ADRENERGIC REGULATION OF SPLENIC FUNCTIONS IN NEONATAL PIGS

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(ABSTRACT)

The purpose of this study was to assess adrenergic control of splenic hemodynamic function and oxygen metabolism in neonatal pigs (NP). Seventeen piglets, 28-45 days of age, were anesthetized with pentobarbitol (30 mg/kg, i.p.) and prepared for measurement of splenic venous outflow. Simultaneous arterial and venous blood samples were analyzed for O_2 content and hematocrit (Hct). Splenic oxygen consumption and extraction were calculated. The effects of adrenergic stimulation on splenic leukocyte migration and proliferation were also assessed. Fluorescence histochemistry of the spleen from NP revealed noradrenergic innervation of the vasculature taken from the hilar portions of the spleen. Norepinephrine (NE) infusion , (2μg/kg/min) caused a significant decrease in splenic venous outflow ($P< 0.01$) with a concomitant significant increase in splenic resistance ($P< 0.005$). Splenic leukocyte migration and proliferation did not change significantly during NE infusion, but the splenic venous Hct was significantly increased ($P< 0.001$). Similar changes were observed with electrical stimulation of the splenic nerve. Pretreating the NP with beta-adrenoceptor blocker, propranolol (1 mg/kg), had no significant effect on these responses. In contrast, these responses were abolished with the addition of alpha-adrenoceptor blocker, phentolamine (1 mg/kg). Splenic O_2 metabolism did not change significantly during nerve stimulation, but splenic venous Hct was significantly increased ($P< 0.05$). These responses were not altered by the adrenoceptor blockade.

It is concluded that activation of the adrenergic system of the spleen causes a significant decrease in splenic venous outflow with a concomitant increase in splenic vascular resistance and this is largely mediated by the activation of the

alpha-adrenoceptor system. Adrenergic stimulation of the spleen did not influence splenic oxygen metabolism in piglets. This may relate to the high red blood cell storage and O_2 availability of the spleen and the low oxygen demand of the organ.

DEDICATION

For James,

"Deep greens and blues were the colors he chose, won't you let them go down in his dreams....rock-a-bye sweet baby James". J.T.

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CHAPTER I

INTRODUCTION

The spleen is the largest of the lymphoid organs that is directly connected to the general circulation. This organ is important in servicing many of the body's needs including: hematopoiesis, antibody production, blood filtration, and reservoir functions as well. The anatomical dissimilarities that exist between the spleens of different species are reflected in the variation in its primary function in these species (11). The size, weight and shape of the spleen vary greatly between different species as well as within the same species under different conditions. The spleen is considerably larger in those individuals who are well fed while it is smaller in those who are malnourished (42). The spleen also enlarges greatly in response to fever and infection while it decreases to up to 1/2 its original size as the human body ages (18).

A. PRINCIPLE AIMS

For years, medical science has been investigating the effects of stress on the human body. Various stresses have been shown to have a deleterious effect on various animal models. This is particularly true in neonates. More recently, a functional link between the nervous and immune systems has been studied extensively. The nervous system can influence the immune system and modulate the cellular function of lymphoid tissue. The affects of adrenergic control of lymphocyte activity have been extensively studied. However, the autonomic control of splenic hemodynamic and metabolic functions has been investigated to a lesser degree. The purpose of this study was to investigate the effects of adrenergic control of splenic hemodynamic and metabolic functions in neonatal pigs. In addition, the adrenergic affects of lymphoid activity in the spleens of these pigs also was assessed.

B. A REVIEW OF THE LITERATURE

1. COMPARATIVE ANATOMY AND PHYSIOLOGY OF THE SPLEEN

a. Gross anatomy of the spleen

The human spleen is soft, highly vascular, of sponge-like consistency and bright red to purple in color. It is oblong and flat and often said to resemble the shape of a coffee bean (fig. 1). The human spleen ranges between 12.7-15.2 cm. in length, 5.1-7.6 cm. in breadth and 2.5-3.8 cm. thick with the average weight of 1 Kg. (18). The canine spleen is bright red in color, long and flat, resembling the shape of a dumbbell (35). (Fig. 2). The size and weight of canine spleen vary greatly with the breed and size of the dog. The canine spleen is approximately 9.7-24 cm. long, 2.5-4.6 cm. wide, and 8-147 gm. in weight (35). The splenic capsule of the equine spleen is quite thick and therefore the spleen is more bluish in color than red. The equine spleen is falciform in shape being widest at the base and pointed at its ventral end (35). (Fig. 3). The equine spleen is approximately 40-67 cm. in length, 17-22 cm. in width at its widest part, 2-6 cm. thick with an average weight of 950-1680 gm. (35). The color of the bovine spleen varies with the sex and age of the animal. The spleen is reddish brown to bluish red in calves, bluish gray in cows, and dark red to reddish brown in steers (35). (Fig. 4). The bovine spleen is long and flat, and approximately 50 cm. in length, 15 cm. in width, 2-3 cm. thick with an average weight of 900 gm. (43). The porcine spleen is bright red in color and long and narrow in shape (35). (Fig.5). The porcine spleen is approximately 24-45 cm. long, 3.2-12.5 cm. wide, with an approximate weight of 90-335 gm. (35). The spleen in the chicken is brownish red and spherical (fig. 6). It is approximately 1.5 cm. in diameter and weighs approximately 3 gm. in the female and 4.5 gm. in the male (43). The size and shape of the spleen of the turkey is similar to that of the chicken while it is smaller in size and more triangular in shape in the duck and goose (10).

