ABSTRACT

INTRARENAL HEMODYNAMIC EFFECTS OF
FUROSEMIDE IN THE DOG

By

Nicholas Thomas Stowe

Furosemide (4-chloro-N-furfuryl-5-sulfamoylanthranilic acid) is an orally effective saluretic-diuretic agent in man and laboratory animals. In addition to having a marked effect on renal sodium transport, the drug increases total renal blood flow. Since furosemide increases renal blood flow without necessarily increasing glomerular filtration rate, the evidence suggests that this drug induces a redistribution of blood flow within the kidney. The purpose of the present work was to determine the role of renal hemodynamic changes in the effect of furosemide on electrolyte and water excretion and on the concentrating mechanism.

Three general groups of experiments were performed. The first group focused on the effect of furosemide in the anesthetized dog. The effect of furosemide and other diuretics on renal tissue electrolyte and water content was also determined. The second group concentrated on

correlating the renal hemodynamic changes produced by furosemide with changes in renal lymph flow and composition. The third series studied the redistribution of renal blood flow produced by furosemide in an isolated dog kidney by means of a dye dilution technique.

A low dose of furosemide (0.1 mg/kg, i.v.) abolished the renal cortico-medullary electrolyte gradient within 10 minutes even when the increase in renal blood flow was prevented; thus demonstrating that an increase in renal blood flow *per se* is not responsible for decreasing the concentrating ability. The abolishment of the electrolyte gradient by furosemide was found to be due to an influx of water rather than a depletion of non-urea solute within the medulla.

Furosemide produced a significant increase in renal hilar lymph flow in hydropenic dogs. In those experiments in which furosemide increased renal blood flow, renal hilar lymph flow also increased. In those experiments in which furosemide did not increase renal blood flow, renal hilar lymph flow did not increase. Changes in lymph flow were more a consequence of changes in renal blood flow than in urine volume. The lymph to plasma ratio of urea was significantly greater than one in control animals. After furosemide, more urea and protein per unit time left the kidney via the hilar lymphatics.

Using a dye (indocyanine green) dilution technique in the isolated kidney, it was determined that the percentage of total renal blood flow which flows through the medullary compartment doubled after furosemide. Analysis of the dye dilution curves showed that after furosemide, an additional component appeared in the washout curve.

The data obtained in these studies strongly support the hypothesis that furosemide induces an intrarenal redistribution of blood flow toward the medulla. The data do not in any way rule out the previous assumption that furosemide inhibits sodium reabsorption in the loop of Henle. Furthermore, the data suggest that the increase in renal blood flow and, more importantly, the redistribution of blood flow produced by furosemide can markedly enhance the effect of the drug, particularly on the concentrating mechanism.

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INTRODUCTION

Chemistry and Pharmacology of Furosemide

Furosemide (4-chloro-N-furfuryl-5-sulfamoylanthranilic acid) is an extremely effective natriuretic-diuretic agent (Muth, 1968). It is a strong acid (pka 3.9, Hajdú and Häussler, 1964) which is transported by the renal tubules by the same transport mechanism that handles para-aminohippurate (PAH) (Gayer, 1964). Since the natriuresis induced by furosemide can be inhibited by probenecid, it appears that adequate cellular concentration (and/or luminal concentration) rather than plasma concentration is a prerequisite to the natriuretic action of furosemide (Hook and Williamson, 1965a).

The distribution and excretion of ^{35}S-labelled furosemide in the rat was studied by Seno, Shaw and Christian (1969). Furosemide was primarily concentrated in the liver and kidney; concentrations in these tissues following a single dose were not significantly different from those observed in chronically treated rats at the termination of drug administration. Nevertheless, 10 days later, the retention of furosemide was significantly greater in the chronically treated rats. When the

excretion of ^{35}S-labelled furosemide and/or metabolites was determined in the urine and feces, four days after the final dose of furosemide, 48% was accounted for in the urine while 52% was found in the feces. This suggests a biliary as well as a urinary route of excretion for furosemide.

The saluretic-diuretic activity of furosemide was first characterized by Timmerman *et al.* (1964). Although structurally similar to the thiazides, the action of furosemide is more rapid in onset and shorter in duration than the thiazides. During maximal diuresis produced by hydrochlorothiazide, addition of furosemide produced a diuretic-saluretic response significantly greater than that produced by hydrochlorothiazide alone (Hook and Williamson, 1965b). During maximal furosemide diuresis, hydrochlorothiazide was without effect. These results suggested that furosemide is capable of inhibiting the reabsorption of sodium by a different mechanism or at a different site than does hydrochlorothiazide. Hook and Williamson (1965a) also suggested that furosemide and ethacrynic acid, though chemically unrelated, share a common mechanism or site of action along the nephron.

Diuretics have traditionally been assumed to exert their effects on renal sodium excretion by interfering with active sodium transport processes at the cellular

level (Bank, 1968). Hook and Williamson (1965c) showed that furosemide decreased the renal medullary sodium gradient. This action, they suggested, was consistent with an inhibition of active sodium transport in the ascending limb of the loop of Henle. Suki *et al.* (1965) showed that furosemide inhibited both solute free water clearance (C_{H_2O}) and the reabsorption of solute-free water ($T^C_{H_2O}$) indicating a marked effect in the ascending limb of the loop of Henle. They concluded that the major site of action of furosemide appears to be the ascending limb of Henle's loop with a probable additional site in the proximal tubule. Other work (Seldin *et al.*, 1966; LaZotte *et al.*, 1966 and Puschett and Goldberg, 1968) has supported the contention that the primary effect of furosemide is to inhibit sodium transport in the ascending limb of Henle's loop.

Furosemide may have an effect on sodium reabsorption in the proximal tubule. Seldin *et al.* (1966) concluded that massive doses of furosemide can produce a net inhibition of sodium reabsorption in this segment of the tubule. They stressed that it is doubtful that this proximal inhibitory effect is actually present in circumstances where potent diuretics are commonly employed such as in edematous states. Seldin *et al.* (1966) concluded that the principle site of inhibition of sodium reabsorption is the ascending limb of Henle's loop. Brenner *et al.*

(1969) stated that in rats, furosemide appeared to retard the absolute rate of sodium reabsorption within the proximal tubule as measured by the shrinking drop technique. However, other investigators (Dirks *et al.*, 1966 and Dirks and Seely, 1970) failed to show any inhibition of fractional reabsorption at this site in the dog. It appears that the effects of furosemide within the proximal tubule are variable, depending on the species studied and experimental conditions.

Hemodynamic Effect of Furosemide

The response to diuretic agents is principally determined by three factors: the locus in the nephron where the drug exerts its inhibitory effect on sodium reabsorption; the potency of its action; and the nature and magnitude of the internal regulatory influences which augment the renal tubular reabsorption of sodium (Seldin *et al.*, 1966). The preceding discussion pointed out that the primary locus in the nephron where furosemide exerts its inhibitory effect on sodium reabsorption is the ascending limb of the loop of Henle. Furosemide is a more potent diuretic than hydrochlorothiazide and approximately equi-potent when compared to ethacrynic acid. As will be shown, furosemide can also influence renal tubular reabsorption of sodium through the hemodynamic effects of the drug.

It is becoming increasingly apparent that changes in intrarenal hemodynamics play a major role in renal sodium handling, independent of changes in the overall rate of glomerular filtration (Epstein *et al.*, 1971). Epstein *et al.* (1971) pointed out that renal hemodynamics may influence renal sodium handling through changes in peritubular hydrostatic and oncotic pressure, thereby controlling the removal of reabsorbate from the peritubular spaces. In addition, changes in sodium balance appear to be associated with significant shifts in both the patterns of the distribution of blood flow within the kidney and the distribution of glomerular filtrate to different nephron populations (Barger, 1966).

Alterations in sodium excretion in the absence of significant changes in glomerular filtration rate could be explained by a redistribution of renal blood flow (Goodyer and Jaeger, 1955). A redistribution of blood flow could result in a shift in activity between long salt-conserving nephrons and short, salt-wasting nephrons. Barger (1966) observed that sodium retention in patients with congestive heart failure occurs when renal blood flow is primarily distributed to the inner cortical and outer medullary region and that natriuresis ensues when blood flow is increased to the outer cortical region. Recent work by Epstein *et al.* (1971) demonstrated that

the diuretic response to ethacrynic acid but not chlorothiazide was associated with a significant change in the pattern of intrarenal hemodynamics.

Furosemide increases total renal blood flow (Hook *et al.*, 1966; Birtch *et al.*, 1967; Ludens *et al.*, 1968). Since the drug increases renal blood flow without increasing glomerular filtration rate, the evidence suggests that furosemide induces a redistribution of blood flow within the kidney. Birtch *et al.* (1967) demonstrated that the administration of furosemide to dogs is associated with quantitatively important changes in intrarenal blood flow distribution. Data obtained utilizing the krypton washout technique suggested that furosemide shunted blood to the outer cortex following vasoconstriction in the juxtamedullary region. Because krypton can be trapped within the countercurrent mechanism of the medulla, the use of this indicator to monitor changes in the deep medullary circulation is questionable. The only reference which Birtch *et al.* (1967) made to the deep medullary circulation was that the vasa recta appeared dilated after furosemide.

Countercurrent Mechanism

Since it has been suggested that intrarenal hemodynamics play a major role in renal sodium handling, independent of changes in the overall rate of glomerular filtration, it is essential to understand the relationship

between renal blood flow and the concentrating mechanism. Although Wirz, Hargitay and Kuhn (1951) were the first to speculate on the countercurrent mechanism, earlier work (Filehne and Biberfeld, 1902; Hirokowa, 1908) showed that the osmotic pressure of samples taken from the medulla of kidneys was higher than that of samples from the cortex. These early workers pointed out that the osmotic pressure of the urine increases considerably during the passage through the loops of Henle and collecting tubules. These findings remained unnoticed until Wirz, Hargitay and Kuhn (1951) presented further cryoscopic data on kidney slices. Their data demonstrated that the osmolality was identical for all adjacent tubular structures at any level in the kidney, and that there was a steadily increasing osmotic gradient from the cortex to the tip of the papilla. This was the basis for a new theory of urine concentration. This theory was based on the premise that the hairpin-like loop of Henle acts as a countercurrent multiplier system (Hargitay and Kuhn, 1951). The countercurrent mechanism can be described in the following manner:

The renal artery divides within the pelvis of the kidney into the interlobar arteries which form the arcuate arteries at the cortico-medullary junction. The afferent arterioles to the glomeruli originate from the interlobular arteries. Nephrons in the cortex have short

loops of Henle as opposed to the juxtamedullary nephrons which have very long loops of Henle. In the cortex, the efferent arterioles which leave the glomeruli form a dense capillary network within the cortex. The efferent arterioles of the juxtamedullary glomeruli form the vasa recta which follow the long loops of Henle into the medulla. It is the long loops of Henle and the vasa recta of the juxtamedullary nephrons which are responsible for urine concentration.

A comprehensive study of the spatial interrelationship between vascular and tubular elements in the medulla of the rat was made by Kriz and Lever (1969). In the outer zone of the medulla, they found vascular bundles which contain alternating descending and ascending vasa recta in a strict geometrical arrangement. In a ring which surrounds these vascular bundles, they found that descending limbs of the loop of Henle alternate regularly with ascending vasa recta. To the periphery of this ring, ascending limbs of the loop were associated with collecting ducts enmeshed in a capillary plexus. In the inner medulla, ascending thin limbs of the loops of Henle retain their association with collecting ducts and descending limbs with ascending vasa recta. The two limbs of the loop of Henle are thus systematically separated from each other, but there is also a close relationship between loops of Henle and surrounding blood vessels. This

definite interrelationship can be summarized by saying that the ascending limbs of the loop of Henle are closely related to the collecting ducts whereas the descending limbs of the loop of Henle appear to be more closely related to venous vasa recta.

A countercurrent multiplier consists of two components: a hairpin loop and an energy consuming process capable of establishing a small difference of concentration between the adjacent limbs of the loop (Kriz and Lever, 1969). In the kidney, the loop of Henle is the hairpin loop. The energy-consuming process is the active transport of sodium out of the relatively water impermeable ascending limb of the loop of Henle. Maintenance of a small difference of concentration between the adjacent limbs of the loop by the active process has been called the "single-effect." This single effect establishes a gradient of perhaps 200 mOs/kg H_2O between the fluid of the ascending limb and interstitium (Gottschalk and Mylle, 1959). This single effect is multiplied as the fluid in the thin descending limb comes into osmotic equilibrium with the interstitial fluid by diffusion of water out of the descending limb (Gottschalk and Mylle, 1959). In addition some sodium chloride probably diffuses into the descending limb thus raising the osmolality of the fluid presented to the ascending limb. This results in the establishment of an increasing osmotic gradient in the

direction of the tip of the papilla; however, at no level is there a large osmotic difference between luminal and interstitial fluid. According to Gottschalk (1964) the magnitude of the longitudinal gradient established will depend on: (1) the magnitude of the single effect at any level, (2) the length of the loop along which it is multiplied, (3) the volume rate of flow through the loop. It is the longitudinal gradient established by the active transport of sodium out of the ascending limb of the loop of Henle which is ultimately responsible for the concentration of urine within the collecting ducts. In the presence of antidiuretic hormone (ADH) water diffuses out of the collecting ducts into the hyperosmotic medullary interstitium until the fluid remaining in the collecting ducts becomes correspondingly concentrated (Gottschalk and Mylle, 1959).

The integrity of the countercurrent mechanism is maintained by the vasa recta and by the lymphatic system. The blood supply and the lymphatic system within the kidney are functionally interrelated. Inasmuch as lymph is an ultrafiltrate of blood, lymph formation within the kidney may also influence the concentrating mechanism. Since the vasa recta and the lymphatic system help to maintain the osmotic gradient within the kidneys, these systems will be discussed in some detail.

Countercurrent Exchangers

The vasa recta participate in the concentrating mechanisms by functioning as countercurrent diffusion exchangers (Gottschalk and Mylle, 1959). It appears that the vasa recta operate in a passive fashion. Ions move along the concentration gradient established between the vasa recta and the interstitium. This passive movement results in the trapping of sodium, urea and other diffusible solutes in the medulla. The trapping of these solutes increases the osmolar gradient within the kidney. The performance of the vasa recta as a countercurrent exchanger can be contrasted with the performance of the loop of Henle as a countercurrent multiplier. The exchanger is capable only of maintaining an existing gradient whereas the multiplier creates the gradient.

The vasa recta function as efficient exchangers because blood flow through the vasa recta is relatively low. Thurau (1964) stated that in the dog, blood flow in the outer and inner medulla account for 6 or 7% of the total renal blood flow. The inner medulla is perfused by approximately 1% of total renal blood flow and has the lowest perfusion rate per unit tissue weight in the kidney. Thurau (1964) stressed the fact that the term low medullary blood flow is strictly relative. It is low only in comparison to the renal cortex. The inner medullary blood flow per unit tissue weight is about

15 times that in resting muscle, 50% that in the brain and 30% that in the heart under basal conditions (Thurau, 1964). It is this relatively low blood flow which maintains the integrity of the countercurrent mechanism. Berliner *et al.* (1958) pointed out that osmotic concentration in the medulla is inversely related to blood flow, if other factors remain constant. For this reason, the ability of a vasoactive agent to alter medullary blood flow can be an important determinant in the action of that drug on electrolyte and water excretion.

The problems inherent in measuring medullary blood flow are numerous because of the inaccessability of the circulation. The recent development of the xenon and krypton washout technique has added much information concerning the intrarenal distribution of blood flow (Blaufox *et al.*, 1970). The very recent use of thermistors (Powers, 1970) and fiber optics (Suki *et al.*, 1970) also appear to offer a means of studying the medullary circulation. Just as means have been found to study the medullary circulation, new means are also being developed to study the lymphatic circulation of the kidney. It is fair to say that today's state of knowledge regarding the function of the lymphatics is comparable to what was known about medullary blood flow 15 years ago. The development of polyethylene and silastic cannulae has greatly facilitated the study of lymph flow and composition.

Lymphatic System

Since the lymphatics within the kidney are so small, anatomical studies are incomplete and controversial. Some investigators (Bell *et al.*, 1968; Keyl *et al.*, 1965) have been unable to find lymphatic vessels in the medulla. This inability to visualize medullary lymphatics may be due to the method of retrograde infusion of a dye into the hilar lymphatics. Valves present in the hilar lymphatics may prevent the entry of the dye into the medulla (Bell *et al.*, 1968). Although the evidence supporting the presence of medullary lymphatics is meager, it is convincing. Rawson (1949), in a study of a pathological human kidney, proposed that two lymphatic systems are present in the kidney. One begins in the cortex and accompanies the interlobular vessels toward the corticomedullary junction; the other starts at the papilla and ascends to join the cortical system at the corticomedullary junction. From there, large trunks follow the arcurate and interlobar vessels to leave the kidney at the hilus. Rusznyak *et al.* (1967) reported that the lymphatics in the rabbit followed the renal blood vessels and drained both cortical and medullary areas. They also showed that in the dog the lymphatics of the capsule and the lymph vascular system of the parenchyma of the kidney were interconnected. Indirect evidence for the existence of two intrarenal lymphatic

networks was recently supplied by Cockett *et al.* (1969) who reported significantly higher glucose levels in capsular lymph than in hilar lymph. Since glucose is actively reabsorbed in the proximal tubule, they reasoned that the cortical interstitium would have the highest concentration of glucose. The glucose level found in hilar lymph was explained by communications between capsular and hilar lymphatic systems. A report by Slotkoff *et al.* (1968) suggested that the cortical and juxtamedullary circulations are functionally independent. If this is true, one could postulate a lymphatic system to serve the cortical circulation and another to serve the medullary circulation. The evidence in the literature appears to favor two lymphatic systems in the kidney; one cortical and one medullary, both interconnected.

Direct attempts to correlate the composition of capsular lymph with the cortical area of the kidney and hilar lymph with the medullary area of the kidney have been unsuccessful. Santos-Martinez and Selkurt (1969) concluded that medullary lymphatics which drain centrifugally toward the juxtamedullary zone would be expected to have their constituents come into equilibrium with the vasa recta by exchange diffusion in the outer medulla. This lymph may then join the lymph that drains the cortex, so that a new equilibrium concentration, near isotonicity with arterial plasma is attained.

Because of the difficulties involved in obtaining renal lymph, estimates of lymph flow rates are highly variable. Keyl *et al.* (1965) reported in studies on unanesthetized dogs that lymph flow from one capsular lymphatic was around 7 µl/min. Hilar lymph flow has been estimated to be 4 to 8 times capsular lymph flow (Keyl *et al.*, 1965). Selkurt (1963) estimated total lymphatic flow to be about 7 ml per hour per kidney. Capsular lymph flow can be increased fourfold by infusion of hypertonic mannitol (LeBrie, 1968). It appears that the kidneys produce approximately as much lymph as urine per hour (Rusznyak *et al.*, 1967).

The composition of renal lymph is another area in which a great deal of controversy exists. All investigators agree that the concentration of protein is less in renal lymph than in arterial plasma. In regard to the urea and sodium concentration, there is no general agreement. It appears that the lymph to plasma ratio for sodium is approximately one. Keyl *et al.* (1965) and Cockett *et al.* (1969) reported no difference in sodium concentrations between capsular and hilar lymph. The data for urea is quite variable. Surgarman *et al.* (1942) reported a lymph to plasma ratio greater than one, while Keyl *et al.* (1965) and Santos-Martinez and Selkurt (1969) reported the lymph to plasma ratio for urea to be less than one. Keyl *et al.* (1965) point out that some of the

discrepancies in the values for the composition of renal lymph may be due to evaporation and impairment of normal renal function. Such errors should affect the protein determinations as well, but since these values are similar, the question of the difference in techniques of measuring urea is raised. It is possible that in addition to the difficulties involved in collecting lymph, the means of determining urea concentrations may differ among investigators and account for the differences observed. Since hilar and capsular lymphatics may be interconnected, lymph from one area of the kidney may be diluted with lymph from another area (Rusznyak *et al.*, 1967).

Several ideas have been proposed concerning the function of the renal lymphatics. Mayerson (1963) suggested three homeostatic functions of the lymphatic system in the kidney. The first is a recirculation function or the return to the blood stream of macromolecules and other plasma constituents which have leaked out. The recirculation function is important because it is known that the loop of Henle, vasa recta and collecting ducts are permeable to water, urea and sodium (Morgan and Berliner, 1968) and protein (Moffat, 1969). The second is a transport function or the carrying of substances to the blood stream from the cells of origin in tissues. Very little is known about the transport function. The

lymphatics may be important in the release of endogenous hormones such as renin (Lever and Peart, 1962). The third is the "safety valve" function. The lymphatics protect the kidney whenever there is a load on the kidney such as in diuresis, increased venous pressure and ureteral obstruction. Closely associated with the "safety valve" function is the suggestion by Bell *et al.* (1968) that the lymphatics may buffer changes in interstitial fluid pressure in the areas adjacent to the juxtaglomerular apparatus. Thus the lymphatics may indirectly influence sodium balance. However, Gazitua *et al.* (1969) showed that significant changes in lymphatic vessel pressure were not observed during infusion of hyperosmotic solutions of dextrose and sodium chloride either at natural or constant flow. If the lymphatics do buffer changes in interstitial fluid pressure, one would have expected to see significant changes in lymphatic pressure during the infusion of hyperosmotic fluids. It does not appear that the lymphatics are directly involved in sodium balance. In essence, the three functions of the lymphatics can be summarized as a third outflow system from the kidney (Cockett *et al.*, 1969).

In addition to the very general functions just listed, several investigators (Cockett *et al.*, 1969; Mayerson, 1963; Tormene *et al.*, 1965) feel that the renal lymphatics subserve an important function for the operation

of the countercurrent multiplier system. Gottschalk and Mylle (1959) pointed out that the osmotic equilibration of vasa recta blood with medullary interstitial fluid in all likelihood is due not only to the diffusion of solute into their descending and out of their ascending limbs, but also results in a large part from the diffusion of water in the opposite direction. This "short circuiting" of water across the tops of the vasa recta helps to maintain the high concentration gradient within the medulla. According to Selkurt (1963), water abstracted from the collecting ducts moves into the vasa recta because of the gradient of chemical potential established by the colloid osmotic pressure of the plasma proteins. If this gradient is altered by the accumulation of proteins in the interstitium, edema results and the integrity of the countercurrent system is interrupted. The lymphatics may drain off the excessive protein filtered by the vasa recta, and thus prevent the accumulation of protein in the interstitial spaces of the medulla (Rusznyak *et al.*, 1967).

One important function of the renal lymphatics may be to maintain a relatively low oncotic pressure in the interstitium, thus permitting the establishment of a gradient with the higher oncotic pressure within the vasa recta. To support this contention, Mayerson (1963) reported that when the main lymphatic trunk from one

kidney was obstructed, the osmotic pressure of the urine of the experimental kidney decreased 25% as contrasted to a rise in the control kidney of approximately the same amount.

