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Circulation of B and T Lymphocytes Through the Canine Kidney in Control and Hydronephrotic States

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CIRCULATION OF B AND T LYMPHOCYTES THROUGH THE CANINE KIDNEY IN CONTROL AND HYDRONEPHROTIC STATES

by

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VITA

The author, John P. Ferron, is the son of Norbert P. and Irene (Carey) Ferron. He was born January 10, 1951, in Youngstown, Ohio.

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INTRODUCTION

In recent years there has been considerable accumulation of data concerning the cells of the lymphomyeloid complex and their role in the immune response. Cells from blood, bone marrow, lymphoid organs and those circulating in post nodal lymph have received the major attention largely because of the ease with which they are obtained. Information is available regarding the relative proportions of the thymus dependent T lymphocytes and the thymic independent B lymphocytes in the above-mentioned tissues in laboratory animals and man. Few studies, however, have involved prenodal or peripheral lymph which drains from the tissues to the regional lymph nodes. A large percentage of the cells in this peripheral lymph have been shown to be lymphocytes (24, 94).

Small lymphocytes have been divided functionally into two sub-populations; the thymus dependent T lymphocyte and the thymic independent B lymphocyte. The precursor T cells, become mature T cells after their surface is somehow altered by the thymus, are responsible for cellular immunity, delayed hypersensitivity, allograft rejection and tumor suppression. The B lymphocytes have been described as being derived from the bursa of Fabricius in birds (18) and in other animals, including man, from bone marrow or gut associated lymphoid tissue. When an immunocompetent B cell is antigenically stimulated, often by an encapsulated bacteria, it differentiates into an antibody producing plasma cell and is therefore responsible for humoral immunity.

Morphologically B and T cells are indistinguishable so surface membrane receptors, unique to each population, are employed to differentiate them. T lymphocytes possess a receptor for unsensitized erythrocytes and when the two are mixed under specific conditions spontaneous rosettes form (33) which serve as a convenient marker for the identification of the T cell population. There have been four surface receptors described for B cells: surface immunoglobulins, either IgM or IgD (65, 69, 70); a receptor for the Fc portion of an immunoglobulin (4, 13, 15, 53, 62, 87, 89); complement receptors (6, 58) and a receptor for the Epstein Barr virus (39). Macrophages also have a receptor for complement but can be distinguished from lymphocytes by their ability to ingest latex particles.

The general purpose of this study was to investigate the cell content of the peripheral lymph, which is draining from the tissues to the lymph node, in control dogs. More specifically the proportions of B and T lymphocytes in the peripheral renal lymph were determined and compared with values obtained from the peripheral blood and thoracic duct lymph in the same animals. The T cell's ability to form spontaneous (E) rosettes with unsensitized guinea pig erythrocytes was used as its marker. B lymphocytes were characterized by their formation of erythrocyte antibody complement (EAC) rosettes (73). After control values had been established, similar experiments were carried out in dogs following unilateral ureteric obstructions and the results compared with those of the controls.

REVIEW OF RELATED LITERATURE

I. GENERAL LYMPHATICS

In 1939 Yoffey and Drinker (94) suggested the following terminology for lymph in the distal portions of the lymphatic circulation and that which empties into the main collecting vessels. They proposed that the lymph which is draining from the tissues and has not yet passed through a lymph node be called peripheral or prenodal lymph. After lymph has passed through one lymph node it is called intermediate lymph. The lymph which has already passed through one or more nodes and is on its way back to the blood stream via the thoracic duct or another main lymphatic channel is referred to as central lymph.

The study of peripheral (prenodal) lymph has in the past been hampered by the technical problems related to its slow rate of flow and its collection. Cannulation of peripheral lymphatic vessels with polyethylene tubing has aided in solving the collection problems. Successful collections of peripheral lymph from several species have been reported (38) and reviewed (95). Studies on the fate of labeled lymphocytes injected into the peripheral lymphatics (63) and subcutaneously (28) also have been published. Collection of prenodal lymph from the limbs, skin, kidney and other organs of sheep were first reported by Smith, McIntosh and Morris (79). Hall and Morris (24) found the cellular content of the prenodal lymph to be much less than that of the efferent

lymph and calculated that the lymphocytes recirculating to a lymph node enter mainly (90%) from the nodal capillary bed directly and the remaining 10% enter by the prenodal lymphatics.

II. RENAL LYMPHATICS

A. CONTROL

Two networks of lymphatics have been described as draining the kidney in dogs and man. The superficial network is seen on the capsule of the kidney and consists of 2-4 collecting lymphatics which leave this organ at the poles and eventually join the other network near the hilar node. The deep network leaves the kidney at the hilus as 6-10 collecting trunks which follow the renal artery and vein. These lymphatics drain into the para-aortic nodes.

The exact parenchymal origins of the two lymphatic networks have been a topic of much debate. The major point of the debate centers on the existence of intralobular lymphatic vessels. Several authors claim to have seen them (40, 55, 57, 74) while others have not been able to demonstrate them (64, 72, 82). Investigations have shown that lymphatics surrounding the glomeruli and tubules will drain into either of the two collecting networks while the lymph vessels in the medulla, if indeed they exist, drain mainly into the hilar collecting system (35, 84). In a recent publication Holmes, O'Morchoe and O'Morchoe (30) reported on finding communicating lymphatics forming direct connections between the hilar and capsular networks in hydronephrosis. They proposed that these communications, although dilated by hydronephrosis, existed under control conditions.

The flow rate in the renal lymphatics has been hard to assess. Most investigators assume there are ten lymphatic vessels draining each kidney and therefore have measured the flow rate by determining the rate from one cannulated vessel and multiplying that value by ten. Yoffey (95) reviewed the literature for the flow rate of renal lymph in dogs and found the range to be 2.2-14 ml/hour. He stated that the flow rate did not seem to be related to sex, age or weight of the animal. By a variety of methods other authors (27, 59, 60) have estimated the renal lymph production in anesthetized dogs to be 0.3 ml/min per 100g kidney. The renal lymph flow from both kidneys has been shown to form an appreciable amount, up to 40%, of the thoracic duct lymph flow (59, 66).

Changes in the rate of renal lymph flow following partial obstruction of the inferior vena cava have been described. Katz and Cockett (36) showed that obstruction of the vena cava caused increased renal venous pressure and thoracic duct flow while decreasing both the flow and sodium concentration of the urine. LeBrie and Mayerson (42) found a five-fold increase in capsular lymph flow after partial obstruction of the inferior vena cava. They stated that this increase in capsular flow often exceeded the urine flow from the same kidney. Associated with this partial obstruction of the inferior vena cava there was also an increase in the lymph protein concentration.

B. HYDRONEPHROSIS

Hydronephrosis is a term used to describe any impediment of free urine flow resulting in dilation of the renal pelvis associated with progressive atrophy and cystic enlargement of the kidney. Laboratory

models designed to induce a state of hydronephrosis have involved ligation of the ureter and Ludwig and Savarykin (45) were the first to describe a distention of the renal lymphatics following such a ligation. Several investigators in the 1950's and 1960's (11, 17, 20, 86) proposed that the renal lymphatics act as a "safety valve" by absorbing urine from the renal pelvis thereby protecting the renal parenchyma from destruction. However, others (27, 29) have shown that although there was a 4-5 fold increase in renal lymph following obstruction, its composition was little affected and differed widely from that of urine in the renal pelvis. Another phenomenon that has been studied is the fact that some materials can be absorbed from the renal pelvis during hydronephrosis into the interstitial spaces of the kidney and eventually leave the kidney by the renal lymphatics (1, 11, 17, 54, 55).

The short-term effect of ureteric obstruction is an increase in renal lymph flow (48, 61, 80, 86) thought to be caused by the alteration of renal hemodynamics (27). Ureteric ligation has been shown to increase the renal blood flow and the interstitial pressure (56, 85). The prolonged effects of ureteric ligation in the unanesthetized animal have also been reported. In the dog, Holmes et al. (29) showed a major diversion of lymph flow from the hilar to capsular network in chronic obstruction. Working with sheep, McIntosh and Morris (50) found that after a 3-4 day time interval the rate of renal lymph flow increased 10-12 times over the control values. After 2-3 weeks the cell content of the renal lymph increased to 50-100 times the control value. This reflected one of the histological changes that occur in hydronephrosis. The cortex and corticomedullary areas were found to be infiltrated

with dense aggregations of lymphocytes, especially beneath the capsule of the kidney. Many lymphocytes were found in small blood vessels, renal tubules and glomeruli. Smith, McIntosh and Morris (80) surmised that this lymphocytic infiltration suggests an increased migration of these cells in response to tissue damage.

III. LYMPHOCYTES

A. GENERAL

In recent years the function of the small lymphocyte in body defense mechanisms has received much attention. It is ironic that these important functions of lymphocytes have evaded investigators for so long. It is now believed that the body is protected from infections and cancer by two types of immunity derived from the small lymphocyte. Although lymphocytes show little morphological heterogeneity, functional subpopulations of these cells do exist. This functional heterogeneity was demonstrated by the existence of two distinct populations of lymphocytes, the thymus dependent T and thymic independent B lymphocytes (12, 52).

Both the B and T subpopulations of lymphocytes are derived from the same stem cell. In 1975, Yoffey (93) described the "transitional" cells as the precursors of the small lymphocytes. Transitional cells are components of the hemopoietic stem cell compartment of the bone marrow and consist of a wide variety of cell sizes. The transitional cell compartment gives rise to cells of the lymphocytic, erythrocytic and granulocytic series. The small transitional cells divide to form small lymphocytes while the larger ones give rise to blast cells of the

various cell series.

The cell surface of the stem cells are somehow altered as they pass through the fetal thymus conferring T lymphocyte characteristics on it. Proof of the thymic involvement has been established by thymectomy studies (47, 51) and by studies of immune deficiency diseases such as DiGeorge's Syndrome or thymic aplasia. After processing, the T cells appear in the peripheral lymphoid tissue, heavily concentrated in the paracortical region of lymph nodes. The T lymphocyte population confers cell mediated immunity which combats fungal and viral infections and is important in delayed hypersensitivity, the rejection of tissue transplants and the suppression of tumors.

The thymic independent B cell population is responsible for humoral immunity which involves the production of specific antibodies against antigens presented to the host. Humoral response is especially effective against encapsulated bacteria and viral reinfections. The B cells are processed in the bone marrow or the gut associated lymphoid tissue in mammals. In birds the bursa of Fabricius is the location of the processing of the B cell population. Glick (18) showed that bursectomy in the chicken resulted in a decreased antibody production which he hypothesized was due to a decrease in the B cell population. B cells are heavily concentrated in the germinal centers of lymphoid tissue and the spleen. With the appropriate antigenic stimulation a B lymphocyte differentiates into an antibody producing plasma cell.

It should be emphasized that in an immunologically competent individual or animal the B and T cells function together. There is some cooperation between these populations in defense mechanisms and many

immune responses involving antibody production by B cells first involves help from T lymphocytes and macrophages in the processing and presentation of the antigen.