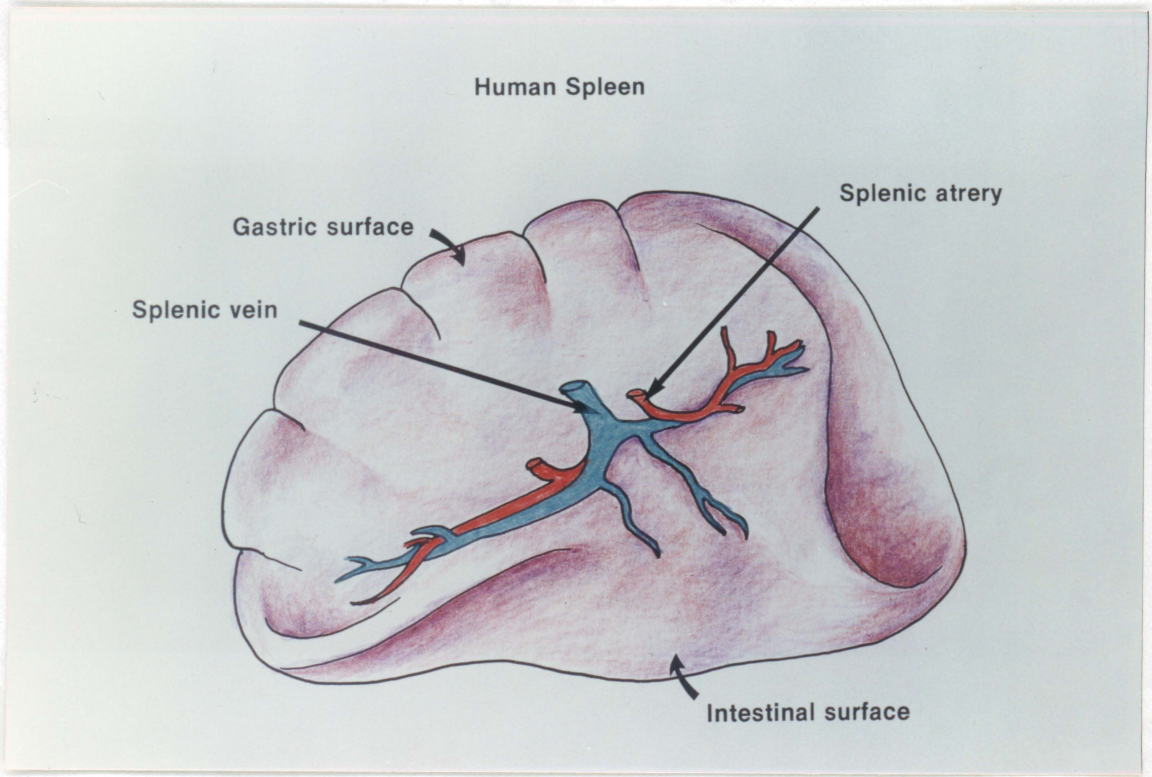

Figure 1 illustrates the visceral surfaces of the human spleen (gastric and intestinal). The human spleen contains arteries (red) and veins (blue) entering and exiting the spleen at the hilus. Although not illustrated here, the human spleen also contains a splenic nerve which accompanies the vessels of the spleen at the hilus. The human spleen is reddish purple in color and resembling the shape of a coffee bean. (Modified from Gray. Gray's Anatomy. Lea & Fibiger, Philadelphia, 1985.)

