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THE RENAL LYMPHATICS.

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# THE RENAL LYMPHATICS

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THE RENAL LYMPHATICS

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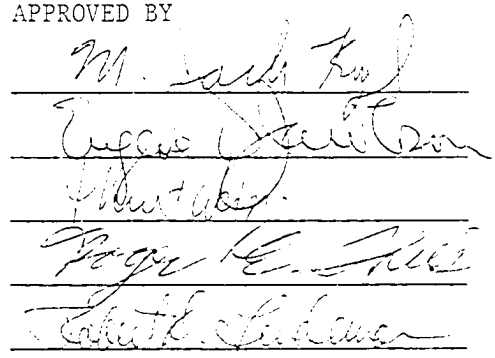

DISSERTATION COMMITTEE

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# THE RENAL LYMPHATICS

## CHAPTER I

## INTRODUCTION

### The Systemic Lymphatics

The mammalian lymphatic system and the importance of lymph in bodily function is a relatively recent discovery. Man, in his earliest beginnings, probably made crude observations concerning the size, location and appearance of most of the organs and systems of the animals he killed for food. The lymphatic system, on the other hand, consists of vessels so tenuous, and fluids so unremarkable, that even the larger lymphatic trunks can be observed only after the most careful dissections or application of special methods. Although lymphatics were probably observed by members of the Alexandrian School (300-250 B.C.), it was not until the 17th century that important treatises on the anatomy and general significance of the lymphatic system were published (29). The anatomy of the more important lymphatic trunks was established by the end of the 18th century, but the bulk of the knowledge concerning the lymphatic system as a whole was yet to be discovered. The excellent work and discerning reviews of Sabin (44) in the early 1900s established that the lymphatic vessels are derived from veins, invading the body in the same manner as do blood vessels. The invasion of the body by lymphatic vessels is, however, incomplete since some structures, such as

alveolae and the hepatic lobules, are reported never to receive them (8,25).

## Organ Lymphatics

According to Drinker (11), lymphatic vessels are mainly found in fibrous planes. In support of this generalization, Lee (25) has found the lymphatic vessels of the liver to be distributed primarily in the connective tissue of this organ and to be absent from the hepatic lobule itself. Lee has suggested that lymphatic vessels may extend up to but probably do not penetrate the physiological units of the internal organs. This concept is substantiated by the study of the lymphatic vessels of the lung by Cunningham (8). He showed that the bronchi, arteries and veins of the lung are accompanied by a rich network of lymphatics, as are the connective tissue septa. Yet the lymphatic vessels of the lung are excluded from the area of the alveolae.

The distribution of lymphatic vessels varies from organ to organ, and the function and distribution of lymphatics within each organ constitutes a separate study.

Among the workers whose efforts have added to knowledge of organ lymph, Drinker and his associates are outstanding. Not the least among the contributions of Drinker (10) is his important investigation concerning the lymphatic system of the heart. Investigations such as these set the stage for later workers, such as Miller, who investigated the possible relationships between the coronary lymphatic system and diseases of the heart (35) and circulation (34).

The lymphatic trunks draining the heart and most organs are small and tenuous and fluid from such vessels is collected only with great

difficulty. The lymphatics of the kidney are no exception.

## Renal Lymph and Lymphatics

Physiological experiments on renal lymph were reported by Ludwig in 1863 (27), but it was not until 1942 that renal lymph was actually collected for analysis (50). Numerous papers have since been published which are primarily concerned with renal lymph and lymphatics. LeBrie and Mayerson (22) and Keyl and co-workers (19) have published studies concerned with the normal composition of renal lymph, while the effects of elevated renal venous pressure were studied by Haddy and co-workers (15). Other studies demonstrate the effects of ureteral obstruction (12, 36) and diuresis (37, 46) on renal lymph, as well as the effects of obstruction of renal lymphatics themselves (2, 16). These investigations indicate that renal lymph is intimately associated with both renal hemodynamics and urine production. It is well accepted that the composition of renal lymph is determined by the character of both tubular reabsorbate and renal blood plasma (7, 17). Yet, little is known concerning the actual intrarenal sources of lymph and how its composition is altered by renal function.

## Exogenous Substances

Renal lymph is apparently altered by the cortical nephron. This was demonstrated by intravenous infusion of both p-amino hippuric acid (PAH) and inulin in experimental animals (19). In these experiments by Keyl and co-workers (19), the renal lymph inulin concentration was 80% of the plasma concentration. The inulin in lymph is derived from blood

plasma, but it is diluted by inulin-free reabsorbate from the renal tubules. Water reabsorption in the cortical nephron thus reduces the lymph inulin concentration by approximately 20%.

Due to protein binding, plasma PAH is 80-90% filterable. PAH is also bound to the protein of lymph, and renal lymph contains approximately 50% the protein concentration of blood plasma. If renal lymph were a simple filtrate of blood plasma, it would contain a PAH concentration near the plasma level. It was found that renal lymph PAH concentration was only 58% of that in plasma. This demonstrates that PAH of renal lymph is not only diluted, as is inulin, but also reduced by active secretion of PAH from renal lymph into the proximal tubule. Renal lymph composition can thus be affected by the activity of the cortical portion of the nephrons of the kidney.

## Endogenous Substances

Different laboratories disagree in their analyses of renal lymph composition (19). As shown in Table 1, LeBrie and Mayerson (22) found a renal lymph to plasma concentration ratio (L/P) for sodium greater than one, whereas Swann and co-workers (51) and Keyl and co-workers (19) found this ratio to be essentially unity. Likewise, Swann and co-workers (51) found an elevated L/P ratio for potassium while LeBrie and Mayerson (22) and Keyl and co-workers (19) did not. The L/P ratios for urea found by Sugarman and co-workers (50) and Swann and co-workers (51) were definitely greater than unity, while that found by Keyl and co-workers (19) was less than unity.

TABLE 1

RENAL LYMPH COMPOSITION AS DERIVED IN FOUR LABORATORIES

| Investigator | L/P $Na^+$(n) | L/P $K^+$(n) | L/P Urea (n) |
|---|---|---|---|
| LeBrie and Mayerson (1959) | 1.11 (27) | 1.02 (12) | --------- |
| Sugarman et al. (1942) | --------- | --------- | 1.45 (11) |
| Swann et al. (1958) | 0.98 ( 5) | 1.26 ( 5) | 1.39 ( 5) |
| Keyl et al. (1965) | 0.97 (13) | 0.94 (14) | 0.89 ( 7) |

Sources of Variation

Renal lymph is thought to be composed of a filtrate of blood plasma along with a component derived from tubular reabsorbate (7, 17). The differences in lymph composition shown in Table 1 might be due to differences in tubular reabsorbate from the various populations sampled by the investigators. The renal handling of sodium, potassium and urea would be expected to vary in accordance with differing diets and result in differing renal lymph compositions. Season of the year, ambient temperature, breed of dog, climate and conditions of animal health are uncontrolled variables which could also affect the results of different studies.

Different experimental techniques could also result in alterations in renal function and thereby cause a consistent change in renal lymph composition. Of the four studies shown in Table 1, only that of Keyl and co-workers (19) reported use of a sustaining infusion of isotonic saline. Such an infusion may be necessary to offset alterations in extracellular fluid volume resulting from blood loss and evaporation during lymph

collections.

The data of Table 1 is further complicated by the fact that both LeBrie and Mayerson (22) and Swann and co-workers (51) utilized lymph collected from capsular collecting trunks, while Keyl and co-workers (19) used only hilar lymph. On the other hand, Sugarman and co-workers (50) derived their data from both capsular and hilar lymph. Such differences in source of lymph could account for variations in composition.

## The Capsular and Hilar Lymphatics

The anatomical arrangement of capsular and hilar lymphatic trunks is shown in Figure 1. In this figure a capsular lymphtic vessel is shown on the surface of the renal capsule. This capsular vessel is shown to follow the curvature of the kidney and to leave the renal capsule at the left. In contrast to the capsular lymphatic, a hilar lymphatic vessel is shown leaving the pelvis of the kidney along the renal artery. Contrary to the findings of Dogiel (9), Parker (38) failed to demonstrate continuity of capsular lymphatics with the renal parenchyma. Other studies indicate that the capsular lymphatics drain the renal cortex while the hilar lymphatics drain both cortex and medulla (23, 50). For example, when a solution of Evans' blue dye is introduced by stab injection into the kidneys of anesthetized dogs, dye introduced into the medullary area always appeared in renal hilar lymph within 0.5 to 4.5 minutes (50). In only 2 of 15 kidneys injected in this manner did dye appear in capsular lymphatic vessels. On the other hand, when injected into cortical tissue in 11 kidneys, dye was observed to enter capsular lymphatics in 8 cases and hilar lymphatics in 7. In five of these 11 kidneys dye left the kidney via both capsular and hilar lymphatic vessels (50). Unfortunately, the specific

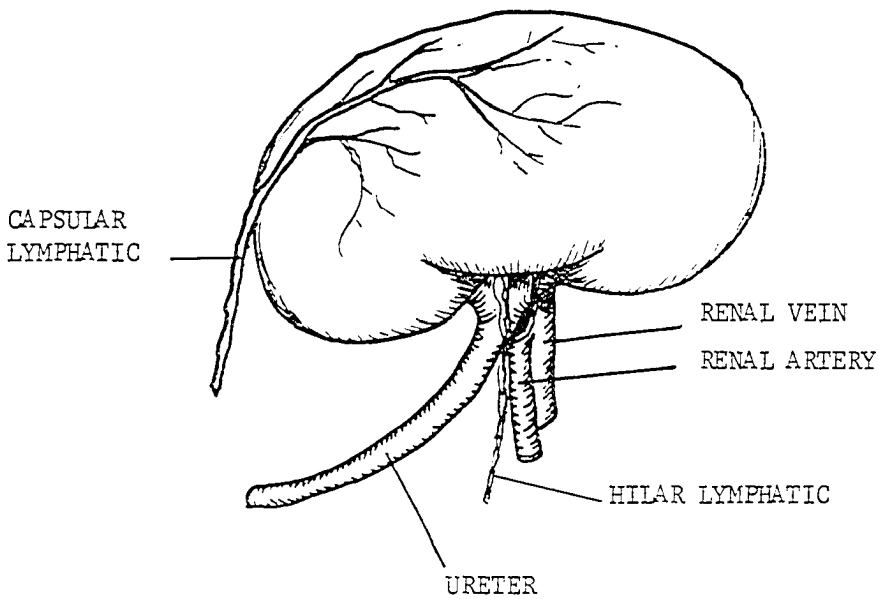


Figure 1. The Canine Kidney Showing Capsular and Hilar Lymphatic Vessels.

sites of injection in these studies were not reported. Such additional data would make it possible to estimate which portions of the renal cortex drained via the two lymphatic outflows.

## Renal Medullary Lymph

Cockett and co-workers (4, 6) have suggested that antibiotic levels in renal hilar lymph may reflect effective drug concentrations in the renal medullary interstitium, and Katz and co-workers (18) have shown that hilar lymph concentrations of the antibiotic, nitrofurantoin, may be higher than those found in capsular lymph. In addition, LeBrie (21) has concluded that much of the protein found in renal lymph is derived from the medulla. L/P ratios for sodium and urea greater than unity for renal lymph may result from a mixing of hypertonic lymph from the renal medulla and isotonic lymph from the cortex (32, 48). In fact, Selkurt (48) has stated, "An interesting avenue of investigation of the countercurrent mechanism thus appears to be afforded by a study of the renal lymphatics". Yet, assuming a component of renal lymph from the medulla neither explains the fact that some investigators do not find elevated L/P ratios for sodium and urea, nor does it account for the two reports of elevated L/P ratios for potassium seen in Table 1 (22, 51). L/P ratios for potassium greater than unity must reflect cortical function since this ion is not concentrated in the medulla as are sodium and urea (47). Furthermore, if any lymph is formed in the renal medulla, then its solute should equilibrate across the wall of the lymphatic vessel as it proceeds toward the corticomedullary border. Lymph emerging from medullary lymphatics would thus have solute concentrations similar to those of blood plasma. If this is the case, then L/P ratios different from unity must arise from

sources other than the countercurrent gradient.

Only 8-10% of the total renal blood flow is distributed to the medulla. Although the blood flow is small, there exists an opportunity for plasma protein to leak out of the vasa recta and pool in the medullary interstitium (32). Gottschalk and Mylle (14) maintain that the gradient of colloid osmotic pressure between the medullary interstitium and the blood plasma within the vasa recta is an important factor in removing water which diffuses from the descending loops of Henle and the collecting ducts. Pooling of plasma protein within the medullary interstitium would decrease the effective colloid osmotic pressure of the vasa recta. Medullary interstitial protein must be maintained at a relatively low concentration for efficient operation of the renal countercurrent mechanism. Protein leaks out of blood capillaries in most tissues of the body, and the lymphatic system returns this protein to the blood (30). Some feel that lymphatic vessels must exist in the renal medulla to carry away plasma protein which would otherwise interfere with the countercurrent system. The low perfusion rate of the renal medulla allows countercurrent exchange to take place resulting in minimal solute loss via the vasa recta. The importance of medullary lymphatics lies not in the blood flow of the medulla, but in their relationship to the countercurrent gradient. Renal lymph often has higher concentrations of sodium and urea than blood plasma, and these higher concentrations may have been derived from the medulla. These unusually high renal lymph sodium concentrations may play a role in the sodium retention of congestive heart failure (3, 31). If renal lymph normally contains renal medullary interstitial fluid with its high concentrations of sodium and urea, the countercurrent

multiplier system must overcome water gains from the vasa recta, descending loops of Henle and collecting ducts, as well as compensate for solute loss via the lymphatic vessels. Functional lymphatics in the medulla may be of considerable importance in removing interstitial protein, yet direct evidence that lymphatics remove medullary interstitial fluid, with its high concentrations of sodium and urea, is lacking.

## The Intrarenal Distribution of Lymphatic Vessels

### Cortical Lymphatics

It is known with some degree of certainty that the intrarenal lymphatics follow the interlobular, the arcuate and the interlobar blood vessels (39). The relationship of lymphatic vessels to other renal structures is highly controversial.