B. SEPARATION OF LYMPHOCYTE POPULATIONS

The isolation of lymphocytes from the blood has developed as an important step in the analysis of the lymphocytic subpopulations. The most commonly employed method is the Ficoll-Hypaque density gradient which was described by Boyum (9) and gives greater than 90% yield of lymphocytes while theoretically not causing a selective loss of a particular lymphocytic subpopulation.

Once the lymphocytic population has been separated from whatever source, various methods for the isolation of the different subpopulations have been developed utilizing such properties as differences in density in albumin gradients, sedimentation velocities, electrophoretic mobilities (14) and adhesion to nylon fibers (16, 21).

In 1973, several reports describing a relatively simple method for distinguishing T and B lymphocyte populations using Scanning Electron Microscopy (SEM) were published (44, 67). It appeared that many B and T derived cells could be recognized on the basis of the morphology of their cell surface. The thymus dependent cells possessed smaller microvilli and were generally smoother than the more villous "hairy" bone marrow derived B lymphocytes. However several authors have shown that the number of villi present was dependent on the processing technique utilized (2) while others said that the number of villi depended on the stage of cell growth (77).

C. SURFACE RECEPTORS ON LYMPHOCYTES

Differences in the cell surface characteristics between the B and T lymphocytes have been useful in separating them into populations that could be studied individually. Some of these surface characteristics may be functionally significant in the immune response such as immunoglobulins while others such as the sheep red blood cell (SRBC) receptor are important as laboratory tools.

Two distinct receptors have been reported in the literature for T lymphocytes. Murine T cells appear to carry the theta antigen (70) on their cell surface but to date it has not been detected in any other species. Jondal (33) was the first to describe the receptor for sheep red blood cells on human T lymphocytes. He showed that under specific conditions human T cells formed spontaneous rosettes with unsensitized SRBC's. The World Health Organization Report (92) stated that most of the T lymphocytes in man form rosettes with sheep erythrocytes and lack surface immunoglobulins. If immunoglobulins are present on T cell surfaces they must be in small numbers because they fail to stain with fluorescein labeled antiserum. Both human and canine T lymphocytes have been shown to form spontaneous rosettes with unsensitized red blood cells from a variety of sources such as ox, pigeon and guinea pig (8, 92). Another commonly employed method for detecting T lymphocytes is to use a heterologous absorbed antithymocyte serum. Schafer (76) has established a method for permanently mounting rosette preparations on slides with the advantage of providing distinct morphological identification of the rosette forming cells. This method also produces a permanent slide mount for future reference.

B lymphocytes can be identified by several unique surface characteristics: the presence of surface immunoglobulin; the ability to bind specific antigens; and the ability to bind complement. Several authors (38, 40, 41) have described surface immunoglobulin on the B cell surface. Of the five classes of immunoglobulins, IgM and IgD are the classes present at the cell surface. Both of these immunoglobulins may be present on the same cell, IgD appearing first in ontogeny (41) and IgM present on the surface of immunocompetent B cells (88). Kunkel (41) has established that these surface immunoglobulins are positioned at the surface with their C-terminal portion buried deeply in the membrane. This allows the antigenic receptors of the immunoglobulin to project above the surface.

Other membrane receptors have been reported for the B lymphocyte population. The Fc receptor has been reported as the binding site for antigen-antibody complexes for both B cells and macrophages (4, 13, 15, 53, 62, 87, 89). Detection of this Fc receptor was accomplished by labeling antigen-antibody complexes with fluorescein or radioactive materials. Surface receptors which interact with components of complement have also been established (6, 58). Erythrocytes sensitized with antibody and complement form EAC complexes which can bind to the complement receptor on the B cell thus forming an EAC rosette (73). Klein et al. (39) has recently described a receptor on B cells for the Epstein Barr virus (EBV).

Lymphocytes that are not positive for either B or T cell markers have been referred to in the literature as 'null' cells (34). The number of null cells is estimated as the difference between 100% and the sum of the percentages of B and T cells (22, 34). Several reasons

for their existence have been proposed in the literature. They may be the result of insufficient rosetting techniques. The null cells may represent a new fraction or subpopulation of lymphocytes for which no markers have yet been described. Finally they may be immature lymphocytes and/or monocyte predecessors. The reason for their existence is still an open question. Also in the literature have been reports of lymphocytes which are positive for both B and T cell populations and it is felt that they represent T or B cells or both (10).

D. THE PROPORTIONS OF B AND T LYMPHOCYTES FROM VARIOUS SOURCES

The relative proportions of B and T lymphocytes in peripheral blood (PB) have been determined for humans, dogs, rats and mice. In human PB the proportions of T and B lymphocytes have been reviewed (75) finding 42-74% of the cells positive for T cell markers and 15-30% B positive. Table 1 summarizes recently published data concerning the lymphocytes in human PB. Table 2 represents data on the proportions of B and T cells in murine PB. Several authors have reported specifically on the PB lymphocytes of dogs (5, 8, 96). Problems have evolved concerning the separation of canine lymphocytes from the blood. Most investigators have employed the Ficoll-Hypaque method described by Boyum (9) for human lymphocytes resulting in high yields (5, 8, 96). Others claim that this density gradient method is often contaminated with granulocytes because of the similarity in size of canine lymphocytes and polymorphonuclear leukocytes (37). They claim the Ficoll-Hypaque method yields a 70% pure lymphocyte population. To improve the purity of yield Whitacre and Lang (91) have suggested feeding the PMN's iron particles to accelerate

their separation (43). The surface membrane characteristics of canine lymphocytes from the peripheral blood have been investigated by employing immunofluorescent staining techniques, rosette formation by erythrocyte (E) and erythrocyte antibody complement (EAC) assays and antithymocyte globulin (ATG) techniques. The relative proportions of canine B and T lymphocytes are shown in Table 3.

The thoracic duct lymph in man contains about 60-75% T lymphocytes (25). Murine thoracic duct lymph contains 60-87% T cells and 10-16% B cells (19, 46, 71, 81). To date there have been no studies published concerning the relative proportion of T and B cells in canine thoracic duct lymph.

The only report in the literature concerning the proportion of B and T cells in peripheral lymph found that 5-15% of the lymphocytes in sheep peripheral lymph were positive for B cell markers (23).

STATEMENT OF OBJECTIVES

GENERAL

The general aim is to provide information on the movement of lymphocytes from peripheral tissues to a regional lymph node and on the functional significance of the movement.

SPECIFIC

1. To use the renal hilar lymph as a source of peripheral (prenodal) lymph in order to study its cellular content.

2. To determine the proportions of B and T lymphocytes in canine peripheral blood, thoracic duct lymph and renal peripheral lymph.

3. To compare the control values of B and T lymphocytes in the peripheral blood, thoracic duct lymph and the renal hilar lymph with experimental values obtained after unilateral ureteric obstruction which induced hydronephrosis.

MATERIALS AND METHODS

This study involved 28 dogs of either sex. The dogs were cared for in the animal quarters and given daily allotments of chow and water according to standards of the American Association for Live Animal Science. All dogs were anesthetized with sodium pentobarbital (Nembutal) given intravenously (35 mg./kg. body weight). The experiments conducted were divided into three groups: (1) a control group of 19 animals; (2) an experimental group with induced hydronephrosis in eight animals and (3) one animal with a plastic cannula chronically implanted into the thoracic duct. Samples were collected from the peripheral blood (PB), the thoracic duct (TD) and the renal hilar lymphatics (RL).

I. SURGICAL PROCEDURES

In Group I the renal lymphatics were exposed through a left loin incision and cannulated with nylon tubing (Portex) I.D. 0.02 inches according to a technique developed in this laboratory. The left carotid artery was used as a source for the peripheral blood samples. This artery was exposed in the anterior triangle of the neck as it passes cephalically in the carotic sheath. A PE 210 cannula was placed in the artery and secured. Blood samples were collected using a syringe attached to this cannula and then placed in heparinized tubes at room temperature. Using the same neck incision, the thoracic duct was located at its junction with the left subclavian and jugular veins. A polyethylene cannula

size PE 90 was placed in the duct and secured. Samples from the thoracic duct and renal lymphatics were collected in cooled heparinized tubes. Normal (isotonic) saline was slowly infused into each animal via a cannula in the saphenous vein attached to a Sage Tubing Pump at a rate of 0.67 ml./min. to replace fluids lost in sample collections and during surgery. To prevent an artificial depletion of lymphocytes due to drainage of the thoracic duct, excess lymph from this source was reinfused into each animal.

Group II consisted of eight dogs with experimentally induced hydronephrosis. The ureter was exposed through an incision over the left iliac crest, the muscle layers split and the ureter identified and ligated with silk suture (Ethicon 0). These operations were performed under semisterile conditions. After the wound was closed, an antibacterial powder, Furcin, was applied and a subcutaneous injection of Flocillin was administered (300,000 u/10 kg. body weight). At various time intervals from 2-5 days postoperatively these dogs were again anesthetized, cannulated in a similar way to the control animals and samples were collected.

Group III consisted of one animal with a chronic implantation of a plastic annula (Silastic) in the thoracic duct. The thoracic duct was exposed by a left lateral thoracotomy in the intercostal space between the eighth and ninth ribs. Cell samples were collected daily from the thoracic duct of the unanesthetized animal for a period of 5 days.

At the termination of each experiment representative tissue samples were collected from the left kidney and also from a left hilar lymph node when possible. These samples were fixed in normal buffered formalin,

embedded in paraffin and cut in 5-10 micron sections. The sections were placed on slides and stained with Hematoxylin and Eosin Y for histological examination.

II. LEUKOCYTE COUNTS

Leukocyte counts were routinely performed on samples from the peripheral blood and thoracic duct. These counts were important for determining the concentration of lymphocytes in each sample. This information was later used to calculate the concentration of erythrocytes used in the rosetting techniques. Counts were performed using a Levy-Hausser Counting Chamber and the conventional method of dilution and hemolysis with 1% glacial acetic acid. The formula employed for calculating the number of leukocytes per cmm. was:

$$\frac{\text{Leukocytes counted X dilution X 10}}{\text{Number of 1 sq. mm. areas counted}}$$

At least eight such 1 square mm. areas were counted.

Since the cellular content in peripheral lymph is so low, a modification of the standard leukocyte counting method was developed in this laboratory to count the cells in the renal hilar lymph. One half ml. of renal lymph was mixed with 0.5 ml. of a 1% glacial acetic acid solution. This mixture was then centrifuged at 1000 rpm for 10 minutes. Next, 0.8 ml. of the supernatant was removed and the remaining concentrated solution placed on the counting chamber and counted in the standard fashion. The same formula was employed to determine the number of leukocytes present.

Differential counts were performed on the leukocytes obtained from

the thoracic duct and peripheral blood. Whenever possible, differential counts were also carried out on samples from the renal hilar lymph.

III. LYMPHOCYTE PREPARATION

The blood and lymph samples collected in cooled heparinized test tubes were prepared for rosetting in the following manner. Blood was diluted 1:1 with phosphate buffered saline (PBS, pH 7.2-7.4). Then 4 ml. of the blood dilution was layered carefully over 3 ml. of Lymphoprep (Nyegaard Corp., New York) in a centrifuge tube. At the same time, 3 ml. of thoracic duct lymph was also layered over 3 ml. of Lymphoprep in another centrifuge tube. Because of the low cellular concentration of the renal hilar lymph these samples were spun down to a button in order to obtain the highest recovery of cells.