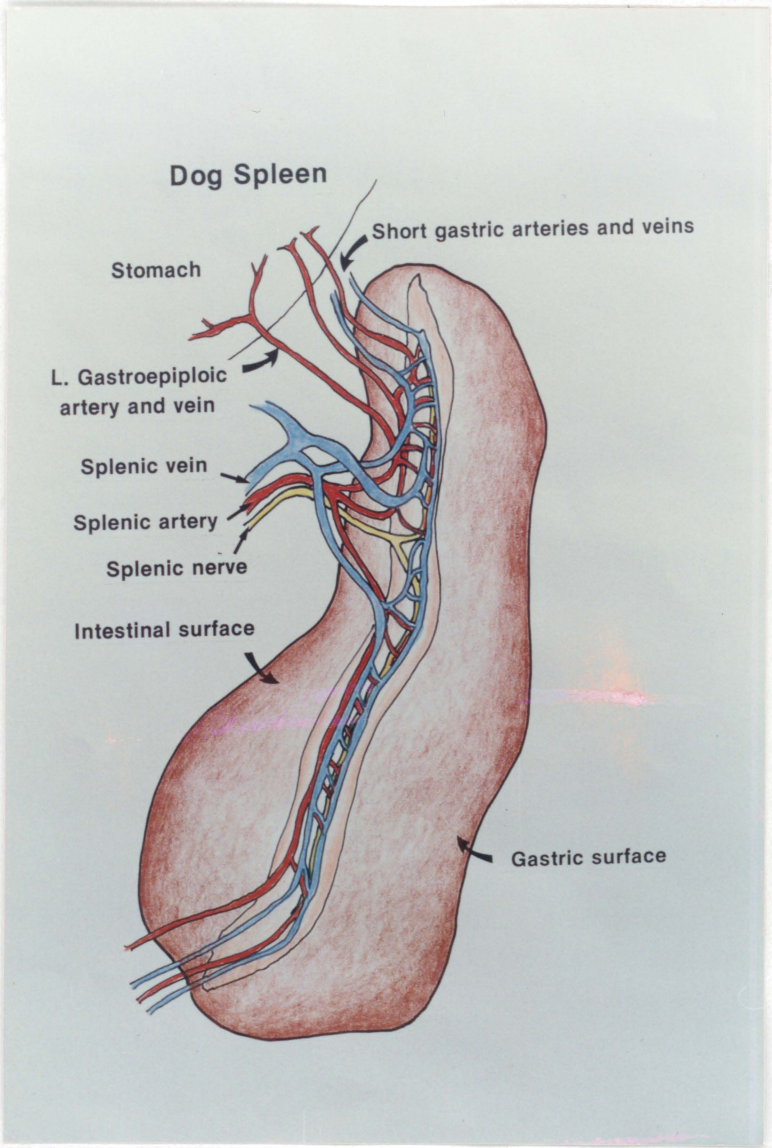

Figure 2 illustrates the visceral surfaces of the canine spleen (gastric and intestinal). The canine spleen contains arteries (red) and veins (blue) entering and exiting the hilus. The splenic nerve (yellow) also enters the canine spleen at the basal end of the hilus. The short gastric arteries and the left gastroepiploic artery supply the ventral extremity of the canine spleen, while the short gastric veins and left gastroepiploic vein drain this extremity. The canine spleen is reddish brown in color and shaped like a dumbbell, being widest at its basal and ventral ends and narrower in its mid-section. (Modified from Nickel and Sack. Viscera of Domestic Animals. Springer Verlag, New York, 1979.)

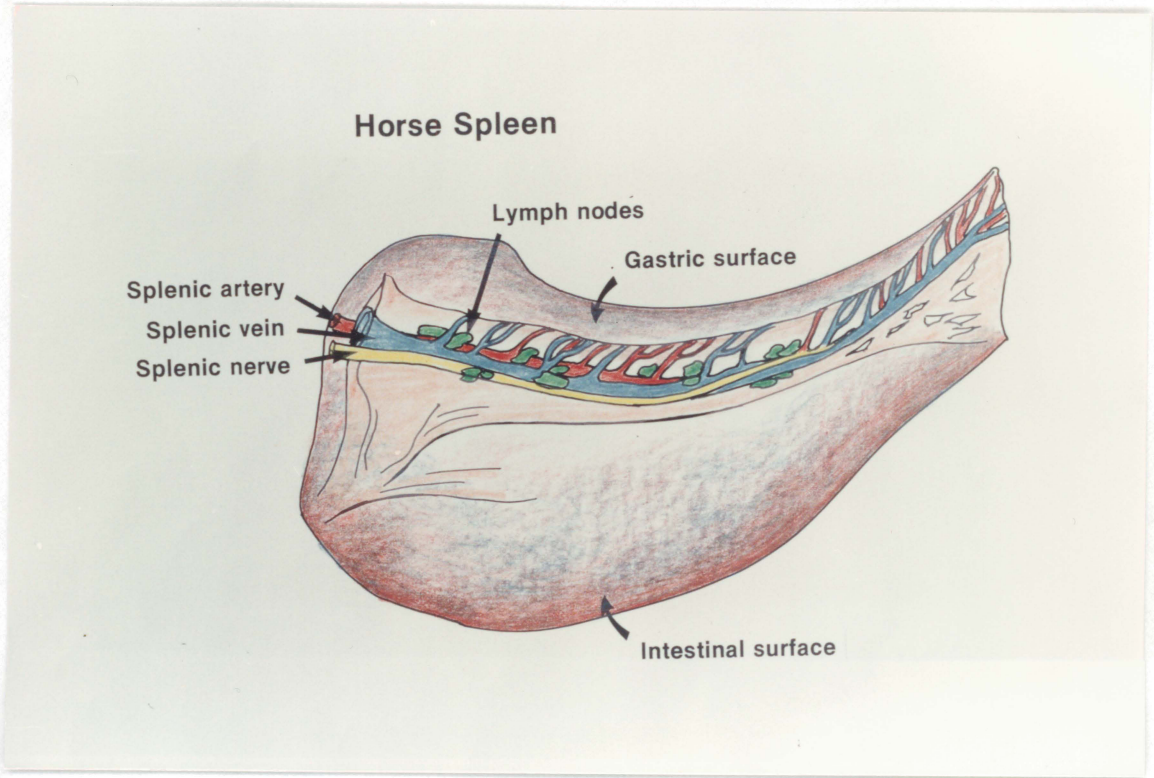

Figure 3 illustrates the visceral surfaces of the equine spleen (gastric and intestinal). Splenic arteries (red), splenic veins (blue) and the splenic nerve (yellow) enter the equine spleen at the basal end of the hilus and branch toward the ventral extremity. Splenic associated lymph nodes (green) are found accompanying the vessels along the hilus. The equine spleen is bluish red in color due to the thickness of its capsule. The equine spleen is falciform in shape, being widest at its basal extremity and narrower at its ventral extremity. (Modified from Nickel and Sack. Viscera of Domestic Animals. Springer Verlag, New York, 1979.)