Stab injection is frequently used to study intrarenal lymphatics, since the numerous valves of the collecting lymphatic trunks usually prevent retrograde injection. The stab injection technique involves injection of India ink or other suitable media directly into the renal tissue. Lymphatic vessels are identified by their uptake of the injected substance. Stab injections involve a high probability of filling many structures other than lymphatics with the injection medium. Interpretation of these structures as lymphatic vessels may have resulted in many conflicting reports concerning intrarenal lymphatic distribution, yet carefully analysed stab injection preparations can be quite valuable. The conscientious study by Peirce (39) is very illuminating. India ink was introduced into both cortex and medulla of living dog kidneys by stab injection. Histological sections revealed that only one out of fifteen injected kidneys was sufficiently free of artifacts for accurate analysis of the

intrarenal lymphatic distribution. Peirce (39) found that the lymphatic vessels of the renal cortex were confined to the interlobular spaces and to the vicinity of the major blood vessels of the kidney. Rigorous criteria were used in this study to eliminate artifacts due to filling of blood vessels and renal tubules. These same criteria may also have eliminated indications of lymphatic vessels within the renal lobules.

Since renal tubules and peritubular capillaries are densely packed within the lobules, it is unlikely that one could insert a needle into their midst without rupturing them and filling them with ink. The large number of ink filled blood capillaries and renal tubules would make any injected lymphatic vessels impossible to identify. Peirce (39) may have drawn his conclusions only from stab injections made into the interlobular spaces. Injected ink would be taken up by the interlobular lymphatic vessels, be conducted to the hilus of the kidney, and appear within hilar lymphatic vessels without filling any intralobular branches of the lymphatic system. Thus, anatomical visualization of the intrarenal lymphatic vessels by means of forward filling is highly informative, but has serious limitations.

Ideally, one would wish to demonstrate lymphatics by introducing into them a contrasting medium by means of the most physiological route, i.e., via the blood vessels of the organ. Yet dyes introduced into the renal artery stain blood capillaries as well as lymphatic vessels. Peirce (39) found that certain dye substances injected into the renal artery were washed out of the capillary system within a short time. These dyes were observable in the intrarenal lymphatics of the kidney, but only in the larger ones. The failure to demonstrate small lymphatic vessels was due

to dilution of the dye by interstitial fluid. The highly dilute dye within the small lymphatics could not be distinguished from the other tubular structures of the kidney. Until a substance is found which binds specifically to the lymphatic vessels, this method will not be too useful. Certain fluorescent dyes may be of utility for future studies, provided these are sufficiently specific for lymphatic vessels and can be applied with advantage to the kidney (45).

## Medullary Lymphatics

Rawson (40) described lymphatic plexuses in both cortex and medulla of the human kidney. These findings were drawn from examination of only one preparation and were possible only by virtue of extensive carcinomatous permeation. It is doubtful if such observations can be applied to the canine kidney or to the normal human kidney. Lymphatics have been found in the renal medulla of the rabbit after acute experimental hydronephrosis (1), but this has never been demonstrated in dog or man.

The electron micrographic studies of Rodin (41) indicate that lymphatic capillaries are quite numerous in the renal medulla and papilla. These findings have not been confirmed by other workers since the criteria by which lymphatic vessels are identified in electron micrographs are not established.

As stated above, Peirce (39) injected ink directly into the renal medulla. In these cases he never found the ink to be taken up by lymphatic vessels, and it was concluded that lymphatics did not occur in the renal medulla.

## Unsolved Problems

The foregoing discussion of previous work concerning the source and composition of renal lymph suggests several unsolved problems.

A. Renal lymph composition is determined by the composition of both tubular reabsorbate and blood plasma. It is not known what contribution, if any, is made to renal lymph composition by renal medullary interstitial fluid.

B. Renal lymph composition is specifically altered by the activity of the tubular mass of the cortex. Peritubular fluid may be taken up by lymphatic vessels within the renal lobules, or on the other hand, tubular reabsorbate may alter renal lymph composition only by diffusion.

C. Variations in L/P ratios for sodium, potassium and urea have been reported from different laboratories. The range of variations in the same laboratory under conditions of constant experimental technique over as long as a year has not been studied.

## Objectives

The experiments reported in this study were designed to provide data to answer the following questions:

1. Can the intrarenal distribution of lymphatic vessels demonstrated by stab injection studies be confirmed by the retrograde injection method?

2. When visualized by retrograde injection, can lymphatic vessels be demonstrated in areas of the kidney where stab injection studies have failed to show their existance?

3. Can the variations in L/P ratios for sodium, potassium and urea among different laboratories be reproduced in one laboratory over a

period of a year using constant experimental techniques?

4. Do the high concentrations of sodium and total solute in the renal medulla alter lymph composition, and if so, by what means?

5. Finally, can our present understanding of renal lymph and lymphatics be synthesized into a single hypothesis?

# CHAPTER II

## METHODS

The experimental animals used in these studies were 117 mongrel dogs obtained from professional dealers and 7 horses and 11 calves obtained through the Department of Veterinary Surgery of Oklahoma State University, Stillwater, Oklahoma.

### Anesthesia

In each experiment, a surgical plane of anesthesia was obtained by an intravenous dose of sodium pentobarbital (Nembutal Sodium, Abbott Laboratories) at 30 mg/kg of body wt. Additional sodium pentobarbital was administered as needed to maintain each animal in a surgical level of anesthesia for up to 4 hours. The level of anesthesia was judged by an absent or weak reaction to noxious stimuli applied to the hind limb or flank, while making certain that respiration was not severely depressed. In addition, each experimental animal was tested for an absent or weak eyelid reflex, but care was taken not to anesthetize the animal so deeply as to abolish the corneal reflex. In these ways, the level of anesthesia could be followed throughout each experiment and additional anesthetic agent administered as needed.

### Surgical Preparation

Animals were placed on a surgical table and secured in a supine

or lateral recumbent position by heavy cords loosely tied to front and hind limbs. In dogs, tracheostomy was performed through a midline incision in the neck and a tracheal cannula was tied firmly in place. In the horse and calf, free breathing was assured by inserting an endotrachial catheter per os. In dogs, an incision was made directly over the femoral artery and vein, and after exposure of these vessels, they were ligated and catheterized with saline filled polyethylene tubing (PE 205, Clay Adams, Inc.). The femoral artery catheter was then attached to a Statham P-23 arterial pressure transducer, and a continuous recording of arterial pressure was made by use of a Grass Polygraph model 5. The femoral vein catheter was used to administer drugs and infusions. In the horse and calf, the jugular vein was catheterized to permit intravenous administration of drugs.

In all animals the left kidney was exposed through a flank incision made just below the rib cage and extending from the level of the lateral spinous processes to within an inch of the rectus abdominous muscle. The incision was carried through the peritoneum, and upon retraction of the abdominal muscles, the left kidney could be visualized at the dorsal extent of the incision. The left kidney, along with an adherent flap of peritoneum was then dissected free of the dorsal body wall and retracted ventrally to expose the hilus. Lateral retraction of the kidney exposed the outer curvature and ventral aspect of the kidney where capsular lymphatic vessels could be visualized. The left ureter was isolated and catheterized at this time. The ureter was ligated and a small incision was made rostral to the ligation. A blunt polyethylene catheter (PE 205, Clay Adams, Inc.) was then inserted into the lumen of

the ureter and slipped forward until the tip could be felt at the pelvis of the kidney. The catheter was then tied firmly in place and was observed carefully to make certain that a free flow of urine was obtained. It was found that moderate traction applied to the catheterized ureter prevented blockage of the catheter tip by the wall of the ureter.

## Lymphatic Catheterization

### Hilar Lymphatics

Careful inspection of the hilar region of the kidney often revealed one to four efferent lymphatic trunks running parallel to the renal blood vessels (Figure 1). One of these hilar lymphatic vessels was dissected free of surrounding tissue and ligated with 4-0 silk. The lymphatic was divided distal to the ligature. The lymphatic vessel was lifted into position for catheterization by gentle traction applied to the 4-0 suture which is left long for this purpose. Polyethylene tubing having an outside diameter of 0.16 mm (PE 10, Clay Adams, Inc.) was carefully cut to leave a bevel at the end intended to enter the lymphatic vessel. The catheter was grasped near the bevel with a small thumb forceps and, bevel down, the point is forced against the lymphatic vessel near the ligature as shown in Figure 2. In a single motion, the catheter pierced the vessel wall and slipped well into the lymphatic. The tubing was tied in place with 4-0 suture. Renal lymph then filled the catheter and dripped from its free end.

### Capsular Lymphatics

The disposition of a capsular lymphatic vessel is shown in Figure 3. These lymphatics lie on the surface of the fibrous capsule and

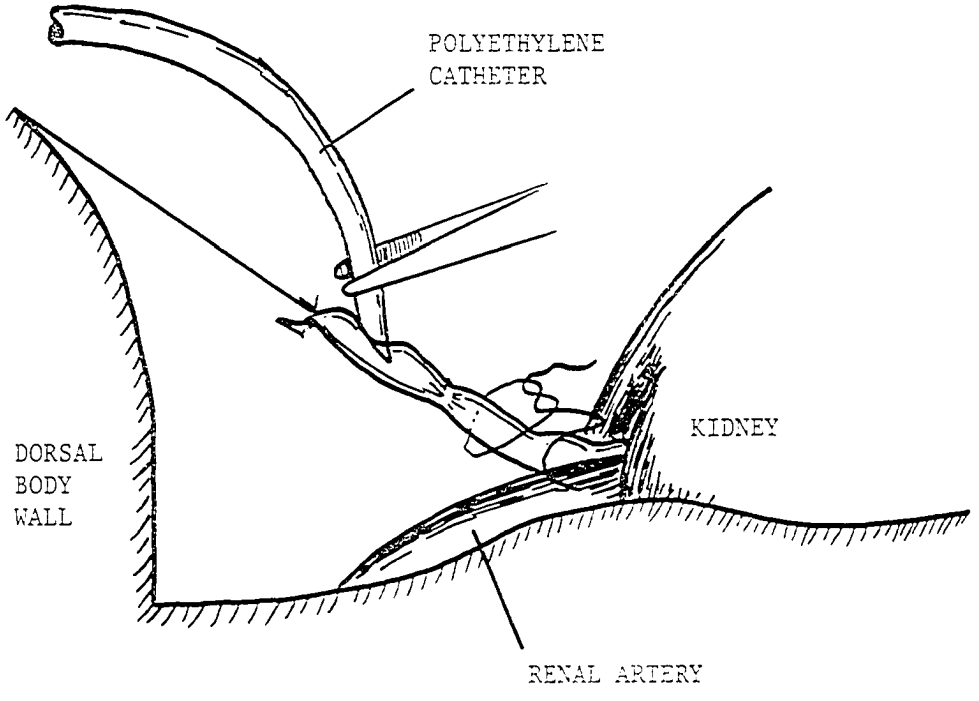


Figure 2. Method of Catheterization of Lymphatic Vessels of the Renal Hilus.

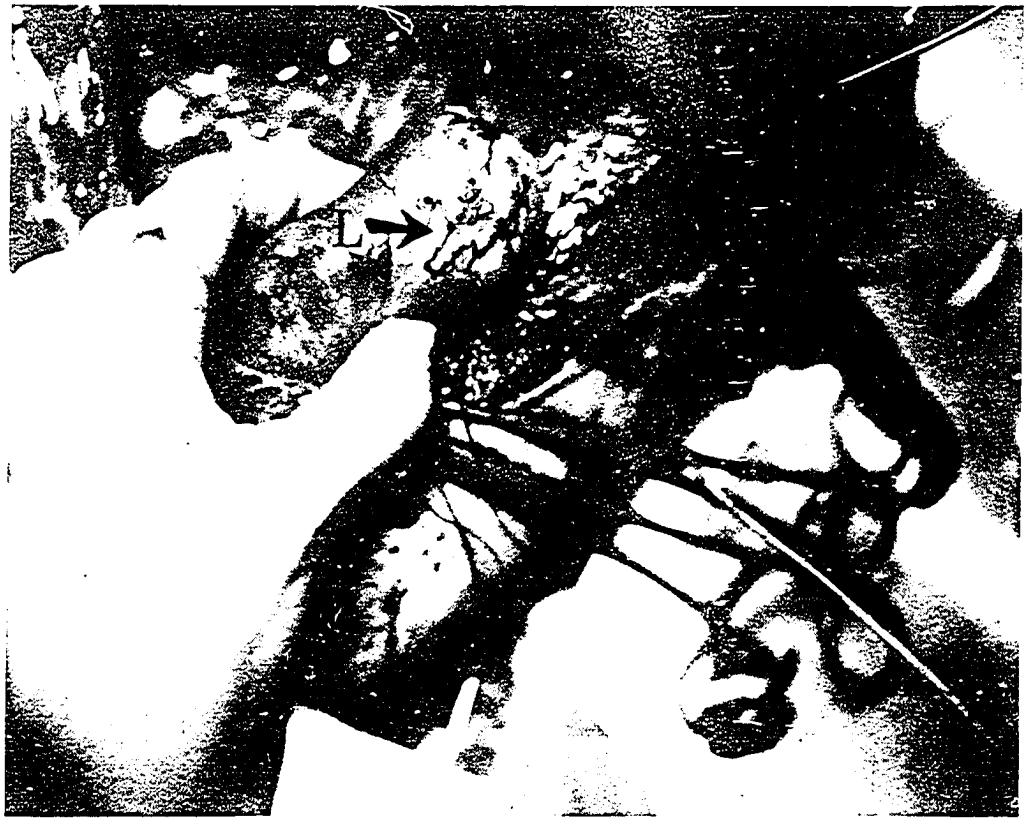


Figure 3. India ink injected capsular lymphatic (L) on the surface of a dog kidney.

drain toward each pole of the kidney. The occurrence and size of the capsular lymphatic vessels are quite variable, and in approximately 20% of the animals they are too small to allow catheterization. When a large capsular lymphatic vessel was found, it was ligated, and a ligature was passed beneath it at the point where the catheter was to be tied in place. A small incision was then made in the wall of the lymphatic and the beveled end of a polyethylene catheter was passed into the vessel in a manner similar to that used to catheterize hilar lymphatics. The catheter was tied in place, and lymphatic fluid was observed to fill the catheter and drip from its distal end.

Immediately upon catheterizing a hilar or both hilar and capsular lymphatic vessels, 1 mg/kg of body wt. sodium heparin (Sodium Heparin, USP, Hynson, Westcott and Dunning, Inc.) was administered intravenously as a 1% solution to prevent clotting of lymph within the catheter.

During all procedures, care was taken to leave the renal nerves and other lymphatic vessels undisturbed.

## Retrograde Injection

### Rationale

The disadvantages of the stab injection method of study were reviewed in the Introduction (p. 10). Retrograde injection of renal lymphatic vessels was chosen for the present study in an attempt to obviate these difficulties. The advantages of the retrograde injection method as used in this study are as follows:

1. The method is specific because injection medium is introduced directly into the lymphatic vessels.

2. Since the retrograde injection method does not involve the introduction of any implement into the kidney substance, disruption of the normal integrity and anatomical relationships of the intrarenal structures is prevented.