The blood and thoracic duct samples were then centrifuged at 1490 rpm (400 x g) for a period of 35-40 minutes. At the end of centrifugation, the lymphocytes at the interface were collected with a Pasteur pipette and washed twice with PBS to remove the platelets. These lymphocytes were then placed in a solution of 90% Hank's Balanced Salt Solution (HBSS) and 10% normal dog serum. Sometimes Minimal Essential Medium (MEM) was substituted for the HBSS. This serum had been previously deactivated at 57°C for 30 minutes. Most of the lymphocyte rosette preparations were stored overnight at 4°C, though the rosette procedure did not differ whether it was carried out immediately or after overnight cryopreservation. On the following day, the samples were removed from the refrigerator, warmed and agitated. Following centrifugation at 500 rpm for 5 minutes, the supernatant was removed and fresh HBSS-serum mixture was

added to the cell button to bring each sample to the standard cell concentration calibrated for the rosetting technique in this laboratory.

A. PROCEDURE FOR ERYTHROCYTE ROSETTES

Human and canine T lymphocytes have been shown to possess a surface receptor for a variety of unsensitized erythrocytes. The canine T cell specifically forms spontaneous rosettes when mixed with either human or guinea pig red blood cells. The rosetting technique used in these experiments was adopted from the work of Bowles, et al. published in 1975 (8).

A 0.2 ml. aliquot of canine lymphocytes from the blood and thoracic duct lymph diluted to 3.0×10^6 cells/ml. were combined with 0.2 ml. of a suspension containing 3.0×10^8 cells/ml. of guinea pig red blood cells. These erythrocytes were stored in a 50% Alsever's solution at $4^{\circ}C$ and on the day of testing they were thawed, washed twice with PBS and resuspended in the HBSS-serum solution (pH 7.3-7.4) at the concentration mentioned above. The guinea pig red blood cells were obtained from International Scientific Products Inc., (Carey, Ill.). To a concentrated sample of renal lymph were added GPRBC's in a concentration of at least 100 RBC; 1 lymphocyte. The samples were all incubated at $22^{\circ}C$ in a 5% CO_2 atmosphere for 30 minutes. The cell mixtures were then centrifuged at 200 x g at room temperature for 5 minutes and half of each harvested pellet gently resuspended in a 0.3 ml. of a 0.01% solution of crystal violet in PBS. A drop of this suspension was added to a hemocytometer and the percentage of lymphocytes binding to three or more erythrocytes was determined microscopically at a magnification of 250x. A total of at least 200 lymphocytes were counted for each sample. The other half of the sample

was placed on a slide using a wide bore Pasteur pipette and the slide tipped to spread the suspension evenly over its surface. This permanent mount of rosettes was air dried, dehydrated in ethanol and stained with methyl green pyronin (MGP). The percentage of lymphocytes forming rosettes in this MGP stained preparation was also determined by counting at least 200 lymphocytes.

B. PROCEDURE FOR ERYTHROCYTE ANTIBODY COMPLEMENT ROSETTES

The B cell population was tested by using sheep red blood cells absorbed with rabbit antisheep red blood cell globulin (7S fraction) and guinea pig complement with components of C1-C4 (Cordis Laboratory, Miami, Florida). This test is referred to as an Erythrocyte Antibody Complement (EAC) complex test. These cells were stored at $4^{o}C$ and were thawed and washed, as described for GPRBC's, on the day of testing. A 0.2 ml. suspension of canine lymphocytes from the blood and thoracic duct lymph were combined with 0.2 ml. of the EAC complex diluted to 3.0×10^{8} cells/ml. Again the cells from the renal hilar lymph were concentrated and an excess of EAC cells added. The cell mixtures were incubated at $37^{o}C$ in a 5% CO_2 atmosphere for 30 minutes followed by centrifugation at room temperature at 200 x g for 5 minutes. Half of each pellet was mixed with 0.3 ml. of a 0.01% solution of crystal violet in PBS and examined using a hemocytometer. The remaining half was mounted on a slide for permanent keeping, stained with MGP and the percentage of rosette forming lymphocytes determined.

C. LATEX PARTICLES

On a few occasions, an attempt was made to distinguish lymphocyte rosettes from those of macrophage origin. To do this the lymphocyte rich pellets collected at the interphase were fed latex particles of a 0.804 μ diameter (Dow Chemical Company, Indianapolis, Indiana) and incubated for 10 minutes at 37°C. After cooling to room temperature, the pellets were mixed with the red blood cells and the standard rosetting technique continued. When the rosettes were counted the macrophage rosettes could be easily distinguished since they had ingested the latex particles into their cytoplasm.

RESULTS

The major difficulty in the interpretation of data concerning lymphocyte investigations, in the past decade, has been the wide variety of techniques used by different research workers. In 1974, the World Health Organization sponsored a workshop on human B and T lymphocytes (92). This workshop made several recommendations in order to standardize techniques, such as rosetting and immunofluorescent staining, used in lymphocyte research. The WHO recommendations concerning the processing and counting of lymphocyte rosettes were used as guidelines in this study.

I. STANDARDIZATION OF TECHNIQUES

The lymphocytes from the peripheral blood of one animal were used in order to standardize the rosetting techniques to be employed in this laboratory. The reproducibility of these methods could be shown and then compared with values collected in other laboratories (5, 8, 96). Blood was drawn from this dog on 3 separate occasions during a one-week period and lymphocytes were separated from the blood and rosetted by the techniques described elsewhere in this text. Erythrocyte (E) rosettes, using guinea pig red blood cells, were employed as markers of the T lymphocyte population. In these preliminary experiments rosettes were stained with crystal violet and counted on a hemocytometer. The percentage of rosette forming cells with T cell markers on the three different days were: (1) 41.2%; (2) 43.2% and (3) 39.5%. These values agreed with

those in the literature for similar studies (5, 8, 96). In this animal, there were no reproducibility tests performed on the peripheral blood lymphocytes bearing complement receptor sites, a marker for a subpopulation of B lymphocytes. The total leukocyte count in the peripheral blood did not change significantly during this time.

A cannula was implanted into the thoracic duct of a second animal and lymphocytes drained daily from this source were used in another standardization study. The Erythrocyte (E) rosetting technique was again used to mark the canine T lymphocyte population. Over a 5 day period, the daily percentages of T positive cells in the thoracic duct of the unanesthetized animal were: (1) 36.2%; (2) 31.4%; (3) 23.2%; (4) 28.1% and (5) 44.6%. The mean percentage for this serial study was 32.7$\pm$ 3.6 (mean $\pm$ S.E.) which did not differ significantly ($p>0.05$) from the mean percentage (41.8 $\pm$ 4.7) of T lymphocytes in the thoracic duct of all the anesthetized control animals in this study. To date, there have been no other reports in the literature on the relative proportion of B and T lymphocytes in the canine thoracic duct.

Two different techniques were employed during the standardization procedures to determine the proportion of rosette forming cells. The first method consisted of counting freshly mounted rosette suspensions stained with crystal violet. The second method involved a permanent mount of rosettes stained with methyl green pyronin (MGP) as described by Schafer, et al. (76). Table 5 is a comparison of the percentages of rosette forming cells obtained by these two methods. The only significant difference ($p<0.05$) found was in the comparison of the T lympho-

cytes in the thoracic duct lymph of dog #577, where the percentage of rosettes on the permanently fixed slides (MGP) was higher than those of the freshly prepared samples. During the course of the experiments, it was learned that lymphocytes could be stored overnight at $4^{o}C$ without causing a significant difference in the rosette formation for either E or EAC rosettes (7, 31, 90). Therefore preliminary studies involved only fresh mounts of samples made on the same day as the collection, while in later studies the lymphocytes had been medium-stored in a HBSS-serum solution overnight at $4^{o}C$. There were no differences noted in comparing rosetting results of freshly mounted and medium-stored rosette preparations.

II. CONTROL ANIMALS

In the control group, nonimmune E rosettes with guinea pig red blood cells were used as markers for canine T lymphocytes. Table 4 lists the percentages of E rosette forming cells in the peripheral blood (BT-44.9 $\pm$ 2.4), the thoracic duct lymph (TT- 41.8 $\pm$ 4.7) and the renal hilar lymph (RT- 43.8 $\pm$ 3.8) of the control animals. Table 7 summarizes the mean percentage and range for each of the above-mentioned groups.

Complement receptor sites on canine B lymphocytes were demonstrated by Erythrocyte Antibody Complement (EAC) rosettes. Table 4 lists the relative proportions of EAC positive lymphocytes in the peripheral blood (BB- 39.6 $\pm$ 4.2), the thoracic duct lymph (TB- 14.2 $\pm$ 2.3) and the renal hilar lymph (RB- 28.5 $\pm$ 3.4). Table 7 summarizes the mean percentage and range of B lymphocytes in the aforementioned samples.

Figure I is a bar graph representing the percentages of T and B positive rosettes in the peripheral blood, thoracic duct and hilar lymph of control dogs. Figure II is a comparison of the distribution of the B and T lymphocyte populations in the three measured fluids. There is a marked and significant difference ($p<0.001$) between the B cell populations of the peripheral blood and the thoracic duct lymph. There is also a significant difference ($p<0.05$) between B cell populations in the renal hilar lymph and the thoracic duct lymph.

III. EXPERIMENTAL ANIMALS

This study involved eight dogs with unilateral hydronephrosis. The hydronephrosis was induced by ligation of the ureter 2-5 days prior to sample collection. Table 6 lists the mean percentages of E and EAC rosette forming cells in the hydronephrotic dogs. Table 7 contains the mean percentage and range of erythrocyte (E) rosettes in the peripheral blood (BT- 43.9 ± 3.4), the thoracic duct lymph (TT- 44.9 ± 6.7) and the renal hilar lymph (RT- 43.6 ± 5.8) and the mean percentages and ranges for EAC forming rosette cells in the peripheral blood (BB- 42.8 ± 8.3), the thoracic duct lymph (TB- 14.2 ± 3.5) and the renal hilar lymph (RB- 28.0 ± 7.8).

Figure III is a bar graph comparing the percentage of T and B lymphocytes in the peripheral blood, thoracic duct lymph and the renal hilar lymph of the control and hydronephrotic groups. There were no significant differences in the results obtained in these two groups. Table 8 is a comparison of percentages and the absolute numbers of B and T lymphocytes in the peripheral blood, and

renal hilar lymph in control and hydronephrotic dogs.

IV. LEUKOCYTE COUNTS

Leukocyte counts were performed on samples of peripheral blood, thoracic duct lymph and renal hilar lymph in control and experimental animals. The mean WBC for blood in control animals was 11,018 cells/mm^3, while in hydronephrotic dogs it was 16,696 cells/mm^3. The canine thoracic duct lymph contained a mean of 9,125 leukocytes/mm^3 in the control animals while in hydronephrotic dogs the WBC was 5,925 cells/mm^3. There was no significant difference ($p>0.05$) when comparing the leukocyte counts in the blood and thoracic duct lymph between the control and hydronephrotic groups. However, the renal hilar lymph of the control dogs contained a mean of 50 cells/mm^3 while the hilar lymph in experimental animals had a mean leukocyte count of 652 cells/mm^3. This was a marked and significant difference ($p<0.001$).