The work of Tormene *et al.* (1965) reaffirms the conclusion that the lymphatic system is indispensable in the regulation of the complex mechanism of urinary concentration and dilution. In 10 dogs, the lymphatics of one kidney were obstructed. This resulted in an almost immediate increase in urine volume and edema in the obstructed kidney. Tormene *et al.* (1965) pointed out that the interstitial edema which is so rapid and obvious cannot be explained by the interstitial stagnation of protein. They calculated the amount of protein drained from the lymphatic system of the kidney to be about $1.2178 \pm .0967$ mg/min. Thus the interstitial stagnation of protein in the space of 10-15 minutes varies from 12.178 to 18.267 mg. They say that this quantity of protein is too small to justify the degree of edema observed. To restate their views, if the renal lymphatics functioned solely to remove the excess filtered protein from the interstitium, obstruction of the lymphatics would lead to only a small increase in interstitial protein concentration. This small increase of extravascular protein could not by itself account for the edema. Consequently, the observation by Mayerson (1963)

that the renal lymphatics maintain a relatively low oncotic pressure in the interstitium, thus permitting the establishment of a gradient with the higher oncotic pressure within the vasa recta, becomes extremely relevant. It is this gradient which is responsible for the "short circuiting" of water across the tops of the vasa recta as suggested by Gottschalk and Mylle (1959). This gradient may be very sensitive to changes in the oncotic pressure of the interstitium. Any alteration which would favor a reversal or even diminution of the normal gradient for water between the interstitium and vasa recta could destroy the functional integrity of the vasa recta as countercurrent diffusion exchangers. Thus, it is not the extra protein *per se* which is responsible for the loss of concentrating capacity of a kidney in lymphostasis. Rather, it is the presence of the extra protein in a lymphatic system which appears to be functionally integrated with the countercurrent multiplier system of the loop of Henle and the countercurrent diffusion exchanger function of the vasa recta. Any alteration in one system affects the other two systems and ultimately the concentrating capacity of the kidney.

This section has attempted to review some of the important aspects of the countercurrent mechanism. It is apparent that this is a complex problem involving a consideration of intrarenal hemodynamics and of the

lymphatic system. The early research concerning the countercurrent mechanism focused upon the single effect, or the active transport of sodium out of the ascending limb of the loop of Henle. The loop of Henle was thought to be the important structure within the medulla. While the active transport of sodium within the loop of Henle is still considered the important process, the relationship of the countercurrent multiplier to other structures within the medulla is now being questioned.

Kriz and Lever (1969) stressed that because of the archectural arrangement of the tubules and blood vessels within the medulla, the single effect or multiplication occurs within a vasa recta-loop of Henle complex. There is probably a series of linked countercurrent processes which lead to the establishment of the concentration gradient within the kidney. Thus there is now a spatial or a dimensional approach to understanding the concentration of urine by the kidney. The loop of Henle must be considered in relationship to the other structures, vasa recta, lymphatics and tubules, which surround it. This approach must also be taken into consideration in defining the action of a drug on the concentrating mechanism of the kidney. Since the concentrating mechanism depends upon many different factors, such as blood and lymph flow, the effect of the drug on these factors must also be

considered. Furosemide is a good example of a drug which affects more than one parameter of the concentrating mechanism.

Specific Objectives

The purpose of the present work was to determine the role of renal hemodynamic changes in the effect of furosemide on electrolyte and water excretion and on the concentrating mechanism. The specific aim was to test the hypothesis that furosemide induces a redistribution of renal blood flow toward the medulla. The hypothesis does not rule out in any way the assumption that furosemide inhibits sodium reabsorption in the ascending limb of the loop of Henle.

To confirm this hypothesis, three general groups of experiments were performed. The first group focused on the effects of furosemide on renal tissue electrolyte and water content in the dog. These effects were also compared to those of other diuretics. The second group of experiments concentrated on correlating the hemodynamic changes produced by furosemide with the effect of the drug on renal lymph flow and composition. The third series involved a study of furosemide in the isolated dog kidney. A dye dilution technique was developed and used to estimate the intrarenal distribution of blood flow before and after furosemide. The data

obtained from these experiments support the hypothesis that furosemide induces a redistribution of renal blood flow toward the medulla.

METHODS

General Methods

Mongrel dogs of either sex, weighing 16 to 30 kg, were anesthetized with pentobarbital sodium (30 mg/kg, i.v.). An endotracheal tube was inserted, and a heating pad was wrapped around the dog's chest to maintain body temperature near 37°C. Four to eighteen hours prior to each experiment, the animal was deprived of food and water and given 5 units of vasopressin (ADH) in oil, intramuscularly. A femoral artery and vein were cannulated with polyethylene tubing to facilitate withdrawal of blood samples and infusion of fluids, respectively. Femoral arterial blood pressure was monitored with a Statham pressure transducer and a Grass recorder.

The left kidney was exposed through a flank incision. The ureter was cannulated with polyethylene tubing, and a flowmeter probe was placed around the renal artery. Total renal blood flow was monitored with a square-wave electromagnetic flowmeter (Carolina Instrument Co.). In some experiments renal venous blood was obtained from a polyethylene catheter placed into the renal vein through the spermatic or ovarian vein.

In most experiments, the drug under study was administered intravenously. In certain experiments, a 24 gauge needle connected to polyethylene tubing was inserted in the direction of flow into the left renal artery near its origin at the aorta. In these experiments, the drug was administered intraarterially through this needle. If urine was collected from the right kidney, the right ureter was exposed through the left flank incision and cannulated with polyethylene tubing.

Urine was collected during either 5 or 10 minute collection periods. Femoral arterial blood samples were obtained at the midpoint of each collection period. Control diuresis was produced by the intravenous infusion of 0.9% NaCl (0.1 or 0.25 ml/kg/min). Inulin (0.8%) was added to all intravenous infusions to monitor the rate of glomerular filtration. p-Aminohippurate (PAH, 0.12%) was also added to the saline infusion to estimate renal plasma flow.

In some experiments, an effort was made to restrict the blood flow in one kidney. This was accomplished in two ways. The first involved altering the resistance of a clamp placed around the aorta just proximal to the left renal artery. The second was accomplished by altering the resistance of a clamp placed around the left renal artery. The perfusion pressure below the clamp was monitored through a needle placed in the renal artery. The

needle was connected via polyethylene tubing to a Statham pressure transducer.

In a large group of experiments, tissue samples were obtained from both kidneys for analysis of electrolytes, urea and water. If the right kidney in a particular experiment was used as a control, it was removed through a flank incision prior to running the experiment on the left kidney of the same animal. Tissue samples were taken from the kidneys in the following manner: Duplicate tissue samples of renal cortex, outer and inner medulla and papilla were rapidly cut and weighed. The wet weight of these tissue samples was approximately 100 mg. Tissue dry weight was determined after drying the samples in an oven at 100°C for 16 hours. Following digestion in concentrated nitric acid, the samples were analyzed for sodium and potassium content by flame photometry. In those experiments in which the urea content of the kidney was determined, two extra tissue samples were obtained from each area of the kidney. These samples were transferred to pre-weighed flasks containing 2 ml water. These flasks were then heated in a boiling water bath for 5 min. The concentration of urea in the supernatant was determined.

Lymph Studies

According to the procedures described in the general methods section, a flowmeter probe was placed around the exposed left renal artery.

A hilar lymph vessel of the same kidney was identified. In all experiments, only the hilar vessel selected for cannulation was ligated. A polyethylene cannula was inserted toward the kidney with the aid of a dissecting microscope and tied in place. To minimize clot formation in lymph, heparin was given intravenously, 1000 USP units per animal. Lymph was collected in small preweighed vials and flow was recorded as mg/min.
Urine and lymph samples were serially collected at 10 minute intervals.

Several types of experiments were conducted. In the first group of 3 animals, furosemide (5 mg/kg, i.v.) was administered after two 10 minute collection periods. Thirty minutes after furosemide, 100 ml of a 12.5% mannitol solution was infused intravenously. Urine and lymph samples were collected for 30 minutes after the infusion of mannitol. In two of these experiments, the hilar lymph vessel was cannulated with PE 10 tubing (I.D. .011"). The lymph vessel in the third experiment, as well as all the subsequent vessels in the entire study, was cannulated with PE 50 tubing (I.D. .023").

In the second group of 16 animals, furosemide was given in doses ranging from 0.3 to 10 mg/kg, i.v. Lymph and urine samples were collected for periods up to 90 minutes after the administration of furosemide.

In the third group of 3 animals, hydrochlorothiazide, 5 mg/kg, i.v. was given. Lymph and urine samples were collected for 40 minutes after the administration of hydrochlorothiazide. The water and electrolyte content of these kidneys was determined.

Isolated Kidney Studies

Much larger mongrel dogs (25 to 35 kg) were generally used in this part of the study. The dogs usually had free access to food and water prior to the experiment. Prior to the removal of the left kidney through an abdominal midline incision, the dog received heparin 500 USP units/kg. Five minutes after heparinization, the kidney was removed from the animal, and the renal artery and vein were cannulated with polyethylene tubing (0.115" I.D. and 0.625" I.D. respectively). The arterial cannula had previously been connected to a cannula placed in the femoral artery of a donor dog. The donor animal was also anesthetized with sodium pentobarbital and received 500 USP units of heparin per kg. The transfer time of kidney from the dog to the donor circulation rarely exceeded 2 minutes. After the circulation was begun, the kidney was placed in a chamber around which warm water circulated. The temperature of the kidney was maintained by this system around 37°C. The isolated kidney was placed at a lower level than the donor dog to maintain perfusion pressure at 100 mm Hg. The ureter of the kidney was cannulated

with polethylene tubing. Renal venous blood was collected and returned to the donor animal via a Sigmamotor pump. Blood was warmed by passing the Tygon tubing through a water bath.

An extracorporeal flowmeter probe was placed in the perfusion circuit between the donor animal and the isolated kidney. Femoral blood pressure of the donor animal was monitored at the flowmeter probe via a Statham pressure transducer. Renal perfusion pressure was monitored by placing a needle connected to a Statham transducer into the polyethylene tubing which cannulated the isolated kidney. Drugs and/or dye were infused into the system via this cannula. Femoral blood pressure, renal perfusion pressure and renal blood flow were all recorded on a Grass recorder.

A priming dose of inulin (7 g) and para-aminohippurate (PAH, .75 g) was given to the donor animal. This was followed by a constant infusion (.25 mg/kg/min) of 12% inulin and 4% PAH in saline. After a 30-minute equilibration period, arterial and venous blood samples were collected at the midpoint of 10-minute urine collection periods. The drug under study was administered either intraarterially or intravenously.

In the experiments in which dye dilution curves were obtained, a saline solution of indocyanine green (Cardiogreen, Hynson, Wescott and Dunning, Baltimore, Md.)

was made just prior to its use in each study. In all but one experiment, the dye was continuously infused for 3 minutes into the kidney, at the rate of 1 mg/min in a volume of 0.5 ml/min. In the one experiment in which a bolus injection of approximately 900 mg of dye was given, the dye was made up using the plasma obtained from the dog from which the isolated kidney was taken.

The dye dilution curves were obtained in the following manner. The dye was infused into the kidney through the needle placed in the renal artery. In one experiment, a bolus injection of the dye was made. Just before stopping the infusion of the dye or before the bolus injection of the dye, serial samples of renal venous blood were collected every 3 seconds for varying lengths of time. The dye did not recirculate. Fresh blood, obtained from other dogs, was used to maintain the venous return to the donor animal.

Standards for the dye were made for each dye dilution curve by adding the dye to blood samples obtained 10 min before each curve was run. The plasma was removed from the standards and read within eight hours in a Hitachi Perkin-Elmer Digital readout spectrophotometer at 820 mμ. The renal venous samples were read in the same manner. When necessary, proper dilutions with plasma were made before reading the dye concentration. In most experiments, the dye concentration was recorded as mg% per timed sample.

In the one experiment in which a bolus injection of the dye was made, the renal venous samples were collected in preweighed tubes. This allowed the calculation of the actual content of dye per timed sample.

The decrease in dye concentration or content as a function of time was plotted on semi-logarithm paper or as the natural logarithms. For analysis of the data, a straight line was drawn by inspection or by a least squares method (Lewis, 1966) through the terminal portion of the curve and extrapolated back to zero time (Component IV in a four component curve). The dye concentrations of the extrapolated line were then subtracted graphically from the original curve to obtain the third exponential (Component III). The second and first exponentials similarly were drawn in sequential fashion (Component II and I). This procedure of curve stripping describes the washout pattern as a sum of exponentials (Thorburn *et al.*, 1963). The number of exponentials which describe a particular is determined by the number of components which are obtained by the above analysis of the washout pattern.

$$A = A_{o'}e^{-k't} + A_{o"}e^{-k"t} \ldots\ldots A_{o}n\text{-}1e^{-k^{n-1}t}$$

Where A = total dye concentration

k', $k"$, k^{n-1} = slope of lines

$A_{o'}$, $A_{o"}$ $A_{o'''}$ = apparent dye concentration present initially in each component.

Analysis of Data

Inulin was estimated by the method of Schreiner (1950) and PAH by the method of Smith *et al.* (1945). Urea was determined by the method of Fawcett and Scott (1960) and protein by an ultraviolet spectrophotometric method described by Waddell (1956). Sodium and potassium were analyzed by flame photometry (Instrumentation Laboratories, internal standard flame photometer). Chloride was estimated with a Buchler-Cotlove Chloridometer. Osmolalities were estimated with an Advanced Osmometer. The data were analyzed statistically using Student's "t" test (Lewis, 1966). The 0.05 level of probability was used as the criterion of significance.

Drugs and Chemical Reagents

The following drugs and chemicals were used: Furosemide (supplied by Hoechst Pharmaceutical Company); Antidiuretic hormone (ADH; pitressin tannate in oil, Parke-Davis and Company); Indocyanine green (Hynson, Westcott and Dunning, Incorporated); Chlormerodrin (Lakeside Laboratories, Incorporated); Hydrochlorothiazide (Merck Sharp and Dohme Research Laboratories); Sodium acetazolamide (Lederle); Strophanthin G (Calbiochem); Glucagon (Eli Lilly and Company); Ethacrynic acid (Merck Sharp and Dohme Research Laboratories); Inulin, para amino hippuric acid (PAH); mannitol

(Nutritional Biochemicals Corporation); Hyland UN-Test kit (Travenol Laboratories, Incorporated); and other standard laboratory reagents readily available from commercial sources.

RESULTS

Effect of Furosemide in the Anesthetized Dog

The intravenous infusion of furosemide (20 μg/kg/min) produced a significant increase in urine flow, sodium excretion and renal blood flow throughout a 20-minute period of infusion (Table 1). Although there were decreases in the clearance of inulin and PAH, these changes were not statistically significant (Table 1). Figure 1 illustrates a typical response obtained during one of five experiments. Shortly after initiating furosemide infusion, there was a transient decrease followed by a marked, sustained, increase in renal blood flow.

Five animals were treated with ADH in oil, and 18 hours later the animals were anesthetized, their kidneys quickly removed and the concentration of electrolytes measured in the various areas of the kidney. In these control animals, the concentration of electrolytes increased from the cortex through the medulla to the papillary tip (Figure 2). Following the infusion of furosemide (20 μg/kg/min) for 20 minutes, this cortico-medullary electrolyte gradient was completely abolished

(Figure 2). When saline alone was infused (0.1 ml/kg/min) the cortico-medullary gradient was reduced (Figure 2).

To access the role of renal blood flow changes in the total natriuretic-diuretic response to furosemide, an attempt was made to keep blood flow constant in one kidney during drug infusion. In four animals, a variable resistance clamp was placed around the aorta between the two renal arteries. After obtaining adequate control samples, furosemide was infused at 20 µg/kg/min. During the drug infusion, the clamp was tightened so that renal blood flow in one kidney remained relatively constant. Such a constriction produced a drop in blood pressure distal to the clamp of approximately 22 mm Hg. As illustrated in Figure 3, there was a significant decrease in inulin clearance during drug infusion. Nevertheless, the magnitude of this change was similar in both kidneys. Urine volume and sodium excretion were significantly increased in both kidneys, but the increment of response was significantly greater in the kidney in which blood flow was not restricted. The cortico-medullary electrolyte gradient was abolished in both kidneys of these animals.

Similar results were obtained in experiments in which the increase in renal blood flow after furosemide was prevented by altering the resistance of a clamp placed around the left renal artery. Table 2 presents

the results of these experiments. After furosemide, urine and sodium excretion was less in the kidney in which renal blood flow was maintained at a constant level. The cortico-medullary electrolyte gradient was also abolished in both kidneys of these animals.

Inasmuch as the cortico-medullary electrolyte gradient is normally measured in terms of tissue wet weight, it was of interest to determine if the changes observed in this gradient were due to a decrease in electrolyte content or to an increase in water content of the tissue. Furosemide (20 µg/kg/min, i.v.) was found to decrease significantly the electrolyte content of the renal papilla within 20 minutes (Figure 4). At the same time there was also a significant increase in the water content of the tissue. Similar results were obtained in the experiments in which blood flow to one kidney was restricted. The kidney with constant renal blood flow appeared to have a slightly higher electrolyte content. However, both kidneys showed a marked increase in water content (Figure 4). In an attempt to magnify the differences between the two kidneys, the same dose of furosemide (20 µg/kg/min, i.v.) was infused for 60 minutes. As illustrated in Figure 5, the infusion of furosemide for 60 minutes resulted in a significant decrease in electrolyte content of the papillary tip of both kidneys (restricted blood flow and unrestricted blood flow) when

compared to control. Nevertheless, this decrease was no different than that produced by infusing saline (0.25 ml/kg/min) for a comparable period of time (Figure 5). In the animals infused with furosemide for 60 minutes, there appeared to be some difference between the electrolyte content in the kidney with increased renal blood flow and the kidney with constant renal blood flow, but this was small and not statistically significant. Saline infusion produced a statistically significant increase in water content of the papillary tip, and furosemide produced an even greater increase in the water content of the papillary tip in both kidneys.

The effect of a 60-minute infusion of furosemide on renal hemodynamics and excretion (Table 3) appeared similar to that seen with only 20 minutes of infusion (Table 1). With only 3 animals and considerable variation, statistical manipulations were of no value. Inulin clearance tended to fall in both kidneys to a comparable degree and the mean increase in sodium excretion and urine volume appeared to be greater from the kidney with unrestricted blood flow.

To gain some information about the effects of furosemide immediately after administration of the drug, a single dose (0.1 mg/kg) was administered intravenously and 10 minutes later the kidneys were removed and the electrolyte and water content of the tissue determined.

Infusion of saline (0.1 ml/kg/min) for 10 minutes produced a significant decrease in tissue electrolyte content. This was not further altered by the administration of furosemide (Figure 6). Within 10 minutes after furosemide, there was a significant increase in water content of the papillary tip which was not different in the two kidneys. The renal cortico-medullary gradient normally observed in control animals was completely obliterated with this dose of furosemide, and this was due almost exclusively to the increase in water content rather than a decrease in electrolyte content in the papillary tip (Figure 6). The hemodynamic and excretory data from these animals is illustrated in Table 4. The clamp between the two renal arteries significantly depressed inulin clearance in the left kidney, whereas in the unrestricted kidney inulin clearance remained constant over the 10-minute period. Similarly, the increase in urine flow and sodium excretion by the left kidney was less than that of the right. Urine osmolality was markedly reduced in both kidneys. Ten minutes after furosemide, urine osmolality in the kidney with unrestricted blood flow was nearly isosmotic. Even when blood flow was held constant, urine osmolality had dropped to 550 mOsm.

In a group of four anesthetized dogs, the urea gradient in the kidney was measured before and 10 minutes after the administration of furosemide (0.5 mg/kg, i.v.).

The results are presented in Table 5. After furosemide, the urea gradient was obliterated. In these same experiments, the amount of urea excreted in the urine doubled in the first 5-minute period after furosemide. However, 10 minutes after furosemide, the amount of urea excreted in the urine was the same as the control values. The increase in water content and decrease in electrolyte content of the papilla was similar to that observed for the 20-minute infusion of furosemide (Figure 4).

Comparison of Furosemide with Other Diuretics

The effect of different diuretics on the water content of the renal papilla was determined. The water content and urine volume changes observed after different procedures are summarized in Table 6. It is apparent that the increase in urine volume observed after the diuretics is not responsible for the increase in tissue water. The administration of ethacrynic acid resulted in the largest change in urine volume (3.2 ml/min). However, the water content of the kidneys exposed to ethacrynic acid was no different than that of the kidneys infused with saline for 60 minutes in which the urine volume changes were only 0.24 ml/min. Likewise, the infusion of mannitol resulted in a large urine volume change (2.3 ml/min) but the increase in the water content of the medulla was small. Although the urine volume changes associated with furosemide were

equal to or less than that of ethacrynic acid and mannitol, the water content of the papilla after furosemide was significantly greater. The water content observed after chlormerodrin and ouabain was similar to furosemide even though the urine volume changes observed after chlormerodrin and ouabain were small (unable to record urine volume after ouabain).

An attempt was made to compare equi-diuretic doses of furosemide and hydrochlorothiazide. The results of these experiments are presented in Figure 7. Equi-diuretic doses of furosemide (0.5 mg/kg, i.v.) in four experiments and hydrochlorothiazide (5 mg/kg, i.v.) in three experiments were used. Tissue samples were taken at the peak urine volume response for each diuretic (furosemide, 10 minutes; hydrochlorothiazide, 40 minutes). These samples were compared to six control animals which were infused with saline at 0.1 mg/kg/min for 10 minutes. Both furosemide and hydrochlorothiazide caused a significant increase in water content of the papilla when compared to saline controls, and the amount of water present after furosemide was significantly greater than the amount present after hydrochlorothiazide.

Lymph Studies

The average lymph flow from a single hilar lymphatic vessel measured during a 10-minute collection period was 32 $\pm$ 6 mg/min. Table 7 summarizes the data for

twenty-four dogs in which control lymph flows were measured. The lymph to plasma ratio for urea was significantly greater than one, and the lymph to plasma ratio for protein was significantly less than one. The lymph to plasma ratio for sodium was not significantly different than one.

Mannitol and Furosemide

Figure 8 represents the results from experiments in which mannitol was administered after furosemide in the same experiment. In the first 10-minute period following the i.v. administration of furosemide, 5 mg/kg, total renal blood flow and urine volume were significantly increased. In addition, furosemide significantly increased renal hilar lymph flow from 21 to 28 mg/min. Thirty minutes after furosemide, 12.5% mannitol solution was infused intravenously. Renal hilar lymph flow increased from 28 to 35 mg/min. The increase in lymph flow following both drugs was transient and renal hilar lymph flow returned to control values within 30 minutes.

Furosemide

Table 8 presents the individual values for lymph flow prior to and during the administration of furosemide (1 mg/kg, i.v.). In each of these dogs, renal hilar lymph flow increased after furosemide. This increase was significantly elevated for 20 minutes but declined toward

control values within 30 to 40 minutes. The specific gravity of the lymph was measured in three of these experiments. Although the specific gravity increased following furosemide, the increase was not enough to warrant corrections in the weight of lymph.

All the data from these experiments are summarized in Table 9. Total renal blood flow was significantly increased during the first 10-minute period following furosemide. Both the increase in blood flow and lymph flow were transient, with the decline in lymph flow lagging behind the decline in blood flow by about 10 minutes. The clearance of inulin characteristically fell after furosemide administration while urea clearance increased. The amount of urea excreted in the urine increased after furosemide. In those experiments in which the concentration of urea was measured in renal venous blood, no significant change in urea concentration was observed following furosemide. The lymph to plasma ratio of sodium appeared to decline after furosemide whereas the lymph to plasma ratio for urea did not change. The lymph to plasma ratio of protein increased after furosemide.

The effects of hydrochlorothiazide (5 mg/kg, i.v.) on renal hilar lymph flow are summarized in Figure 9. In contrast to furosemide and mannitol, renal blood flow did not significantly change from a control value of

222 ml/min after hydrochlorothiazide. Renal hilar lymph flow also did not change from a control value of 31 mg/min. The values for hydrochlorothiazide were obtained 10 minutes after the drug was given and represent the peak values.