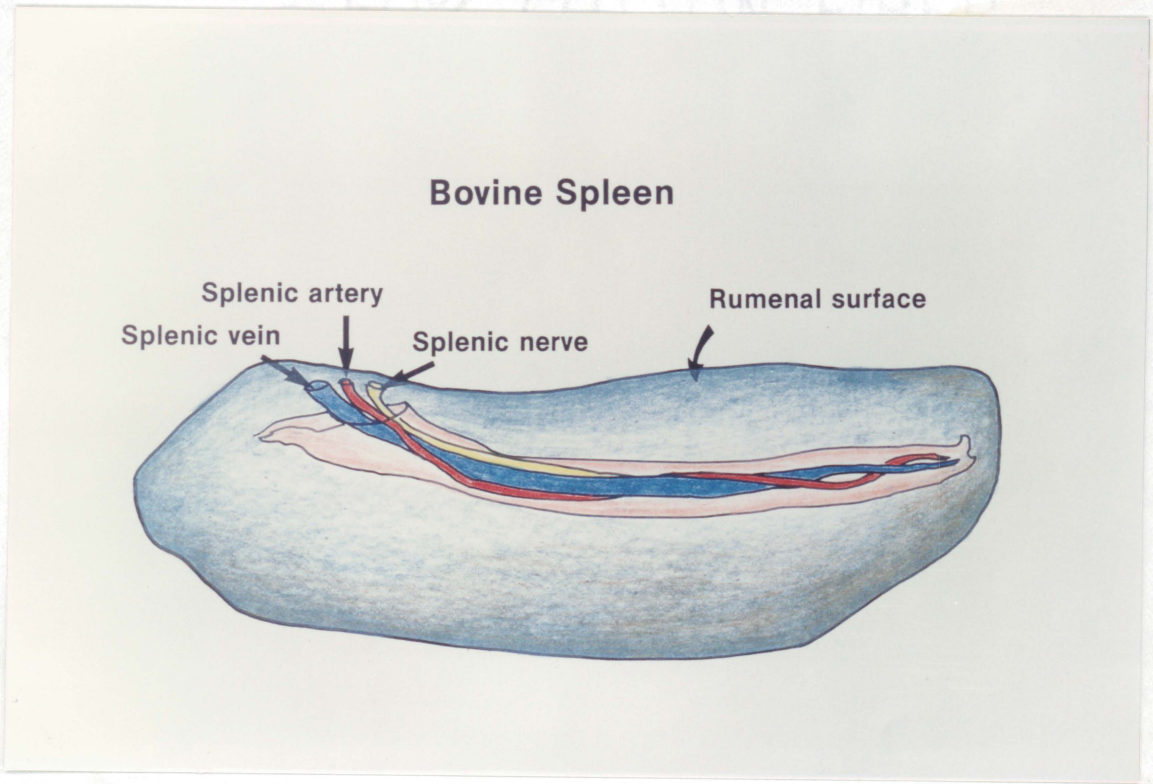

Figure 4 illustrates the rumenal surface of a cow's spleen. The splenic artery (red), splenic vein (blue), and splenic nerve (yellow) enter the cow's spleen at the basal end of the hilus. The cow's spleen is bluish gray in color and long and flat in shape. (Modified from Nickel and Sack. Viscera of Domestic Animals. Springer Verlag, New York, 1979.)

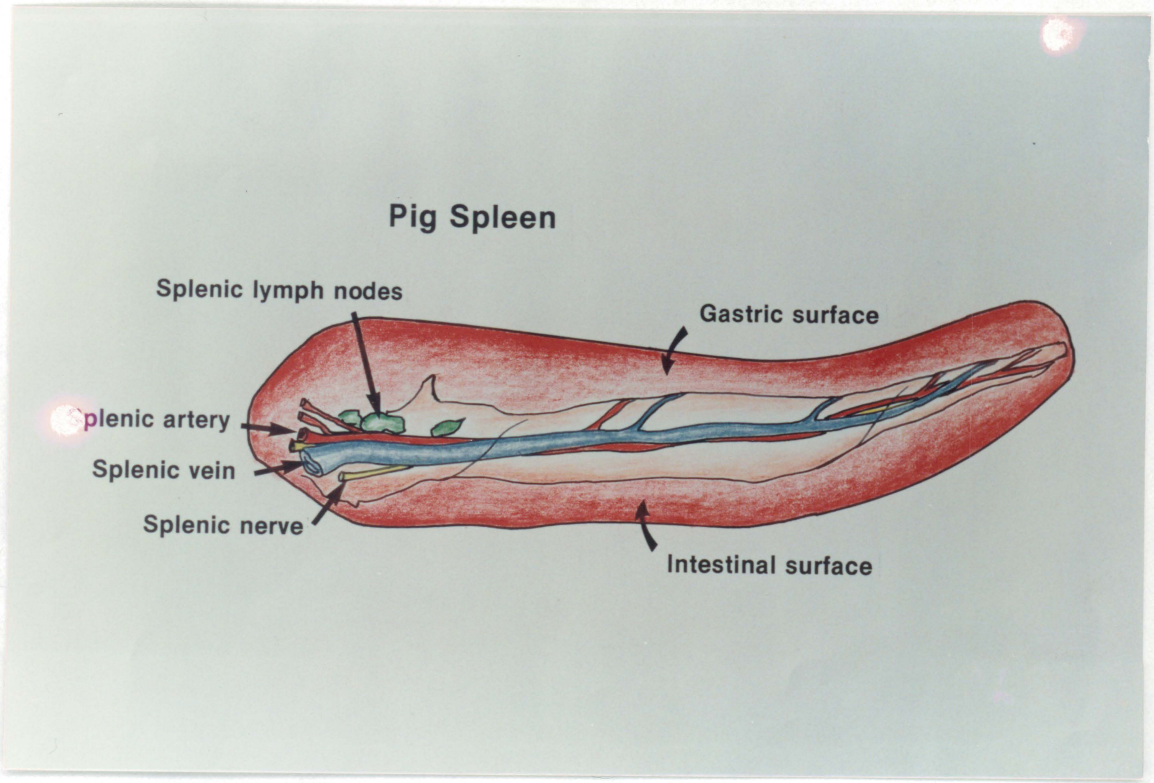

Figure 5 illustrates the visceral surfaces of the porcine spleen (gastric and intestinal). The splenic artery (red), splenic vein (blue), and splenic nerve (yellow) enter the porcine spleen at the basal end of the hilus and branch towards the ventral extremity of the spleen. Splenic associated lymph nodes accompany the vessels along the hilus. The porcine spleen is bright red in color and long and narrow in shape. (Modified from Nickel and Sack. Viscera of Domestic Animals. Springer Verlag, New York, 1979.)