Differential counts were performed on smears from the peripheral blood of all and the renal hilar lymph from some control and hydronephrotic animals. This study did not concern itself with differential counts from the thoracic duct lymph. In the peripheral blood of control animals about 50-70% of the leukocytes were neutrophils, about 20-30% were lymphocytes and the remainder were monocytes, eosinophils and an occasional basophil. The differential count on the peripheral blood in the hydronephrotic animals reflected a state of inflammation with an increase in the number of neutrophils.

In the renal lymph from control dogs 90% of the white blood cells

were lymphocytes and 10% were neutrophils with an occasional monocyte. However, the differential counts in the renal lymph of hydronephrotic animals showed that 60% of the cells were lymphocytes and 40% were neutrophils.

The absolute number of lymphocytes in the peripheral blood and renal hilar lymph of control and hydronephrotic dogs was calculated from knowing the mean leukocyte counts, mean percentage of rosette forming cells and the mean differential count. Table 8 is a comparison of the mean percentages of rosette forming cells and absolute lymphocyte counts in control and hydronephrotic dogs. In the peripheral blood, the absolute number of T and B lymphocytes was not significantly different in the control and hydronephrotic studies. In the renal hilar lymph, however, there was a marked increase in the lymphocyte traffic of T and B cells in the hydronephrotic group even though the relative percentages were the same.

V. TISSUE SPECIMENS

Tissue from the left kidney of each animal was obtained at the end of each experiment, fixed in formalin, embedded in paraffin, cut into 5-10 micron sections and stained with Hematoxylin and Eosin Y for histological examination. Tissues from the control group were examined to rule out any pathological process and then served as comparisons for the experimental group.

The changes observed in tissue sections from the hydronephrotic kidneys correlated well with those reported in the literature (17, 20, 48).

There was marked congestion of the glomerular tufts and in the 4 and 5 day studies there was distortion of the glomerular capsular spaces. Early changes in the renal tubules included dilatation and fatty changes in the epithelial cells. The interstitial tissue became fibrotic with an infiltration of lymphocytes and other inflammatory cells. As the hydronephrosis progressed, there was marked dilatation of both capillaries and arterioles.

DISCUSSION

The general purpose of this study was to provide information on the movement of lymphocytes from the tissues to a regional lymph node. The hilar lymph system draining from the kidney was used as the source of peripheral lymph, which is lymph draining from the tissues prior to entering the first lymph node. It was anticipated that some insight into the functional significance of this lymphocyte traffic could be gained if the kidney was altered or damaged. Therefore, the cell content of renal peripheral lymph was studied in a control group and compared with values obtained after unilateral ureteric obstruction, which induces a pathological state known as hydronephrosis. A previous study of induced hydronephrosis in sheep (80) had shown a progressive infiltration of lymphocytes into the renal parenchyma as the duration of the obstruction increased to 3 weeks. New information on the separation of lymphocytes into functional subpopulations inspired this investigation to determine which subpopulation responds to this altered state of renal structure and function.

Although morphologically nondistinguishable, small lymphocytes have been functionally divided into two populations, the thymic dependent T cells and the thymic independent B cells (12, 52). Surface receptors, unique to each population, have been employed in the differentiation of these populations in man and a variety of research animals. B and T cell receptors have recently been reported for canine lymphocytes (5, 8, 96).

While sheep red blood cells are employed for rosette formation by human T lymphocytes, guinea pig and human erythrocytes have been found to form the highest percentage of spontaneous rosettes with canine T lymphocytes (8). In the present study, two markers of surface receptors were used to distinguish canine lymphocyte subpopulations in the peripheral blood, thoracic duct lymph and peripheral (renal hilar) lymph. The T cell population was detected by its ability to form spontaneous erythrocyte (E) rosettes with guinea pig red blood cells. Complement receptors on B lymphocytes were detected by using erythrocytes coated with antibody and complement. This complex forms EAC rosettes under the appropriate conditions.

The peripheral blood of the dog contains two distinct populations of lymphocytes (5, 8, 96). The thymic independent B cell population consists of cells with detectable surface immunoglobulin and complement receptors. Investigators have reported that 18-46% of peripheral blood lymphocytes possess surface immunoglobulin while 35-60% of the same cells also rosetted with EAC complex cells. Control values in this study were consistent with those found in the literature (Table 3). The percentage of EAC positive cells in canine peripheral blood were also found to be comparable to EAC rosette forming cells in human peripheral blood (Table 1). T cells comprise the second population of lymphocytes in canine peripheral blood and were detected in vitro by their formation of spontaneous rosettes with washed guinea pig erythrocytes. The published values of canine peripheral blood thymic dependent cells are 32-41%. Again the results of rosette tests performed in this laboratory were consistent with those

reported by others (Table 3). The Erythrocyte (E) rosette test had a high reproducibility and offered a convenient means by which the percentage of T lymphocytes in canine peripheral blood could be determined. The absolute number of B and T lymphocytes in the peripheral blood of hydronephrotic dogs did not differ significantly from the controls (Table 8).

The cellular content of the thoracic duct lymph in the anesthetized dog has been reported in the literature. A study by Haynes and Field (26) determined that the number of leukocytes present ranged from 500 - 12,250 cells/mm^3, the majority of which were mature lymphocytes (87%), with some large mononuclear cells (5.2%) and polymorphonuclear leukocytes (1.2%), whilst others (94) have reported a mean leukocyte count of 7,800 cells/mm^3. The mean leukocyte count of 9,125 cells/mm^3 in the control dogs of this study was consistent with the values in the literature.

The relative proportions of B and T lymphocytes in human and murine thoracic duct lymph have also been reported (19, 25, 46, 71, 81). However, to date there have been no reports on the percentage of T and B lymphocytes in canine thoracic duct lymph or renal hilar lymph. Using erythrocyte (E) and erythrocyte antibody complement (EAC) rosette techniques, the control values in canine thoracic duct were found to be 42% T cells and 14% B cells. The low EAC positive cell percentage was similar to the low values reported in human and murine thoracic ducts (Tables 1 and 2). In this laboratory, the thoracic duct lymph of the dogs was also found to contain a large number of blast cells. Jondal (32) found the sheep red blood cell (SRBC) receptors on human T lymphocytes to be present on both resting and blast-transformed (phytohemagglutinin stimulated) T cells.

There have been no data published concerning canine lymphoblasts and a more detailed study of these cells was not included in this study.

Lymph draining from the tissues which had not yet entered the first lymph node was termed peripheral lymph by Yoffey and Drinker (94). The cell content of this lymph has been studied from a variety of sources such as limbs, liver, testis, thyroid and kidney. Smith, McIntosh and Morris (80) concluded that the cell content of peripheral lymph was similar regardless of the tissue drained. Mature lymphocytes were the principle cells found (80%) in this lymph, with some macrophages (5-20%) and plasma cells also present. The presence of macrophages in the peripheral lymph distinguished it from central lymph, according to these authors and these phagocytic cells were thought to originate either from blood monocytes or tissue histiocytes. The same authors showed that the leukocyte count of renal peripheral lymph in conscious sheep ranged from 100-700 cells/mm^3 of which 75-85% were lymphocytes and 15-20% macrophages (80).

The peripheral (hilar) lymph of the kidney of control anesthetized animals was found to contain a mean of 50 cells/mm^3 of which 90% were lymphocytes and the remaining 10% were polymorphonuclear leukocytes, with an occasional monocyte. The proportion of E rosette forming cells present in the peripheral renal lymph of the control group was about 44%. This value corresponds to the mean percentage of T cells in the peripheral blood (45%) and the thoracic duct lymph (42%). The mean percentage of complement receptor EAC cells, marking a B cell population, in the renal hilar lymph of control animals was 28.5%. This B cell population correlates well with the 39.6% B cell population in the peripheral blood, but

differs significantly ($p<0.05$) from the B cell population in the thoracic duct lymph.

Lymphocytes that are not positive for either B or T cell markers have been referred to in the literature as 'null' cells (22, 34). In this investigation the percentage of null cells in the peripheral blood, thoracic duct lymph and renal hilar lymph did not differ in the control and experimental groups. The percentages of cells with no detectable E and EAC markers for each of the samples were: (1) 15% in peripheral blood; (2) 42% in thoracic duct lymph and (3) 28% in renal peripheral lymph. Several theories have been proposed to explain the existence of the null cell. These cells may result from insufficient rosetting techniques since rosettes are very sensitive to changes in temperature and pH. These cells may also represent a pool of immature T or B lymphocytes which have not developed their surface membrane receptors. In this study, the EAC rosettes only detected the subpopulation of the B cell population with complement receptor sites and no single receptor on B lymphocytes has proven sufficient to mark the entire population.

Obstruction of the free urine flow from the kidney caused a pathological state known as hydronephrosis. Depending on the length of the obstruction, several progressive changes became noticeable. Grossly, the kidney increased in size with accumulation of urine in the renal pelvis. Externally, there was a dilatation of capsular lymphatics and engorgement of the capsular blood vessels. As the vessels of the capsular lymphatic system increased in diameter, there was a progressive decrease in the caliber of the hilar vessels. Holmes, et al. (30) have reported

connections between the two lymphatic systems in the hydronephrotic kidney and showed, as did the present study, that after one week of obstruction the hilar system is almost completely collapsed, with a diversion of renal lymph flow from the hilar to the capsular system. Early observable histological changes in hydronephrosis included marked congestion of glomerular tufts, distortion of the capsular spaces and dilatation of the tubules, especially those of the collecting system. Changes which occurred after one week of obstruction showed cloudy swelling of tubular cells, fibrosis of the interstitial tissue and a 'round cell' infiltration (20). Smith, McIntosh and Morris (80) in 1970, observed that within 24-48 hours of ligation of the ureter in sheep, the cell content of the renal lymph began to increase and reached a maximum after three weeks of a 50-100 fold increase over the control values. This increased cell content was comprised of both lymphocytes (95%) and macrophages (5%). The cortex and corticomedullary tissue were found to be extensively infiltrated with lymphocytes, these cells forming dense aggregations beneath the capsule, around the glomeruli and throughout the connective tissue. These authors concluded that the increased number of lymphocytes in peripheral lymph reflected this infiltration of the hydronephrotic kidney without evidence of any immunologic reaction.

The leukocyte counts from the peripheral blood in the hydronephrotic group indicated a slight neutrophilia. This increase in the circulating leukocyte pool may reflect a bacterial infection or inflammation from the previous surgery, or tissue necrosis or uremia due to the obstructed free flow of urine. In two to five days the leukocyte count in the

renal hilar lymph increased 10-12 times over the control values, but the differential counts showed a marked increase in the number of neutrophils, indicative of acute inflammation. However, there was a 9 fold increase in the absolute number of lymphocytes in the renal lymph of the hydronephrotic dogs. The hydronephrotic and control animals contained similar proportions of T and B lymphocytes in the peripheral blood, thoracic duct and renal peripheral lymph.