The results of seventeen experiments in which the effects of furosemide (0.3 mg to 10 mg/kg, i.v.) on renal blood flow and lymph flow were examined are summarized in Table 10. In six experiments, there was no increase in renal blood flow nor hilar lymph flow. In the eleven experiments in which renal blood flow increased, there was also a significant increase in renal hilar lymph flow. In both groups, urine volume was significantly increased. These same data are represented in Figure 10. The absolute values of lymph flow and renal blood flow before and after furosemide for each experiment was plotted. Such a plot shows that in those experiments in which renal blood flow increased,hilar lymph flow also increased. When renal blood flow did not increase,hilar lymph flow also did not increase. Furthermore, if the increase in renal blood flow was small, the increase in hilar lymph flow was usually small. Figure 10 also displays the extreme variation in control lymph flow values and the variation in response to furosemide.

Effect of Furosemide in the Isolated Kidney

Renal function in the isolated kidney was studied during the infusion of saline (0.5 ml/min). The results of these experiments are summarized in Table 11. The glomerular filtration rate (as measured by the clearance of inulin) was slightly lower in the isolated kidney than in the kidney of an anesthetized dog (Table 1). Renal blood flow averaged 2 to 3 ml/g kidney. Renal blood flow and renal vascular resistance did not significantly change during the 40-minute experimental period. Urine volume and sodium excretion decreased slightly. When the infusion of saline was begun, urine osmolality decreased. Arterial osmolality and arterial hematocrit remained relatively constant during the experiment.

The tissue contents of urea and water after the infusion of saline in the isolated kidney are summarized in Table 12. The values obtained in the isolated kidney were compared to those of the other kidney of the same dog from which the isolated kidney was obtained. After the infusion of saline, the urea gradient in the isolated kidney was still present.

When furosemide (1 mg/kg, dose based on weight of animal which supplies the circulation to the isolated kidney) was infused intraarterially for a 10-minute period, the results listed in Table 13 were obtained.

After furosemide, urine volume and sodium excretion were significantly elevated. Tissue urea and water concentrations in kidneys from these same experiments are summarized in Table 14. The tissue urea gradient based on dry weight was destroyed after furosemide. There also was a large increase in tissue water after furosemide. In renal papillary tissue, the water (g per 100 g dry solid) increased from 513g $\pm$ 79 to 948g $\pm$ 59 after furosemide.

Effect of Furosemide on Hilar Lymph Flow in the Isolated Kidney

A hilar lymphatic vessel of an isolated kidney was cannulated in four experiments and lymph flow determined (Table 15). In all the experiments, furosemide (100 to 500 mg) was administered intravenously to the animal supplying the circulation to the isolated kidney. Two of the four experiments responded with an increase in renal blood flow of approximately 10 ml/min. In these two animals, there was a slight increase in hilar lymph flow. In the other two kidneys, which did not respond with an increase in renal blood flow, hilar lymph flow also did not increase. Thirty minutes after the administration of furosemide, a 12.5% mannitol solution was administered to the donor animals in three of the four experiments. Hilar lymph flow increased slightly.

Dye Dilution Studies in the Isolated Kidney

Two different concentrations of indocyanine green were infused into the renal artery of an anesthetized dog. Total blood flow of the same kidney was recorded with an electromagnetic flowmeter probe placed around the artery. Figure 11 is a photograph of the record of this experiment. Renal blood flow decreased to about one-half of its original value and remained depressed during the infusion of 10 mg/min of dye. When 5 mg/min was used, no such depression was observed.

Figure 12 depicts the washout of indocyanine green from an isolated kidney after an intraarterial infusion of the dye (1 mg/min) for 3 minutes. The perfusion pressure (50 mm Hg) and renal blood flow (10 ml/min) were purposely maintained at the low values to facilitate a long collection period. During the 46-minute collection period, the dye did not recirculate through the kidney. Because of the low blood flow, these data were not analyzed.

The reproducibility of the washout of indocyanine green is demonstrated in the experiment illustrated in Figure 13. Two experiments were performed in the same isolated kidney, and essentially the same pattern of washout was observed.

The effect of furosemide on dye washout was determined in a group of five experiments. First, a

control washout pattern was obtained. Then, furosemide, in doses ranging between 50 and 100 mg, was administered over a 10-minute period. At the end of the drug infusion period, a second washout pattern was obtained. The results of one such experiment is depicted graphically in Figure 14. The data from the five experiments are summarized in Table 16. Renal blood flow and urine volume increased after furosemide. When the washout patterns were analyzed, two components, one very fast and one slower, were found. In these experiments, furosemide significantly reduced the half time ($T_{1/2}$) for component II, but did not alter the fast component, component I.

An experiment was designed in which more parameters of the isolated kidney could be measured during the collection of the dye washout. In this experiment, each determination was made following an intraarterial injection of a bolus of dye (900 mg) dissolved in saline. A summary of this experiment is presented in Table 17. The control washout was run between collection periods 1 and 2. Furosemide was administered between periods 2 and 3, and the drug altered washout was determined between periods 3 and 4. Renal blood flow was constant during the control washout (Figure 15). Although renal blood flow increased during the intraarterial administration of furosemide (Figure 16), it remained constant during the

actual collection for the drug-altered washout (Figure 17). Arterial hematocrit also remained constant during both washouts (Table 17). A characteristic response to furosemide, in terms of changes in blood flow, urine volume, and clearance of inulin was observed in the isolated kidney (Table 17).

During the control washout, 95% of the dye was recovered in 4 minutes; in the post-furosemide washout, 93% of the dye was recovered during the 4 minutes. Figures 18 and 19 show the data obtained before and after furosemide, respectively. Data in Figure 18 were fitted with a 3 component exponential equation:

$$A = 0.437e^{-0.364t} + 0.011e^{-0.038t} + 0.0008 \quad (1)$$

Where A = mg apparent dye recovered at times, t.
Data in Figure 19 were fitted with a 4 component exponential equation:

$$A = 0.456e^{-0.584t} + 0.050e^{-0.131t} + 0.0004e^{-0.022t} + 0.0002 \quad (2)$$

The $T_{1/2}$ for the components for both equations are presented in Table 18.

Furosemide caused the appearance of an additional component in the washout pattern. It appears that the component marked "II + III" in Figure 18 breaks up into two separate components, II and III, after furosemide in Figure 19. Furosemide also appeared to decrease the $T_{1/2}$

for the very fast component, component I (Table 18). Even though the initial concentration of the dye was slightly higher in the second curve, the dye was washed out much faster than in the control curve.

The dye dilution curves for the control washout (Figure 18) and for the drug altered washout (Figure 19) were plotted on arithmetic paper (Figures 20 and 21 respectively). Using equations (1) and (2) obtained from the exponential analysis of the curves, a theoretical washout curve was determined. When the theoretical curves were plotted with the experimental curves a very close agreement between curves was observed (Figures 20 and 21). Using the exponentials obtained for the fastest component of each curve (the first component of equations 1 and 2),

$$A = 0.437e^{-0.364t} \quad (3)$$

$$A = 0.456e^{-0.584t} \quad (4)$$

the dye dilution curves (Figures 20 and 21) were partitioned into cortical (shaded) and medullary components. From this type of exponential extrapolation before the drug (Figure 20) it was determined that 94% of the total renal blood flow flows through the fast component (cortex) while only 6% flows through the slow component (medulla). However, after furosemide (Figure 21) the percentage of total renal blood flow through the medulla approximately

doubled (from 6% in the control curve to 11% in the drug altered curve). The cortical component decreased to 89% after furosemide.

Another way of expressing this same data was to relate the cortical and medullary percentages in each curve to the total renal blood actually measured (Table 17). After furosemide, medullary blood flow increased 107% (from 8 ml/min to 18 ml/min). Cortical blood flow also increased but by only 7% (from 136 ml/min to 146 ml/min).

From the collection of timed samples following a bolus injection of the dye, total circulating blood volume of the kidney was determined. This technique involves the determination of the shape of the dye dilution curve if the injection had been maintained at a constant rate instead of being abruptly terminated (Lewis, 1953). Figures 22 and 23 graphically represent the technique employed to obtain the curve of continuous infusion for the control curve and the drug altered curve respectively. Using this technique, the vascular volume obtained before furosemide was 16% of wet kidney weight and the volume after furosemide was 17%.

DISCUSSION

Furosemide produced an increase in renal blood flow whether administered intravenously or intraarterially (Figures 1 and 16, respectively). The pattern of blood flow change, a transient constriction followed by a dilation, was identical in the kidney of an intact anesthetized animal (Figure 1) and in an isolated kidney (Figure 16). The elevation of renal blood flow after furosemide can be maintained for at least 60 minutes by replacing urine volume (Table 3). Since the effect of furosemide on renal blood flow in the anesthetized dog is related to dose and to the initial state of hydration of the animal (Ludens *et al.*, 1968a), hydropenic animals were used in most of the studies to maximize the vascular effects of furosemide.

An attempt to determine the role of hemodynamic changes in the natriuretic-diuretic response to furosemide was made by maintaining blood flow to one kidney constant after furosemide (Figure 3 and Table 3). Urine volume and sodium excretion were significantly increased in both kidneys, but the increment of response was

significantly greater in the kidney in which blood flow was not restricted (Figure 3). When renal blood flow was held constant, renal perfusion pressure fell approximately 22 mmHg. Ludens *et al.* (1968b) found that a fall in perfusion pressure of this magnitude in an untreated animal decreased sodium excretion by only 22 μEq/min. The data suggested, therefore, that the observed differences in electrolyte and water excretion between the two kidneys were due to differences in blood flow. However, in the collection periods immediately following initiation of furosemide infusion, there appeared to be a greater fall in inulin clearance in the kidney with restricted blood flow. This suggested, then, that the differences in electrolyte and water excretion could have been due to the fall in perfusion pressure in the kidney with restricted blood flow rather than due to the increased blood flow in the unrestricted kidney. Furthermore, Knox *et al.* (1969) found that decreases in perfusion pressure of similar magnitude can have a marked effect on proximal tubular function. In an effort to minimize the fall in perfusion pressure, the renal artery instead of the aorta was clamped after furosemide (Table 3). Differences in the effect of furosemide on water and electrolyte excretion between the two kidneys was observed. Again this difference could be explained by changes in glomerular filtration rate. Although these (the clamp)

experiments did not separate the hemodynamic factor from the natriuretic-diuretic response to furosemide, they demonstrated that it is not an increase in renal blood flow *per se* which obliterates the cortico-medullary electrolyte gradient. Even though the increase in renal blood flow after furosemide was prevented, the medullary concentration gradient was abolished (Figures 3, 4, 5, and 6). Although the differences in electrolyte and water excretion can be explained by the changes in glomerular filtration rate, such changes cannot explain the large increase in renal tissue water content observed after furosemide (Figures 4, 5 and 6).

In a previous study of the effect of furosemide on the renal medullary sodium gradient, a relatively large dose (25 mg/kg) was employed (Hook and Williamson, 1965c). The present study shows that a dose of furosemide as low as 0.1 mg/kg, i.v. abolished the electrolyte concentration gradient within 10 minutes (Figure 6). However, as pointed out by Goldberg (1966), it is important to distinguish between solute content and solute concentration when discussing the cortico-medullary gradient. Such a distinction must be made because changes in the renal cortico-medullary electrolyte gradient may be due to solute depletion and/or water accumulation. The studies described here demonstrated that the abolishment of the electrolyte gradient in the dog kidney by furosemide

can be explained in terms of the change in solute concentration but not in terms of the change in solute content. Dow and Irvine (1967) reported comparable results in the rat. Furthermore, these studies show that the concentration changes occur within 10 minutes and do so even when the increase in renal blood flow is prevented (Figure 6).

Appelboom *et al.* (1965) reported that not more than 10% of medullary tissue is occupied by urine during osmotic diuresis in the dog. Therefore, the increase in renal papillary tissue water content after furosemide cannot be explained by the diuresis. The present studies which compared different diuretics (Table 6) support this contention because there was no correlation between urine volume changes and water content of renal papillary tissue. Specifically, it was possible to compare the response to furosemide and hydrochlorothiazide since the urine volume changes with these two diuretics were equal (Table 6, Figure 7). The increase in water content after furosemide was significantly greater than that after hydrochlorothiazide. The observation that the increase in water content after hydrochlorothiazide is less than that after furosemide has been reported by several investigators (Goldberg, 1966; Dow and Irvine, 1967; Cannon *et al.*, 1968). This observation is important in view of the recent observation that chlorothiazide did not alter

the intrarenal distribution of blood flow (Epstein *et al.*, 1971). Neither blood flow rates nor blood flow distribution was changed. Since the urine volumes were equal, the differences in water content between hydrochlorothiazide and furosemide is probably due to the intrarenal hemodynamic effects of furosemide. Furthermore, Dirks *et al.*, (1966) and Knox *et al.*, (1969) demonstrated that the administration of furosemide to dogs does not result in increased delivery of sodium from the proximal tubule. Therefore, the increased water in the medulla would not appear to be due to an increased volume of fluid in the tubular system. It appears, therefore, that the increased fluid is vascular and/or interstitial. Thurau (1964) suggested that the vascular volume per unit tissue is greater in the inner medulla than in the cortex. Consequently, an increase in medullary blood flow could contribute significantly to the increased water content of the medulla. Urine osmolality dropped from 1300 mOSm to 500 mOSm within 10 minutes following furosemide (Table 4). The effective osmotic gradient across the epithelium of the collecting ducts was abolished at a time when the water content of the medulla was increased, whereas electrolyte content remained unchanged from the saline control. Unless furosemide inhibits ADH it is difficult to deny the premise that the increased water content is a contributing factor in decreasing urine concentrating capacity.

Since the increase in renal papillary water content after furosemide cannot be explained by the diuresis, the water must be within the vascular and/or interstitial compartments. If the water were interstitial, intrarenal hemodynamic changes might be reflected in the composition and flow of renal lymph. The kidney is drained by about eight to ten lymphatics (Mayerson, 1963). Since the lymphatics, like venules, are thin walled, lymph flow is sensitive to intraluminal and extraluminal pressure changes (Rusznyak *et al.*, 1967). Consequently, any increase in pressure or resistance in one lymphatic can result in a shunting of lymph flow to other lymphatic vessels where the resistance to flow is less. A polyethylene cannula can in many ways (bore, length, position) increase the resistance in the cannulated lymphatic. Because of resistance factors, lymph flow out of one cannulated lymphatic does not represent the normal flow through the intact lymphatic. Therefore the value of total lymph flow cannot be extrapolated from the flow through one small cannula. Indeed, the considerable variation in lymph flow observed between experiments is probably due to the resistance offered by each cannula. Such factors as anesthesia, diet and the state of hydration of the animal can also influence lymph flow (Mayerson, 1963; Henry *et al.*, 1969).

The present experiments demonstrated that furosemide as well as mannitol can increase renal hilar lymph flow. In those experiments in which renal blood flow increased after furosemide (Table 10), renal hilar lymph flow also increased. This observation of an increase in hilar lymph flow is substantiated by a preliminary report of an increased thoracic duct lymph flow after furosemide (Szwed *et al.*, 1971). O'Morchoe *et al.* (1970) also recorded hilar lymph flow in anesthetized dogs and reported that lymph flow was slightly reduced after furosemide. The animals used in their study were not hydropenic and renal blood flow was not recorded. Because of the experimental conditions employed by O'Morchoe *et al.* (1970), an increase in renal blood flow after furosemide would not be expected. These results (O'Morchoe *et al.*, 1970) are consistent with the data obtained from the five animals in which furosemide did not increase renal blood flow (Table 10). In these animals, renal hilar lymph flow also did not increase.

It is apparent from the present work (Table 10) and that of others (LeBrie, 1968, and O'Morchoe *et al.*, 1970) that diuresis *per se* is not the main factor responsible for the increased output of lymph after furosemide. This concept is important because it implies that the volume of fluid in the interstitium increased. On the basis of the Starling-Landis hypothesis, an

increase in blood flow through a vascular bed would result in an increase in capillary filtrate. Part of this capillary filtrate will be removed from the interstitium by the lymphatic system. Therefore, it is logical to assume that if furosemide increases renal blood flow, renal lymph flow will also increase. Such an assumption requires that the factors (mean capillary pressure, tissue colloid osmotic pressure, tissue pressure, and plasma colloid osmotic pressure) considered in the Starling-Landis hypothesis remain constant (Guyton, 1961). The effect of hydrochlorothiazide on lymph flow illustrates this concept very well. Since the thiazides do not alter total renal blood flow or the intrarenal distribution of bloow flow (Epstein *et al.*, 1971), the drug should not increase hilar lymph flow. Indeed, hydrochlorothiazide did not increase lymph flow (Table 10, Figure 10).

The composition of hilar lymph before and after furosemide was analyzed to determine the effect of intrarenal hemodynamic changes on the composition of lymph. On the basis of anatomical studies (Bell *et al.*, 1968), it has been suggested that renal lymph is derived primarily from fluid formed in the periarterial spaces along with a component derived from the vicinity of Bowman's capsule. Although the precise anatomical areas which the capsular and hilar lymphatics drain is not known, several investigators (Santos-Martinez and Selkurt,

1969; O'Morchoe *et al.*, 1970 and Tormene *et al.*, 1965) reported that the medulla is a significant source of renal hilar lymph. If hilar lymphatic vessels drain fluid from the medullary interstitium, the hilar lymph to plasma ratios for urea and sodium would be greater than one. The lymph to plasma ratios for urea and sodium during a saline infusion were both greater than one (Table 7). The urea ratio was significantly different than one. After furosemide, the lymph to plasma ratio for sodium appeared to decline. This decline could be explained by either the increase in lymph volume or by the blockade of sodium transport by furosemide.

In contrast to sodium, the movement of urea in the mammaliam kidney is passive in nature (Koike and Kellog, 1963). Urea plays an important role in the production of a concentrated urine in mammals. Urea is not in diffusion equilibrium in the various medullary structures. Its concentration is highest in fluid from the collecting ducts, intermediate in plasma from the vasa recta and lowest in fluid from the loop of Henle (Gottschalk, 1964). Berliner *et al.* (1958) postulated that diffusion of urea out of the collecting ducts and passive accumulation in medullary interstitial fluid by the vasa recta permits urea to add to the osmolality of the urine without being balanced by an equivalent amount of sodium chloride. The urea in the urine is osmotically

balanced by a nearly equal concentration of urea in the interstitium; therefore, urea can be excreted largely in water already obligated for the excretion of other urinary solutes (Berliner and Bennett, 1967). A large part of the urea lost from the collecting ducts is captured in the descending limb of the loop of Henle and recirculated through the distal convolution into the collecting ducts. In nondiuretic rats,only a small percentage of the filtered urea is excreted in the final urine.

Any increase in urine flow will wash out the urea gradient (Goldberg, 1966). Since the movement of urea is passive, an increase in blood flow through the vasa recta and/or an increase in urine flow through the collecting ducts will prevent the trapping and accumulation of urea in the medullary interstitium. The washout of urea is characterized by a transient increase in the amount of urea excreted in the urine. Shannon (1938) used the word "exaltation" in referring to this effect. This phenomenon was observed after furosemide (Table 9). Since the lymph to plasma ratio of urea did not change after furosemide, more urea per unit time left the kidney. This increase in urea excretion parallels the "exaltation" of urea in the urine (Table 9). It appears that the hilar lymph, as does the urine, serves as a pathway for the washout of urea (Table 5). Urea also leaves the

kidney via the venous blood. Since renal blood flow is large, it is difficult to detect a transient increase in the renal venous blood concentration of urea. The washout of urea from the medulla is consistent with the diuresis observed after furosemide. In addition, an increase in blood flow through the medulla would result in the formation of more lymph which in turn would also contribute to the washout of urea from the medullary interstitium.

The lymph to plasma ratio for protein increased after furosemide (Table 9). The increase of this ratio in the presence of an increase in lymph volume indicated that more protein left the kidney. Such an increase could result either from a faster washout of the interstitial protein pool or from an increased rate of protein extravasation; either of which might result from an increase in medullary blood flow (O'Morchoe *et al.*, 1970).

Furosemide clearly increases renal hilar lymph flow. The changes in the lymph to plasma ratios for urea, sodium and protein suggest that the drug induces a redistribution of blood flow toward the renal medulla. However, since there is at present no convincing anatomical evidence which relates the hilar lymphatics as specifically draining the medulla, the present lymph studies provided only circumstantial evidence for a redistribution of blood flow toward the medulla. The

data obtained from the lymph studies do, however, support the concept that changes in renal hilar lymph flow are more a consequence of changes in renal blood flow than in urine volume.

Unfortunately the classical methods of determining renal blood flow, such as PAH extraction, do not provide adequate information concerning the intrarenal distribution of blood flow (Aukland *et al.*, 1970). Within the past 10 years the xenon or krypton washout technique has been used to estimate the intrarenal distribution of blood flow (Blaufox *et al.*, 1970). However, since these gases are highly diffusable, they can easily be trapped within the countercurrent mechanism of the medulla. Consequently, it may be questioned whether the use of these indicators provides information concerning changes in the deep medullary circulation.

Indocyanine green, a dye which binds with plasma proteins, has been widely used for many years in dye dilution studies to obtain information on cardiac output (Sheppard *et al.*, 1968). Since most of the dye stays within the vascular compartment, it is ideal for the study of the medullary circulation because it will not be trapped like xenon in the countercurrent mechanism. To prevent the recirculation of the dye, an isolated kidney system was developed (Tables 5, 11 and 12). Since the kidney in this system responded to furosemide in the

same manner as a kidney in an intact anesthetized dog (Tables 5, 13 and 14), it was valid to relate the data obtained from the isolated kidney to the anesthetized dog.

The mechanism by which furosemide induces a redistribution of renal blood flow is unclear. Since furosemide increases renal blood flow without increasing glomerular filtration rate, a post-glomerular vasodilation is suggested. A dilation of the efferent arteriole and/or the vasa recta could explain the observed decrease in glomerular filtration rate after the administration of furosemide. Measurement of the distribution of renal blood flow by the microsphere technique (Katz *et al.*, (1970) and by the krypton washout technique (Birtch *et al.*, 1967) show that quantitatively important shifts in the intra-renal distribution of blood flow occur after furosemide. The data obtained from the microsphere technique and the krypton washout technique suggest that a large proportion of the blood is shunted within the cortex, but neither technique can determine changes in the deep medullary circulation. The dye dilution data obtained in the present experiments indicate that the cortex is not the only area to receive an increased blood supply following furosemide. It appears, however, that there is a dis-proportionately greater increase in medullary blood flow after furosemide.

Thurau (1964) stated that in the dog, blood flow rates in the outer and inner medulla account for 6 or 7% of the total renal blood flow. The integrity of the countercurrent mechanism is maintained by the relatively low blood flow through the medulla. If blood flow through the medulla or vasa recta increases, the vasa recta no longer function as countercurrent exchangers; consequently, the osmotic gradient maintained by the countercurrent mechanism is rapidly destroyed. This increase in medullary blood flow could increase the water content of the papillary tip and decrease the efficiency of the countercurrent multiplier system.

Exponential analysis of the dye dilutions curves obtained before and after furosemide, show that furosemide caused the appearance of another component (Figures 18 and 19). The work of Ochwadt (1963) suggests that this new component which appeared after furosemide represents a non-cortical compartment. Ochwadt (1963) using Cr^{51}-red cells and I^{131}-albumin obtained washout curves in an isolated kidney preparation similar to the one employed in these studies. From the analysis of the timed samples, he plotted a red cell and albumin concentration curve. Both curves could be fitted with a 3 component exponential equation. The fastest component, component I, corresponded to the larger part of the renal cortex. This compartment was perfused with 79% of the total blood flow. It was

assumed that the second component corresponded to the juxtamedullary region and perhaps part of the outer medulla. The third component, he felt, represented circulation within the medulla. In the control curve obtained in this study, a similar number (3) of components was found. Since it appears that the second component of the control curve (component II + III, Figure 18) was split into two components (components II and III, Figure 19) after furosemide, it may be that the juxtamedullary and deep medullary circulations were both affected. It is possible that the studies of Birtch *et al.* (1967) did not show any changes in the medullary circulation because of the trapping of the krypton within the medullary interstitium by the countercurrent mechanism.