3. The retrograde injection method involves positive pressure filling; therefore, the injection mass is directed toward the end lymphatics rather than away from them as in a forward filling technique. This offers an opportunity for filling lymphatics within renal lobules if they exist there.

4. Artifacts due to lymphatic rupture during retrograde injection are easily recognized. Rupture results in filling of interstitial spaces yielding a characteristic diffuse pattern which cannot be mistaken as a lymphatic structure.

## Injection Method

Retrograde injection of renal lymphatics was accomplished by introducing a 0.16 mm O.D. polyethylene catheter (PE 10, Clay Adams, Inc.) into a lymphatic vessel on the surface of the renal capsule. India ink was introduced into the lymphatic catheter from a 1 c.c. tuberculin syringe. The ink was allowed to flow into the lymphatic vessel via the catheter. The lymphatic vessel was kept filled with ink by advancing the syringe plunger as needed. The lymphatic vessel was subjected to gentle stroking in a retrograde direction which allowed the ink to pass the valves filling a length of lymphatic. Injection pressure was derived solely from the peristaltic effect of retrograde stroking. Such an injected lymphatic is shown in Figure 3. Figure 4 is a schematic representation of the preparation. The catheter is secured in the lymphatic

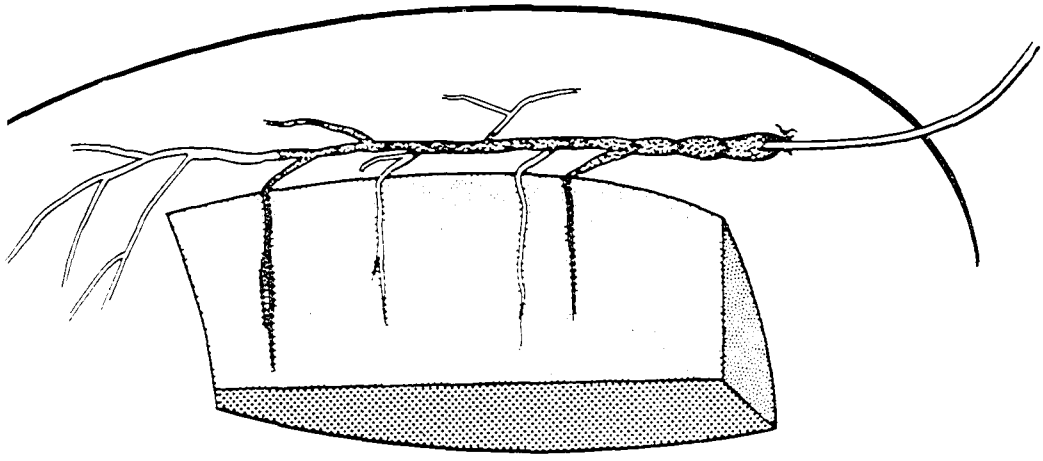

Figure 4. Schematic Diagram of Capsular Lymphatic Vessel During Retrograde Injection.

lumen by a ligature seen at the right in Figure 4. A length of lymphatic vessel is seen to fill with injection medium, as do communicating lymphatic vessels of the renal parenchyma. The left kidneys from 25 dogs, with their injected lymphatics, were removed from the animals and prepared for microscopic examination. Similar procedures yielded only one capsular injection out of 7 attempts in the horse, since capsular lymphatics in this species are extremely small.

A single hilar injection was obtained in a calf, an animal in which capsular lymphatics were never observed. In this case injection was possible by virtue of a fortuitous patency or lack of lymphatic valves.

## Histological Preparation

Blocks of tissue were cut from the injected kidneys and placed in buffered 10% formalin. After fixation for 24 hours, the blocks were removed from the formalin and sectioned without further treatment. Sections of suitable thickness (approx. 0.5 mm) were made with a Stadie microtome, an instrument designed to prepare sections of living tissue for metabolic studies. Sections were individually dehydrated by passage through graded concentrations of ethyl alcohol (70%, 80%, and 95%), and finally in acetone. The sections were cleared in xylene and mounted unstained on microscope slides and sealed with synthetic resin (Permount, Fisher Scientific Co.).

## Examination of the Sections

Sections were studied individually to ascertain the course of the injected intrarenal lymphatic vessels and their relationship to the

other intrarenal structures. Sections with excessive extravasation of injection medium were excluded from consideration.

Photomicrographs were prepared using a 35 mm SLR camera with Kodachrome II film and a B and L student model microscope.

## Lymph Composition Studies

### Sample Collection

Collections of lymphatic fluid were begun 20 minutes after heparinization to insure that the heparin had equillibrated throughout the circulation. Renal lymph was collected by inserting the free end of the lymphatic catheter into a 2.5 ml. graduated centrifuge tube containing approximately 0.5 ml. of mineral oil. All lymph collections were made under mineral oil to prevent evaporation of water during the collection periods which varied from 20 min. to 3 hours. Only one lymph sample was collected from each dog.

Lymph and urine were simultaneously collected, and blood was sampled via the femoral arterial catheter at equal intervals throughout each collection period. A sustaining infusion of 0.9% saline was given intravenously at 2 ml./min. throughout each experiment to offset body fluid losses due to evaporation and seepage.

### Capsular-Hilar Collections

In 6 dogs simultaneous collection of capsular and hilar lymph was possible. Each of these 6 animals produced two samples of renal lymph, each collected as described above. Care was taken in each case to assure that the time course of the capsular lymph collection coincided exactly with that of the hilar lymph collection.

Although 4 out of 5 dogs may yield satisfactory hilar lymph collections, only 1 out of 4 may yield a suitable volume of capsular lymph. When catheterization of these vessels is accomplished, the lymph flow obtained is frequently so sluggish that the 0.5 to 1.0 ml of lymph necessary for analysis cannot be obtained. The expenditure of time and money necessary to collect a large volume of data comparing simultaneously collected capsular and hilar lymph was prohibitive.

### Alterations in Urine Concentration

In certain cases it was desirable to obtain renal lymph samples while urine of low total solute was being produced. Preparations producing urine of minimal osmolality were obtained by hydrating dogs with 50 ml. of tap water per kg. of body wt. given by stomach tube both on the evening preceding the experiment and again on the morning of the experiment.

### Analyses

Electrolytes were measured with a Baird flame photometer model DB-4, requiring 1.5 ml of sample, or KY-3, requiring 0.5 ml of sample, using a lithium internal standard. The internal standard method of flame photometry is an ingenious way to obtain highly accurate and stable analyses. Each sample is appropriately diluted with deionized water to which is added a known constant concentration of lithium ion. This solution is atomized and fed into the flame as a finely divided aerosol. The characteristic emissions of sodium, potassium and lithium are selected by optical filters and directed upon light sensing devices which convert the emission intensities into electrical signals. The

emission intensity of either sodium or potassium is compared with that of the lithium standard within the circuitry of the flame photometer; the meter deflection noted is the result of the balance between the emission intensity of the ion to be measured and that of the lithium standard. Since the emission of the ion to be measured is always read against the emission of the internal standard, variations in atomization and flame intensity are partially compensated. The Baird flame photometer must be calibrated daily, and known standards must be interspersed with samples to insure maximum reproducibility. If these precaustions are followed recovery studies reveal errors of less than $\pm$ 2%.

Urea was measured by the colorimetric method of Rosenthal (42). This method utilized a diacetyl monoxime reagent to develop color. The relationship of optical density to urea concentration is linear only in a limited range of concentrations when this method is used. A standard curve must therefore be generated from a large number of closely spaced dilutions. In this way an exact plot of optical density against urea concentration can be obtained. This method is found to yield reproducibility of 99%, and has been found quite suitable as long as known standards are used frequently to revalidate the standard curve. This procedure required 0.2 ml of sample.

Osmolality was measured using a Mechrolab model 301 osmometer. This device senses vapor pressure differences between a standard solution (distilled water) and the test solution (lymph, plasma or urine). This device must be calibrated with solutions of known osmolalities and its response has been found to be linerarly related to osmolality within its usable range. The reproducibility of the Mechrolab osmometer model 301 has been found to be within 99%. A sample volume of 0.1 ml is required.

In order to maintain a high degree of accuracy, commercial control sera such as Versatol (Warner Chilcott) or Lab-Trol (Dade Reagents Inc.) or solutions of known concentrations were analysed along with unknown samples.

## The L/P Ratio

Raw data were tabulated and ratios for lymph to arterial plasma (L/P) or urine to arterial plasma (U/P) calculated. The use of such erived values is justified below. Previous work concerning renal lymph showed that this fluid is composed of both tubular reabsorbate and an ultrafiltrate of renal blood plasma. Therefore, variations in plasma composition will result in similar alterations in renal lymph composition. The present study, however, is concerned less with the relationship of renal lymph to plasma composition than with the functions peculiar to the nephron. By the use of the simple expedient of a ratio one answers the question: What is the direction and magnitude of the difference between lymph composition and plasma composition? The ratio presents the lymph concentration of a substance as a fraction of the plasma concentration of that substance at a time when that plasma is perfusing the organ producing the lymph. Concentrations of sodium, potassium and urea vary among plasma samples from different dogs. Previous studies have established the following normal ranges in plasma of mongrel dogs used in this laboratory: sodium, 127-159 mEq/L, potassium, 2.4-3.9 mEq/L; urea nitrogen, 5-27 mg/100 ml. The L/P ratios were used to present variations in lymph composition which are independent of variations in plasma composition among dogs. The L/P ratios reflect only alteration in lymph composition due to the functions of the nephron population of the kidney. This study is

concerned with these specific lymph composition changes and the intrarenal structures that produce them.

## Statistical Treatment

The statistics used were limited to common techniques (49). Probability values shown in Tables 2 and 3 were derived by use of the Student's t, calculated by the formula: $t = \frac{\bar{x} - \mu}{S_{\bar{x}}}$. $\mu$ in this formula, which is the population mean, is taken to equal 1.00; $\bar{x}$ is the experimentally derived mean; $S_{\bar{x}}$ is the standard deviation of the mean or the standard error.

A probability value of 0.05 or less was considered to indicate "statistical significance."

Additional statistical calculations relating lymph and plasma protein concentrations to L/P ratios for solute in sample water were performed. These calculations were obtained through the Biostatistics Unit of the University of Oklahoma Medical Center.

# CHAPTER III

# RESULTS

## Anatomical Findings

It was stated in the Introduction (p. 11) that the study of renal lymphatics by stab injection is highly informative, but has serious limitations. The retrograde injection method was used to obviate these limitations. Ink was introduced into the canine kidney via capsular lymphatics since the valves of the hilar vessels preclude retrograde injection.

### The Capsular Lymphatics

The capsular lymphatic trunk of the canine kidney is a small irregular tube filled with clear colorless fluid. Two such capsular lymphatic vessels are often found draining toward opposite poles of the kidney. After ligation the capsular lymphatic vessel swells markedly, and frequent narrowings of the lymphatic wall appear where the valves are located. The direction of flow in these vessels is easily determined since the vessel collapses immediately on the outflow side of the ligature. As the capsular trunk is being injected, as described in Methods (p. 21-23), it is difficult to force ink past the valves. On the other hand, filling occurs quickly and easily in the minute lymphatic vessels branching from the sides of the main trunk. This may indicate an absence of functional valves in these smaller lymphatic vessels.

Figure 3 shows an injected capsular lymphatic on the surface of a dog kidney. This lymphatic has a typically irregular path and a few smaller branches. Other small lymphatic branches, not seen by gross inspection, leave the capsular trunks to enter the renal parenchyma.

## The Interlobular Lymphatics

Lymphatics entering the kidney can be followed in sagittal sections of the kidney, such as the one shown in Figure 5. The injected intrarenal lymphatics labeled "L" are tubular structures filled with black injection medium. Microscopic examination reveals blood vessels, labeled "B", and glomeruli (dense spots) as red and translucent in appearance due to trapped red blood cells. The lymphatic vessels shown in Figure 5 follow a pattern which was seen repeatedly in these preparations. The lymphatic vessels enter the renal parenchyma through the renal capsule shown at the top of Figure 5. Upon entering the kidney, the lymphatic vessels branch into a narrow anastomosing plexus. Typically, the lymphatic plexus is confined to the immediate vicinity of an interlobular blood vessel, following it from the capsule to the corticomedullary border. For example, lymphatic vessels lie very close to a cortical blood vessel in Figure 5, and even follow its branches. Since the lymphatics of the kidney are closely associated with the major blood vessels, lateral branching in the cortex is seen only in special instances, i.e., the periglomerular lymphatics discussed later in this section.

The intimate relationship of lymphatic capillaries to the cortical blood vessels is shown in more detail in Figure 6. In Figure 6, the anastomosing ink-filled lymphatic capillaries labeled "L" form a plexis around a blood vessel labeled "B" and closely follow its branches. The


Figure 5. A sagittal section through cortex and outer medulla of a dog kidney. A capsular lymphatic (C) has been injected with India ink. Intrarenal lymphatics may be seen as a network of vessels (L) following interlobular blood vessels (B). X 10.

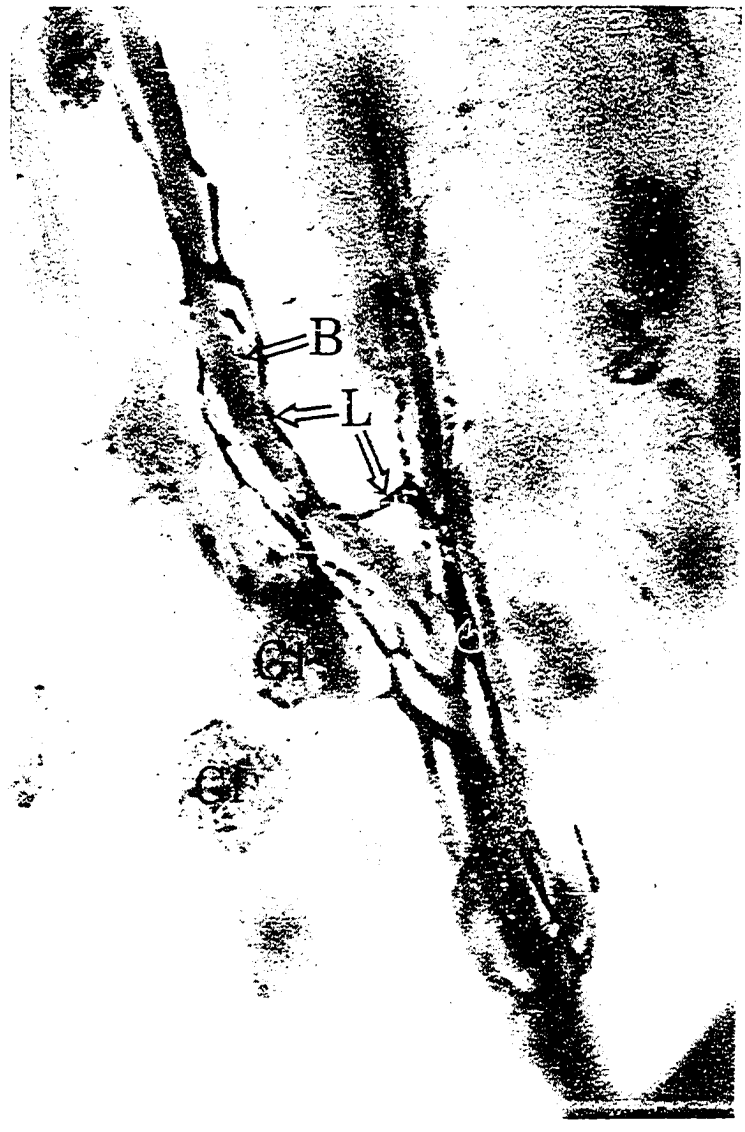


Figure 6. Lymphatic plexus (L) around a blood vessel (B) in the cortex of the dog kidney. Several glomeruli (Gl) may be seen. X 75.

narrow columns of lymphatic vessels observed in the cortex of the canine kidney are even clearer in the calf kidney injected via a hilar lymphatic. A section from this preparation is shown in Figure 7. The renal lobules appear as light colored bands radiating from the corticomedullary border to the capsule. The darker bands observed between the lobules are the interlobular areas containing the major cortical blood vessels and connective tissue. Injected lymphatics labeled "L" in Figure 7 are found in this interlobular connective tissue.