The increased renal lymph flow in short term ureteric obstruction has been described by other authors (48, 61, 80, 86). It was once believed (11, 17, 20, 86) that the renal lymphatics of the hydronephrotic kidney acted as safety valves by absorbing urine from the renal pelvis. O'Morchoe et al. (27, 29) have shown that the composition of the renal lymph differs widely from that of the urine in the pelvis, and they concluded that the increased lymph flow is probably due to alterations in the renal hemodynamics. The findings of this study are consistent with the idea that increased lymph flow is related to the changes produced by the increased pressure on the circulation in the kidney parenchyma. The round cell infiltration in hydronephrosis is probably not due to an immunologic change, but reflects an inflamed area where tissue necrosis is also taking place. This is reflected in the 10-12 fold increase in the cell content in the renal hilar lymph especially with an increase in the percentage of neutrophils. The 9 fold increase in absolute number of B and T lymphocytes indicated a significant change in the lymphocytic traffic in the renal hilar lymph draining a hydronephrotic kidney.

CONCLUSIONS

The general purpose of this study was to provide information on the movement of lymphocytes from the peripheral tissues to a regional lymph node. The following conclusions were drawn from the data collected in this endeavor:

(1) The Erythrocyte (E) and Erythrocyte Antibody Complement (EAC) rosette techniques provided a reliable and reproducible means of detecting two populations considered to be canine T and B lymphocytes, respectively, from the peripheral blood, thoracic duct lymph and renal hilar lymph.

(2) Most T lymphocytes, by definition, form spontaneous rosettes when mixed with washed erythrocytes. In this study, EAC rosettes were used as a marker of B cells. It is important to realize that cells with complement receptors may form a subpopulation of the total B cells but is the most reliable test at the present time.

(3) In canine peripheral blood from both the control and experimental groups, the mean percentages of rosette forming cells with T markers were 45%, while 40% possessed B markers and 15% were null cells. In the canine thoracic duct lymph, the mean percentage of T positive cells was 42%, while 14% had detectable complement receptors (B cells) and 44% were null cells. These values did not change in the hydronephrotic group.

(4) The mean leukocyte content in the renal hilar lymph of control animals was 50 cells/mm^3, which were largely (90%) lymphocytes with the

remaining cells (10%) either neutrophils or monocytes. After ureteric obstruction of two to five days' duration, the cell content of renal lymph increased 10-12 times (mean of 625 cells/mm^3). However, differential counts showed that there were now 40% neutrophils and 60% lymphocytes. There was a 9 fold increase in the absolute number of B and T lymphocytes in hydronephrosis.

(5) The relative proportions of T and B cells were not different in the control and experimental groups. The rosette forming cells were 44% positive for T cell markers, 28% positive for B cell markers and 28% null cells.

(6) There was a significant difference ($p<0.05$) found in comparing the B cell population in the three sample sources studied. The mean percentages of complement binding cells in the peripheral blood (39.6%) and renal hilar lymph (28.0%) were markedly different than the 14.2% of EAC positive cells in the thoracic duct lymph.

(7) Several noticeable histological changes occur in the first five days of ureteric obstruction including marked congestion of the glomerular tufts, distortion of the glomerular capsular spaces, dilatation of the tubules and an infiltration of the renal parenchyma by inflammatory cells, especially 'round cells' (lymphocytes). This infiltration of the kidney by inflammatory cells is reflected in the 10-12 fold increase in the cell content of the renal hilar lymph. In the first five days there is an increase in the percentage of neutrophils indicating an acute inflammatory process. The mean percentages of B and T lymphocytes in the hydronephrotic group did not differ from values obtained from the

control group, but there was a 9 fold increase in the absolute lymphocyte traffic.

(8) The probable cause of increased flow and increased cell content of the renal lymph in hydronephrosis is due to hemodynamic changes. Pressure from the accumulating urine in the renal pelvis compresses both blood vessels and renal parenchyma causing hypoxia with eventual cell destruction. The inflammatory cells respond to this inflammation.

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LIST OF ABBREVIATIONS

SRBC	SHEEP RED BLOOD CELL
EAC	ERYTHROCYTE ANTIBODY COMPLEMENT
SIg	SURFACE IMMUNOGLOBULIN
N.D.	NOT DETERMINED
n.s.	NOT STATED
HEAC	HUMAN ERYTHROCYTE ANTIBODY COMPLEMENT
HTLA	HUMAN THYMIC LYMPHOCYTE ANTIBODY
Imm-flu	IMMUNOFLUORESCENT STAINING
GPRBC	GUINEA PIG RED BLOOD CELL
ATG	ANTI-THYMIC GLOBULIN
MGP	METHYL GREEN PYRONIN
S.E.	STANDARD ERROR
WBC	WHITE BLOOD CELL
RFC	ROSETTE FORMING CELL

TABLE 1

HUMAN PERIPHERAL BLOOD LYMPHOCYTES

REFERENCES		% T cell	TEST	% B cell	TEST
Jondal	1972	68.09	SRBC	30.18 27.6	EAC SIg
Papamicheal	1972	29.0	SRBC	20-30	n.s.
Bentwich	1973	77.1	SRBC	20-30	anti-Ig
Mendes	1973	55.4	SRBC	14.4	HEAC
Brown,Greaves	1974	68.0	SRBC	29.6	n.s.
Dickler	1974	77.5 75.8	SRBC SRBC	20.2 15.6	SIg EAC
Jønsson	1974	60.6	SRBC	27.5	EAC
Edelson	1974	65.0 84.0	SRBC HTLA	12.0 20.0	EAC SIg
Whiteside	1975	48.98	SRBC	16.76	EAC
Kunkel	1975	82.0	SRBC	7.8 11.1	IgD IgM
Rowland	1975	42-74	n.s.	15-30	n.s.
Birkeland	1975	63.0	SRBC	13.5	HEAC
Schafer	1975	62.0	SRBC	N.D.	

TABLE 2

MURINE PERIPHERAL BLOOD LYMPHOCYTES

REFERENCES		% T cell	TEST	% B cell	TEST
Raff	1971	70.0	Θ	33.0	MBLA
Balch	1974	55.0	horse antisera to TDL		N.D.
Goldschneider	1973	61.0	n.s.	40.0	n.s.

MURINE THORACIC DUCT LYMPHOCYTES

REFERENCES		% T cell	Test	% B cell	Test
Raff	1971	85.0	Θ	14.0	MBLA
Sprent	1973	82.0	anti Θ C3H	16.0	anti FyG
Goldschneider	1973	87.0	n.s.	11.0	n.s.
Mandel	1974	60.0	LyA	15.0	k
		75.0	MSLA	10.0	u

TABLE 3

CANINE PERIPHERAL BLOOD LYMPHOCYTES

REFERENCES		% T cell	TEST	% B cell	TEST
Beall	1974	36.7± 4.4	HRBC	18.4±2.9	Imm-flu
Zander	1975	38.4	HRBC	45.0	EAC
		68.0	ATG	30.5	Ig
Bowles	1975	39.8±7.6	HRBC	35-60	EAC
		32.4	GPRBC		
	serial dog	41.4	HRBC	49.3	EAC
				46.6	Imm-flu

TABLE 4

MEAN PERCENTAGES OF ROSETTE FORMING CELLS IN CONTROL DOGS

	ERYTHROCYTE ROSETTES			EAC ROSETTES		
Dog#	Blood	Thoracic Duct	Renal	Blood	Thoracic Duct	Renal
2	39.2	--	--	--	--	--
88	41.3	15.5	--	--	--	--
192	--	--	--	57.5	6.2	--
417	--	24.0	--	--	15.5	30.0
416	--	--	57.0	--	--	17.0
464	36.2	55.2	--	56.6	34.7	--
561	47.0	61.9	47.5	--	--	--
500	54.3	--	--	45.7	--	--
595	51.7	60.6	63.8	45.4	16.8	20.8
594	40.2	37.9	50.6	20.0	4.7	37.7
623	38.0	42.6	48.2	12.1	1.9	30.4
613	52.9	13.7	--	47.6	20.3	40.8
647	35.3	74.0	32.5	25.2	20.4	29.1
645	62.4	--	54.5	57.3	--	42.9
569	59.8	53.6	39.3	48.9	17.8	--
738	--	56.7	--	--	14.0	--
739	36.0	26.0	36.1	25.9	11.9	28.0
718	42.5	32.1	25.3	35.9	10.0	8.2
577	37.0	40.1	27.3	36.6	10.5	--
870	--	32.7	--	--	--	--
MEAN	44.9	41.8	43.8	39.6	14.2	28.5
S.E.	2.4	4.7	3.8	4.2	2.3	3.4

TABLE 5

A COMPARISON OF FRESH AND PERMANENT (MGP) MOUNT ROSETTE PREPARATIONS

ERYTHROCYTE ROSETTES

Dog#	Blood T		Thoracic duct T		Renal T	
	Fresh	MGP	Fresh	MGP	Fresh	MGP
577	39.8	39.7	25.8	40.8	--	--
	34.5	41.8	28.5	32.0	--	--
	39.5	34.3	24.9	46.7	--	--
	40.9	29.2	28.6	38.4	--	--
	38.9	42.5	27.2	42.9	--	--
561	46.4	47.3	--	--	--	--
623	55.5	33.9	--	--	50.4	42.0
717-H	--	--	--	--	61.4	62.3

TABLE 6

MEAN PERCENTAGES OF ROSETTE FORMING CELLS IN HYDRONEPHROTIC DOGS

		ERYTHROCYTE ROSETTES			EAC ROSETTES		
Dog#	Day	Blood	Thoracic Duct	Renal	Blood	Thoracic Duct	Renal
821	2	54.6	60.1	38.3	--	--	--
911	2	37.6	35.1	26.9	75.8	9.0	10.5
908	3	37.4	57.1	22.5	47.5	31.4	41.6
910	3	38.7	40.7	53.8	23.3	10.5	--
717	4	40.5	46.9	55.7	50.7	8.7	45.0
914	4	39.5	56.5	59.1	37.4	13.9	34.4
940	4	63.4	58.4	64.0	21.8	11.4	8.5
943	5	39.2	49.1	28.5	--	--	--
MEAN		43.9	44.9	43.6	42.8	14.2	28.0
S.E.		3.4	6.7	5.8	8.2	3.5	7.8

TABLE 7

MEAN PERCENTAGES AND RANGES OF ROSETTE FORMING CELLS

	CONTROL		HYDRONEPHROSIS	
	MEAN ± S.E.	RANGE	MEAN ± S.E.	RANGE
Blood T	44.9±2.4	(35.3-62.4)	43.9±3.4	(37.4-63.4)
Thoracic T duct	41.8±4.7	(13.7-61.9)	44.9±6.7	(4.1-60.1)
Renal T	43.8±3.8	(25.3-63.8)	43.6±5.8	(22.5-64.0)
Blood B	39.6±4.2	(12.1-57.5)	42.8±8.2	(21.8-75.0)
Thoracic B duct	14.2±2.3	(1.9-34.7)	14.2±3.5	(8.7-31.0)
Renal B	28.5±3.4	(8.2-42.9)	28.0±7.8	(8.5-45.0)

TABLE 8

A COMPARISON OF THE MEAN PERCENTAGES OF ROSETTE FORMING LYMPHOCYTES AND THE ABSOLUTE NUMBER OF LYMPHOCYTES IN CONTROL AND HYDRONEPHROTIC DOGS

PERIPHERAL BLOOD

	MEAN WBC ($cells/mm^3$)	MEAN % RFC	ABSOLUTE # LYMPHOCYTES ($cells/mm^3$)
CONTROLS	11,020	T-44.9%	T- 1,480
		B-39.6%	B- 1,320
HYDRONEPHROSIS	16,700	T- 43.9%	T- 1,170
		B- 42.8%	B- 1,140

RENAL LYMPH

	MEAN WBC ($cells/mm^3$)	MEAN % RFC	ABSOLUTE # LYMPHOCYTES ($cells/mm^3$)
CONTROLS	50	T- 43.8%	T- 20
		B- 28.5%	B- 13
HYDRONEPHROSIS	650	T- 43.6%	T- 170
		B- 28.0%	B- 110

Figure 1

Bar graph of the percentages of rosette forming cells in control dogs.