Exponential analysis of the washout curves provided information concerning relative changes of intrarenal blood flow distribution. Using the same washout data, but a different method of evaluation (exponential extrapolation of the fastest component), it was determined that medullary blood flow doubled after furosemide (Figures 20 and 21). This method is valid because it was also determined that vascular volume did not change after the administration of furosemide (Figures 22 and 23).

Evidence that the method of exponential extrapolation can be used to determine the percentage of total renal blood flow going to the cortex and medulla comes

primarily from the work of Reubi *et al.* (1966). They compared this method with other dye dilution, thermo-dilution and isotopic washout techniques and found little disagreement. To further validate this method, Reubi *et al.* (1966) devised a model of the circulatory conditions thought to exist in the kidney. In the model, two parallel circulations, cortical and medullary, were made. The cortical circulation had a flow of about 10 times greater than that of the medullary circulation. Cortical volume was about four times greater than medullary volume. Total flow and the partial flows could be measured directly, and dye dilution curves were recorded. The dilution curves were similar to those obtained in the kidney. When these model curves were analyzed by exponential extrapolation, the calculated flow rates agreed with the measured flow rates.

Analysis of washout patterns regardless of the label employed are predicated on the observation that the fast component corresponds to the cortical compartment which normally is perfused by 80 to 90% of the total renal blood flow (Thurau, 1964). The fast component of the washout curve was shown by autoradiography to correlate with the disappearance of radioactivity from the cortical region of the kidney (Thorburn *et al.*, 1963). Recently, Blaufox *et al.*(1970), using a gamma-camera, demonstrated that the disappearance of radioactivity in the normal

kidney proceeds from the cortex inward to the medullary region. This disappearance has been shown to correspond in time to the first component of the washout curve. Nevertheless, these workers conceded that the autoradiographic studies, although highly suggestive that the first component of the curve represents cortical blood flow, do not directly prove this. They simply reveal the coincident appearance of radioactivity in these areas with the timing of the components of the washout curve.

The distribution of red blood cells within the kidney can be determined by a freezing technique (Ulfendahl, 1962). After rapid freezing, slices from different areas of the kidney are taken and analyzed for ^{51}Cr-labelled red blood cells. Using this technique in six preliminary experiments, the distribution of red blood cells was measured within rabbit kidneys before and after furosemide. When the data was based on a dry kidney weight basis, the red blood cell volume increased after furosemide. Since these data are preliminary, they were not formally presented. They are noted because distribution experiments like these will provide data based on anatomical evidence. However, until such techniques are developed, only relative estimates of the intrarenal distribution of blood flow can be made.

SUMMARY AND CONCLUSIONS

In hydropenic animals, furosemide produced an increase in renal blood flow which could be maintained by replacing urine volume. A very low dose of furosemide (0.1 mg/kg, i.v.) abolished the renal cortico-medullary electrolyte concentration gradient within 10 minutes. The abolishment of the electrolyte concentration gradient in the dog kidney was due to an influx of water with no change from saline controls in the non-urea solute content. In a study which compared several different diuretics, no correlation between urine volume changes and water content of the renal papilla was observed. When furosemide was compared to hydrochlorothiazide, at equi-diuretic doses, furosemide caused a significantly greater increase in papillary water content.

The effect of furosemide on renal hilar lymph flow and composition was investigated. In those experiments in which furosemide increased total renal blood flow, there was also an increase in renal hilar lymph flow. When furosemide did not increase renal blood flow, there was no increase in hilar lymph flow. Hydrochlorothiazide did not increase hilar lymph flow. Changes

in renal hilar lymph flow were more a consequence of changes in renal blood flow than in urine volume. The lymph to plasma ratio of urea in the control state was significantly greater than one. A transient increase in the concentration of urea was observed in the lymph as well as in the urine after furosemide.

The redistribution of renal blood flow produced by furosemide was estimated in an isolated kidney by means of a dye (indocyanine green) dilution technique. The isolated kidney was able to concentrate urine and maintain total renal function for periods up to 90 minutes without displaying an increase in renal vascular resistance. Renal blood flow in this system averaged 2 to 3 ml/min/g kidney weight. Furosemide produced an increase in renal blood flow and urine volume. Using the dye dilution technique, it was determined that the percentage of total renal blood flow which flows through the medullary compartment doubled after furosemide. Analysis of the dye dilution curves also showed that after furosemide, another component appeared in the washout curve.

The evidence obtained from the tissue and electrolyte studies, the lymph experiments and the dye dilutions studies strongly support the hypothesis that furosemide induces a redistribution of blood flow toward the medulla. The data do not in any way rule out the previous assumption

that furosemide inhibits sodium reabsorption in the ascending limb of the loop of Henle. Furthermore, the data suggest that the increase in renal blood flow and, more importantly, the redistribution of blood flow produced by furosemide can markedly enhance the effect of the drug, particularly on the concentrating mechanism.

Table 1. Effect of 20 min infusion of furosemide (20 μg/kg/min, i.v.)[a]

	Control	Furosemide
Renal Blood Flow (ml/min)	182±25	222±28[b]
Inulin Clearance (ml/min)	32± 7	25± 6
PAH Clearance (ml/min)	104±30	98±25
Sodium Excretion (μEq/min)	103±50	276±97[b]
Urine Volume	0.7±0.3	2.3±0.7[b]

[a]Control values represent the average of two 10-min collection periods prior to furosemide. Furosemide values represent the average of two 10-min collection periods during drug infusion. Values in Table represent mean ± S.E. of 5 animals.

[b]Significantly different than control ($p<.05$), Student's "t" test, paired comparison.

Table 2. Effects[a] of furosemide (20 μg/kg/min, i.v. for 20 min)

	Unrestricted Blood Flow	Constant[b] Blood Flow
Urine Volume (ml/min)	1.34 (0.50)	0.85 (0.41)
Inulin Clearance (ml/min)	30 (21)	14 (9)
Sodium Excretion (μEq/min)	122 (68)	64 (44)

[a]Values represent the difference (± S.E.) between the 10 min control period prior to furosemide and the control period 10 min after furosemide for 4 experiments.

[b]Renal blood flow was held constant during the infusion of furosemide by clamping the renal artery. Blood flow to the other kidney in the same dog was not restricted.

Table 3. Effect of 60 min infusion of furosemide (20 µg/kg/min, i.v.) at constant renal blood flow[a]

		Control	Furosemide
Inulin Clearance (ml/min)	Left[b]	40± 5	27± 4
	Right[c]	39± 3	26± 6
PAH Clearance (ml/min)	Left	128± 22	101± 7
	Right	136± 21	120± 18
Sodium Excretion (µEq/min)	Left	48± 21	452±191
	Right	68± 8	570±242
Urine Volume (ml/min)	Left	0.5±0.2	3.3±1.0
	Right	0.7±0.1	4.4±2.0

[a]Control values represent the average of three 10-min collection periods prior to furosemide. Furosemide values represent the average of six 10-min collection periods during drug infusion. Values in table represent mean ± S.E. of 3 animals.

[b]Left = data from kidney with blood flow held constant.

[c]Right = data from kidney with blood flow unrestricted.

Table 4. Early (10 min) effects of furosemide (0.1 mg/kg, i.v.) at constant renal blood flow[a]

		Control	Furosemide
Inulin Clearance (ml/min)	Left[b]	30± 7	20± 5[e]
	Right[c]	29± 7	30± 6
PAH Clearance (ml/min)	Left	46± 14	46± 14
	Right	55± 6	59± 11
Sodium Excretion (μEq/min)	Left	40± 9	78± 1[e]
	Right	44± 9	231± 32[e]
Urine Volume (ml/min)	Left	0.4±0.1	0.9±0.3
	Right	0.4±0.1	2.2±0.4[e]
Urine Osmolality[d] (m OS moles)	Left	1301± 59	550± 64[e]
	Right	1346±104	376± 21[e]

[a]Control values represent the average of four 5-min collection periods prior to furosemide. Furosemide values represent the average of the two 5-min collection periods after the drug. Values in table represent mean ± S.E. of 4 animals.

[b]Left = data from kidney with blood flow held constant.

[c]Right = data from kidney with blood flow unrestricted.

[d]Control osmolalities represent the mean ± S.E. of two 5-min collection periods prior to furosemide. Furosemide values represent the mean ± S.E. of the urine collected in second 5-min period after furosemide.

[e]Significantly different than control ($p<.05$), Student's "t" test, paired comparison.

Table 5. Renal tissue urea values in the anesthetized dog after furosemide[a]

	Urea µMoles per g dry solid		Urea µMoles per g tissue water	
	Control[b]	Furosemide[c]	Control	Furosemide
Cortex	26± 7[d]	19± 8	7± 2	4± 2
Outer Medulla	57± 13	20± 7	10± 4	13±10
Inner Medulla	307± 88	48±16	39±19	6± 2
Papilla	931±259	158±52	190±57	17± 4

[a]Furosemide (0.5 mg/kg, i.v.) was administered.

[b]Control values represent values obtained from immediate removal of right kidney.

[c]Furosemide values represent tissue values obtained 10 min after the administration of furosemide.

[d]Values represent mean ± S.E. of 4 experiments.

Table 6. Comparison of renal papillary tissue water after different diuretics

Type of Experiment	Water Content of Papilla g water per 100 g dry solid	Urine Volume (ml/min)	N
Dogs treated with ADH (antidiuretic hormone)	466± 34	NM[a]	7
Saline 10 min[b] (0.1 ml/kg/min, i.v.)	572± 33	NM	6
Saline 60 min (0.25 ml/kg/min, i.v.)	589± 48	0.24	3
Diamox 30 min (10 mg/kg, i.v.)	587± 74	NM	2
Hydrochlorothiazide 20 min (3 mg/kg, i.v.)	572± 47	0.18	2
Hydrochlorothiazide 40 min (5 mg/kg, i.v.)	671± 36	1.8	3
Glucagon 10 min (0.1 mg/kg, i.v.)	722±162	0.22	2
Chloromerdrin 30 min (1 mg/kg, i.v.)	925± 75	0.84	2

Ethacrynic acid 20 min (5 mg/kg, i.v.)	642± 31	3.2	4
Mannitol 10 min (12.5%, i.v.)	676± 23	2.3	4
Ouabain 30 min (5 µg/min, i.a.)	970± 86	NM	5
Furosemide 10 min (0.5 mg/kg, i.v.)	811± 92	2.0	6
Furosemide 20 min (20 µg/kg/min, i.v.)	839± 42	2.0	5
Furosemide 60 min (20 µg/kg/min, i.v.)	834± 66	3.2	4
Hemorrhage (no urine formed)	506± 18	NM	2
Dead controls 20 min	544± 53	NM	2

[a]NM = not measured.

[b]Time indicates when values (water content and urine) were obtained after each procedure.

Table 7. Summary of control values during collection of hilar lymph

	A	B	N
Lymph wt. (mg/min)	32± 7	30± 5	24
Renal Blood Flow (ml/min)	249± 18	249± 18	24
Urine Volume (ml/min)	0.31±0.04	0.32±0.04	24
C_{Inulin} (ml/min)	47± 7	48± 3	24
Urea L/P	1.21±0.07[a]	1.22±0.06[a]	20
Sodium L/P	1.06±0.03	1.03±0.04	17
Potassium L/P	1.09±0.05	1.04±0.04	17
Protein L/P	0.44±0.04[a]	0.49±0.04[a]	15
Kidney wt. (g)	84±7		20

A, B - Successive 10 min collection periods.

N - number of animals included in study.

[a]Significantly different than 1.00, ($p<.05$).

L/P - represents lymph to arterial plasma concentration ratio

Table 8. Effect of furosemide (1 mg/kg, i.v.) on single hilar lymph flow

Expt No.	Control Periods A	B	After Furosemide C	D
1	6.1[a]	2.3	5.5	14.0
2	9.2	8.0	16.4	17.0
3	13.3	13.2	21.4	32.0
4	27.7	30.0	32.3	33.7
5	71.6	63.6	77.5	74.0
Mean	25.6	23.4	30.6[b]	34.1[b]
(S.E.)	(12.1)	(11.1)	(12.5)	(10.7)

A-D - 10 min successive collection periods.

[a]Lymph flow measured as mg/min.

[b]Significantly different than control period B ($p<.05$).

Table 9. Summary of effects of furosemide (1 mg/kg, i.v.)

	Control Periods		After Furosemide	
	A[a]	B	C	D
Renal Blood Flow[b] (ml/min)	287±16	281±15	333±11[e]	309±14
Urine Volume (ml/min)	0.2 ± 0.1	0.3 ±0.1	2.9 ±0.7[e]	4.6 ±0.7[e]
C_{Inulin}[b] (ml/min)	33±6	48± 4	31± 6[e]	30± 5[e]
C_{urea}[d] (ml/min)	17±4	32± 7	32± 9	26± 4
$U_{urea}V$[d] (μM/min)	50±8	87±13	93±18	73±14
Urea L/P[c]	1.14±0.14	1.17±0.13	1.16±0.08	1.14±0.07
Sodium L/P[c]	0.98± .08	1.11±0.11	1.05±0.10	1.02±0.11
Protein L/P[c]	0.46±0.10	0.49±0.05	0.58±0.05	0.49±0.05

[a]Each value represents mean ± S.E. determined during a 10 min collection period.

[b]N = 5

[c]N = 4

[d]N = 3

[e]Significantly different than control period B ($p<.05$).

L/P - Lymph to arterial plasma ratio.

Table 10. Comparison of blood, lymph and urine flow changes produced by furosemide[a]

	↑ RBF (N=11)		no ↑ RBF (N=5)	
	C	F	C	F
RBF (ml/min)	254±20	288±27[b]	250±61	226±62
Lymph wt (mg/min)	22±5	29± 6[b]	47±21	44±22
UV (ml/min)	0.4±0.1	3.2±0.5[b]	0.3±0.1	1.8±0.5[b]

[a]Doses range between 0.3 mg to 10 mg/kg, i.v.

[b]Significantly different than control period ($p<.05$).

C = Values obtained in the 10 min collection period prior to furosemide.

F = Values obtained in first 10 min period after furosemide.

RBF = renal blood blow.

UV = urine volume.

↑RBF = Grouped data from 11 experiments in which furosemide increased total renal blood flow.

no ↑RBF = Grouped data from 5 experiments in which furosemide did not increase total renal blood flow.

Table 11. Results of saline infusion in the isolated kidney

Periods (5 min)	RBF (ml/min)	Res (units)	UV (ml/min)	C_{In} (ml/min)
1	160±36[a]	0.23±0.04	0.23±0.04	19±7
2	156±23	0.22±0.01	0.22±0.01	17±5
3	163±26	0.19±0.02	0.19±0.02	21±3
4	165±24	0.18±0.01	0.18±0.01	21±2
5	161±34	0.13±0.01	0.13±0.01	18±4
6	168±40	0.14±0.02	0.14±0.02	26±9
7	163±36	0.14±0.02	0.14±0.02	18±1
8	170±36	0.15±0.01	0.15±0.01	19±5

[a]Values represent mean ± S.E. of 3 experiments.

[b]Infusion of saline (0.5 ml/min) intraarterially begun in period 5 and continued through period 8.

RBF = renal blood flow.

Res = renal vascular resistance

UV = urine volume

C_{In} = clearance of inulin

$U_{Na}V$ = urinary sodium excretion

Osm = osmolality

Ht = hematocrit

$U_{Na}V$ (μEq/min)	Urine Osm (mOsm)	Arterial Osm (mOsm)	Arterial Ht (%)
29±12	1066±133		
25±10	1093±184	325±6	43±2
18± 7	1072±207		
15± 7	1055±192	312±3	43±1
20± 6	1034±175		
14±11	884±213	316±7	43±1
12±10	846±213		
8± 5	826±224	307±3	42±2

Table 12. Tissues content of urea and water in the isolated kidney after the infusion of saline

	Urea µMoles per g dry solid		Urea µMoles per g tissue water		Water g water per 100 g dry solid	
	Control[a]	Saline[b]	Control	Saline	Control	Saline
Cortex	71± 20[c]	39± 20	18± 4	9± 5	393±39	403± 6
Outer Medulla	98± 31	107± 52	21± 7	16± 8	466±26	670±62
Inner Medulla	369±104	198± 39	62±23	29± 8	632±79	727±69
Papilla	993± 93	570±237	191±25	85±29	534±72	638±54

[a]Control values obtained from the other kidney of the dog from which the kidney used in the isolated set up was obtained.

[b]Saline values represent values determined in isolated kidney 20 min after infusion of saline (0.5 ml/min).

[c]Values represent mean ± S.E. for 3 experiments.

Table 13. Summary of infusion of furosemide in the isolated kidney[a]

	Control[b]	Furosemide[c]
Renal Blood Flow (ml/min)	150± 8	167±10
Urine Volume (ml/min)	0.5±0.1	2.1±0.4[d]
Inulin Clearance (ml/min)	19± 2	12± 2
Sodium Excretion (μEq/min)	81±12	277±53[d]
Urine Osmolality (mOsm)	680±65	444±83[d]

[a]Furosemide (1 mg/kg donor wt) was infused during a 10 min period.

[b]Control values represent mean ± S.E. of four 5 min control periods for 3 experiments.

[c]Furosemide values are the mean ± S.E. of four 5 min periods after furosemide.

[d]Significantly different than control ($p<.05$).

Table 14. Tissue content of urea and water in the isolated kidney after furosemide[a]

	Urea µMoles per g dry solid		Water g water per 100 g dry solid	
	Control[b]	Furosemide	Control	Furosemide
Cortex	95± 44	89± 41	349±11	437±31
Outer Medulla	280±219	157±107	459±43	598±40
Inner Medulla	974±482	118± 83	567±37	913±84
Papilla	674±339	89± 79	513±79	948±59

[a]Furosemide (1 mg/kg donor wt) was infused intraarterially during a 10 min period. Values represent mean ± S.E. for 3 experiments.

[b]Control values obtained from the other kidney of the dog from which the kidney used in the isolated set up was obtained.

[c]Values obtained 20 min after infusion of furosemide.

Table 15. Collection of lymph from an isolated kidney

	Control	Experimental	Δ[c]
Furosemide[a]			
↑ RBF[b]	19[d]	22	3
(N=2)	(2)[e]	(10)	(1)
no ↑ RBF	20	17	-3
(N=2)	(7)	(6)	(2)
Mannitol (12.5%)	16	22	6
(N=3)	(6)	(4)	(3)

[a]Dose between 100-500 mg i.v. to donor animal.

[b]Renal blood flow (RBF) increase of 18 ml/min.

[c]Difference between control and experimental value.

[d]Lymph flow recorded as microliters per minute.

[e]Standard error of mean.

Table 16. Summary of intraarterial infusion of furosemide in an isolated kidney[a]

	Control[b]	Furosemide[c]
Renal Blood Flow (ml/min)	144±11	163±16
Urine Volume[d] (ml/min)	0.10±0.01	0.81±0.05[e]
Component I $T_{1/2}$[f] (sec)	6.3 ±0.5	5.6 ±0.6
Component II $T_{1/2}$ (sec)	50.6±6.5	39.0±4.3[e]

[a]Between 50-100 mg furosemide was infused intraarterially during a 10 min period.

[b]Control values represent the mean ± S.E. of values obtained in 10 min period before furosemide.

[c]Furosemide values represent values obtained 10 min after the intraarterial infusion of furosemide.

[d]Recorded in 3 experiments.

[e]Significantly different from control ($p<.05$) paired comparison based on 3-5 experiments.

[f]$T_{1/2}$ for component I and II represents the half-times ($T_{1/2}$) mean ± S.E. of components of control and furosemide altered washout pattern as determined by exponential analysis of the data.

Table 17. Results of a single experiment in which a bolus injection of dye was given before and after furosemide

	1	C_1 ↓1	2	F ↓	3	C_2 ↓2	4
Renal Blood Flow (ml/min)	135		145		164		170
Urine Volume (ml/min)	.19		.18		2.3		2.9
Sodium Excretion (μEq/min)	10		4		274		316
Inulin Clearance (ml/min)	13		24		12		20
PAH Clearance (ml/min)	51		79		41		86
PAH Extraction (%)	76		78		74		73
Urine Osmolality (mOsm)	787		904		334		305
Arterial Hematocrit (%)	44		43		44		43

Values determined during 5 min collection period

C_1 = Washout #1 determined between periods 1 & 2.

C_2 = Washout #2 determined between periods 3 & 4.

F = Furosemide 50 mg during a 10 min intraarterial infusion between periods 2 & 3.

Table 18. Results of component analysis of dye disappearance curves obtained before and after furosemide

Equation of data before furosemide (Figure 18)

$$A = 0.437e^{-0.364t} + 0.011e^{-0.038t} + 0.0008$$

Equation of data after furosemide (Figure 19)

$$A = 0.456e^{-0.584t} + 0.050e^{-0.131t} + 0.004e^{-0.022t} + 0.0002$$

Where A = dye concentration and t = time

Half times ($T_{1/2}$ in sec) for each component

Component	I	II	III	IV
Control	1.9	18.0		α[a]
Furosemide	1.2	5.3	30.8	α[a]

[a] $T_{1/2}$ very long - assume to be infinite.

Figure 1. Effect of furosemide on renal blood flow in an anesthetized dog (18 kg). The top tracing represents total renal blood flow (RBF) and the bottom tracing femoral blood pressure (FBP). Furosemide was infused intravenously (20 µg/kg/min) during the 20 min period between the arrows. Maximal increase in blood flow was 33 ml/min. Drop in systolic pressure was reversed by flushing cannula with saline.

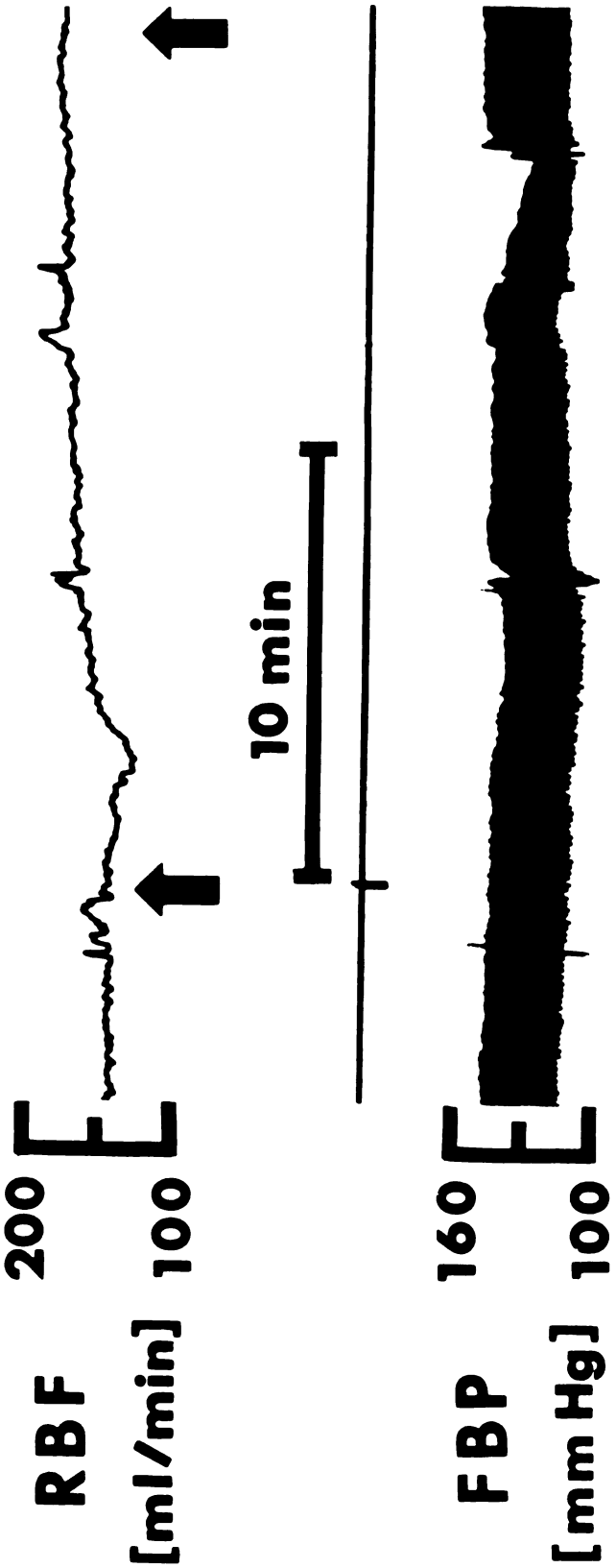

RBF
[ml/min]
200
100
10 min
FBP
[mm Hg]
160
100

Figure 2. Effect of furosemide (20 μg/kg/min, i.v. for 20 min) on renal tissue electrolyte content. The ordinate represents the sum of the concentrations of Na and K times 2. The abscissa represents the areas of the kidney from which the tissue slices were taken. Each point represents the mean ± S.E. of 5 (furosemide), 5 saline infused (0.1 ml/kg/min) 7 (control) animals. Absence of a vertical bar indicates S.E. contained within radius of the point.