## Periglomerular Lymphatics

Lymphatic capillaries are found in the immediate vicinity of glomeruli. These periglomerular lymphatics appear to surround the glomerulus in close association with Bowman's capsule. These extensions of the interlobular lymphatic plexuses are shown in Figure 8 and are labeled "L" in this figure. The close association of lymphatic vessels with Bowman's capsule in the horse kidney is shown in Figure 9. The lymphatic vessel, labeled "L" in Figure 9, is closely applied to the outer side of Bowman's capsule, labeled "BC" and appears to encircle the glomerulus.

Lymphatic capillaries are observed along afferent arterioles and in intimate association with the parietal layer of Bowman's capsule, but lymphatic vessels are never found to enter the glomeruli themselves.

## The Corticomedullary Border

Careful evaluation of the retrograde injection preparations indicates a rich lymphatic plexus at the corticomedullary border around the arcuate vessels. These lymphatic plexuses continue along the interlobar

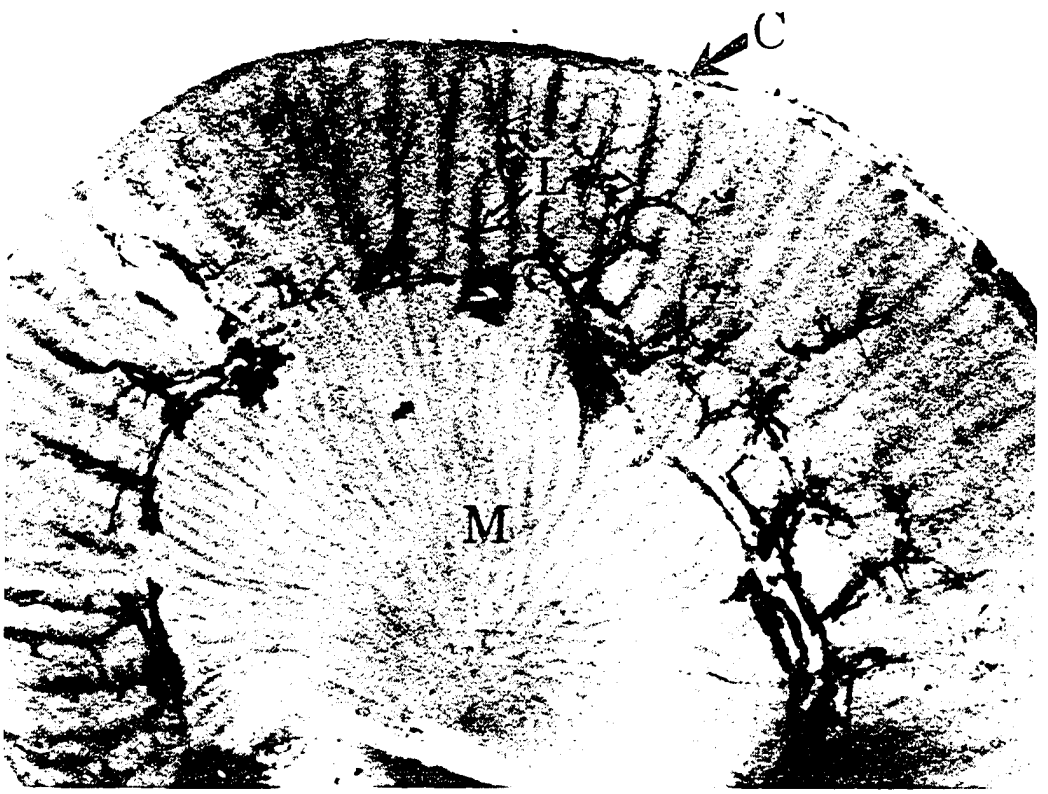


Figure 7. A saggital section through a lobe of a calf kidney which has been injected with India ink via a hilar lymphatic. The lymphatics (L) form a plexus around the cortico-medullary border and ascend to the capsule (C) along blood vessels. There is an absence of injection medium in the medulla (M). X 10.

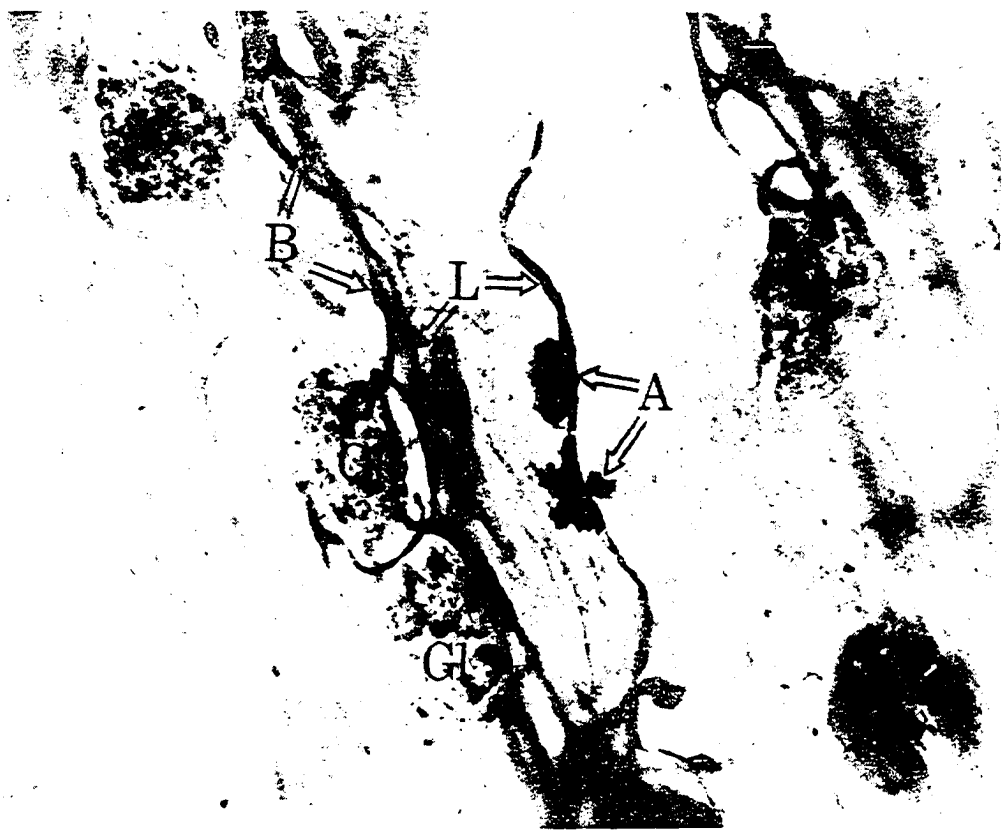


Figure 8. Section through the cortex of a dog kidney. Two glomeruli (Gl) with attendant lymphatics (L) may be seen as well as other lymphatics around blood vessels (B). Two artifacts (A) caused by lymphatic rupture. X 125.


Figure 9. Section through cortex of horse kidney. India ink filled lymphatic (L) surrounding glomerulus (G). X 125.

blood vessels and join at the hilus to form efferent lymphatic trunks. Figure 10 shows the pattern of lymphatic drainage at the base of the cortex. In this figure numerous lymphatic vessels, labeled "L" , at the corticomedullary border converge, leaving the renal parenchyma between adjacent papillae. The relationship of the interlobular lymphatic networks to that at the corticomedullary border is shown in Figure 7. The injection medium spreads laterally throughout the plexus at the corticomedullary border, then fills the interlobular plexuses of the cortex. The interlobular plexuses are thus in communication with the hilar lymphatic trunks as well as those of the capsule.

### The Renal Medulla

The lymphatic plexuses shown at the corticomedullary border in Figures 7 and 10 are in sharp contrast to the lack of injected lymphatics in the medullary areas, labeled "M" in these figures. Medullary lymphatic vessels were never observed in retrograde injection preparations. If such vessels exist, it must be concluded that they cannot be demonstrated by this method.

### Lymphatic Rupture

It was stated in Methods (p. 21) that artifacts due to lymphatic rupture during retrograde injection are easily recognized. Figure 8 shows two such artifacts, labeled "A" in this figure. The characteristic appearance of such artifacts is adequate to prevent their misinterpretation.

## Renal Lymph Composition

Renal lymph concentrations of sodium, potassium and urea differed

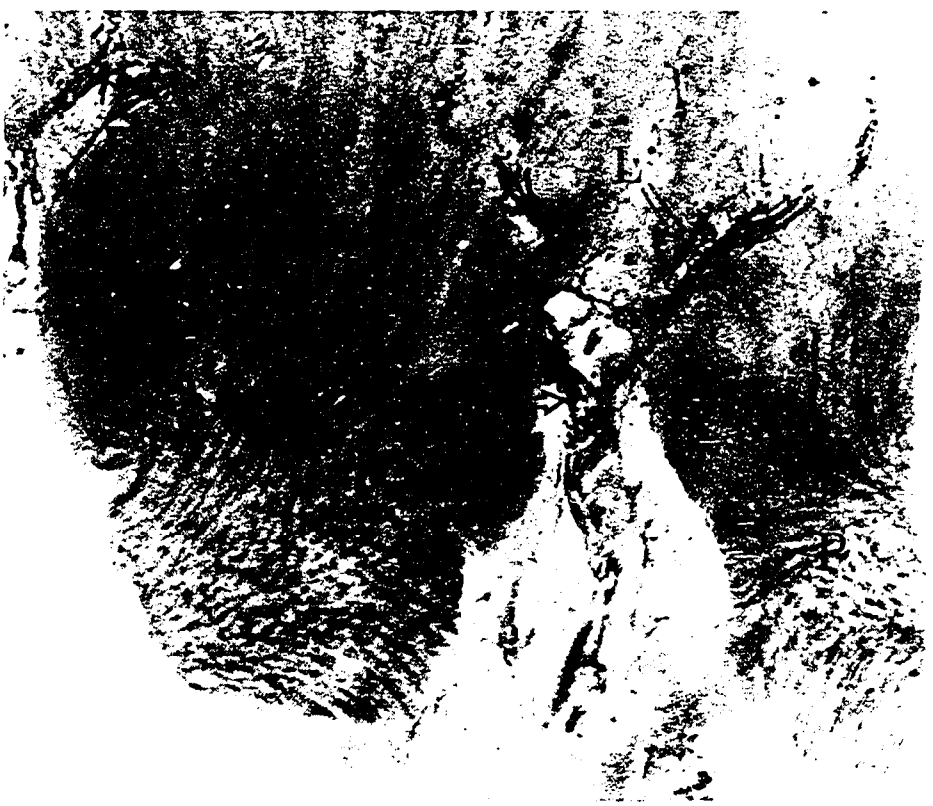


Figure 10. A saggital section through a dog kidney. The cortico-medullary border is at the top of this photo-micrograph. Lymphatics (L) at the cortico-medullary border leave the kidney between adjacent papillae (P). Note absence of injection medium in medullae. X 10.

from those of blood plasma. Yet these composition differences were not consistent from one group of animals to another, although the experimental techniques were identical. Attempts to link renal medullary function to lymph composition were unsuccessful.

The data from the analysis of renal lymph and arterial plasma during acute experiments in dogs are presented in Figures 11, 12 and 13. The lymph to plasma (L/P) ratios in these figures represent individual animals, since duplicate samples were excluded from this study. Mean L/P ratios and ranges presented in part A of these figures were calculated for the animals used during each month. Months are listed along the ordinate at the left, and the number of animals included in each mean are listed along the ordinate at the right. The L/P ratios, in 0.05 units, are plotted as a frequency distribution in part B of Figures 11, 12 and 13. L/P ratios for both parts A and B are shown along the abscissa of part B.

## Sodium

Figure 11-A presents the L/P ratios for sodium from 71 animals. For 8 consecutive months, representing a total of 52 animals, the mean L/P ratios were greater than unity. The mean ratio for November, when the initial experiments were done (5 animals) was less than unity, while those for October (5 animals) and December (9 animals) were near unity.

The frequency distribution curve of Figure 11-B indicates a narrow range of variation among the individual L/P ratios, 82% of which were greater than unity.