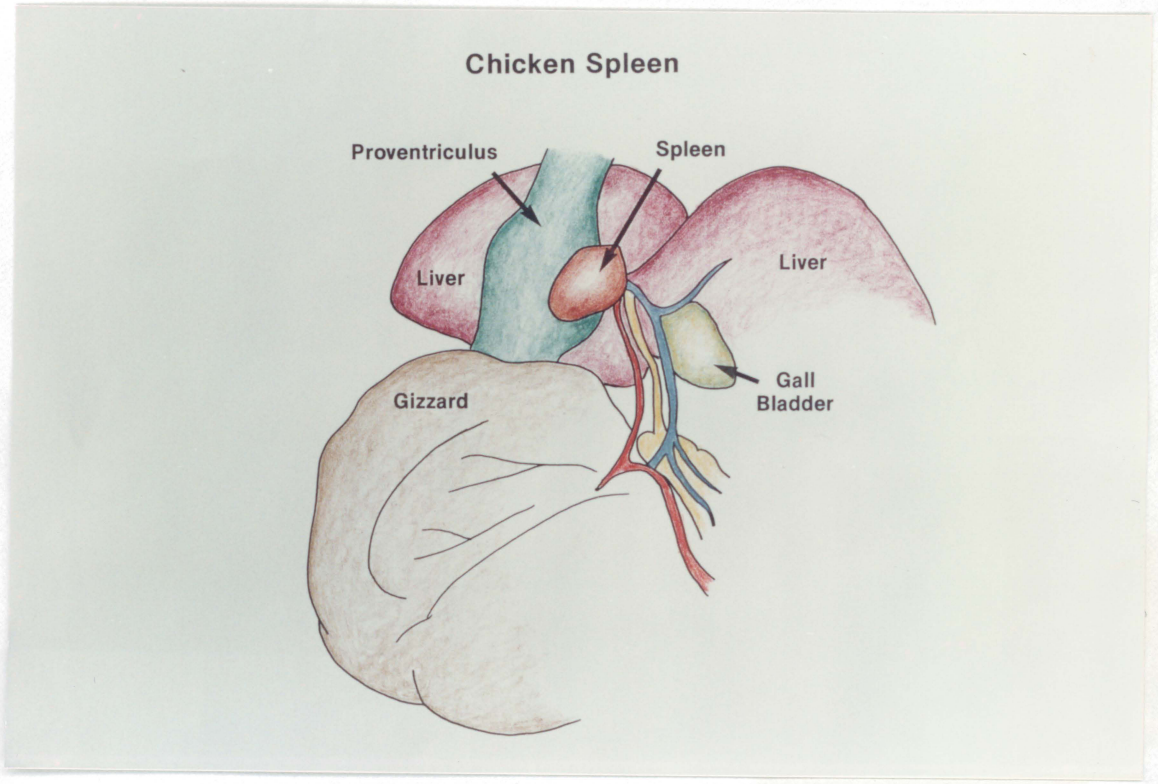

Figure 6 illustrates the location of the spleen in the chicken. The chicken's spleen is located at the right surface of the junction between the proventriculus and the gizzard. The chicken's spleen is reddish brown in color and spherical in shape. (Modified from Nickel and Sack. Viscera of Domestic Animals. Springer Verlag, New York, 1979.)

The parietal surface of the human spleen is smooth, convex and bluntly rounded. This surface lies close to the ribs and thoracic abdominal wall where it directed upward and backward to the left. The upper end of the parietal surface however is directed slightly inward toward the midline (18). The visceral surface of the spleen is broad and concave and divided into two unequal parts by a longitudinal groove called the hilus. The hilus is that portion of the spleen where nerves and vessels enter and exit the spleen. This groove separates the intestinal and gastric portions of the visceral surface. The gastric (or rumenal) portion of the visceral surface projects forward and inward, molding itself on to the greater curvature of the stomach (or rumen) and the tail of the pancreas. The intestinal portion of the visceral surface is directed inward and downward rendering it in close contact with the upper portion of the left kidney, the colon, the greater omentum and the small intestine (18). The cranial border of the spleen is concave and thin and found wedged between the diaphragm and the greater curvature of the stomach. The caudal border is thin also, but is convex in shape instead. The basal extremity is beveled and rests upon the splenic flexure of the colon. This extremity is in close contact with the left kidney, the saccus cecus of the stomach, the tail of the pancreas and the left crus of the diaphragm. The ventral extremity is small and is found in various positions. Typically, it is tipped dorsally behind the ninth and eleventh ribs, directed inward toward the vertebral column (18).

In general, the mammalian spleen is located deep with in the left hypochondriac region between the left part of the greater curvature of the stomach and the diaphragm (fig. 7). Here, it is protected from external trauma by the ribs and is therefore not palpable when of normal size. The position of the spleen in relation to the bony thorax varies with respect to the size and shape of the thorax itself. The spleen tends to be situated deeper in longer more narrow thoracic cavities than in shorter more wide ones (42). The spleen is fixed in position not only by the pressures exerted on it by the neighboring organs, but by a vast, intricate ligamentous network. Except for the hilus of the spleen and those regions encompassing the tail of the pancreas and basal extremity, the spleen is totally surrounded by peritoneum. The peritoneum is adhered to the splenic capsule by three peritoneal folds which are all part of the