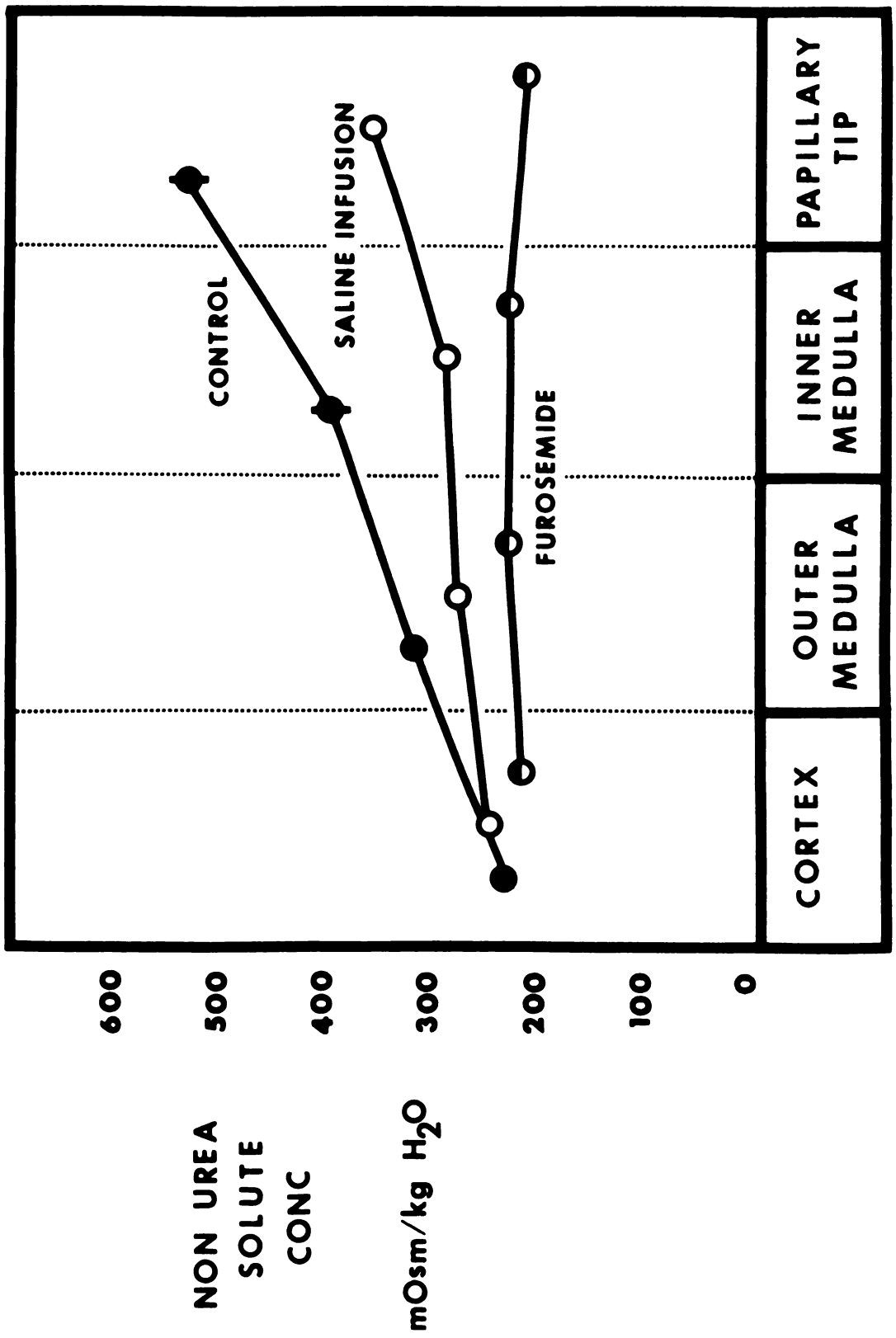

CONTROL
SALINE INFUSION
FUROSEMIDE
CORTEX
OUTER MEDULLA
INNER MEDULLA
PAPILLARY TIP
NON UREA SOLUTE CONC
mOsm/kg H_2O
600
500
400
300
200
100
0

Figure 3. Effect of furosemide (20 μg/kg/min, i.v. for 20 min). Renal blood flow to one kidney was held constant with a variable clamp. The open circles represent the data from the kidney in which blood flow was restricted throughout the 20 min infusion period and the closed circles represent the data from the other kidney. "Control" points represent the mean of two 10 min periods prior to the infusion of furosemide periods. Each point represents the mean ± S.E. of 4 experiments. The abscissa of the lower portion of the figure represents the areas of the kidney, C = cortex, OM = outer medulla, IM = inner medulla, PT - papillary tip.

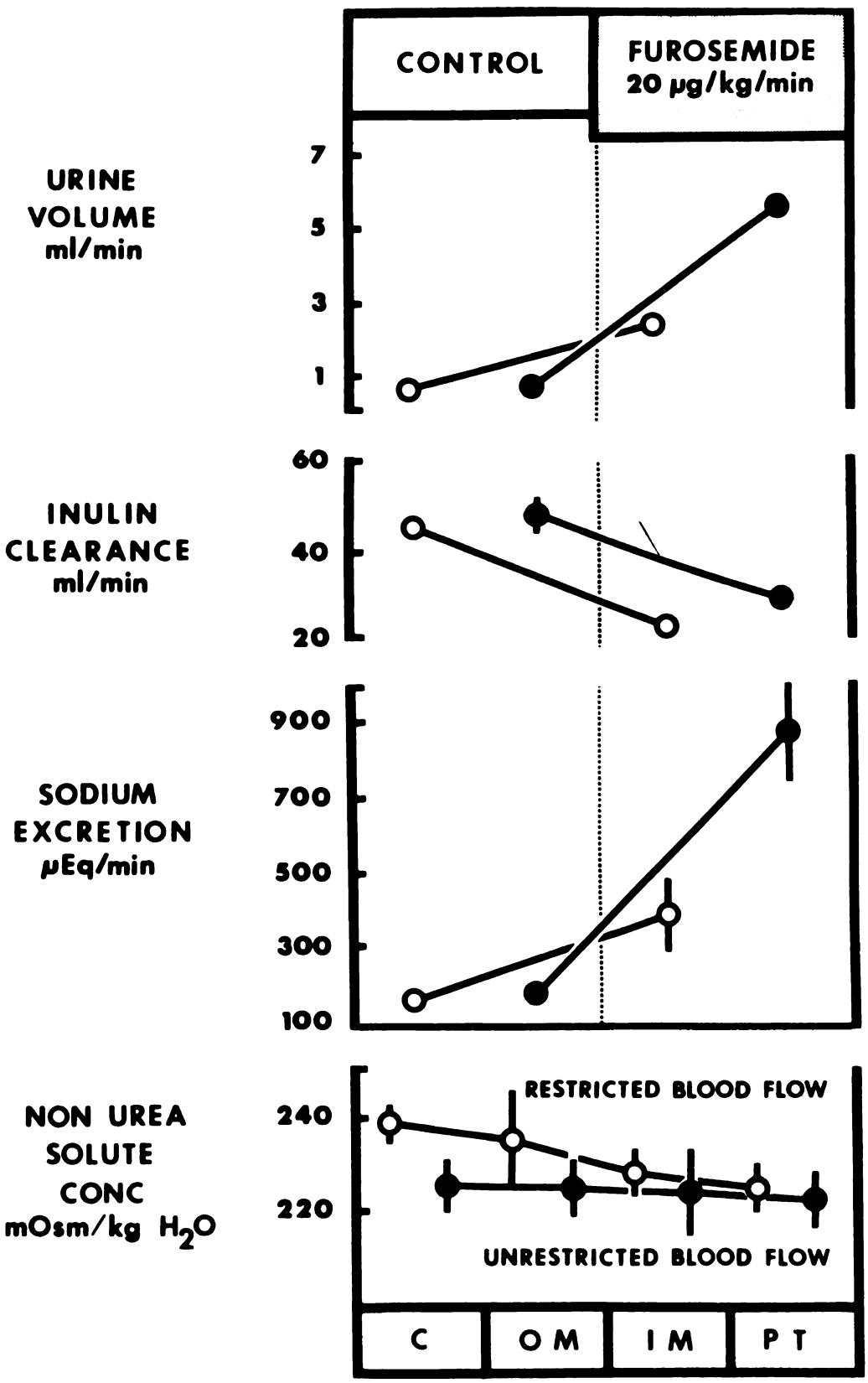

CONTROL
FUROSEMIDE
20 µg/kg/min
URINE
VOLUME
ml/min
7
5
3
1
INULIN
CLEARANCE
ml/min
60
40
20
SODIUM
EXCRETION
µEq/min
900
700
500
300
100
NON UREA
SOLUTE
CONC
mOsm/kg H2O
240
220
RESTRICTED BLOOD FLOW
UNRESTRICTED BLOOD FLOW
C
OM
IM
PT

Figure 4. Effect of 20 min infusion of furosemide on electrolyte (Na + K) and water content in the renal papillary tip. "Control" bars represent the mean ± S.E. of 7 animals pretreated with ADH only. "Furosemide" bars represent 5 animals in which furosemide was infused with no restriction of renal blood flow. "Restricted" and "unrestricted" bars represent separate kidneys of 4 animals in which blood flow to one kidney was restricted during infusion of furosemide.

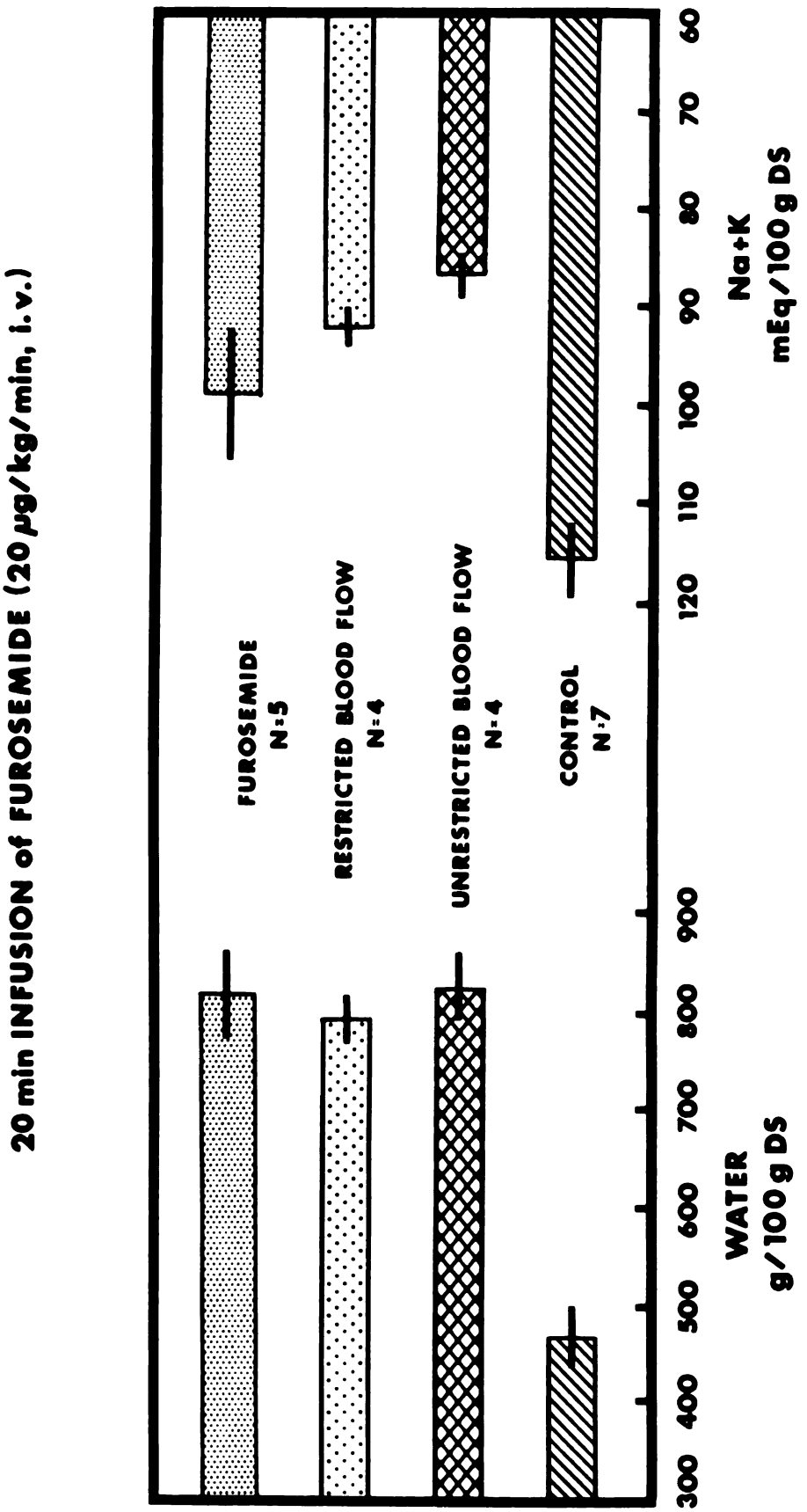

20 min INFUSION of FUROSEMIDE (20 µg/kg/min, i.v.)
FUROSEMIDE
N:5
RESTRICTED BLOOD FLOW
N:4
UNRESTRICTED BLOOD FLOW
N:4
CONTROL
N:7
300 400 500 600 700 800 900 120 110 100 90 80 70 60
WATER
g/100 g DS
Na+K
mEq/100 g DS
PAPILLARY TIP

Figure 5. Effect of 60 min infusion of furosemide or saline on electrolyte (Na + K) and water content in the renal papillary tip. "Saline infusion" bars represent 3 dogs infused with saline (0.25 ml/kg/min). Other symbols defined in Figure 4.

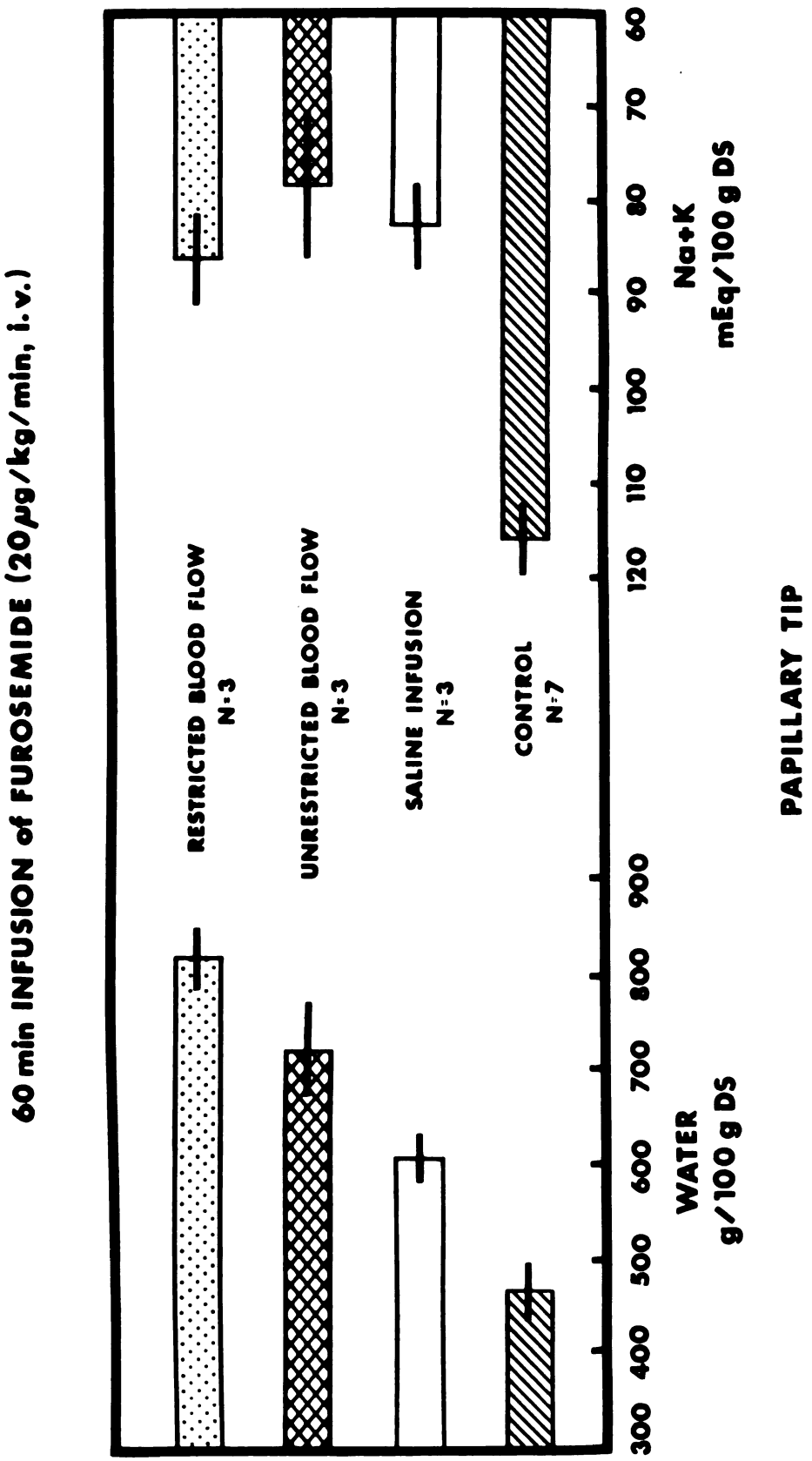

60 min INFUSION of FUROSEMIDE (20μg/kg/min, i.v.)
RESTRICTED BLOOD FLOW
N=3
UNRESTRICTED BLOOD FLOW
N=3
SALINE INFUSION
N=3
CONTROL
N=7
300 400 500 600 700 800 900
WATER
g/100 g DS
60 70 80 90 100 110 120
Na+K
mEq/100 g DS
PAPILLARY TIP

Figure 6. Effect of furosemide (0.1 mg/kg, i.v.) on electrolyte (Na + K) and water content in the renal papillary tip. Tissue was obtained 10 min after injecting furosemide during a saline infusion (0.1 ml/kg/min). Symbols defined in Figures 4 and 5.

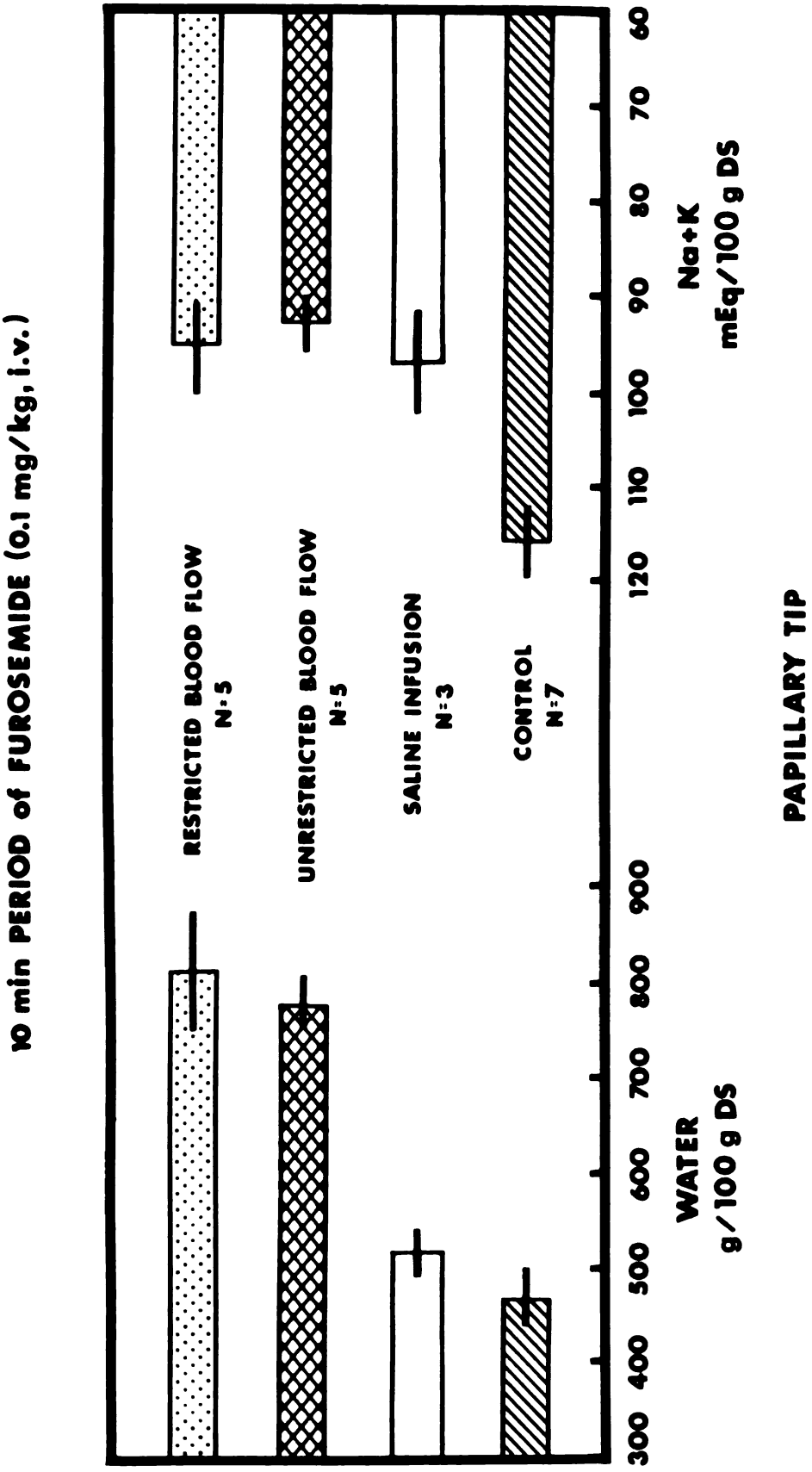

10 min PERIOD of FUROSEMIDE (0.1 mg/kg, i.v.)
RESTRICTED BLOOD FLOW
N=5
UNRESTRICTED BLOOD FLOW
N=5
SALINE INFUSION
N=3
CONTROL
N=7
300 400 500 600 700 800 900
120 110 100 90 80 70 60
WATER
g/100 g DS
Na+K
mEq/100 g DS
PAPILLARY TIP

Figure 7. Comparison of water content of the renal papilla after furosemide and hydrochlorothiazide. Renal papillary tissue samples were obtained from the following experiments for analysis of tissue water (grams per 100 grams dry solid). Each bar represents mean ± S.E.