The mean Sodium L/P ratio (1.05) for 82 animals is shown in the first column of Table 2. This mean is statistically different from

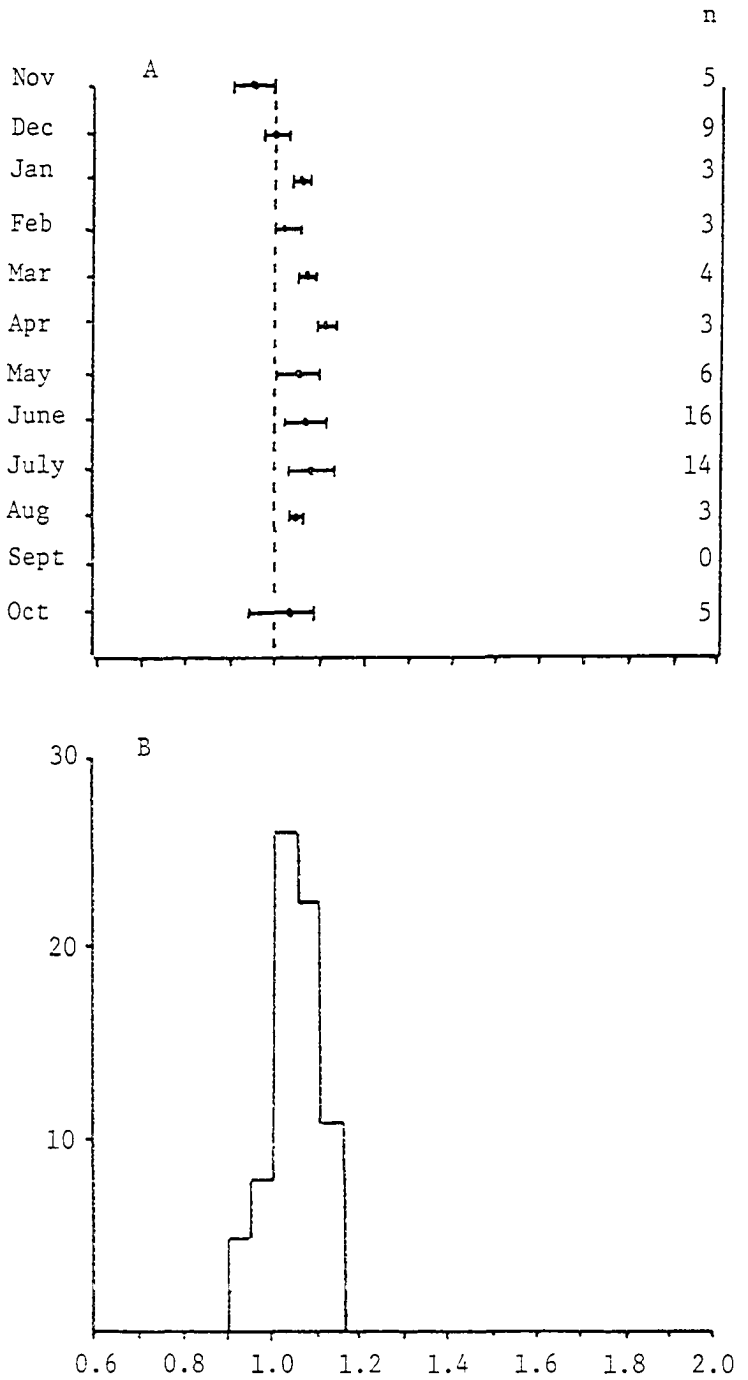


Figure 11. Seventy-one L/p Ratios for Sodium Presented as Time Related Groups.

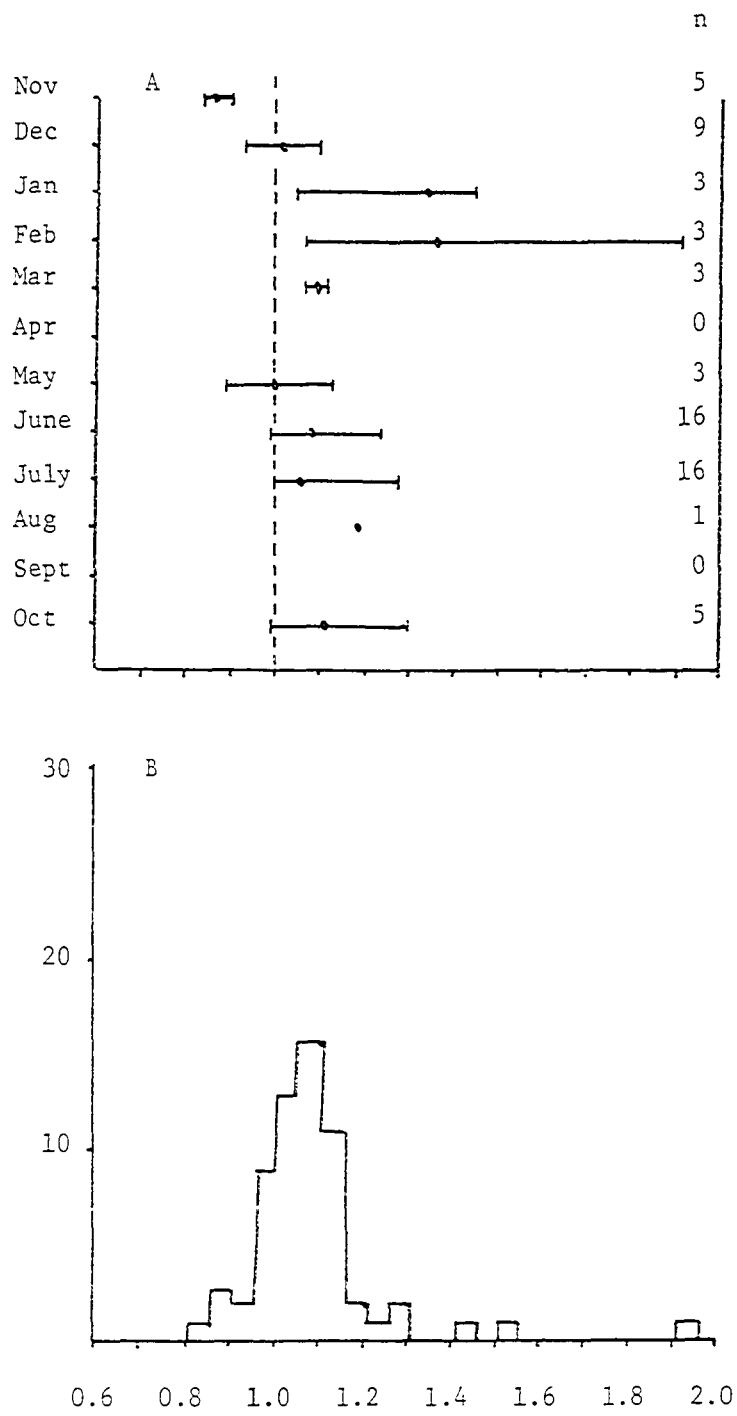


Figure 12. Sixty-four L/p Ratios for Potassium Presented as Time Related Groups.

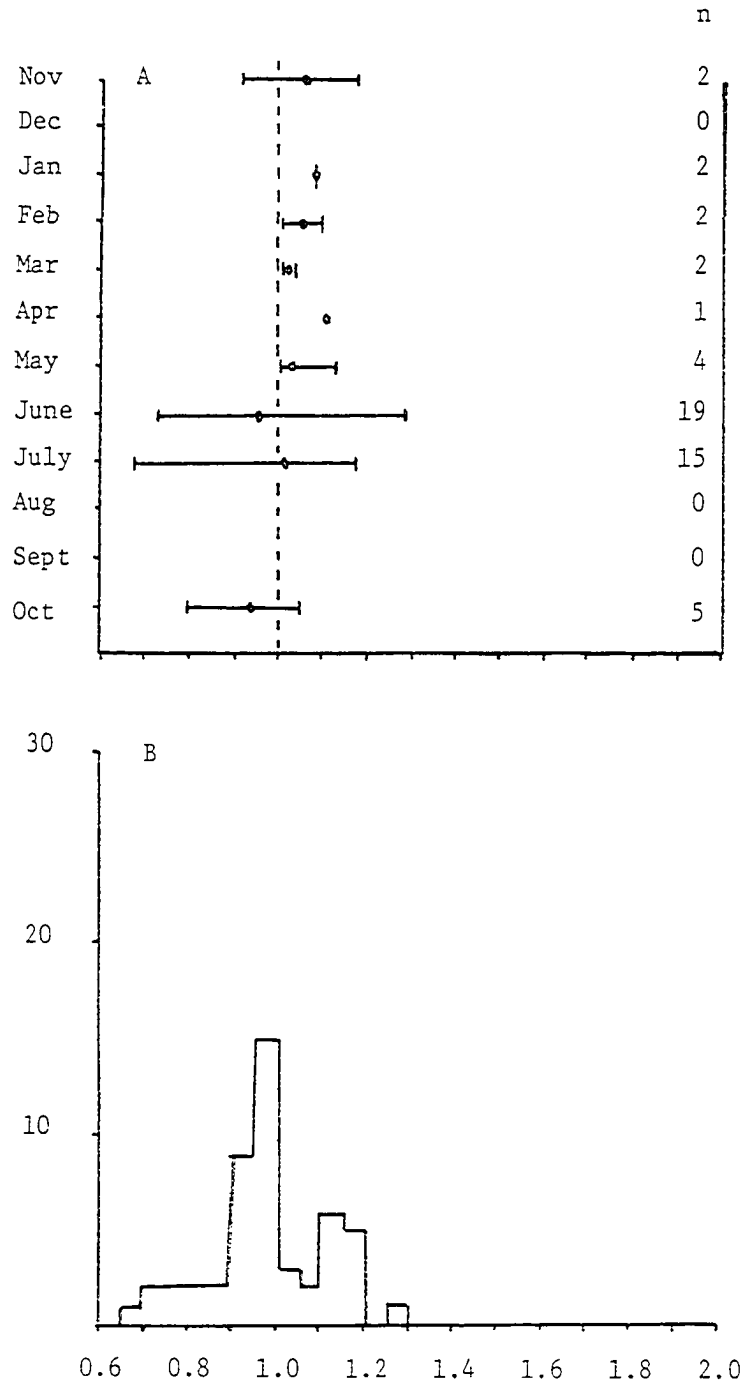


Figure 13. Fifty-two L/p Ratios for Urea Presented as Time Related Groups.

TABLE 2

RENAL LYMPH TO ARTERIAL PLASMA CONCENTRATION RATIOS (L/P) FOR Na, K AND UREA

| | Na | K | Urea |
|---|---|---|---|
| Mean L/P Ratio | 1.05 | 1.07 | 0.99 |
| Range | 0.91-1.15 | 0.83-1.91 | 0.67-1.28 |
| n | 82 | 72 | 58 |
| S.E. | 0.006 | 0.018 | 0.016 |
| P ( $\mu^* = 1.00$) | <.001 | <.001 | <.50 |

*Assumed mean of the population from which sample was drawn.

unity, as shown in the bottom line of this table.

## Potassium

Variations similar to those of sodium are shown in Figure 12-A which presents the L/P ratios for potassium from 64 dogs. The ranges of L/P ratios are greater than those for sodium, but groups of potassium L/P ratios obtained within a limited time period have similar L/P ratios as was observed for sodium. All mean L/P ratios for potassium were greater than unity except the mean for the 5 animals studied in November, which was less than unity. The ratios for January and February, representing a total of 6 animals, were both greater than 1.30, whereas most of the other ratios were 1.10 or less. The mean L/P ratios for potassium derived from 3 animals in May and from 9 animals in December were both near unity.

The frequency distribution curve of the L/P ratios for potassium shown in Figure 12-B indicates a greater range of values than was noted for sodium. 79% of the potassium L/P ratios fell above unity and several of these values are greater than 1.40.

The second column of Table 2 presents the mean value for 72 potassium L/P ratios (1.07). This mean is statistically different from unity, even though the standard error is much greater than that for sodium.

## Urea

L/P ratios for urea derived from 52 animals are shown in Figure 13. An unequal distribution among the means for the months is shown in Figure 13-A. These mean L/P ratios are greater than unity except those

for June (19 animals) and October (5 animals). In addition, all 11 L/P ratios for urea obtained from January to May are either unity or greater than unity, while the ranges of L/P ratios for the 41 animals used in June, July, October and November extend both above and below unity.

The frequency distribution curve in Figure 13-B shows a very broad distribution of L/P ratios for urea. 67% of the individual L/P ratios for this substance were less than unity and values as low as 0.70 were found.

The total mean L/P ratio for urea (0.99) is shown in the third column of Table 2. This mean value is not statistically different from unity as shown in the last line in this Table.

## Capsular Versus Hilar Lymph Composition

Capsular and hilar renal lymph were collected simultaneously in 6 additional experiments. The individual L/P ratios and capsular to hilar lymph concentration ratios for sodium and potassium derived from each of these 6 animals are shown in Table 3. Each line in this Table presents the data derived from one dog with the means for all dogs presented in the bottom line.

No sodium L/P ratios of less than 1.04 were found, and both mean L/P ratios were significantly greater than 1.00. On the other hand, the mean capsular to hilar sodium concentration ratio (0.98) is not significantly different from 1.00.

The L/P ratios for potassium shown in Table 3 vary much more than those for sodium. The mean L/P ratios for potassium are higher than those for sodium, primarily as a result of experiment 2 in which both capsular and hilar L/P ratios were 1.86. Neither the capsular nor hilar mean

TABLE 3

RENAL LYMPH TO ARTERIAL PLASMA (L/P) RATIOS FOR Na AND K DERIVED FROM SIMULTANEOUSLY COLLECTED CAPSULAR AND HILAR LYMPH

| | Sodium | | | Potassium | | |
|---|---|---|---|---|---|---|
| Experiment No. | Capsular L/P | Hilar L/P | Capsular Hilar | Capsular L/P | Hilar L/P | Capsular Hilar |
| 1 | 1.06 | 1.11 | 0.95 | 0.99 | 1.05 | 0.94 |
| 2 | 1.12 | 1.11 | 1.01 | 1.86 | 1.86 | 1.00 |
| 3 | 1.06 | 1.10 | 0.97 | 1.05 | 1.09 | 0.96 |
| 4 | 1.06 | 1.06 | 1.00 | 0.78 | 0.80 | 0.97 |
| 5 | 1.04 | 1.11 | 0.94 | 1.06 | 1.03 | 1.03 |
| 6 | 1.04 | 1.04 | 1.00 | 1.05 | 1.05 | 1.00 |
| Mean | 1.06* | 1.09* | 0.98 | 1.13 | 1.15 | 0.98 |

*Mean is significantly different from 1.00 when tested at the 0.05 level of confidence.

L/P ratio were significantly different from unity.

Table 3 demonstrates that when the capsular L/P ratio for potassium was elevated above unity, the hilar L/P ratio for this ion was also elevated. Low capsular ratios for potassium were likewise accompanied by low hilar L/P ratios for this ion. The mean capsular to hilar ratio (0.98) was identical to that for sodium and was not significantly different from unity.

## Renal Concentrating Ability

It was pointed out in the Introduction (p. 8) that there is a question of whether renal lymph composition is altered by medullary function. If such alterations occur, they would vary as medullary function varies. This relationship was tested by analysing renal lymph from animals in different states of hydration. In these experiments, changes in medullary function were detected by alterations in urine osmolality. Data derived from 13 animals in various states of hydration and dehydration are presented in Figure 14. One sample each of renal hilar lymph and urine was collected simultaneously from each animal. Arterial blood was sampled periodically. Lymph, Plasma and urine were analysed for total solute concentration, and sodium concentrations were determined in plasma and lymph. In Figure 14, the experiments are arranged in order of increasing urinary osmolality with the experiments showing low urinary concentrations at the left. For these 13 experiments, the L/P ratios for sodium and total solute remain almost constant in the presence of 3-fold differences in urine osmolality.