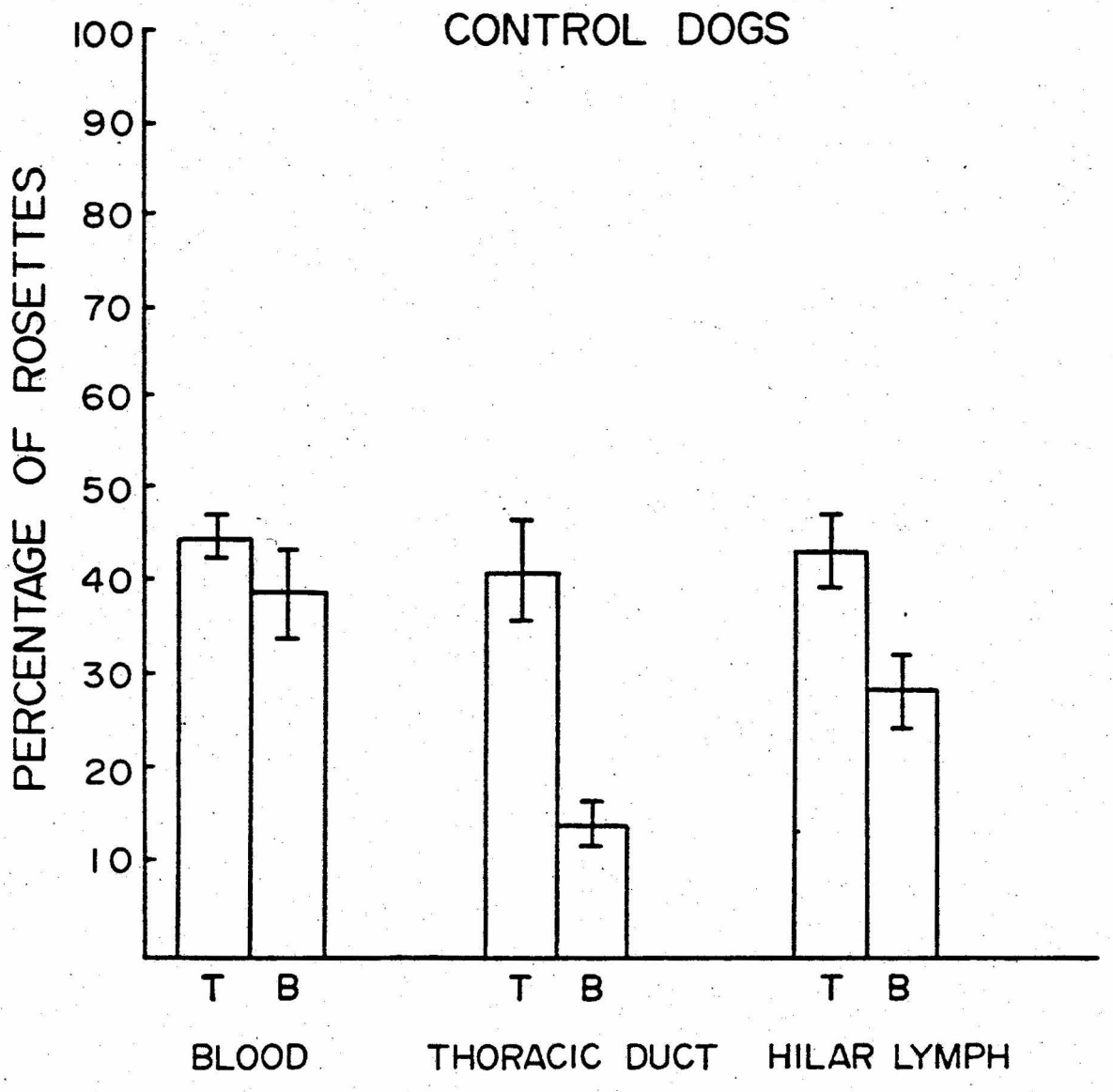
CONTROL DOGS
PERCENTAGE OF ROSETTES
100
90
80
70
60
50
40
30
20
10
T B
T B
T B
BLOOD
THORACIC DUCT
HILAR LYMPH

FIGURE 1

Figure 2

Comparison of the T and B lymphocyte distribution in control dogs.

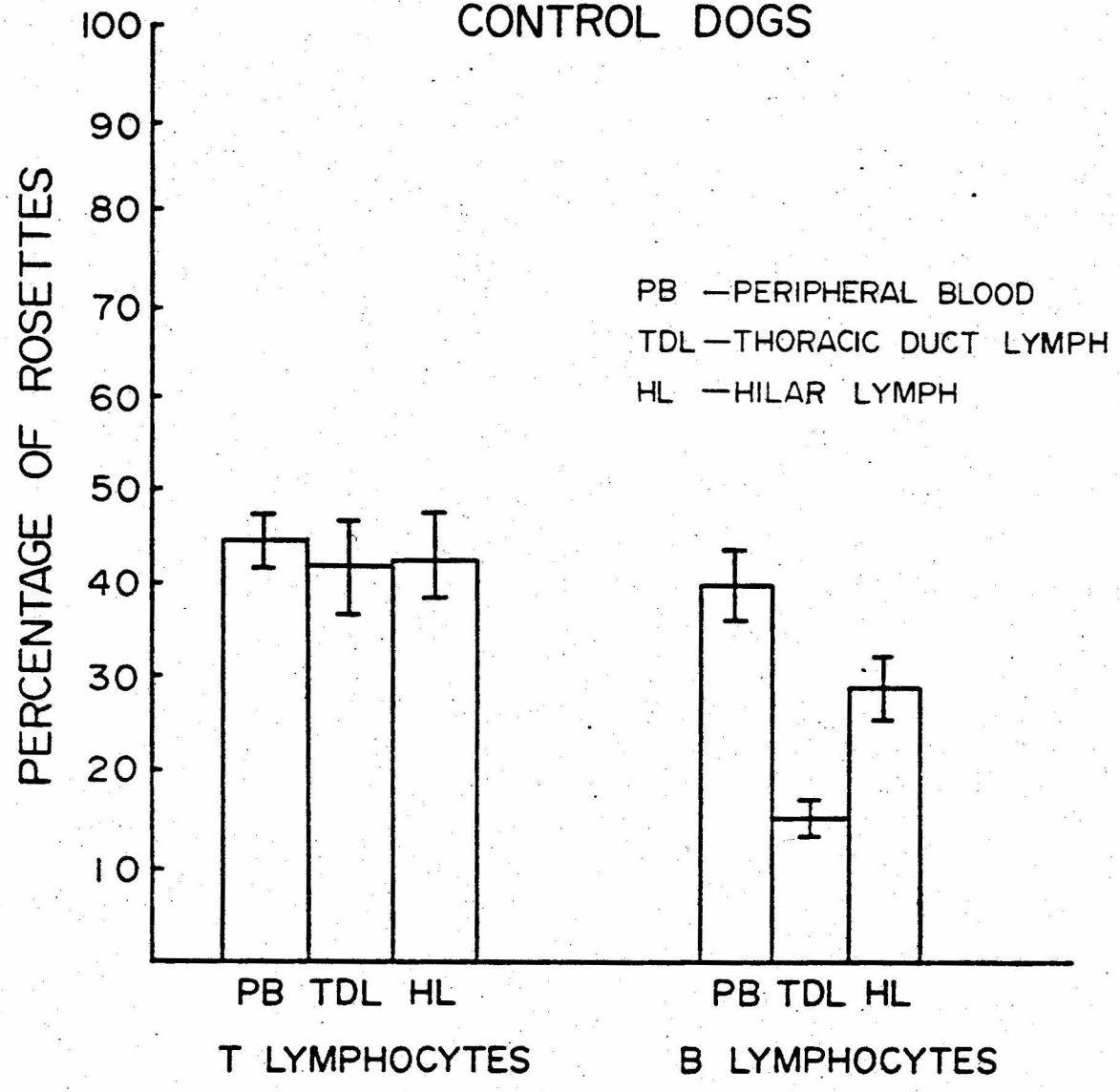
CONTROL DOGS
PERCENTAGE OF ROSETTES
100
90
80
70
60
50
40
30
20
10
PB —PERIPHERAL BLOOD
TDL—THORACIC DUCT LYMPH
HL —HILAR LYMPH
PB TDL HL
T LYMPHOCYTES
PB TDL HL
B LYMPHOCYTES

FIGURE 2

Figure 3

Comparison of the percentages of T and B lymphocytes in control and hydronephrotic dogs.