S = 6 animals in which saline 0.1 ml/kg/min was infused i.v. for 10 min.
H = 3 animals which received hydrochlorothiazide (5 mg/kg, i.v.). Tissue samples obtained 40 min after drug.
F = 4 animals which received furosemide (0.5 mg/kg i.v.). Tissue samples obtained 10 min after drug.

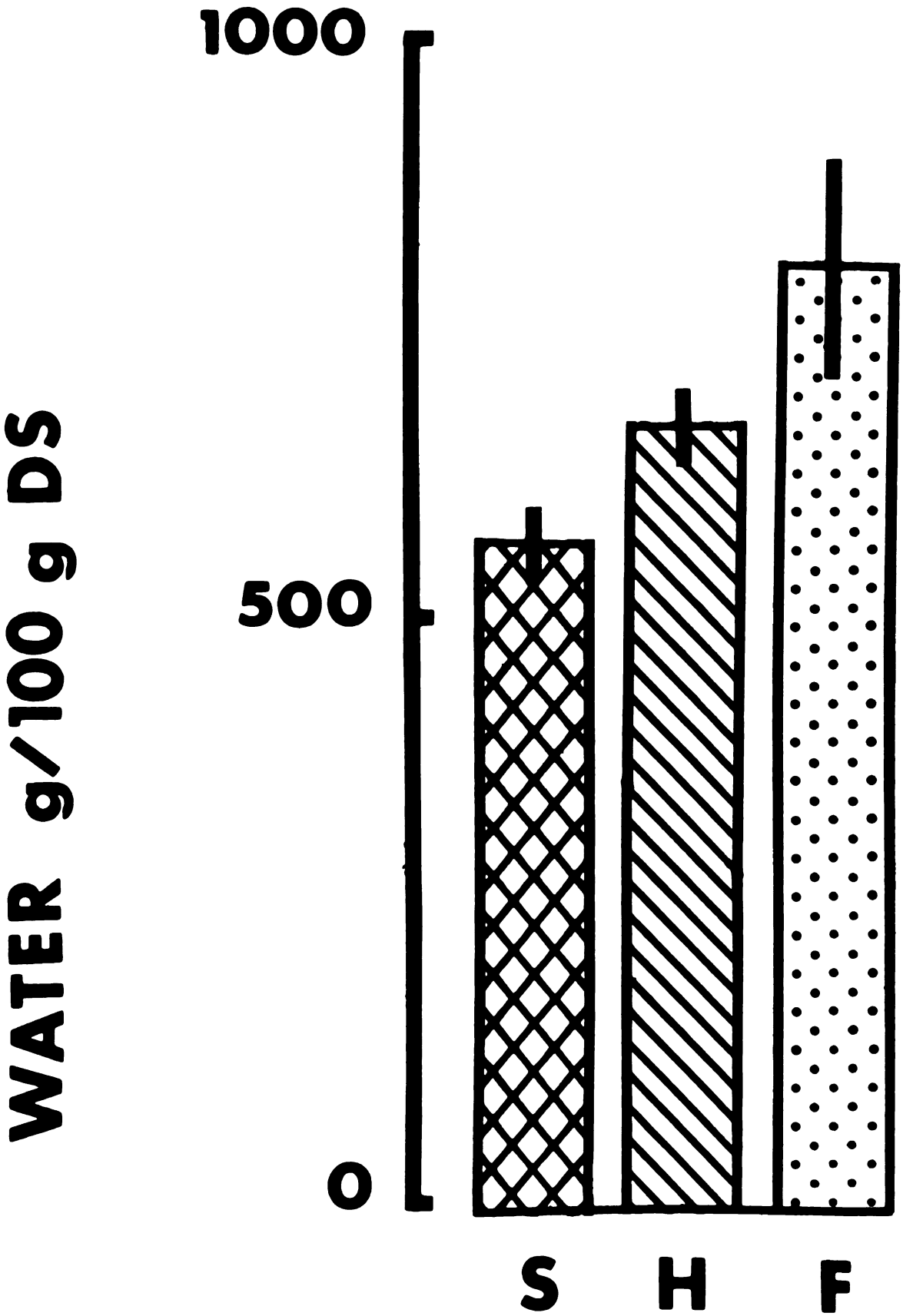

1000
500
0
WATER g/100 g DS
S
H
F

Figure 8. Increased renal hilar lymph flow induced by furosemide and mannitol.
C = values (mean ± S.E.) for 10 min control period prior to furosemide.
F = values obtained first 10 min period following the i.v. administration of furosemide (5 mg/kg).
M = values obtained 20 min after the i.v. infusion of 100 ml 12.5% mannitol solution.
RBF = renal blood flow.
UV = urine volume.
N = 3

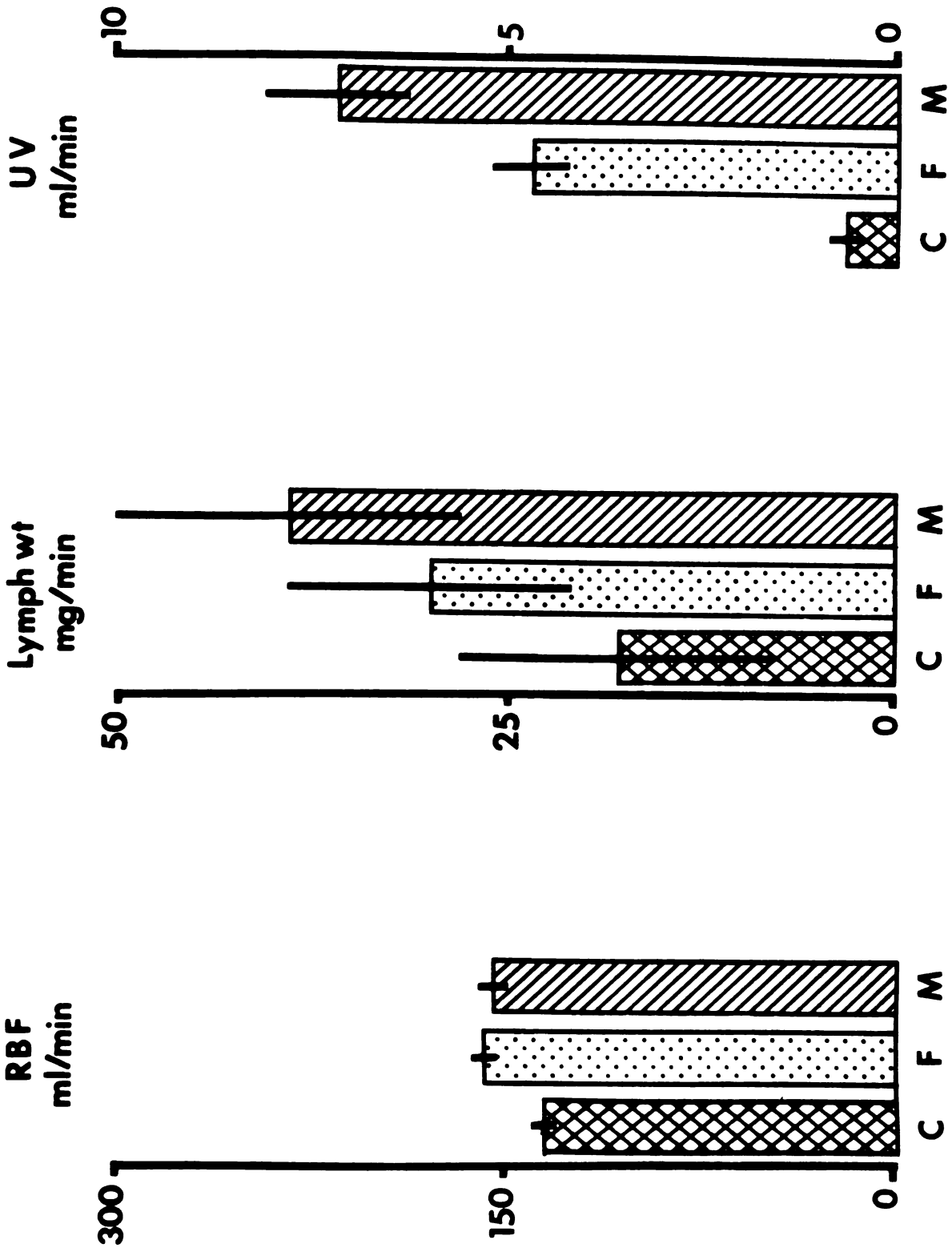

UV
ml/min
10
5
0
C F M
Lymph wt
mg/min
50
25
0
C F M
RBF
ml/min
300
150
0
C F M

Figure 9. Effect of hydrochlorothiazide on renal hilar lymph flow.

Hydrochlorothiazide (5 mg/kg, i.v.) did not alter renal hilar lymph flow in 3 experiments.
C = control values (mean ± S.E.) obtained in 10 min collection period prior to administration of hydrochlorothiazide.
H = values obtained in a 10 min period 10 min after administration of hydrochlorothiazide (H). Values represent peak values obtained.
RBF = renal blood flow.
UV = urine volume.

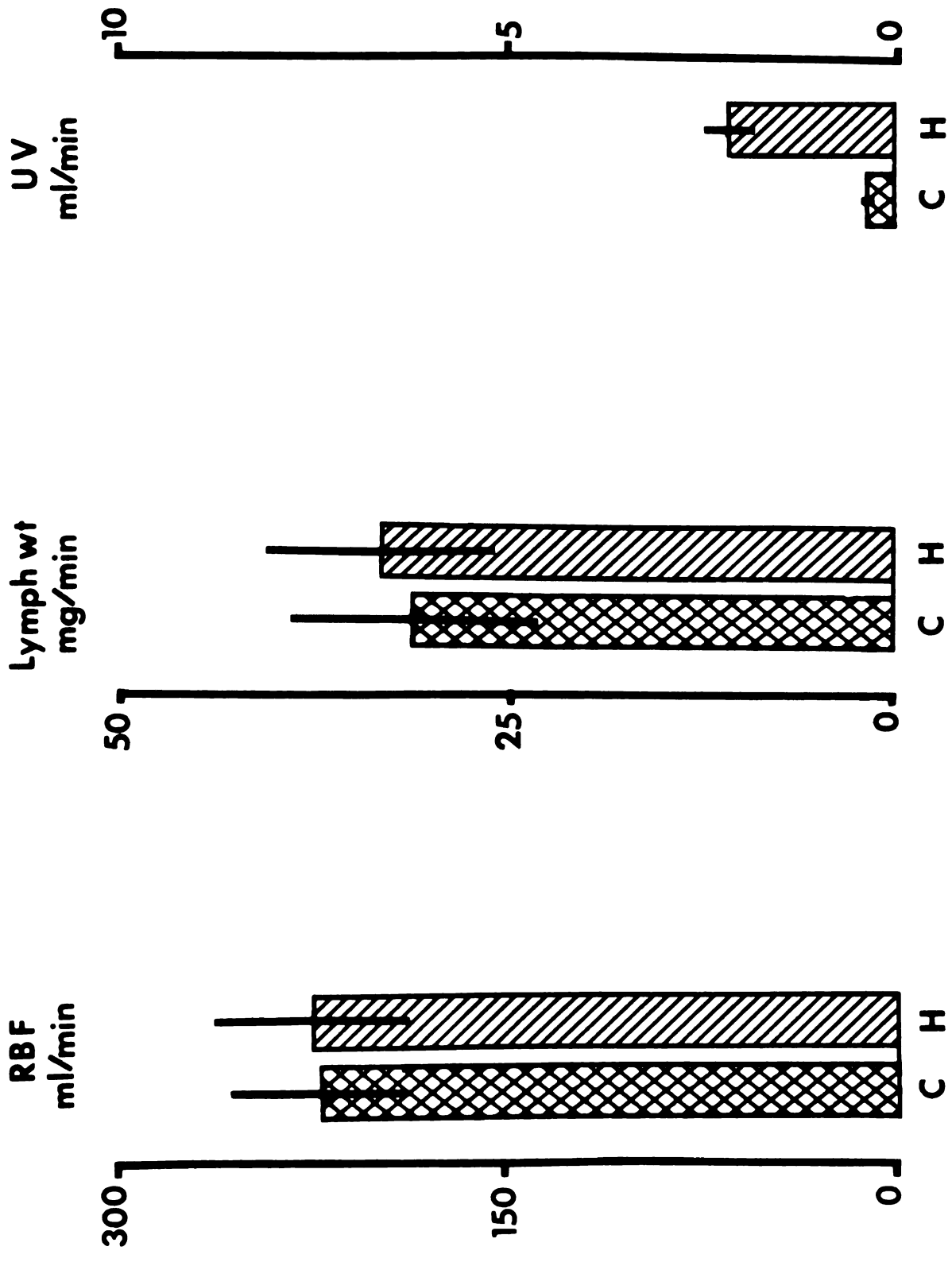

RBF
ml/min
300
150
0
C H
Lymph wt
mg/min
50
25
0
C H
UV
ml/min
10
5
0
C H

Figure 10. Plot of absolute values of lymph weight and renal blood flow before and after furosemide.

Control values (solid circles) and values obtained after furosemide (open circles) were plotted for each experiment. In those experiments in which renal blood flow increased, these values were connected with a solid line. In those experiments in which renal blood flow did not increase, these values were connected with a data line. The data is the same as that presented in Table 10.

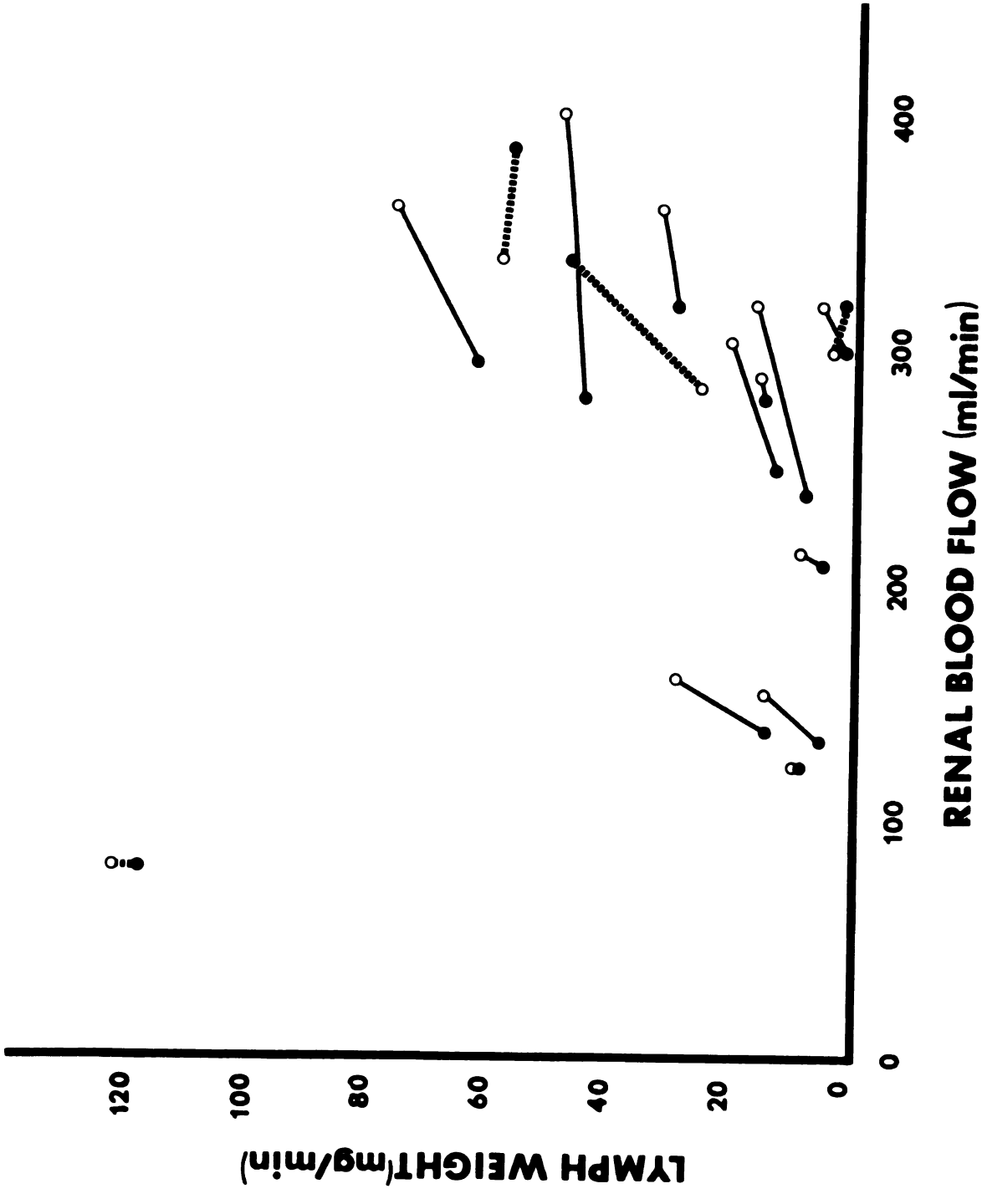

LYMPH WEIGHT(mg/min)
120
100
80
60
40
20
0
0
100
200
300
400
RENAL BLOOD FLOW (ml/min)

Figure 11. Effect of indocyanine green on renal blood flow (RBF). Top tracing is femoral blood pressure (FBP) of anesthetized dog.

Panel A shows renal blood flow changes following the intraarterial infusion of indocyanine green 5 mg/min. Infusion run for 10 min period illustrated between the two arrows.

Panel B. An infusion of indocyanine green 5 mg/min has no effect on RBF.

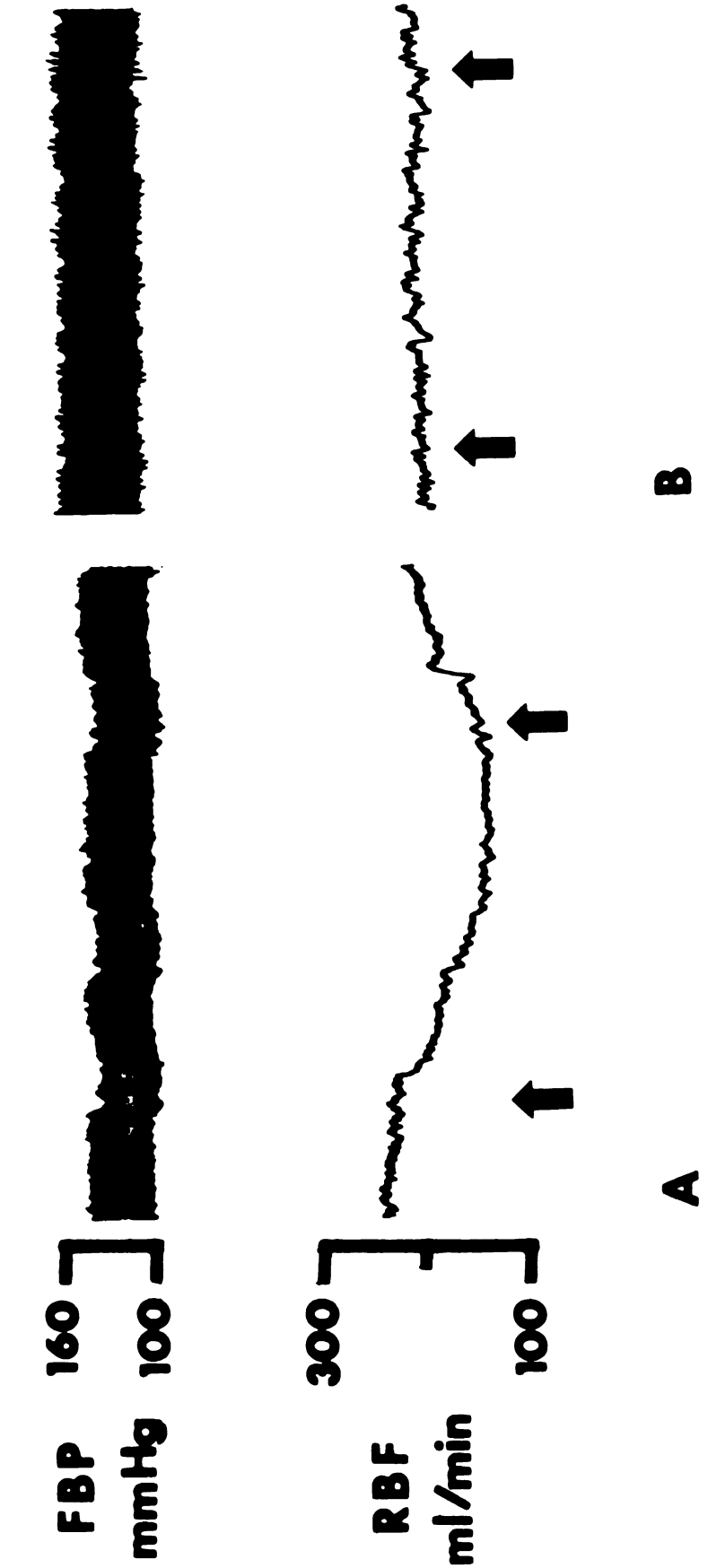

FBP
mmHg
160
100
RBF
ml/min
300
100
A
B

Figure 12. Washout pattern following 3 min infusion of the dye into the renal artery. Each point represents the concentration of dye contained in each timed sample.

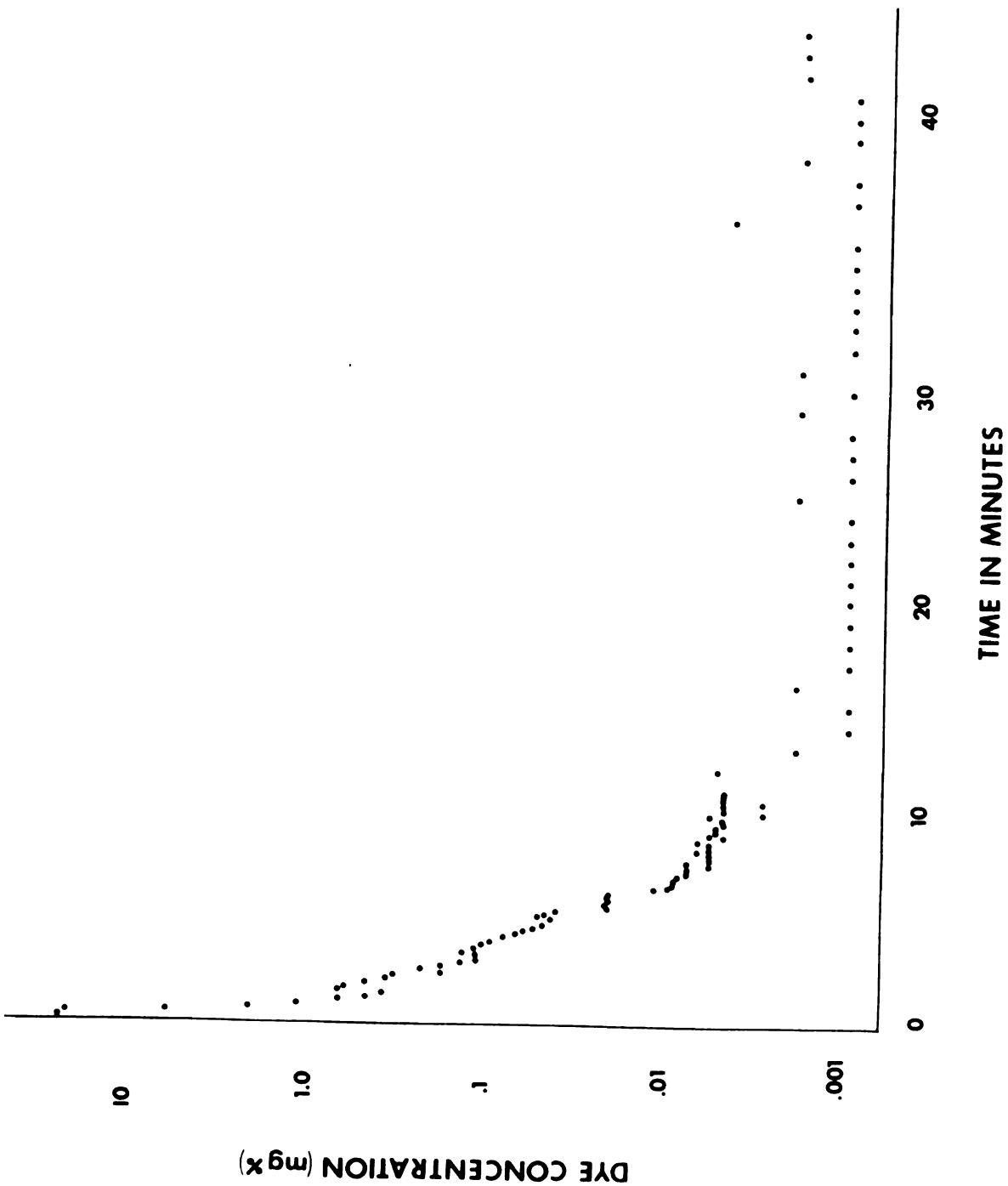

DYE CONCENTRATION (mg x)
10
1.0
.1
.01
.001
0
10
20
30
40
TIME IN MINUTES

Figure 13. The washout of indocyanine green from one isolated kidney following intraarterial infusion of the dye (1 mg/min) for 3 minutes. A 30 min period elapsed between the first experiment (solid circles) and the second experiment (open circles).