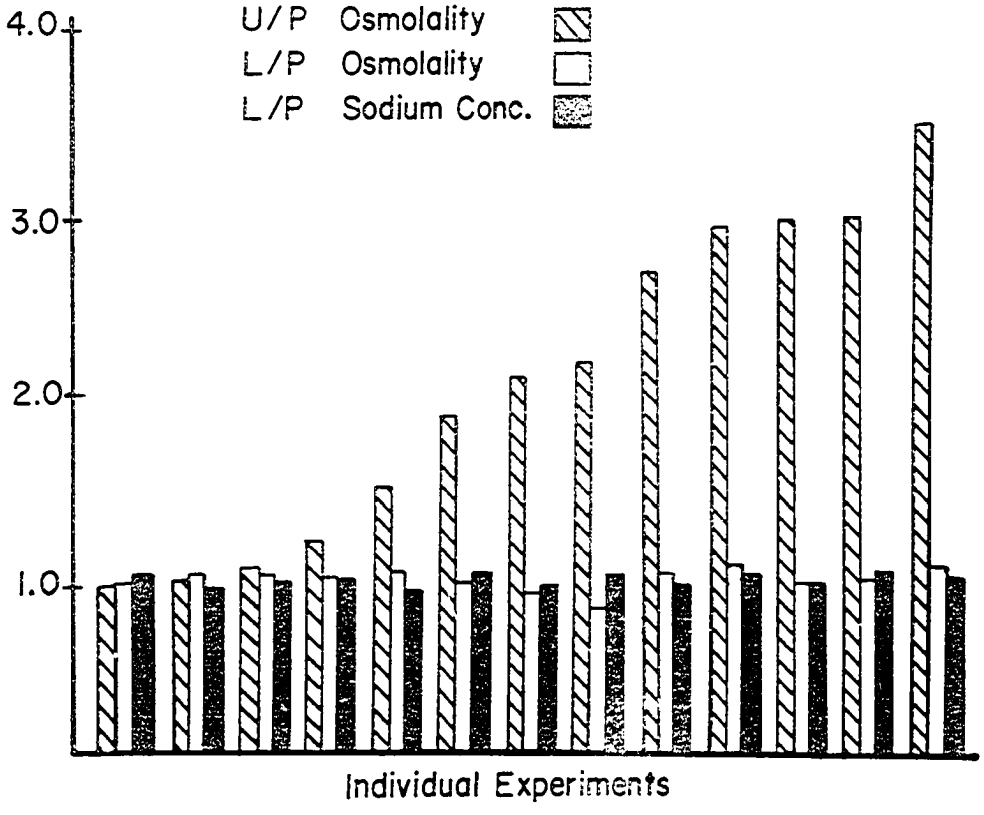


Figure 14. Urine to plasma (U/P) osmolality ratios plotted with lymph to arterial plasma (L/P) ratios for $Na^+$ concentration and osmolality. Experiments are arranged in order of increasing U/P osmolality.

## L/P Ratios For Lymph and Plasma Water

The L/P ratios presented above have been calculated on the basis of the concentrations of the various substances measured in terms of whole plasma and lymph, i.e., no corrections for protein volumes were made. These data can therefore be compared directly with those published by other investigators. The author is unaware of any study in which renal lymph composition was presented in terms of lymph water. Protein concentrations were not determined in the present study, since sample volumes were insufficient to allow this additional determination. Yet the protein concentration of renal lymph has been measured, and such results can be used here. According to Keyl and co-workers (19) lymph contains 1.71 and plasma contains 5.04 grams of protein per 100 ml. (means for 17 animals). These data are summarized in Table 4.

TABLE 4

LYMPH AND PLASMA PROTEIN CONCENTRATIONS RECALCULATED FROM DATA OF KEYL AND CO-WORKERS (19)

| | n | Mean Protein g/100 ml | Range | Standard Error |
|---|---|---|---|---|
| Plasma | 15 | 5.0 | 3.5-6.5 | 0.24 |
| Lymph | 15 | 1.9 | 1.2-3.0 | 0.13 |

Each plasma protein value, in g/100 ml, subtracted from 100 yields the water content of the plasma sample as a per cent. A plasma correction factor ($f_p$) may be derived by dividing 100 by the per cent water content of the sample. Solute concentration in the plasma sample multiplied by

$f_p$ equals solute concentration in plasma water. Renal lymph also contains protein, and a similar factor ($f_L$) may be derived from each lymph protein value. The ratio $f_L/f_p$ then yields a correction factor (f) which can be applied to the L/P ratio. The renal lymph and plasma protein data of Keyl and co-workers (19) yields the following analysis:

$$\bar{f} = 0.9677$$

$$s_{\bar{f}} = 0.002127$$

$$t_{.05} \ (df = 14) = 2.145$$

Therefore: $0.9631 \ (\bar{f}_l) \leq F \leq 0.9723 \ (\bar{f}_u)$

In these calculations $\bar{f}$ is the mean correction factor derived from 15 animals; $s_{\bar{f}}$ is the standard error of the mean; t is the tabulated t applicable to this problem; F is the true correction factor. Although the true correction factor is not known, the data of Keyl and co-workers (19) provides a range within which it probably lies. The lower limit of the correction, $\bar{f}_l$, will be applied to the data whose lower limit is greater than one, and the upper limit, $\bar{f}_u$, will be used for those data whose lower limit is less than one. The L/P ratio data for sodium, potassium and urea derived in the present study has been re-analysed using the corrections described. This data is presented in Table 5. The confidence limits for all 3 parameters shown in Table 5 include unity. Using the corrections, it cannot be concluded that these L/P ratios are greater than unity. Protein concentrations for each animal used in the present study are not available. Therefore, neither a firm statistical model nor reliable probability statements can be established.

TABLE 5

CORRECTED L/P RATIOS FROM TABLE 2

| | $Na^+$ | $K^+$ | Urea |
|---|---|---|---|
| Experimental Mean | 1.049 | 1.071 | .990 |
| Standard Error | .005496 | .01792 | .04995 |
| $t_{.05}$ | 1.99 | 2.00 | 2.00 |
| df | 81 | 71 | 57 |
| Confidence Limits | 1.038-1.060 | 1.035-1.107 | .890-1.090 |
| Corrected Mean | 1.01* | 1.03* | .963** |
| Corrected Confidence Limits | .9997-1.021* | .9997-1.066* | .865-1.060** |

*Lower limit of correction factor used.
**Upper limit of correction factor used.

The present discussion is conservative; the results do not signify that real differences exist in the L/P ratios relative to unity. By the same token, there is no firm foundation for believing that they are equal to one. It is concluded that mean renal L/P ratios for sodium and potassium derived over an extended period of time, using a large number of animals, are greater than unity. These elevated mean ratios are, however, primarily a result of differences in lymph and plasma protein concentrations.

Application of a protein correction factor to Figures 11, 12 and 13 would give slightly greater importance to the L/P ratios less than 1.00 and slightly less importance to ratios greater than 1.00. The unequal distributions related to time would still remain. The differences in L/P ratios found in Figures 11, 12 and 13 are not due to differences in

the L/P ratio for protein, since the effect of such protein differences would be the same for all dissolved substances. The mean potassium L/P ratios for January and February are twice those found for sodium and urea for these months, while all three parameters were very similar during May, July and December. Furthermore, the mean urea L/P ratio for June and October were less than unity while those of sodium and potassium were both greater than unity. Similar observations can be made for individual dogs in Table 3. Since the variations of the mean L/P ratios for sodium, potassium and urea in Figures 11, 12 and 13 and for sodium and potassium in Table 3 do not always vary together, then their variation is not due to a common factor, such as the L/P ratio for protein.

# CHAPTER IV

# DISCUSSION

## Renal Lymphatic Distribution

The present study has shown that renal lymphatics are distributed as follows:

1. In the interlobular spaces.
2. In the immediate vicinity of glomeruli.
3. Along the arcuate blood vessels at the corticomedullary border.
4. Not in the renal medulla.

## Retrograde Injection Method

The data derived from retrograde injection studies substantiate the findings of Peirce (39) except for his failure to find periglomerular lymphatics. It is nevertheless important that similar conclusions are reached by two different methods of approach. Stab injection techniques, such as used by Peirce (39), can be expected to deliniate only those lymphatic vessels distal to the injection site. The retrograde injection method is superior, because it fills the lymphatic vessels with a definite pressure directed toward the end lymphatic capillaries themselves. Therefore, all lymphatic vessels in the area of injection have an opportunity of being filled with injection medium. One would never expect to

fill all the lymphatics of a given area by this method. Intrarenal morphology and local pressures during retrograde injection vary greatly and result in injections of varying degrees of completeness. Retrograde injection produces only a sampling of the lymphatic vessels present rather than a complete injection. The advantage of the method is that the sample should contain lymphatic vessels of all types, whereas stab preparations demonstrate only those distal to the injection site.

## Renal Lobule

The interlobular distribution of lymphatic vessels is relevant to the nature of the renal lobule. After maceration in dilute acid, the kidney can be divided into radiating fasciculi. One such fasciculus is demonstrated in Figure 15, adapted from the work of Traut (52). This drawing shows that the lobule, the structural unit of the kidney, has three or more triangular sides, the bases being at the surface of the cortex, and the apices lying within the papilla. All glomeruli of the structural unit are located in the planes of these triangular sides. Filtrate from these glomeruli enters renal tubules located wholly within the lobule and is pooled in the centrally located collecting ducts of the structural unit. These collecting ducts then join through a common duct at the tip of the papilla. The medullary portion of the renal lobule is composed of the central collecting duct tree surrounded by closely packed loops of Henle and the vasa recta. The entire blood supply of the renal lobule is derived from the interlobular arteries located in the connective tissue septa of the cortex which bind together the lobules to form the kidney. Except for the arcuate lymphatics, the only consistently demonstrable lymphatic vessels are found within these septa, and around the glomeruli

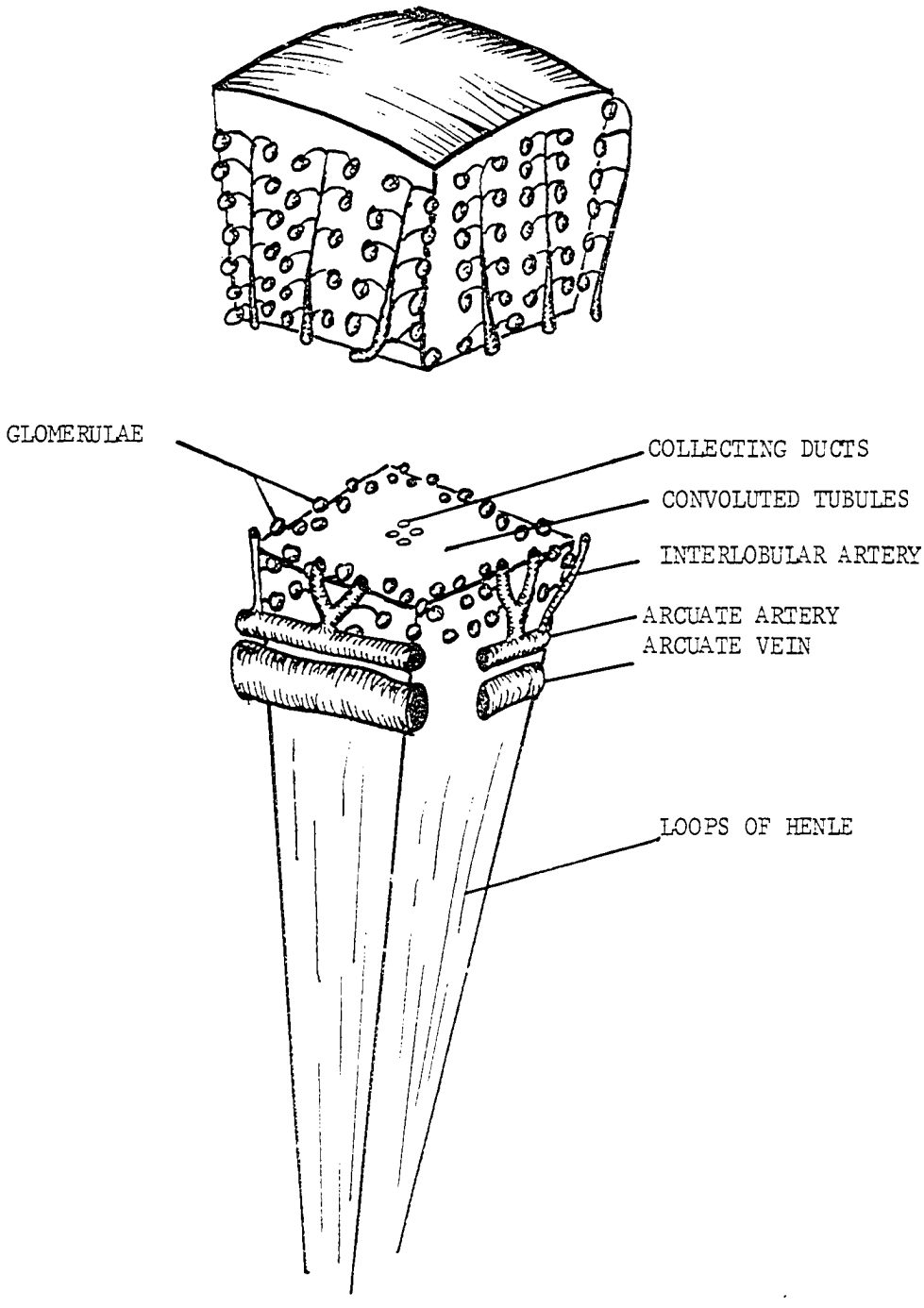


Figure 15. The Functional Unit of the Kidney. (Adapted from Traut, H. F., Contrib to Embryol. 15: 103-120, 1923.)

of their walls.

## Interlobular Lymphatics

It is not surprising to find lymphatics limited to the interlobular spaces, since this was predicted by Lee (25) from his study of the lymphatics of the liver. As demonstrated in Figures 5, 6, and 7, the distribution of the renal lymphatics is primarily along the interlobular vessels. Therefore, it is likely that the lymphatics drain the interlobular spaces of fluid formed by the high hydrostatic pressure of the arteries of the kidney. This is comparable to the function of lymphatics in most arterial systems (24, 33, 43). Retrograde injection data, as well as the findings of Peirce (39), indicate that lymphatic vessels do not penetrate the lobules of the kidney. The renal lymphatics thus drain an intrarenal compartment anatomically distinct from the tubular mass; yet diffusion of solute or solvent between the lymph compartment and the tubular mass must occur, causing lymph composition to differ from that of arterial blood plasma. The interlobular lymphatics deviate from their radial orientation only to follow the afferent arterioles to the area of the glomeruli.