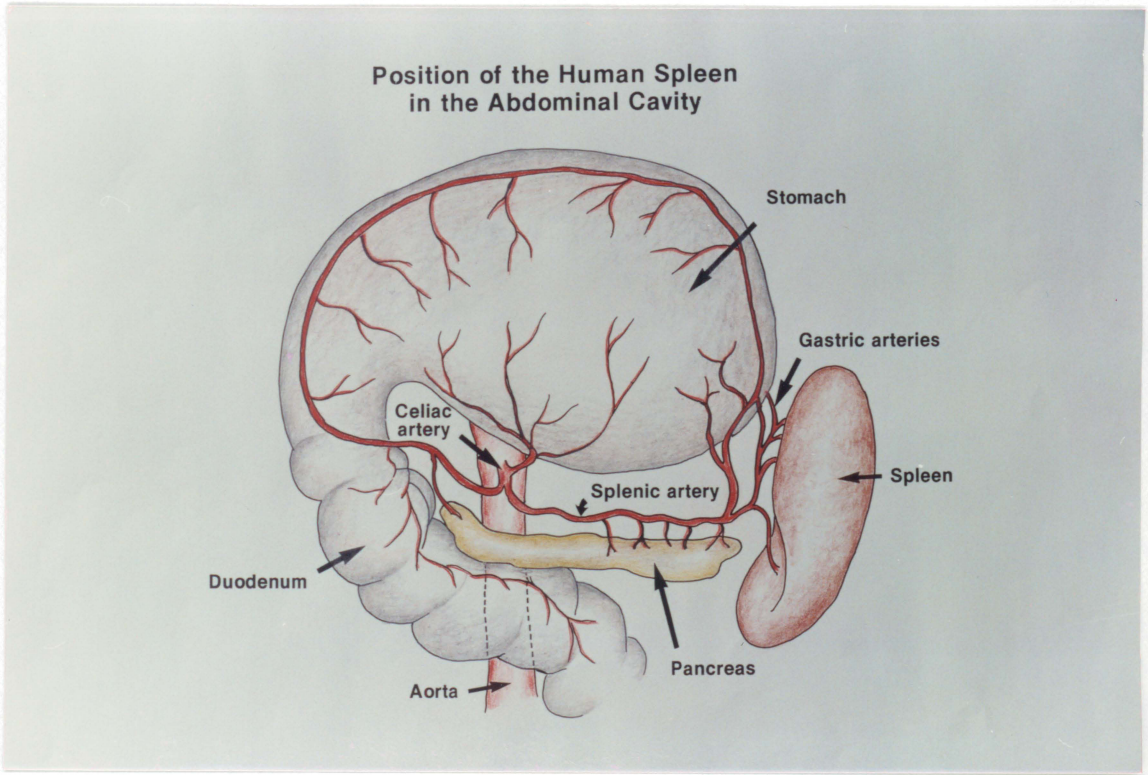

Figure 7 illustrates the position of the human spleen in the abdominal cavity. The human spleen is located deep within the left hypochondriac region near the greater curvature of the stomach. The splenic artery (red) arises from the coeliac trunk of the abdominal aorta and spirals on the cranial edge of the pancreas towards the spleen. The splenic artery branches several times prior to entering the hilus of the human spleen. (Modified from Gray. Gray's Anatomy. Lea & Fibiger, Philadelphia, 1985.)

greater omentum. The position of the spleen depends predominantly upon three things: the expansion of the stomach, the spleen's capacity to become engorged, and the amount of mobility allowed by the ligaments fixing it in place. capacity to become engorged, and the extent of mobility allowed by the ligaments fixing it in place. The ventral end of the canine spleen is allowed excellent mobility due to the loose attachment of the gastrosplenic ligament to the greater curvature of the stomach (43). Although this gives the canine spleen extensive mobility, it still predominantly follows the movements of the stomach. The equine and human spleens are permitted good to fair mobility by the somewhat loose attachment of the gastrosplenic ligament while the bovine spleen is firmly fixed against the rumen and is not permitted the extent of mobility seen in the dog and the horse (11). The bovine spleen, as well as the spleens of all ruminants, has lost its characteristic connection with the greater omentum due to the expansion of the greater curvature of the gastric primordium to form the ruminoreticulum (11). The porcine spleen is permitted extensive mobility. The porcine spleen is attached so loosely to the stomach by the gastrosplenic ligament that torsion of spleen is not uncommon (11). The avian spleen is situated at the right surface of the junction between the proventriculus and the gizzard and is permitted good to fair mobility (fig. 6).

The human spleen is extremely vascular (fig. 8). The splenic artery arises from the celiac trunk of the abdominal aorta and spirals on the cranial edge of the pancreas towards the spleen (42). (Fig. 7). It divides into six or more segmental arteries just prior to entering the hilus which supply the upper and lower poles of the spleen (42). The splenic capsule reflects inward upon the vessels at the hilus forming a trabecular sheath which also contains the splenic veins and nerves (18). These trabecular arteries continue to divide as they move outward away from the inner portions of the spleen. As these arterial branches enter the pulp of the spleen, the tunica adventitia of these pulp arteries becomes infiltrated with lymphocytes and they become follicular arteries. The follicular arteries enter the red pulp of the spleen as brush arteries which in turn divide into small tufts of husk capillaries which empty into the venous sinuses of the red pulp of the spleen. The venous sinuses (large thin walled vessels with irregular lumens) anastomose throughout the red pulp and