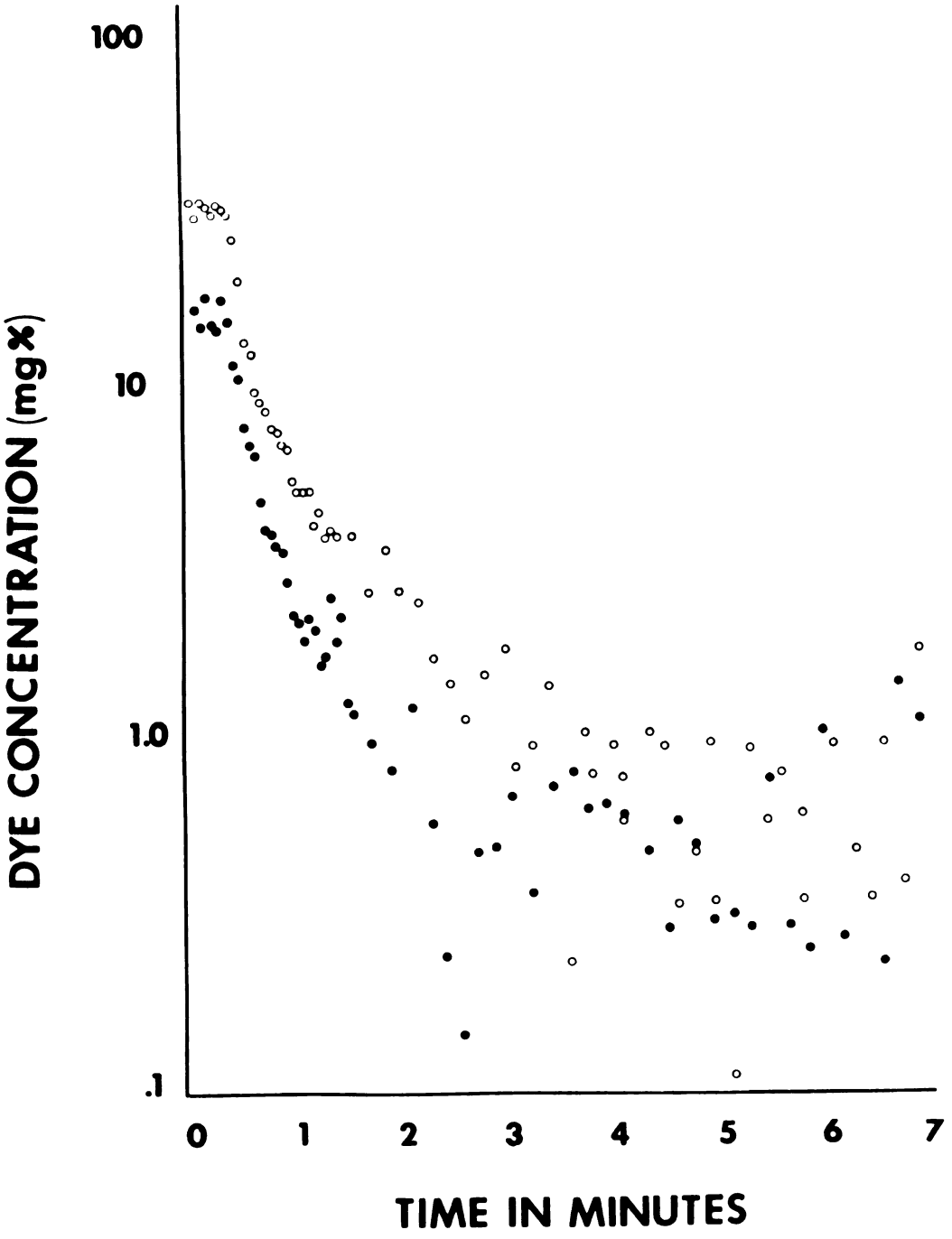

100
10
1.0
.1
DYE CONCENTRATION (mg%)
0
1
2
3
4
5
6
7
TIME IN MINUTES

Figure 14. Alteration of washout pattern by furosemide. Solid circles represent values obtained before furosemide was infused; open circles represent values obtained after a 10 min infusion of 50 mg furosemide.

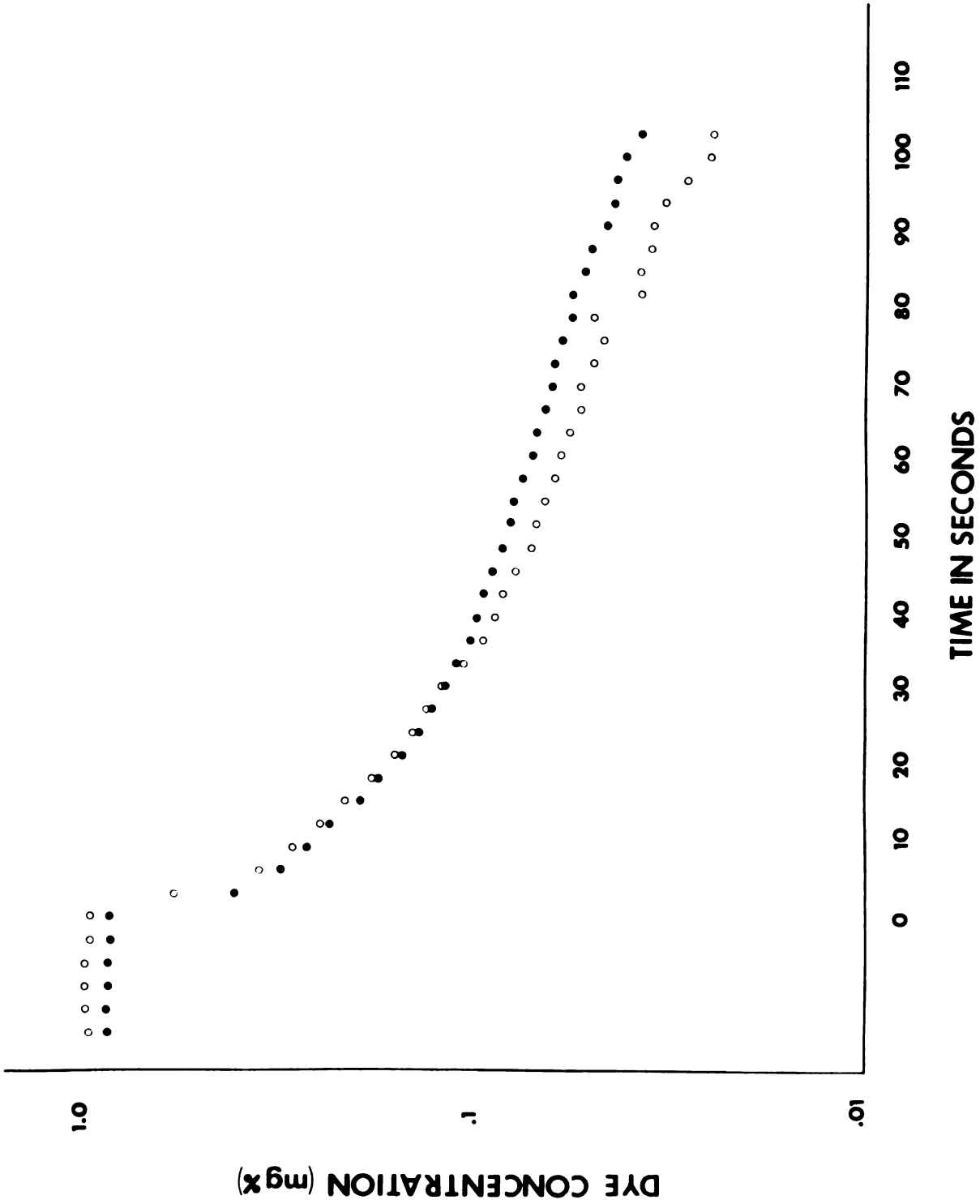

DYE CONCENTRATION (mg%)
1.0
.1
.01
0
10
20
30
40
50
60
70
80
90
100
110
TIME IN SECONDS

Figure 15. Bolus injection of dye for control washout pattern. Top tracing is femoral blood pressure (FBP) of donor animal. Middle tracing is renal blood flow (RBF) recorded with an electromagnetic flowmeter. Bottom tracing is renal perfusion pressure (RPP). Intraarterial injection of approximately 900 mg dye was made at arrow. Collections of venous sample was made every 3 sec.

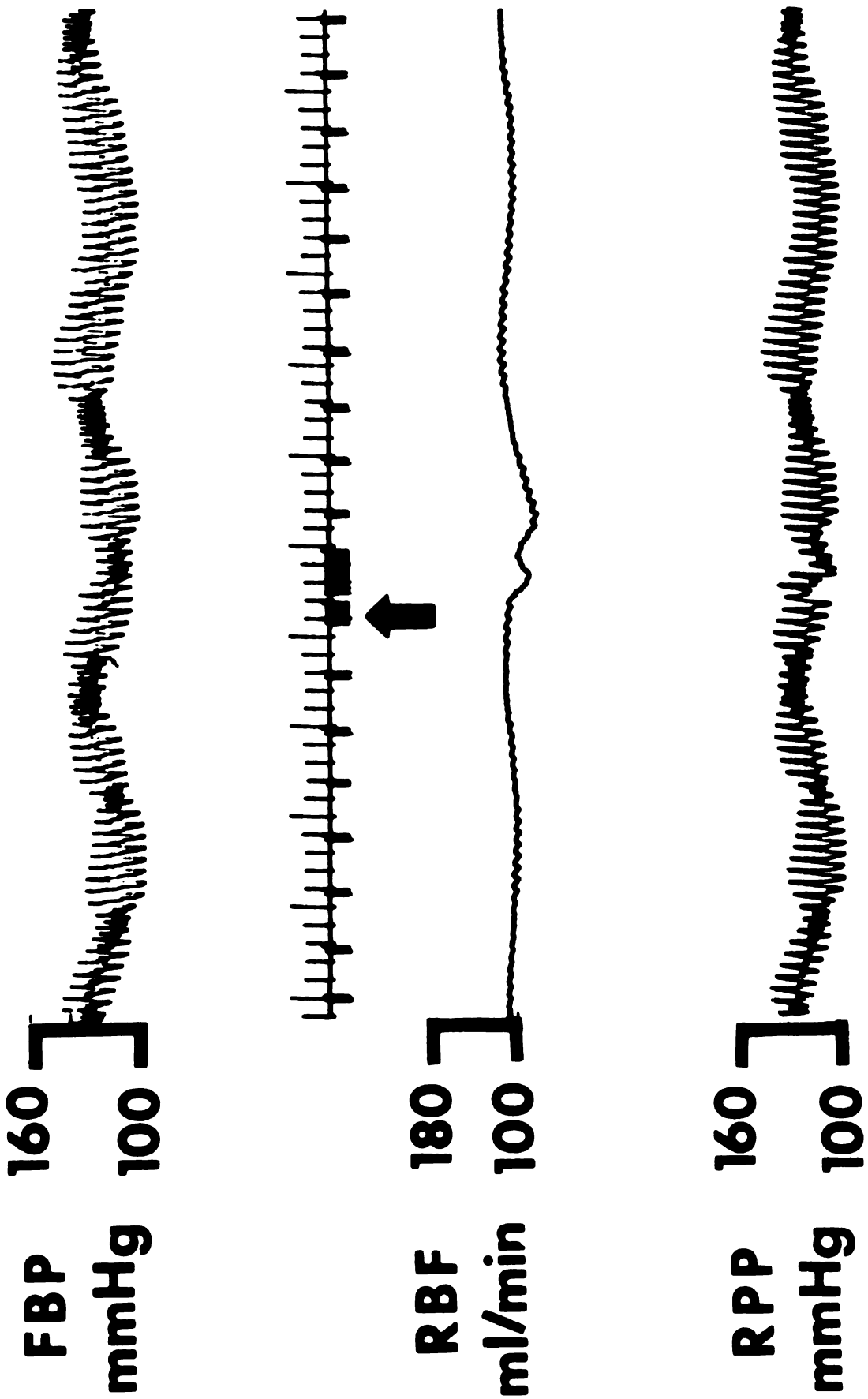

FBP
mmHg
160
100
RBF
ml/min
180
100
RPP
mmHg
160
100

Figure 16. Intraarterial administration of furosemide.

Infusion of 50 mg of furosemide was begun at first arrow and ended 10 min later at second arrow. Renal blood flow increase by approximately 20 ml/min. Rise in middle of blood flow recording occurred when arterial blood samples was obtained through the extracorporeal flowmeter. Descriptions of tracings same as in Figure 15. Disruption of RPP tracing due to infusion of furosemide through same cannula used to record RPP.

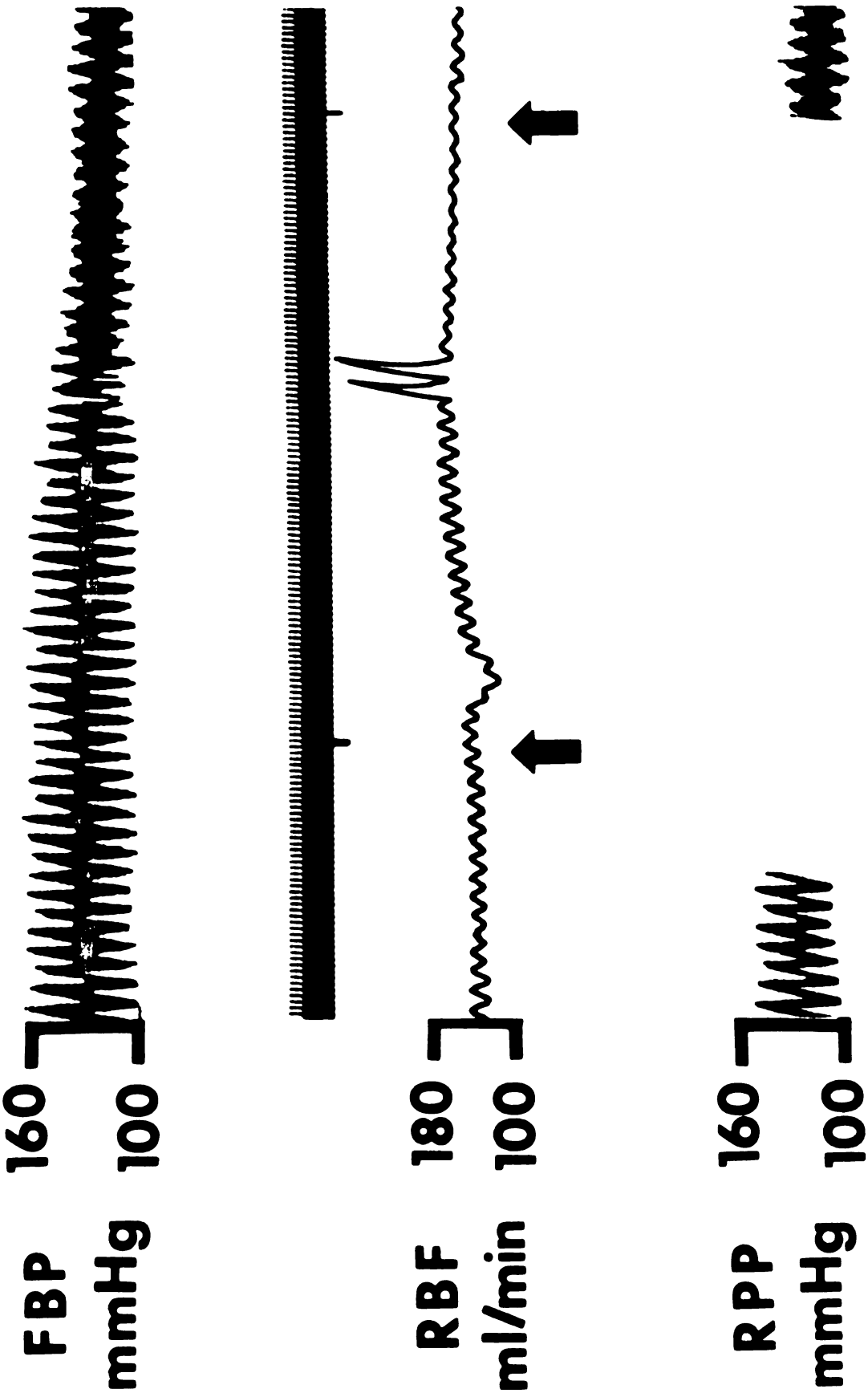

FBP
mmHg
160
100
RBF
ml/min
180
100
RPP
mmHg
160
100

Figure 17. Bolus injection of dye after furosemide. Approximately 900 mg of dye was given intraarterially at arrow. Descriptions of tracings same as in Figure 15.

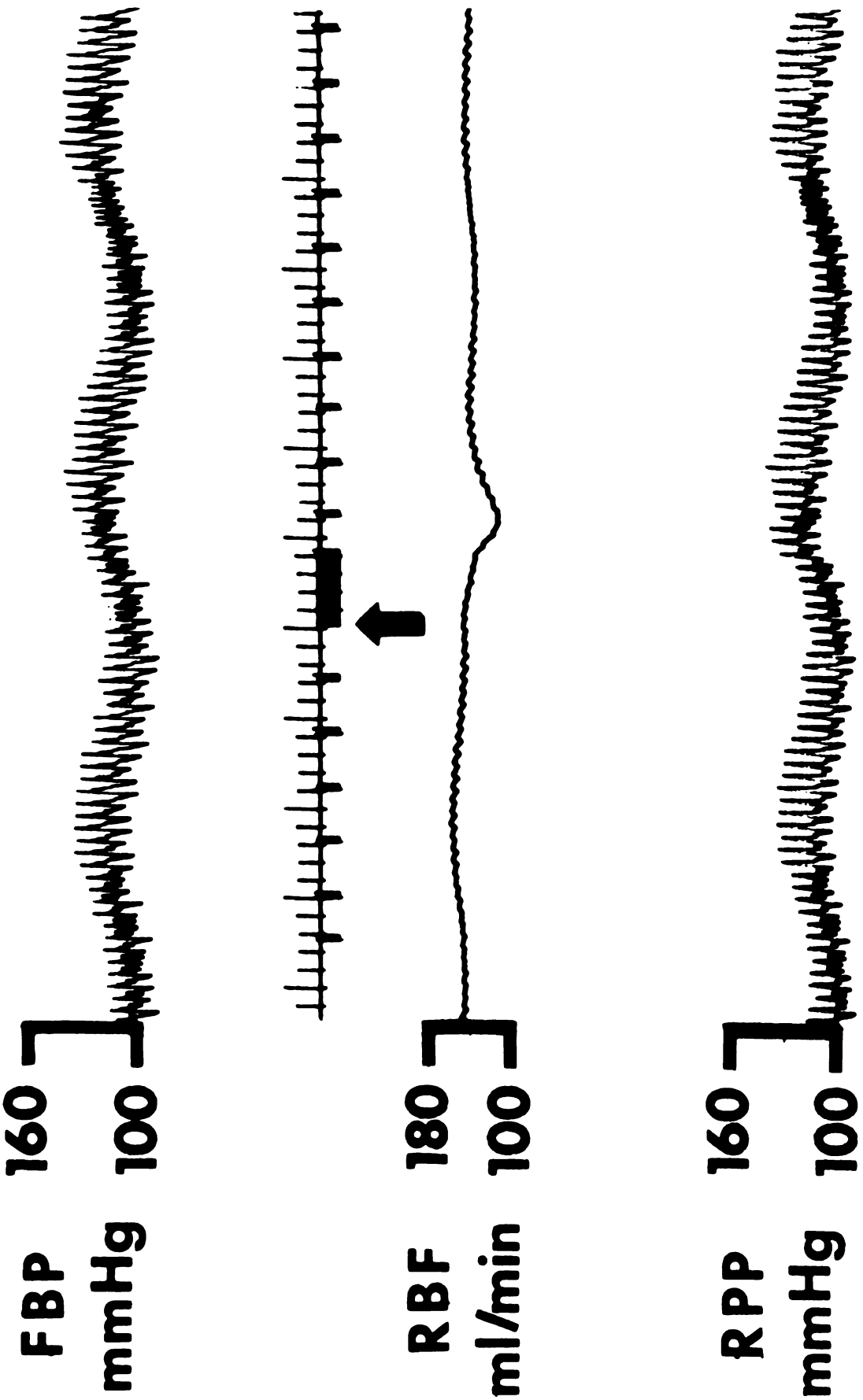

FBP
mmHg
160
100
RBF
ml/min
180
100
RPP
mmHg
160
100

Figure 18. Washout pattern following injection of the dye into the renal artery. Each point represents the actual dye content contained in each 3 sec sample of renal venous blood. Graphic representation of the resultant exponentials is shown by lines marked component I, II + III, and IV.