## Periglomerular Lymphatics

The periglomerular lymphatics were not found by Peirce (39). The reason for the absence of injected periglomerular lymphatics in his preparations is discussed below. These lymphatics represent the most lateral collection point or the interlobular lymphatic plexus, and are, therefore, true end lymphatics.

If these lymphatics are to pick up ink after stab injection, the ink

would have to be deposited in the interstitial spaces in their immediate neighborhood. The ink thus deposited around the periglomerular lymphatics would obscure them even though they were well filled. Peirce (39) himself states that intrarenal lymphatics can be studied only some distance from the stab injection site.

Previous investigators do not agree concerning the presence or absence of lymphatic vessels in glomeruli or around Bowman's capsule (39). In the present study periglomerular lymphatics were observed in both dog and horse, but injected preparations failed to reveal penetration of lymphatics into the glomerulus.

Lymphatic vessels cannot be demonstrated around every glomerulus in the area of an injection; yet this does not necessarily mean that fluid formed in the area of the glomerulus is normally an insignificant portion of total renal lymph formation. It should be emphasized that neither retrograde injection nor any method thus far devised to visualize renal lymphatics can demonstrate all the lymphatics of an organ and their terminal ramifications. It has been postulated that the renal lymphatics function to relieve intrarenal pressures under conditions of diuresis and ureteral obstruction and to maintain a minimal interstitial pressure (12, 13, 36). These ideas may have particular importance when applied to the area of the glomerulus. Development of high interstitial pressure in the vicinity of the glomerulus would oppose filtration and interfere with function of the juxtaglomerular apparatus. Since Tobian (53) has reported that the cells of the juxtaglomerular apparatus are sensitive to the degree of stretch, the maintenance of a constant interstitial pressure would be important in the efficient operation of such a pressoreceptor. On the other hand, if

fluid of high oncotic pressure were to collect in the area of Bowman's capsule, excessive diffusion of filtrate through the visceral layer of Bowman's capsule would occur.

## Arcuate Plexus

The present study demonstrates a rich plexus of lymphatic vessels at the corticomedullary border (Figure 7 and 10). The intrarenal lymphatics form a continuous plexus extending from the glomeruli through the interlobular spaces to join the arcuate plexus. The latter plexus continues along the interlobar blood vessels, finally forming the lymphatic trunks of the hilus. This finding suggests that the renal lymphatics are distributed primarily along the major blood vessels of the kidney. Neither the present study nor that of Peirce (39) has demonstrated lymphatic vessels accompanying blood vessels or any other structure beyond the glomeruli.

## Renal Medulla

Since the blood supply of the renal medulla is entirely postglomerular, it is not surprising that lymphatic vessels are not found there. Nevertheless, negative findings from retrograde injection preparations are inconclusive. It is possible that either pressure relationships or morphological peculiarities of the canine kidney during retrograde injection prevent filling of medullary lymphatics. Injection pressure may be largely dissipated by the resistance of the cortical lymphatics. If the lymphatic vessels of the medulla were rather small or contained valves, there would not be sufficient pressure to fill them with ink.

Although some studies describe lymphatics in the renal medulla

(5,40), Peirce (39) was unable to duplicate these findings in the dog when he injected an India ink suspension directly into the medullary tissue. Peirce (39) reported that lymphatics were never observed unless the ink was deposited in the neighborhood of a large blood vessel. He concluded that these studies show that medullary lymphatics are absent from the canine kidney. This stab injection study is not subject to the criticisms of retrograde injection method. It thus provides the best evidence at the present time concerning the absence of lymphatic vessels in the canine renal medulla.

## Renal Lymph Composition

The time related presentation of renal lymph composition shown in Figures 11, 12 and 13 can be compared with the findings of other investigators.

### Comparison with the Findings of Previous Investigations

Data gathered for this study between the months of January and August demonstrates that the L/P ratio for sodium is definitely greater than unity in 52 animals. This is similar to the findings of LeBrie and Mayerson (22) in 27 animals shown in Table 1. In the same table, Swann and co-workers (51) and Keyl and co-workers (19) found the L/P ratio for sodium to be no different from unity in 5 and 13 animals respectively. This same relationship is shown in Figure 11 for the 19 animals used in October, November, and December.

In Table 1, it is seen that LeBrie and Mayerson (22) and Swann and co-workers (51) obtained L/P ratios for potassium greater than unity with 12 and 5 animals respectively. The data presented in this study

demonstrates such elevated potassium ratios during the months of January, February and March. On the other hand, Keyl and co-workers (19) found the L/P ratio for potassium to be near unity (14 animals). This situation is shown for the months of May and June in Figure 12 for 22 animals.

Similar relationships are demonstrated for urea. Two of the investigations shown in Table 1 found the urea L/P ratio elevated (50, 51) while one found this ratio to be less than unity (19). Both of these findings are shown for different time periods in Figure 13.

## Possible Contribution of Technical Errors To Reported Differences

Unless renal lymph is collected under mineral oil or in closed vessels, evaporation of water will concentrate the specimen. Renal lymph flow varies from 1 to 50 µL/min. At a flow of 25 µL/min. a volume of lymph less than 0.25 ml would be exposed to air for the first 10 minutes. A droplet of fluid of this size has a very large surface area to volume ratio, and evaporation would result in significant concentration changes in the fluid (19). If uncontrolled, such evaporation could account for the differences in lymph composition shown in Table 1 as well as those of the present study. Lymph was collected under mineral oil in the present study, and in that of Keyl and co-workers (19), whereas LeBrie and Mayerson (22) utilized a stoppered test tube to receive renal lymph. Neither Swann and co-workers (51) nor Sugarman and co-workers (50) reported how evaporation of lymph water was prevented. Since lymph was invariably collected under mineral oil in the present study, evaporation of the sample did not alter the results presented.

Contamination of lymph glassware may result in a high L/P ratio

for sodium and potassium but not urea. Figures 11, 12 and 13 show that variations for all 3 parameters were observed. Care was taken to eliminate contamination of lymph, plasma and urine samples in the present study; therefore, variation from this source added minimally to the variances of the data presented.

Since renal lymph must be collected in small quantities, micro and ultramicro methods are developed for chemical determinations on lymph. The accuracy of such methods is equal to that of similar methods using larger quantities of sample. Adequate controls, using commercial standards, will keep errors within acceptable limits: yet errors due to special properties of lymph cannot be eliminated in this way. Renal lymph has less protein than plasma, and is thus less viscous. Lower viscosity will cause lymph to drain from pipettes with greater freedom and with less residual fluid left within the pipette bore. Elevated L/P ratios resulting from this source of variation are similar for all substances measured. Elevated L/P ratios for sodium shown in Table 1 are accompanied by ratios for potassium near unity while the reverse is true when the ratio for potassium is elevated. No conclusion can be drawn from the elevated L/P ratio for urea (1.41) published by Sugarman (50). Variations in the L/P ratios of the present study are not due to errors of this nature. Figures 11 and 12 show that the ratios for potassium in January and February were several times greater than those for sodium while in March the sodium and potassium ratios were quite similar. Further, most of the L/P ratio ranges shown for potassium in Figure 12 are greater than those for sodium in Figure 11. This shows that potassium is subject to a source of variation of which sodium is independent. Similar

observations can be made from Table 3. L/P ratios for potassium in this table varied both above and below unity, while the ratio for sodium was consistently greater than 1.04.

## Lack of a Medullary Component in Renal Lymph

The anatomical data of Peirce (39) and that derived for this study show that lymphatic vessels are primarily distributed in the interlobular spaces of the kidney. Intralobular function then affects renal lymph composition only by diffusion of solute and solvent between the interlobular spaces and the tubular mass. The anatomical studies discussed show that medullary lymphatic vessels probably do not exist, but it is possible that renal medullary function might alter renal lymph composition indirectly by diffusion.

One of the objectives of this study is to obtain a working hypothesis describing how renal physiology alters renal lymph composition. To do this it is necessary to determine whether or not renal lymph is altered, even though indirectly, by the fluids made hypertonic by the medullary countercurrent multiplier system.

## Renal Concentrating Ability

There is no known substance which is a "marker" for medullary interstitial fluid. Indirect methods are therefore used to determine the effects of medullary function on renal lymph composition.

The elevated osmolality of the medullary interstitium is necessary for the production of highly concentrated urine. Much of this high osmolality is due to sodium which is concentrated by the countercurrent multiplier system. If sodium is added to renal lymph from the hypertonic

medullary interstitial fluid causing an alteration in the L/P ratio for this ion, then this effect should be maximal at such times as the kidney is producing highly concentrated urine. Conversely, the high medullary interstitial osmolality and sodium concentration are reduced when urinary concentration is reduced. The effect of the renal medulla on lymph sodium concentration and osmolality should be minimal during these times when the kidney is producing urine of lowered osmolality.

Figure 14 shows that no such relationship exists between urinary concentrating ability and the L/P ratios for sodium and osmolality. The 13 data triplets recorded in Figure 14 were gathered from 13 different dogs in widely varying degrees of hydration and dehydration. Since these animals varied greatly in their state of hydration, it is expected that their urinary osmolality would also vary to a large extent. The data of these experiments are arranged in order of increasing urinary osmolality with animals having low urinary concentration placed at the left of the graph.

Those animals whose urinary concentration was near that of plasma had been given large quantities of water by stomach tube the evening before the experiment and again on the morning of the experiment. Although such animals were well hydrated, their urine was not markedly hypotonic. Failure to produce hypotonic urine results from antidiuretic hormone which is released in response to anesthesia. Hypotonic urine was never obtained during these experiments because of anesthesia induced antidiuretic hormone release.

Figure 14 shows that osmolalities of more than three times that of plasma were obtained. These high urine concentrations indicate high

medullary solute concentration did not result in L/P ratios different from those observed when urinary osmolality was similar to that of plasma. It is concluded that lymph composition relative to that of plasma is not altered by solute concentration in the renal medulla. If the physiology and anatomy of this region allowed solute to leave fast enough to alter lymph composition, a high degree of inefficiency would result. Such solute loss could severely compromise the ability of the mammalian kidney to concentrate urine.

## Composition of Capsular Versus Hilar Lymph

The experiments in which both capsular and hilar lymph were collected were designed to provide evidence that renal lymph composition is not independent of the composition of renal medullary interstitial fluid.

Renal hilar lymph is derived directly from lymphatic plexuses around the arcuate vessels of the kidney located at the corticomedullary border. If sodium, highly concentrated by the countercurrent multiplier system, becomes mixed with lymph formed in the renal cortex, this mixing would be most likely to occur in the plexus around the arcuate blood vessels. This is logical since this plexus is the one nearest the renal medulla. Renal hilar lymph proceeds from its site of formation in the cortex through the interlobular lymphatics to the plexus around the arcuate blood vessels and out of the kidney via the hilar lymphatics. Anatomical studies and the observation that PAH is extracted from hilar lymph demonstrate that cortical lymph is drained in this manner (19). Fluid of high sodium concentration derived from the medulla entering the lymphatics around the arcuate vessels would be conducted out of the kidney via hilar lymphatics. Lymphatic fluid formed in the renal cortex is

divided between two outflows, i.e., the hilar and the capsular efferent trunks. The intrarenal distribution of lymphatic vessels indicates that lymph formed in the area of the cortex nearest the renal capsule is conducted from the kidney via capsular lymphatics. Conversely, lymph formed deeper in the renal cortex would leave the kidney via the plexus around the arcuate vessels and the hilar lymphatic trunks. The division of cortical lymph between capsular and hilar routes of exit is determined by the balance between the hilar lymphatic pressure and resistance and the capsular lymphatic pressure and resistance. Since hilar lymph flow is known to be considerably greater than capsular lymph flow (19), most of the lymph formed in the kidney leaves the renal parenchyma via hilar vessels. The fluid of the capsular lymphatics consists of a small portion of lymph formed rather high in the renal cortex and distant from the medullary and papillary regions.

It was noted earlier that Sugarman (50) recovered Evans blue dye from capsular lymphatics in only 15% of the kidneys in which the dye was injected into the renal medulla (p. 6). Since up to 0.5 ml. of dye solution was injected, such an excess of interstitial volume would force bulk flow of fluid upward toward the corticomedullary border via all routes available. It is also possible that a diffusable foreign substance such as Evans blue deposited in the medulla would be taken up by descending loops of Henle and be carried into the cortex by the tubular urine. Arriving in the cortex, the dye may be free to diffuse out of the distal convoluted tubules and into the interlobular spaces to be taken up by lymphatic vessels. Although the mechanism(s) whereby Sugarman's findings occurred may be open to speculation, there is little doubt as to their significance. Substances from the medulla which enter renal

lymph are found primarily in hilar lymph, and only in unusual cases in capsular lymph. Thus if the renal lymph sodium concentration is altered by virtue of a medullary component, as many have suggested (22, 48), then this alteration should be found only in hilar lymph in the majority of cases. Since potassium is not concentrated in the medullary interstitial fluid, then the potassium concentrations in capsular and hilar lymph would be similar.

The data presented in this study demonstrate that the concentrations of sodium and potassium in hilar lymph are no different than those in capsular lymph. The sodium L/P ratios for both capsular and hilar lymph were greater than unity in these experiments even when approximate corrections are made for lymph and plasma protein concentrations. These data show that sodium ion may be added to lymph within the kidney and that this addition results in lymph sodium concentrations greater than those in plasma. Further, this addition is made to capsular and hilar lymph in almost equal proportion. A similar argument can be advanced concerning potassium, except that much greater variability among individual samples is noted. Table 3 shows that this variability is a biological function and not a characteristic of the method of measurement. The great variability of the L/P ratios for potassium is seen in the capsular as well as the hilar ratios. This table reveals that the capsular and hilar ratios for potassium vary together and in the same direction. The variation noted among L/P ratios for potassium is therefore a function of variation in the handling of potassium in the cortical portion of the nephron.

## Possible Contribution of the Donnan Effect

The anatomical findings reported in this study suggest that renal lymph may be formed in at least 2 separate regions of the renal cortex, i.e., in the area of the glomerulus and in the interlobular spaces. It is quite probable that lymph derived from such different areas would differ somewhat in composition.