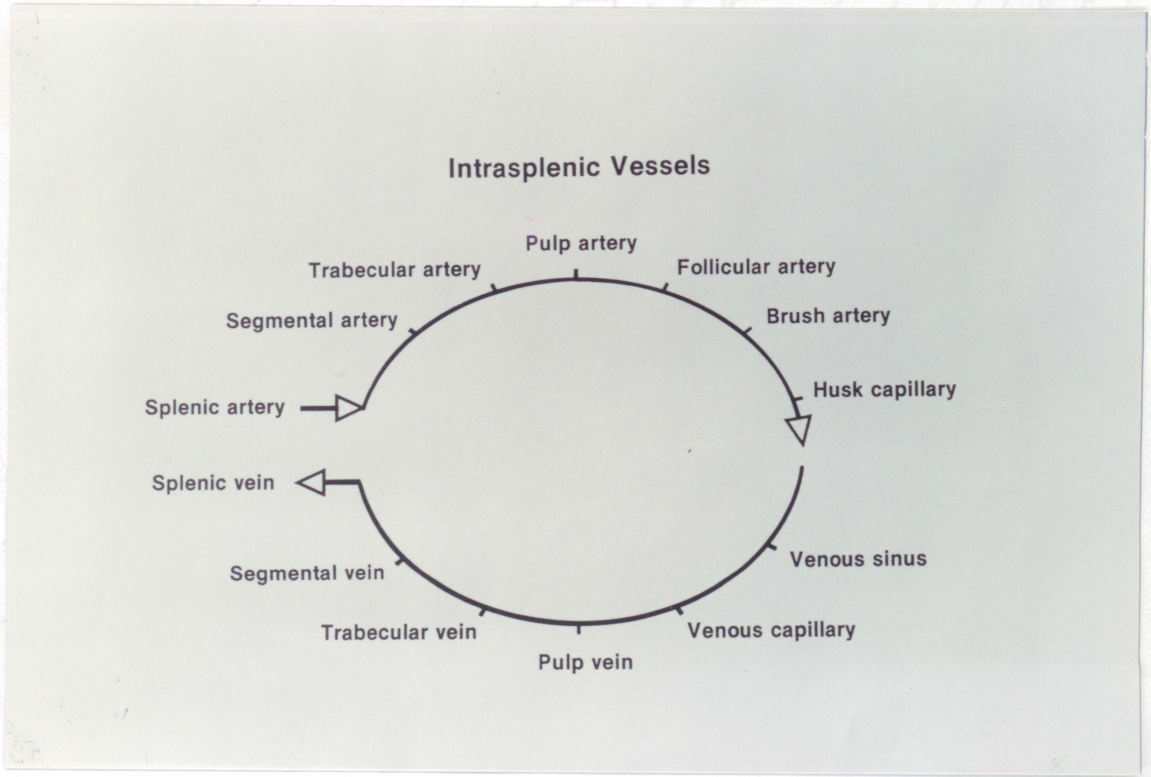

Figure 8 illustrates the direction of arterial and venous blood flow through the spleen. (Modified from Seufert and Mitrou. Surgery of the Spleen. Thieme, Inc., New York, 1986.)

eventually empty into pulp venules which regain their capsular tissue composition and then unite to form larger trabecular veins. These trabecular veins divide into six or more segmental veins which unite at the hilum to form the splenic vein which empties into the hepatic vein and ultimately into the general circulation (42).

b. Histology of the spleen

The spleen is covered by two coats which constitute the splenic capsule. The external serous coat of the spleen is smooth and thin and encompasses all areas of the spleen except those opposing the diaphragm, the stomach and the hilus. The external serous coat comprised of flattened mesothelial cells that are adhered to the internal coat of the splenic capsule. The internal coat of the capsule is made up of connective tissue composed of elastic fibers and smooth muscle cells. The internal fibro-elastic coat of the capsule contributes to the elasticity of the spleen and allows for its great variation in size. The smooth muscle of the external serous coat enables the spleen to contract (9,18). The distribution of smooth muscle and the relative thickness of the capsule is different in different species with swine having the splenic capsule with the most smooth muscle content and the human, bovine, and avian capsules containing only a small amount of smooth muscle (11). The canine and equine splenic capsules are made up of numerous elastic fibers and a considerable amount of smooth muscle (43).

The trabeculae not only function as a guide for the arteries and veins but give structure to the spleen as well (9,18). The trabeculae ramify throughout the parenchyma of the spleen and unite to form areolae in which the pulp of the spleen is contained (fig. 9). The tissue composing the trabeculae entering the hilus of the spleen is continuous with that of the splenic capsule. As the trabecular arteries enter the red pulp of the spleen, the connective tissue surrounding the arteries becomes transformed into a thick lymphoid material. The adventitia surrounding the arteries is substituted for reticular tissue which is

