from the tubular lumen must be removed by some mechanism, or back diffusion will balance efflux. However, little attention has been given to the manner in which the circulation may modify the various aspects of the transport processes.

The data presented in this paper indicate that blood flow in the juxtamedullary cortex, and even more strikingly in the peritubular capillaries of the outer medulla, are reduced by ethacrynic acid and furosemide. Whether the decreased flow is the direct effect of the diuretic agents on specific blood vessels of the kidney, or secondary to depression of metabolism in localized regions cannot be ascertained from these observations. However, the similarity of vascular changes induced, and the diuresis and natriuresis produced by other vasodilator agents, which are not known metabolic inhibitors (e.g. acetylcholine, prostaglandin), suggest that the direct vascular effect may be an important component of the action of the drugs. It does not seem likely that an increase in medullary intrarenal pressure, the result of increased volume of fluid in the collecting ducts, could be responsible for the decreased outer medullary flow since the silastic injections were made after removal of the kidneys and drainage of blood and urine from the organs. The small immediate reduction in total renal blood flow (Doppler) noted after injection of furosemide and ethacrynic acid would be consistent with a direct effect of the drugs on blood flow in this region. The subsequent increase in cortical flow produced an overall elevation of renal blood flow. It is interesting to note in the observations of Washington and Holland (16) on the effects of ethacrynic acid on urinary $P_{o2}$ that urinary oxygen tension fell immediately after injection, and then rose above control values. The fall in urinary $P_{o2}$ may be the result of decreased oxygen supply in the juxtamedullary cortex and outer medulla; the subsequent rise in oxygen tension may result from the marked increase in tubular fluid passing through the outer medulla which may now have decreased metabolism.

The specific vessels in the juxtamedullary cortex (Fig. 3B, JC) and outer medulla (Fig. 3B, MC) in which flow is reduced by the diuretic drugs appear to arise as side branches from the efferent arterioles of the juxtamedullary glomeruli as they pass into the outer medulla to form the vasa recta. This peritubular capillary network has been described by True et al. (17, Fig. 37D), and by Fourman and Moffat (18, pl. 3, no. 10) who called attention to the "dense capillary plexus of characteristic 'frizzled' appearance surrounding the vasa recta which are collected together in bundles." The marked dilatation of these efferent arterioles (Fig. 3B, upper arrows) and vasa recta induced by the diuretic drugs suggest that the decreased resistance in these vessels may be responsible for the relative increase in resistance in the peritubular capillaries which supply the tubules and collecting ducts in this region. Several groups (1, 18, 19) have now demonstrated that the vasa recta are not interspersed between the loops of Henle and collecting ducts in the outer medulla, and Thorburn et al. (1) have shown that the vasa recta in this region are enclosed in a thick connective tissue sheath. Some branches to the peritubular plexus may be given off by the vasa recta, and many of the vasa recta may terminate by breaking up into coalescing branches sur-
Ludwig's diagrammatic illustration of the course of the blood vessels within the cortex proper (20); m, the space occupied by the medullary radius; b, that occupied by the convoluted canals; ai, arteria interlobularis; vi, vena interlobularis; va, vas afferens glomeruli; ve, vas efferens glomeruli; gl, glomerulus; and zv, venous twig of the interlobular vein. (20)

rounding the ducts at the junction of the outer and inner medulla (Fig. 3B, lower arrows), but the major capillary supply of the tubules of the proximal portion of the outer medulla appears to come from a circuit parallel to the vasa recta. As Fourman and Moffat indicated, "It is an interesting possibility that there may be two circulatory pathways through the medulla, namely a direct route via the main vasa recta to the plexus at the tip of the papilla and thence back to the veins and an indirect pathway through the outer and inner medullary plexuses and their tributaries."

The more complex vascular architecture of the cortex proper, and the absence of an all-or-none effect, makes it more difficult to delineate clearly the vascular effects of ethacrynic acid and furosemide in this area. However, anatomic studies now in progress suggest that the increased flow in the cortex is the result of dilatation of the capillary network in the pars radiata. Ludwig (20) had directed attention to the difference in appearance of the blood vessels in the pars convoluta and the pars radiata, noting that "the meshes of the vascular net surrounding the convoluted tubes of the cortical portion are narrow and somewhat circular in shape; those in the network traversing the medullary radius are broader, and are drawn out in the direction of the course taken by the straight
tubules" (Fig. 5). The silastic specimens made after infusion of the diuretics show relatively poor filling in the region of the convoluted tubules, and dilatation of the vessels in the medullary rays, the blood supply of the collecting ducts. Thus, it appears that the blood flow to the convoluted tubules, both in the cortex proper and in the juxtamedullary cortex is reduced, as is the blood flow in the peritubular capillaries of the outer medulla; blood flow to the pars radiata is increased. The decreased blood flow to the convoluted tubules may be related to the report ed decrease in sodium reabsorption in the proximal tubules (3). Studies are now under way to determine more precisely the significance of the alterations in blood flow distribution in relation to salt and water balance.

Acknowledgments

We thank Dr. William Simon for designing the camera which enabled us to obtain sufficient depth of field at high magnification to photograph the silastic injection specimens. We are also grateful to Miss Susan Rakatansky for her assistance. Mr. F. W. Smith for his devoted care of the animals, and to Dr. William Simon for designing the camera which enabled us to obtain sufficient depth of field at high magnification to photograph the silastic injection specimens. We are also grateful to Miss Susan Rakatansky for her assistance.

The furosemide used in these studies was provided by the Hoechst Pharmaceuticals, Inc., and the ethacrynic acid by Merck Sharp and Dohme Research Laboratories.

References

Redistribution of Renal Blood Flow Produced by Furosemide and Ethacrynic Acid
A. G. BIRTHCH, R. M. ZAKHEIM, L. G. JONES and A. C. BARGER

doi: 10.1161/01.RES.21.6.869

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1967 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/21/6/869

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/