Since the visceral layer of Bowman's capsule is known to be permeable to water, periglomerular lymph may be derived from the glomerular filtrate. Such lymph would be expected to have a very low protein content. Low protein lymph thus derived would be conducted to the interlobular lymphatic plexus to mix with the protein containing fluid formed by transudation of fluid from the high-pressure vessels of the cortex. Lymphatic capillaries are notoriously permeable since even red blood cells and carbon particles enter lymphatic vessels with ease. Yet it is quite possible that as the lymphatic capillaries converge upon larger lymphatics at the corticomedullary border, permeability is gradually lost, first to particulate matter, then to protein. A progressive, graded decrease in lymphatic permeability would result in a rapid diffusion of water into or out of the lymphatic vessel in response to any oncotic pressure gradients which might exist. Such water movements would equalize protein concentrations within and without the lymphatic vessels.

If some force is available to prevent diffusion of water in response to oncotic pressure differences, an alteration in the ionic composition of the lymph could result. The nondiffusable protein anion present in higher concentration on one side of the membrane would cause an unequal distribution of the diffusable ions of the system. The result would be a slightly higher concentration of cations on the side of the

membrane having the higher protein concentration and a higher concentration of anions on the side where protein is more dilute. Unfortunately, only the final result can be known, since sampling procedures and measurement techniques are not yet available for determining whether such conditions exist, or if they exist, to what extent their existence alters renal lymph composition.

The retrograde injection preparations presented in this study suggest that there may be ample opportunity for protein concentration differences to alter renal lymph composition. On the other hand, these anatomical studies also suggest that renal lymphatic fluid from all portions of the cortex and of every protein concentration converge upon the lymphatic trunks leaving the cortex between adjacent papillae along the interlobar blood vessels. Renal hilar lymph would then be a mixture of two fluids. It would contain lymphatic fluid which has equilibrated with extracellular fluid of the cortex and also the fluid with which the equilibration was made. Since the interstitial fluid through which the lymphatic channels pass is also taken up by lymphatic vessels to be emptied into the common pool at the corticomedullary border, composition changes occurring at one point in the system may be obliterated at another. It is demonstrated that capsular renal lymph is derived directly from the small lymphatic plexuses of the interlobular spaces and thus mixing of fluids of different compositions should be minimal. Although direct evidence is entirely lacking, the finding that capsular lymph concentrations of sodium and potassium are indistinguishable from those of hilar lymph suggests that Donnan effects, if they occur, may be largely dissipated by the casual nature of the renal lymphatic system.

Further investigation into this facet of lymph formation must await the development of much more sophisticated experimental techniques than those presently available.

## Total Means for Sodium, Potassium and Urea

Table 2 demonstrates the mean relationships of renal lymph concentrations of sodium, potassium and urea to those of plasma when large numbers of animals are used over an extended period of time. The mean values shown in this table represent the most accurate estimates of the parameters measured yet available. These data are valuable when exercising judgement as to whether or not a particular sample is an unusual one with respect to the average. Accurate estimates of overall mean L/P ratios would be expected to be of value, not only in helping to analyse data of future projects, but also in evaluating the findings of others.

In using the data presented in this study, it must be kept in mind that the L/P ratios presented were calculated on the basis of concentrations in whole lymph and plasma. These values are therefore somewhat greater than would be expected if calculated on the basis of lymph and plasma water.

## Variations in L/P Ratios Due to Renal Cortical Function

Variations in L/P ratios for sodium, potassium and urea among different laboratories were reproduced in these experiments over a period of a year using constant experimental techniques. Renal function is reflected in renal lymph composition. This is adequately demonstrated by studies utilizing inulin and PAH in which renal lymph composition was altered by both active and passive transport systems of the kidney (19).

If secretory and reabsorptive activity of the renal tubule alters renal lymph concentrations of PAH and inulin, then they can also alter lymph concentrations of sodium, potassium and urea. Changes in renal handling of these substances would change their concentrations in renal lymph.

The time related graphs presented in Figures 11, 12 and 13 show that L/P ratios for sodium, potassium and urea less than unity may be found. This indicates that these substances may be extracted from renal lymph. L/P ratios slightly less than unity for positive ions might be expected because of a Donnan effect between blood plasma and the plasma filtrate portion of lymph. Since the protein concentration of lymph is almost half that of blood plasma, one would expect only a small effect to be exerted. Since urea is a non-ionic substance, its distribution would not be altered by the protein anion of plasma.

Variations in composition. The wide ranges of the L/P ratios shown in Table 2 and indicated in Figures 11, 12 and 13 are quite striking. It is not possible at this time to eliminate the possibility that a portion of renal lymph is derived from the medulla; yet it has been demonstrated that if any lymph is formed in the medulla it does not alter renal lymph composition. It follows, then, that if renal lymph is observed to have concentrations of sodium, potassium or urea higher or lower than one would predict for a simple filtrate or blood plasma, then some function within the renal cortex has altered the composition of the lymph before it was collected. As indicated previously (p. 50) the differences between lymph and plasma protein concentrations can account for an elevated L/P ratio for solutes. The effects of protein volume differences must be accounted for before lymph composition can be considered to

be unusual. Data presented in this study shows that L/P ratios for sodium, potassium and urea may vary with time. It is concluded that there are potential sources and sinks for sodium, potassium and urea within the renal cortex which can alter renal lymph composition. The body of literature defining accurately the various activities of the different portions of the functioning nephron is as yet small. Even so, some tentative conclusions can be drawn from what has thus far been found.

Sources and sinks for sodium. Approximately 70% of the sodium filtered by the glomerulus is believed to be reabsorbed by active transport in the proximal tubule (26); yet the proximal sodium transport mechanism has been shown to establish only a very small gradient, and this only under highly unusual conditions (55). A significant gradient for sodium probably seldom occurs across the proximal tubular epithelium during normal renal function. Thus the proximal tubule is a poor candidate for either a sodium source or sink.

The nephron is capable of transporting sodium against a high gradient, but the most important of these mechanisms are located in the ascending loop of Henle and the collecting duct (47). Both of these structures are located in the renal medulla and do not effect renal lymph composition. There is, however, a source for sodium in the cortex which might supply this ion at a high enough concentration to elevate the sodium L/P ratio. This is the distal convoluted tubule. Tubular fluid to plasma ~F/P) ratios as low as 0.09 have been reported in this region (28). The TF/P ratio for sodium apparently decreases along the length of the distal tubule indicating that sodium is being reabsorbed at a greater rate than water (28). Since sodium is known to be reabsorbed

against a high gradient in the collecting ducts of the medulla, there is a possibility that the cortical portion of the collecting duct system also might add to the sodium pool which can alter lymph composition. The sodium transport mechanisms of the distal tubule and collecting duct are known to be under hormonal control. Changing levels of aldosterone might alter the distal sodium transport systems sufficiently to account for much of the variation observed in L/P ratios for this ion. A sink to which renal lymph might lose sodium, or a source of sodium-poor water which might dilute the renal lymph sodium concentration, might also be located in the distal tubule. It is known that the ascending loop of Henle actively transports sodium against a high gradient into the medullary interstitial fluid while retaining water in the lumen. The resulting hypotonic fluid of low sodium concentration is delivered to the distal convoluted tubule of the cortex. In the presence of ADH, this fluid equilibrates with the interstitial fluid of the cortex. In the absence of stimulus for sodium reabsorption, this sodium-poor water deposited into the cortical interstitial fluid could produce a diffusion gradient for sodium resulting in loss of this ion from lymph. Malnic (28) has reported TF/P ratios as high as 1.25 for sodium in the distal tubule. It is thus seen that there are mechanisms entirely within the renal cortex which can be invoked to explain sodium L/P ratios both greater than unity and less than unity.

Sources and sinks for potassium. Unlike sodium, a significant potassium gradient may be observed across the proximal tubular epithelium. The rante of proximal TF/P ratios observed by Malnic for potassium was from 0.45 to 0.87 in rats (28). As with sodium, potassium continues to

be reabsorbed in the distal convoluted tubule, but in this case, even higher gradients are found. Potassium is not only reabsorbed in the distal tubule, but may also be secreted into the urine at this point. Thus TF/P ratios for distal tubular fluid potassium have been found to range from 0.18 to 4.45 (28). There are, therefore, adequate mechanisms within the renal cortex to explain lymph concentrations of potassium both greater than and less than that of blood plasma.

Sources and sinks for urea. It is well known that the fluid within the distal convoluted tubule has a urea concentration many times that of the glomerular filtrate (54). TF/P values for urea in this segment of the nephron have been found to be as high as 10.5 (20). Micropuncture data derived by Lassiter and co-workers (20) indicate that water is usually reabsorbed at a greater rate than urea in the distal tubule. Such observations might account for L/P ratios for urea of less than unity, but as yet no source for elevated renal lymph urea concentrations has been reported.

It must be emphasized that the various functions of the cortical nephron which may alter renal lymph composition must, as yet, be only a matter of conjecture. There is as yet no evidence which links tubular reabsorption or secretion of sodium, potassium or urea to renal lymph composition. Since cortical water reabsorption and proximal PAH secretion are reflected in renal lymph composition, it is evident that renal lymph can be altered by gradients developed within the cortex. Even so, many carefully controlled experiments are needed to define the relationship of renal lymph composition to the renal handling of sodium, potassium and urea. The author inteds only to point out that, in most cases, there

are secretory and reabsorptive mechanisms wholly within the renal cortex which might account for the ranges of renal L/P ratios reported in the present study.

# CHAPTER V

## CONCLUSIONS AND HYPOTHESIS

The retrograde injection studies described in this study have established that the lymphatic vessels of the renal cortex are distributed primarily within the interlobular spaces. In addition, no evidence for the existance of medullary or intraglomerular lymphatic vessels was found. These findings are in complete agreement with the findings of Peirce (39) who used a radically different technique. Retrograde injection technique has, however, demonstrated periglomerular lymphatic capillaries which were not shown by the stab injection study of Peirce (39). The conclusions from the present study represent a careful evaluation of both retrograde injection preparations and the stab injection work of Peirce (39). Whereas neither method is valid for areas of the kidney in which lymphatics are not observed, the lymphatic distribution as presented in the present study appears to be more convincing than any previous descriptions.

Renal lymph composition, as derived in this study, varies in a manner independent of experimental technique. Differences in the findings of various laboratories have been duplicated in this study by computing mean L/P ratios for sodium, potassium and urea from small numbers of animals which were used during limited time periods. This finding led to the hypothesis that the variation among these means derived within

limited time periods results from differences in tubular reabsorption and secretion in the kidneys of the animals studied.

Experiments expected to demonstrate the effects of renal medullary function on renal lymph composition yielded entirely negative results. These findings led to the conclusion that if any portion of renal lymph is derived from the medulla, this medullary component does not alter lymph composition.

## Hypothesis

No adequate hypothesis has as yet been published which defines the origin of renal lymph and how renal physiology alters renal lymph composition. The conclusions derived from the present studies make possible the following hypothesis:

Renal lymph is formed within the interlobular spaces of the renal cortex by transudation of fluid from the high pressure arteries of these areas. Therefore, the initial renal lymph is no more than a protein-poor filtrate of renal arterial blood plasma. Similar fluid is formed in the neighborhood of the glomeruli along the walls bounding the interlobular spaces. Periglomerular lymph may be formed by transudation from afferent arterioles or by filtration through the parietal layer of Bowman's capsule itself. The initial lymph formed in this manner leaves the renal parenchyma via two routes. The direct route of exit is upward through perforating capsular lymphatics which join the capsular lymphatic trunks. The second, less direct route, is downward toward the corticomedullary border where the interlobular lymph is mixed with transudate from the larger arcuate arteries. The lymph then leaves the renal parenchyma via the hilar lymphatic vessels. Renal lymph composition may be altered by

renal function, providing that diffusion gradients exist between the interstitial fluid of the tubular mass within the lobule and that of the interlobular spaces. It is hypothesized that all differences in concentrations of freely filterable substances between renal lymph water and arterial blood plasma water are due to diffusional exchanges between these two compartments and to the Donnan phenomenon.

## Implications of the Hypothesis

This hypothesis predicts that any substance which is rapidly secreted into the tubular fluid will be found in renal lymph in relatively low concentrations. After the present experiments were completed, evidence was published that supports this prediction (6). Penicillin is known to be rapidly cleared from blood by active secretion in the proximal tubule. Cockett and co-workers (6) have recently shown that renal lymph concentrations of this substance are one-fifth to one-tenth of those found in blood plasma. It is expected that further evidence for rapidly secreted substances will be found in the future.

According to the hypothesis one would expect substances which are reabsorbed from the urine would cause only small alterations in lymph composition even though reabsorbed with great avidity. This follows from the fact that no more than the filtered quantity of any substance can be reabsorbed, and filtered solutes will normally be reabsorbed along with 80 to 90% of the filtered water. This situation does not produce the high diffusion gradient necessary for large alterations in lymph composition. Therefore, the L/P ratio for PAH is dramatically different from unity, while those for sodium, potassium and urea are not.

Finally, the reabsorption of water can alter renal lymph

composition only in such cases as reabsorption dilutes the interstitial concentration of some filterable solute. It has already been shown that renal lymph inulin concentration is less than that found in blood plasma (19). The same relationship would be expected for other non-reabsorbable sugars and for creatinine.

# CHAPTER VI

## SUMMARY

A retrograde injection method was used to study the intrarenal distribution of lymphatic vessels. The present study is not conclusive concerning areas in which injected lymphatics were not observed; yet the results of this study considered together with those of other investigators yields a more valid description of the distribution of the intrarenal lymphatic vessels than has previously been available. It was concluded that the renal lymphatics are distributed primarily within the interlobular spaces, and along the arcuate vessels. An additional distribution of lymphatics was found in the immediate vicinity of Bowman's capsule, but not in the medulla.

Renal lymph and arterial blood plasma were collected from a large number of dogs over an extended period of time. These samples were analyzed for sodium, potassium and urea, and these parameters were expressed as L/P ratios. Evaluation of these data revealed that variation existed among small groups of data gathered within a limited time period. This variation was quite similar to that observed among different laboratories for the parameters studied. It was concluded that differences in renal reabsorption and/or secretion in different groups of dogs results in L/P ratios for sodium, potassium and urea which are greater than, equal to or less than unity.

Two different sets of experiments were described, each being carefully designed to demonstrate the effect of medullary function on renal lymph composition. No such effect could be detected with either type of experiment. It was therefore concluded that renal lymph composition is independent of renal medullary function.

L/P ratios for sodium and potassium derived from experiments conducted over a long period of time were significantly greater than unity. It was concluded that these ratios are elevated primarily as a result of differences between lymph and plasma protein concentrations.

The conclusions drawn from the present study made possible the statement of an hypothesis concerning how renal lymph is formed and how renal function alters lymph composition. The implications of this hypothesis are discussed.

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