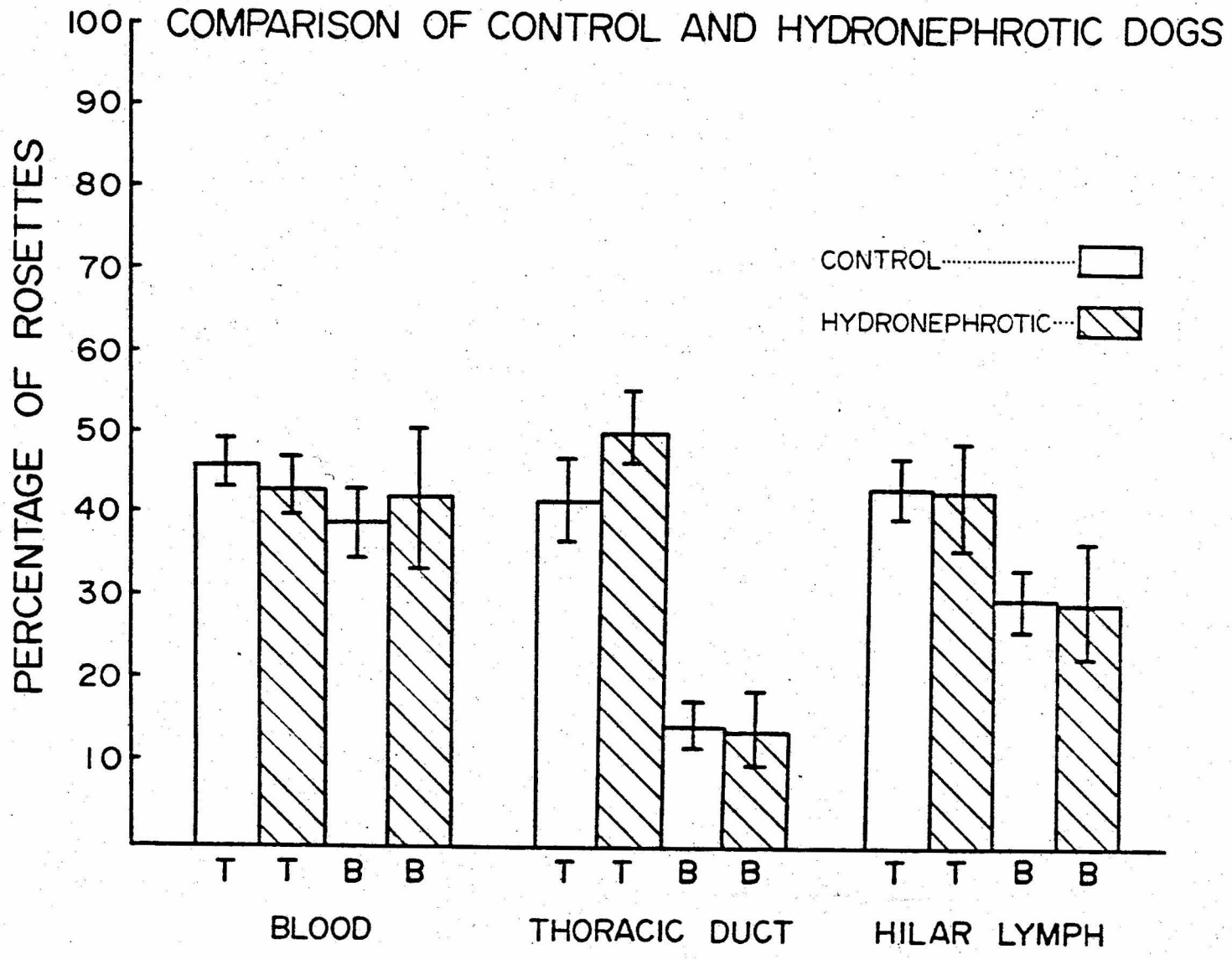
COMPARISON OF CONTROL AND HYDRONEPHROTIC DOGS
PERCENTAGE OF ROSETTES
100
90
80
70
60
50
40
30
20
10
CONTROL
HYDRONEPHROTIC
T T B B
BLOOD
T T B B
THORACIC DUCT
T T B B
HILAR LYMPH

FIGURE 3

FIGURE 4

A canine lymphocyte (1) which has formed a rosette with some of the surrounding erythrocytes. This is from a permanent mount stained with methyl green pyronin (MGP). Magnification 400x.

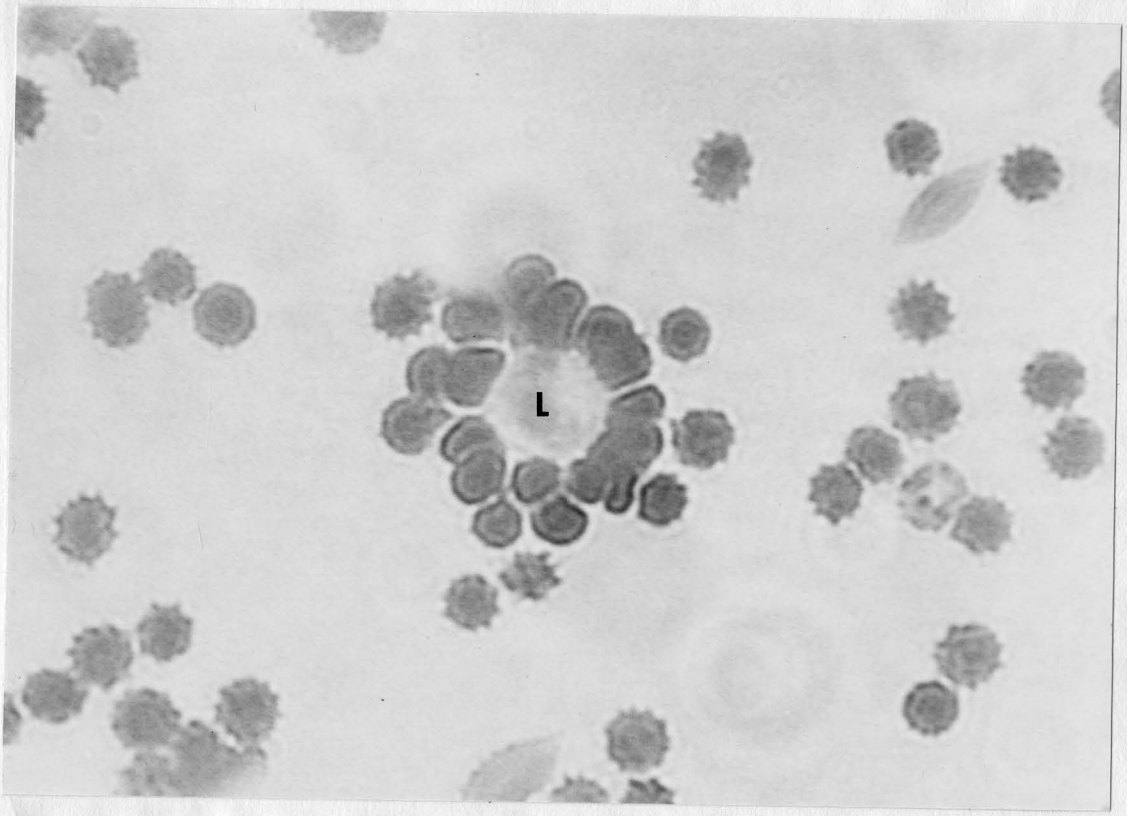

FIGURE 4

FIGURE 5

A view of the renal cortex from a control animal showing the normal structure of the glomeruli (G) and the renal tubules (RT). Magnification 63x.

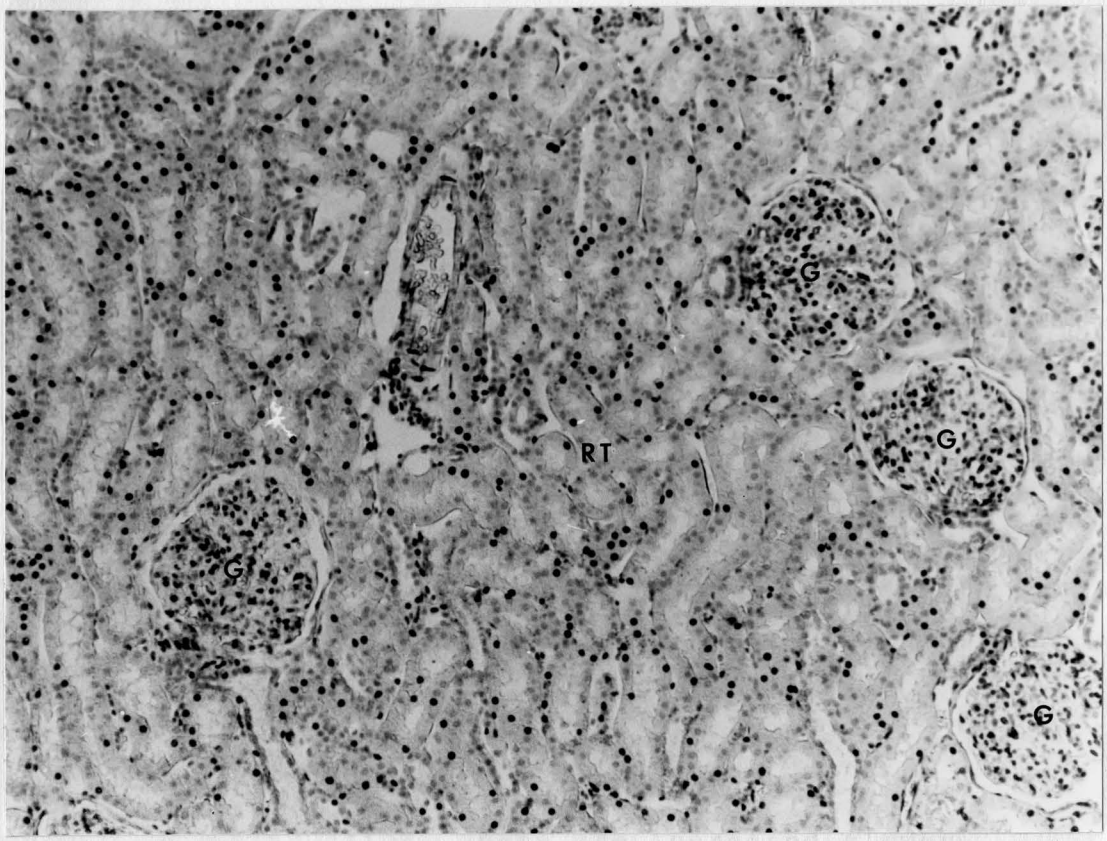

FIGURE 5

Figure 6

The cortex of a hydronephrotic kidney from an animal with a 5 day ureteric obstruction. There are increased glomerular capsular spaces (G) and dilatation of the renal tubules (RT).
Magnification 25x.

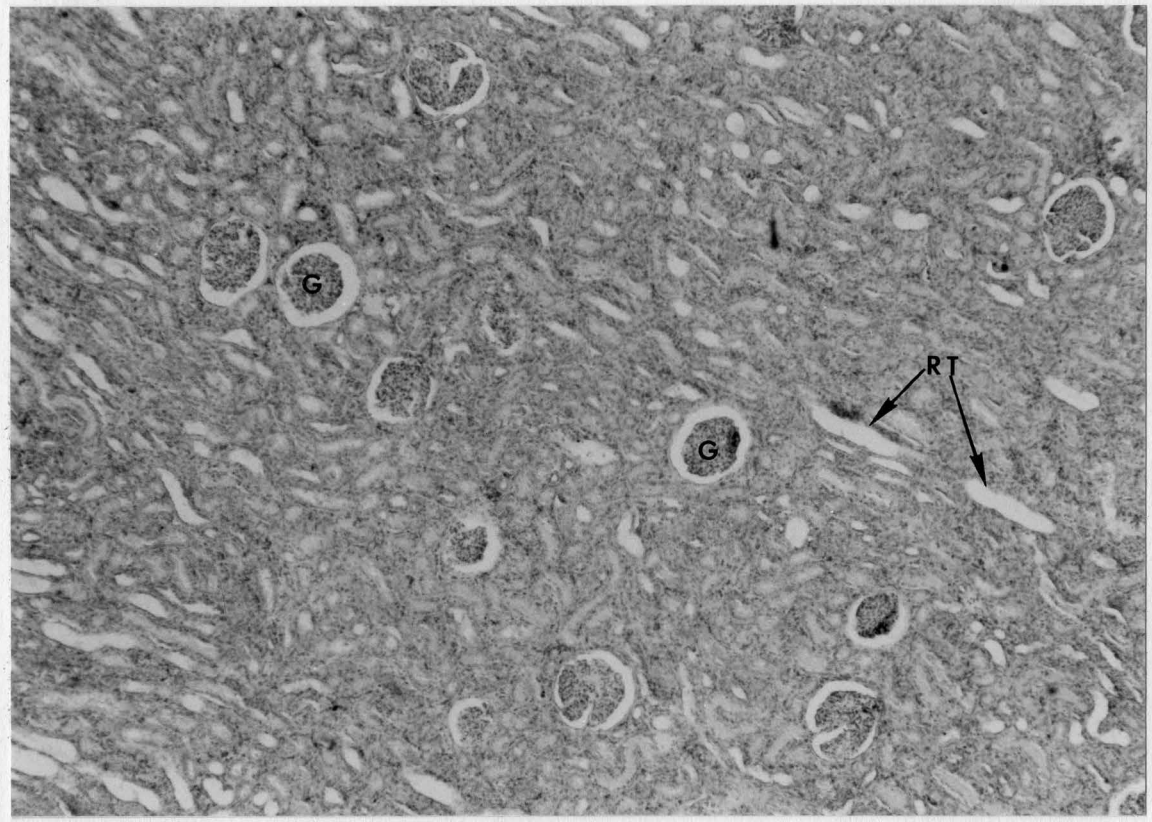

FIGURE 6

FIGURE 7

The glomerulus (G) from a 5 day hydronephrotic kidney showing a marked increase in the glomerular capsular space (CS).