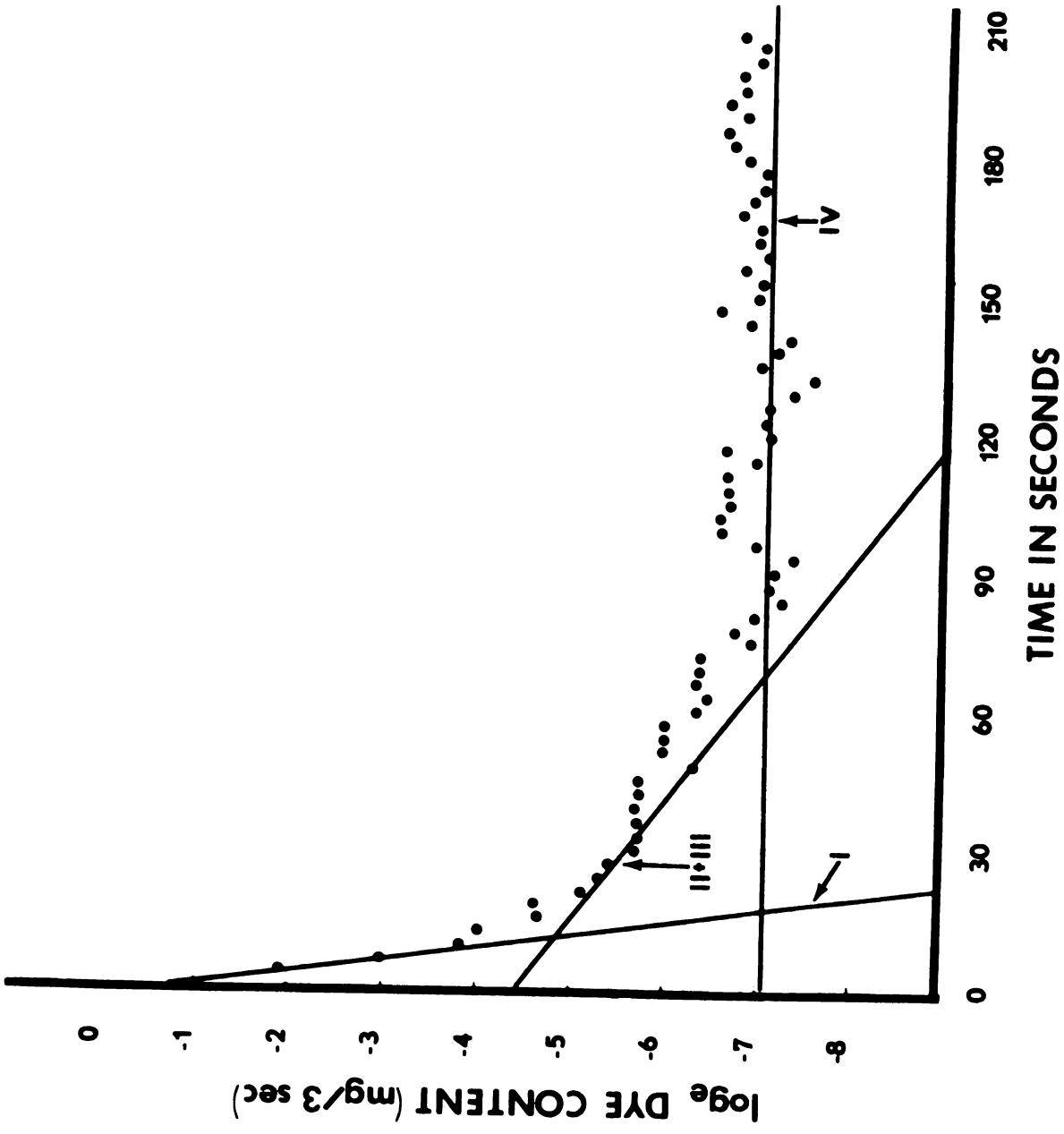

log$_e$ DYE CONTENT (mg/3 sec)
0
-1
-2
-3
-4
-5
-6
-7
-8
TIME IN SECONDS
0
30
60
90
120
150
180
210
I
II+III
IV

Figure 19. Effect of furosemide on the washout pattern following injection of the dye into the renal artery. Each point represents the actual dye content contained in each 3 sec sample of renal venous blood. Graphic representation of the resultant exponentials is shown by lines marked component I, II, III and IV.

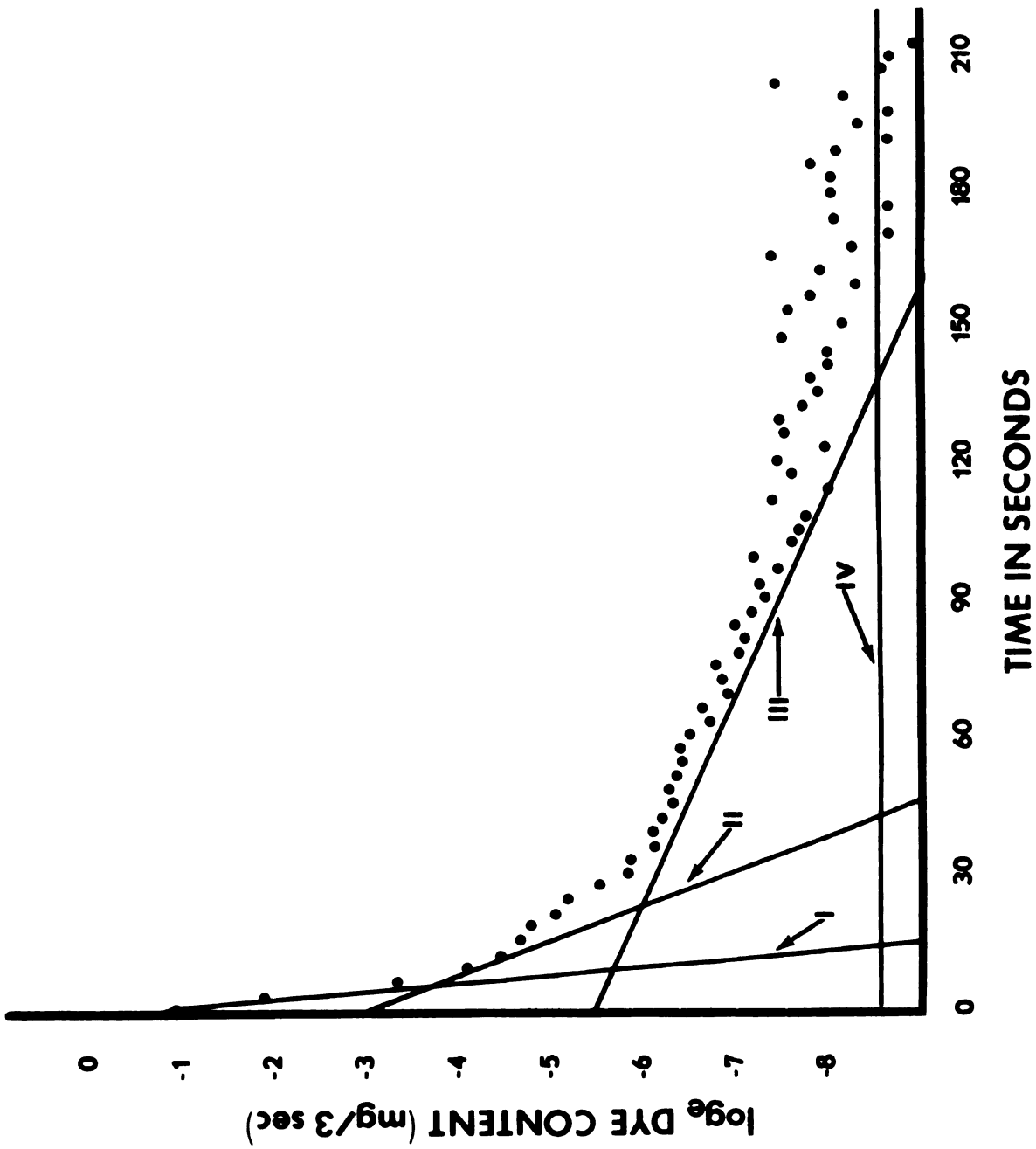

log$_e$ DYE CONTENT (mg/3 sec)
0
-1
-2
-3
-4
-5
-6
-7
-8
TIME IN SECONDS
0
30
60
90
120
150
180
210
I
II
III
IV

Figure 20. Graphical representation of dye dilution curve before furosemide. Solid line and circles represent observed values of dye in the renal venous blood. Dotted line represents theoretical disappearance curve obtained from the exponential analysis of curve in Figure 18. Shaded area represents exponential extrapolation of component I (cortex). Area between shaded area and dotted line represents other components (medulla).

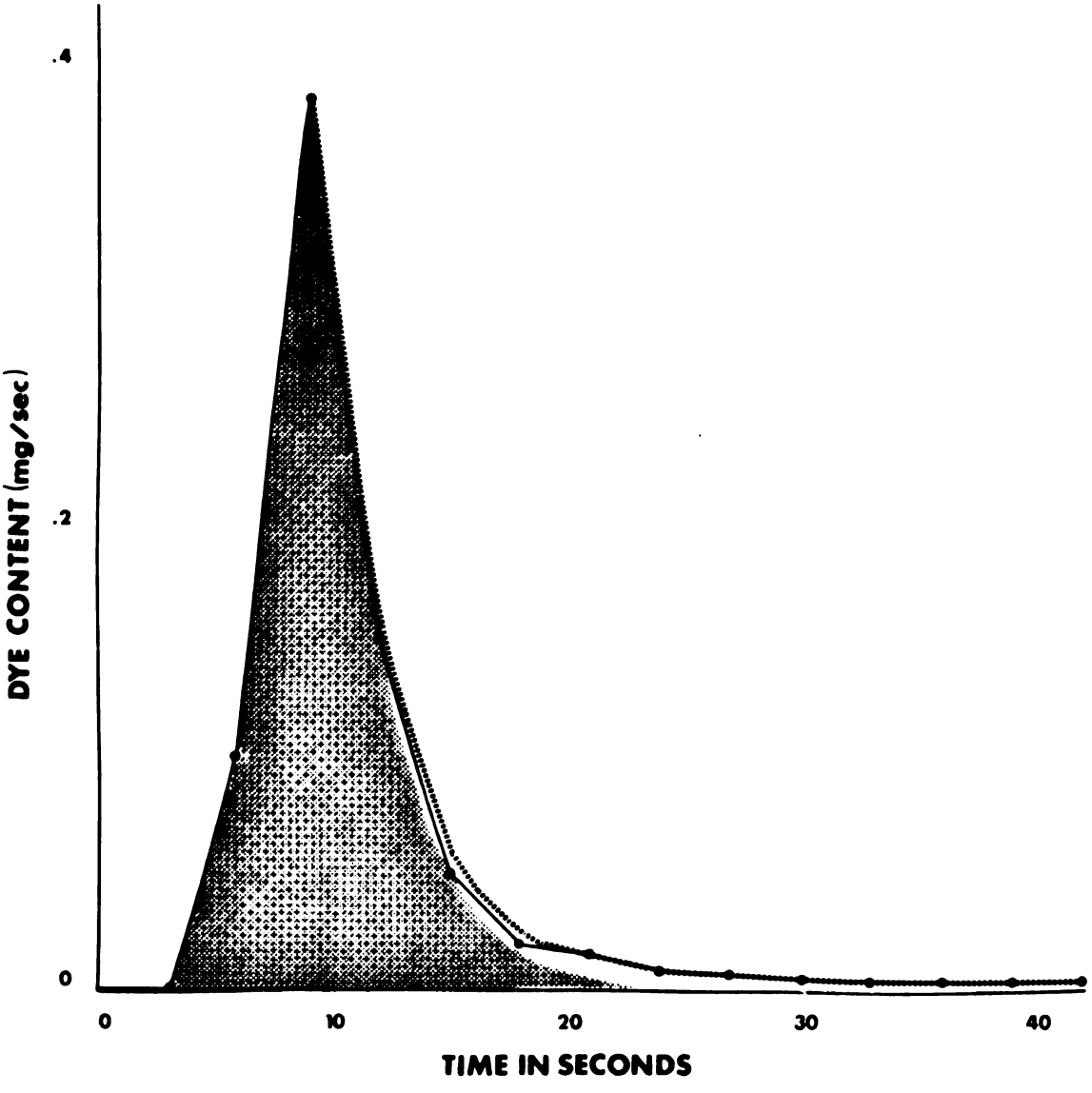

.4
.2
0
DYE CONTENT (mg/sec)
0
10
20
30
40
TIME IN SECONDS

Figure 21. Graphical representation of dye dilution curve after furosemide. Solid line and circles represent observed values of dye in the renal venous blood. Dotted line represents theoretical disappearance curve obtained from the exponential analysis of curve in Figure 19. Shaded area represents exponential extrapolation of component I (cortex). Area between shaded area and dotted line represents other components (medulla).

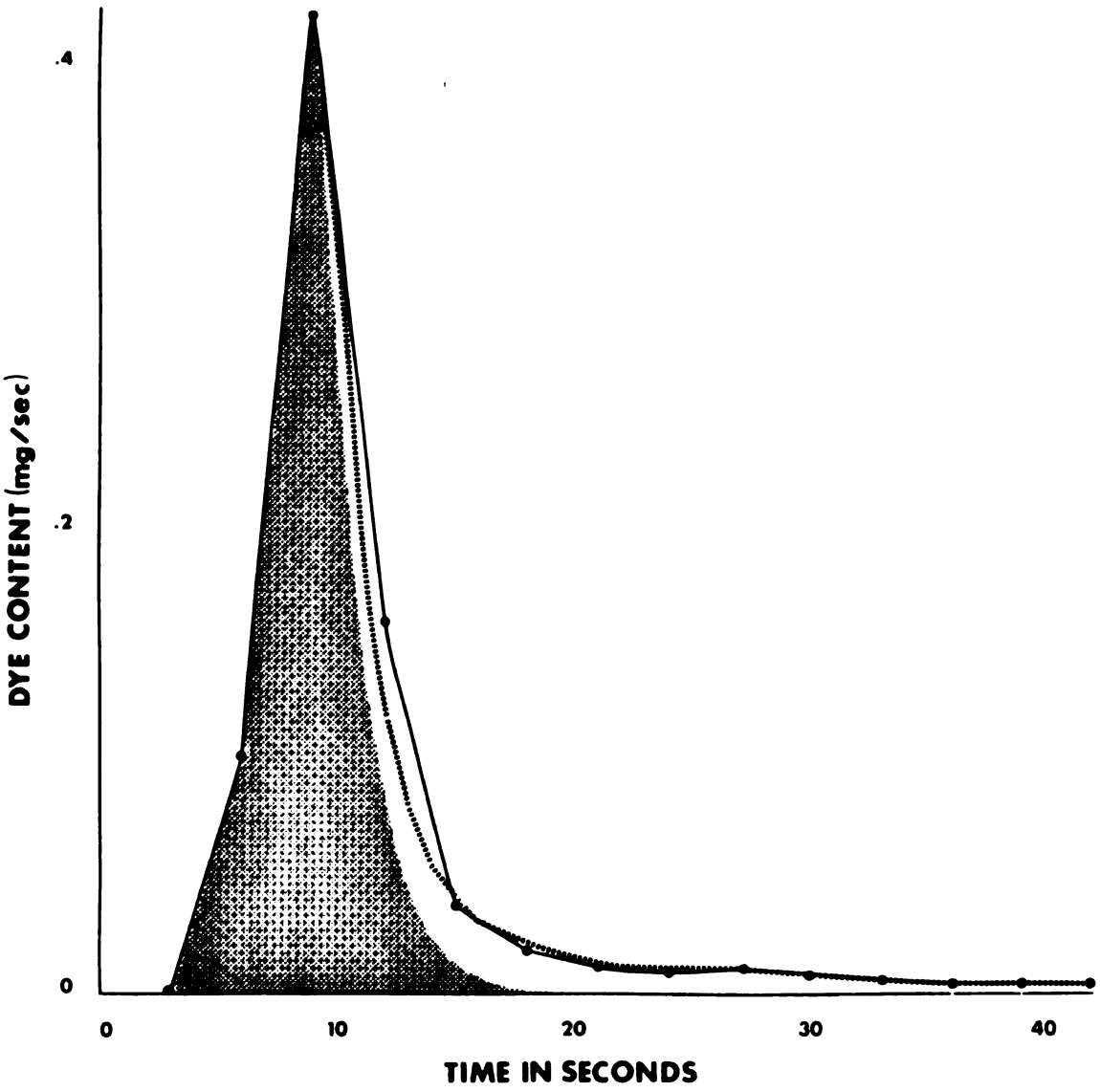

.4
.2
0
DYE CONTENT (mg/sec)
0
10
20
30
40
TIME IN SECONDS

Figure 22. Summation of points on single rapid injection curve to obtain curve of continuous infusion. Shaded area represents dye dilution curve obtained before furosemide. The dotted line represents the plateau the dye infusion would have reached if the infusion had not been stopped.

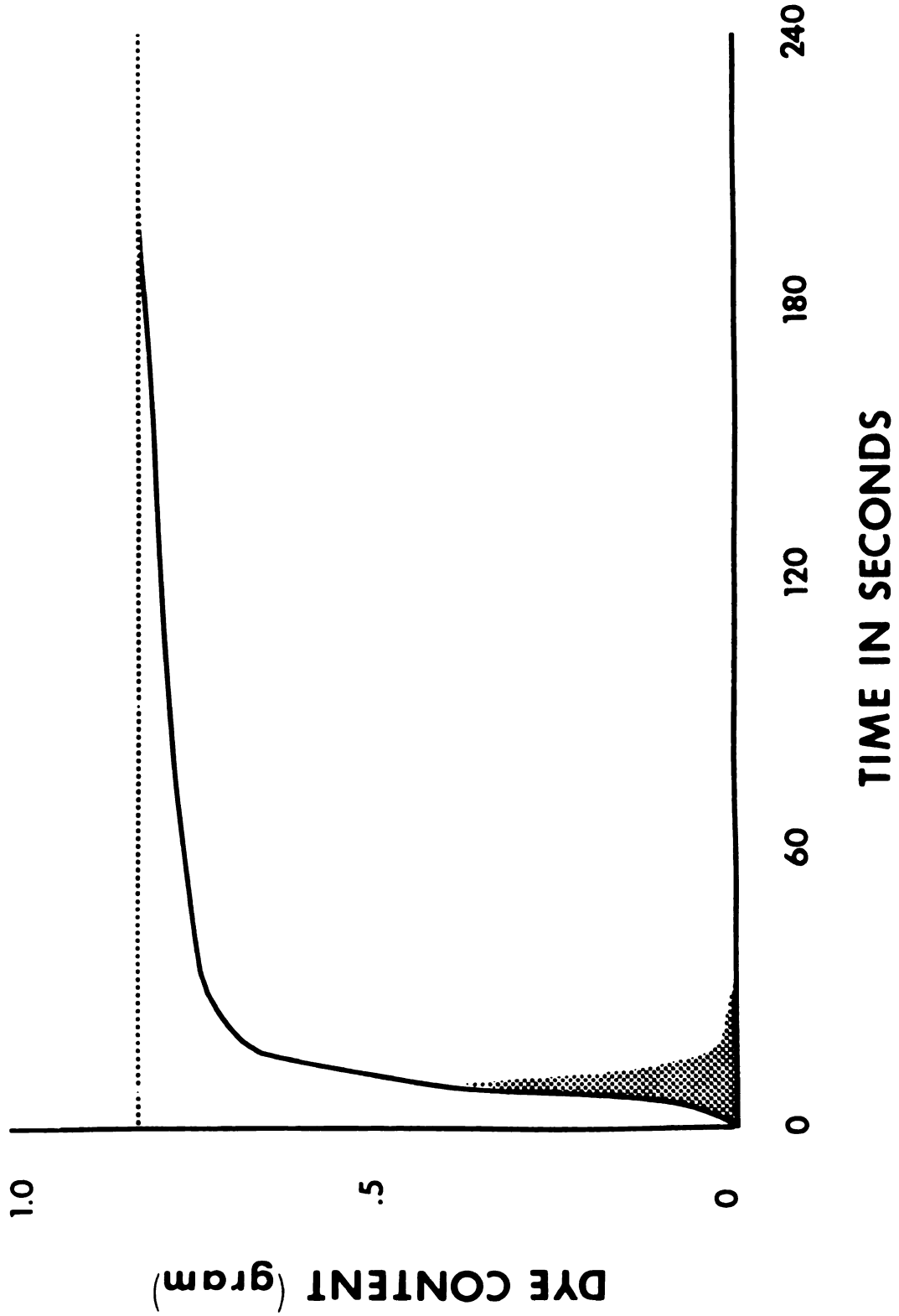
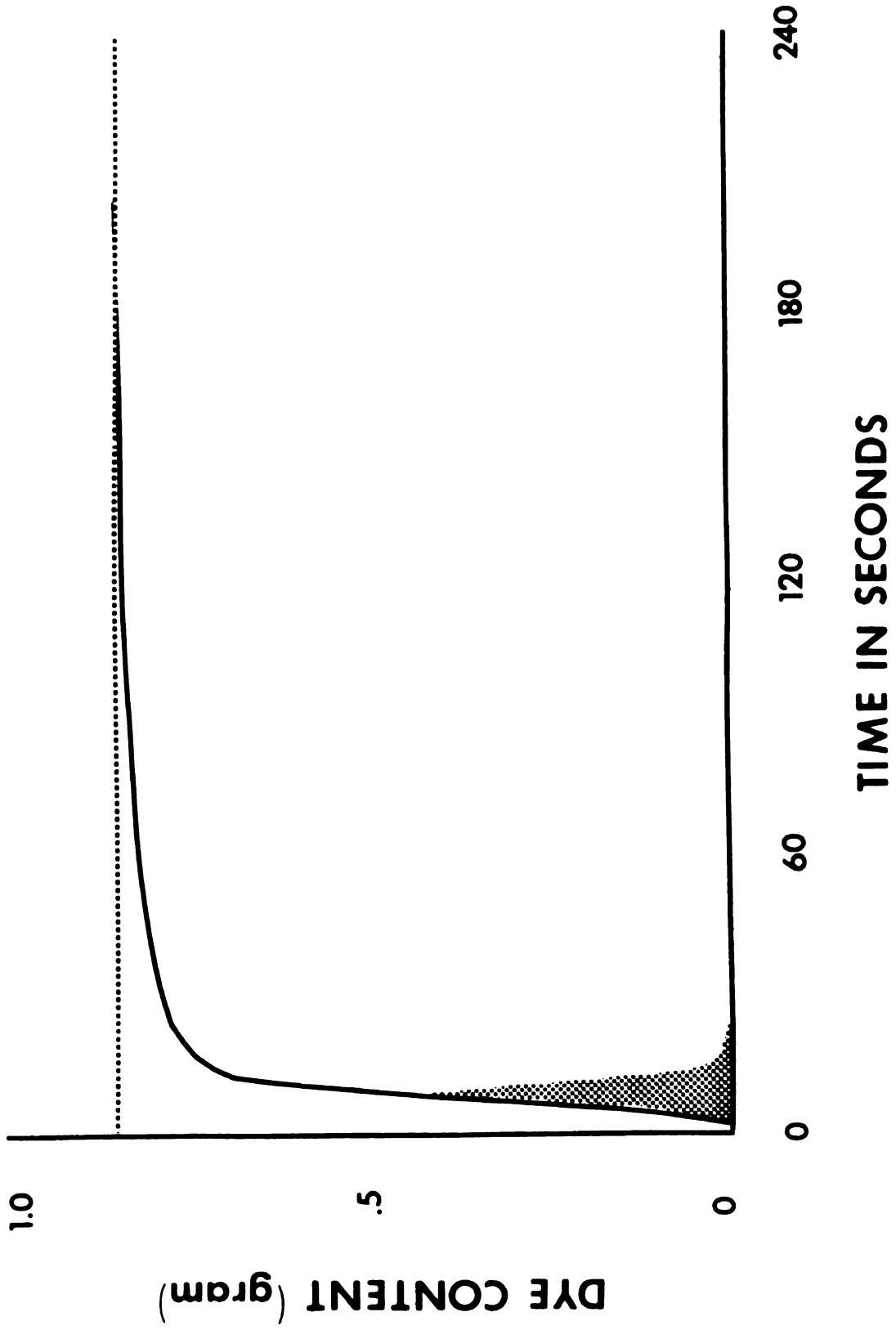

DYE CONTENT (gram)
1.0
.5
0
0
60
120
180
240
TIME IN SECONDS

Figure 23. Summation of points on single rapid injection curve to obtain curve of continuous infusion. Shaded area represents dye dilution curve obtained after furosemide. The dotted line represents the plateau the dye infusion would have reached if the infusion had not been stopped.


DYE CONTENT (gram)
1.0
.5
0
0
60
120
180
240
TIME IN SECONDS

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