Magnification 160x.

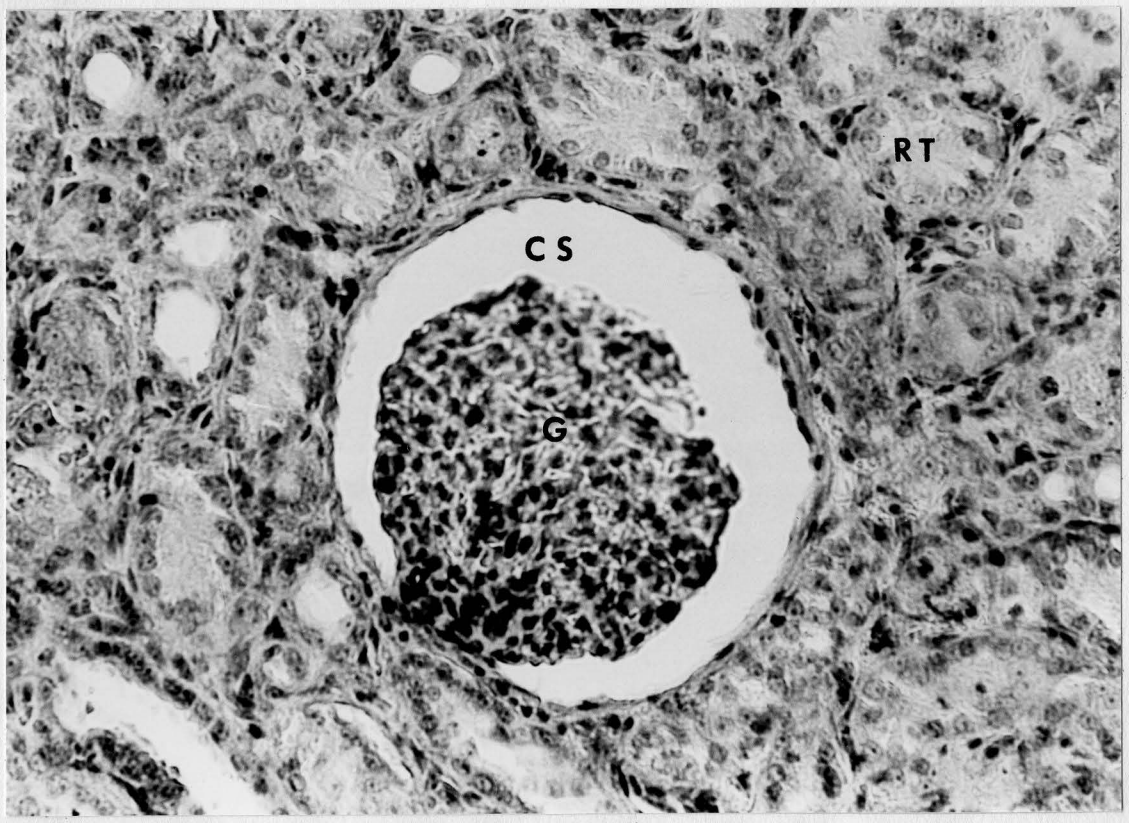

FIGURE 7

FIGURE 8

The cortex of a 4 day hydronephrotic kidney showing dilatation of the venules (V) and several glomeruli (G).

Magnification 63x.

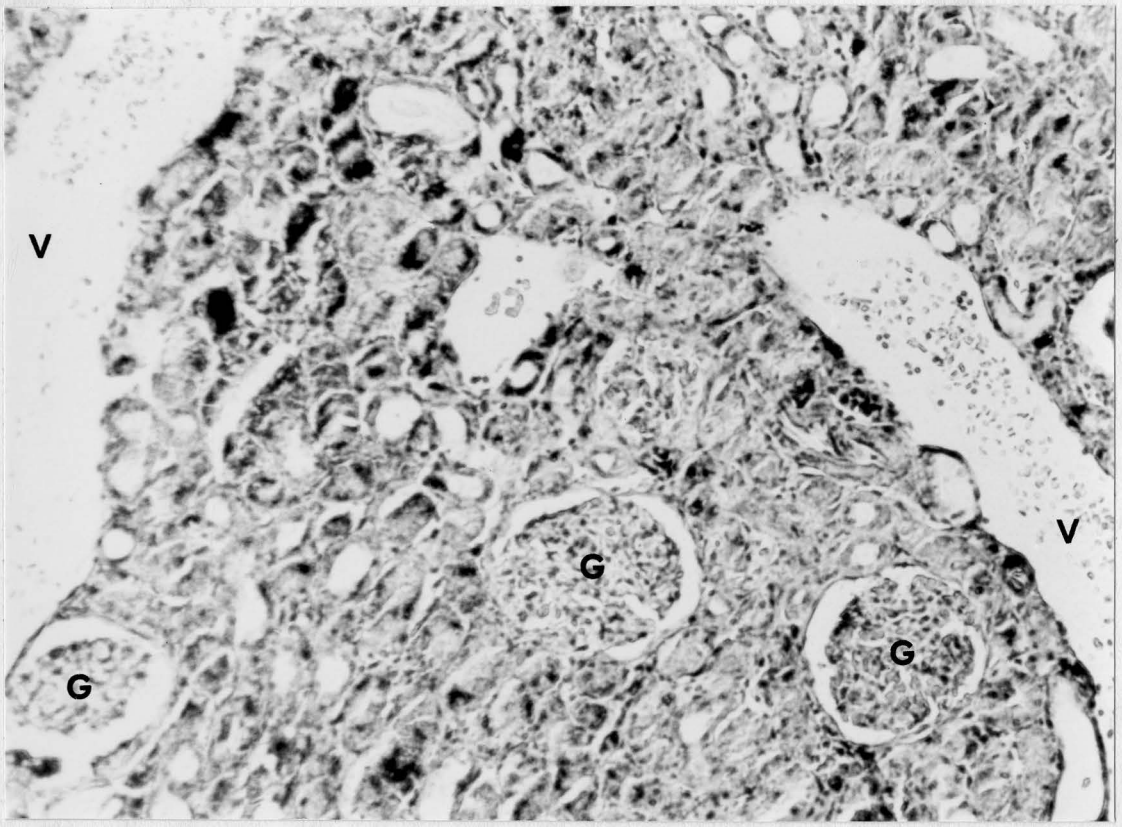

FIGURE 8

FIGURE 9

The renal medulla from a 5 day hydronephrotic kidney showing interstitial accumulations of round cells (lymphocytes) at the arrows. Magnification 63x.

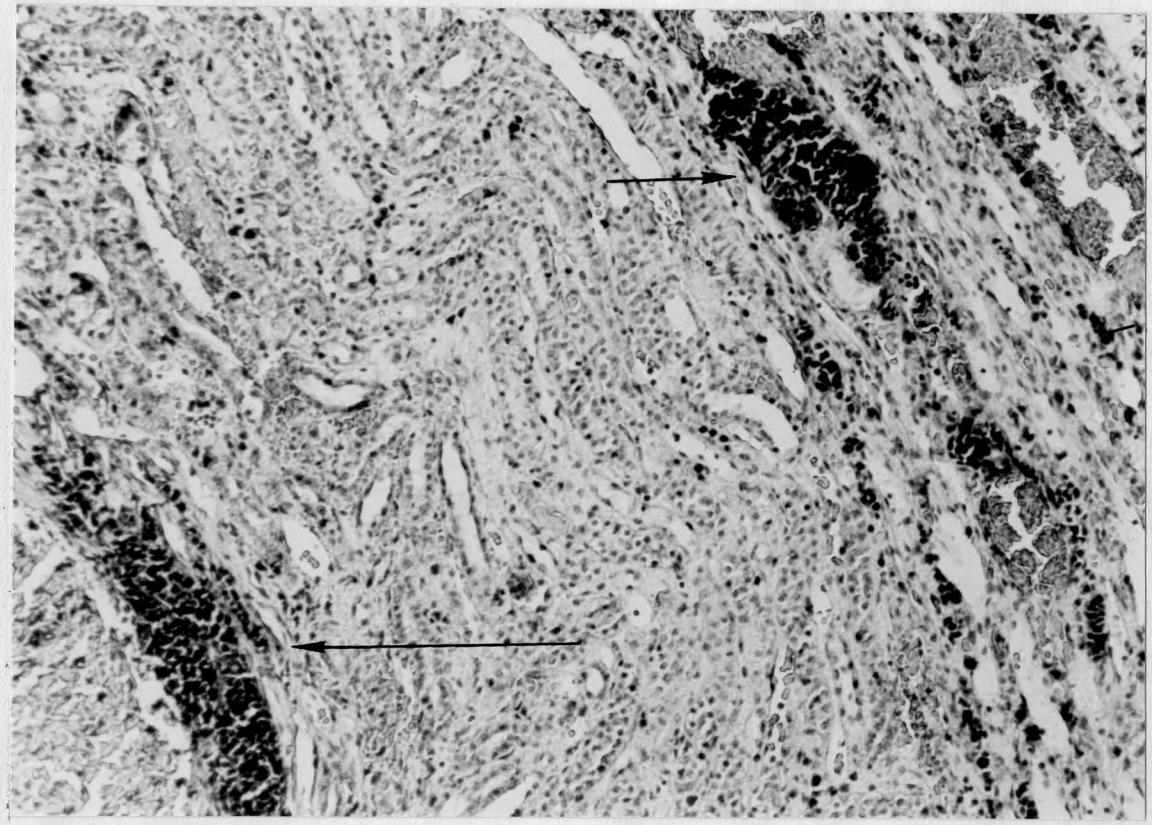

FIGURE 9

APPROVAL SHEET

The thesis submitted by John P.Ferron has been read and approved by the following Committee:

Patricia J. O'Morchoe, M.D., Chairman

C.C.C. O'Morchoe, M.D., Ph.D.

Leslie Emmert, Ph.D.

John Robinson, M.D.

The final copies have been examined by the Chairman of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to form and content.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science in Anatomy.

May 9, 1977

Date

Patricia J. O'Morchoe

Chairman's signature