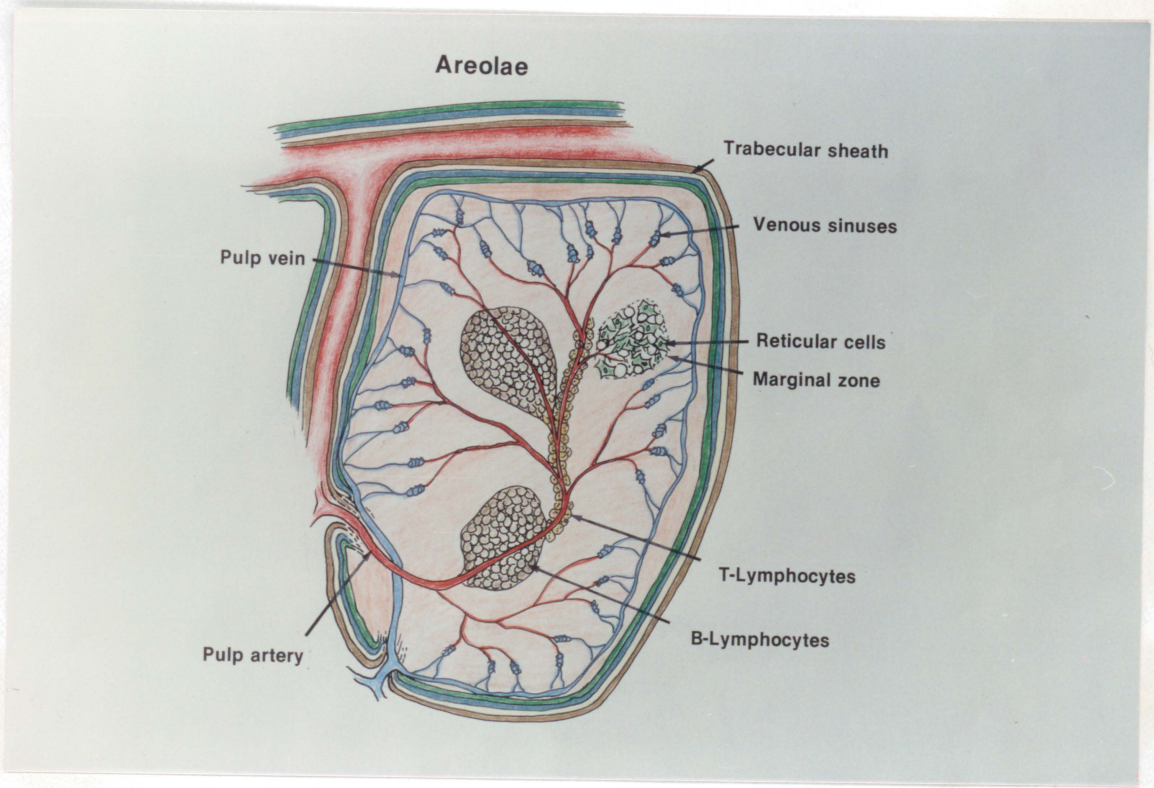

Figure 9 illustrates the histology of the pulp within the areolae of the spleen. The pulp arteries become infiltrated with T-dependent lymphocytes in the periarterial sheath as they make their transition into follicular arteries. B-dependent lymphocytes accumulate along the walls of the follicular arteries forming malpighian bodies. The marginal zone is located between the white and red pulps and is composed of reticular tissue. The pulp venules draining the venous sinuses unite to form pulp veins which empty into trabecular veins. (Modified from Leeson and Leeson. A Brief Atlas of Histology. W.B. Saunders Co., Philadelphia, 1979.)

infiltrated with lymphocytes (30). The T-cells found in the periarterial sheaths are predominantly helper cells (48). This lymphatic tissue is also composed of small and medium lymphocytes, monocytes and plasma cells (9,18). At various points along the walls of the follicular arteries, this lymphatic tissue accumulates and forms small lymphoid nodules or malpighian bodies. It is unclear as to why the lymphatic tissue expands at specific locations along the vessel wall. It is interesting to note, however, that the lymphocytes comprising these nodules are of the B-dependent type rather than the T-dependent type of the reticular ensheathment (30). This lymphoid material terminates as the arterioles break up into capillaries. The random distribution of lymphoid tissue throughout the spleen in the tunica adventitia of the follicular arteries and in the malpighian bodies constitutes the white pulp of the spleen and gives the spleen its immunological characteristics. The morphology of the white pulp varies with the age of the individual. The splenic white pulp is fully developed in the neonate and continues to develop up until the time of puberty where it peaks. Following puberty, the splenic white pulp gradually involutes, eventually becoming inactive with senescence (35). The morphology of the white pulp not only varies with age, but with the presence of antigenic stimulation as well. Germinal centers are absent in the immunologically unstimulated adult spleen while found in abundance in the neonate (35).

The marginal zone is located between the white pulp and the red and encompasses that region containing the brush arteries and the husk capillaries (fig. 9). The cellular composition of the brush arteries is for the most part continuous with the follicular arteries with the exception of the predominance of B-dependent lymphocytes and macrophages. The husk capillaries loose their investment of smooth muscle but are ensheathed, however, in porous epithelial tissue that is continuous with that of the sinuses (18). The spleen lacks afferent lymph vessels but contains efferents in the capsule and larger trabeculae at the base of the marginal zone. When the veins constrict, the plasma is forced out of the venous sinuses and cords and is carried to the thoracic duct by way of the trabecular and capsular efferent lymph vessels in the marginal zone (2,9).

The red pulp of the spleen is composed of two structures: large thin walled vessels with irregular, distensible lumens called venous sinuses, and intervening plates of cells that surround the sinuses called cords (fig. 10). The venous sinuses are wide vascular channels lined with specialized reticular tissue. These cavernous spaces are continuous with the reticular tissue of the pulp and are lined with elongated, longitudinally oriented endothelial cells. The ends of these cells are tapered and separated from each other by narrow gaps, thus rendering the sinuses their porous nature (9). Although these cells are not truly phagocytic, they appear to possess phagocytic properties due to their close association with macrophages whose pseudopodia protrude between the lining into the lumen of the sinuses (42). The few T-cells found near the sinuses and cords of the red pulp are predominantly suppressor/cytotoxic cells. The small venules draining the venous sinuses also are lined with these endothelial cells and acquire the fibro-muscular trabecular tissue layer as the trabecular veins are formed. The splenic cords are made up of reticular fibers composed primarily of reticular cells, monocytes, erythrocytes and other leukocytes (2). These reticular cells are phagocytic, but possess less phagocytic capacity than those reticular cells associated with the lining of the sinuses (9,42).

The splenic plexus is formed by branches from the coeliac plexus, the left semilunar ganglia, and the right pneumogastric nerve (18). Most of the splenic nerve fibers are post ganglionic sympathetic fibers. However, some sensory nerve fibers have been found in the splenic plexus. These sympathetic nerve fibers enter the splenic capsule with the splenic arteries (fig. 11). They continue with these vessels from the capsule into the trabeculae and form a subcapsular plexus and an associated trabecular plexus. Fibers from the trabecular and vascular plexuses follow the follicular artery deep into the white pulp of the spleen (33). Varicosities branching from the vascular plexus enter into the periarterial sheaths where T-dependent lymphocytes predominantly reside. The varicosities do not enter into the malpighian bodies and terminate abruptly at the marginal zones. Only a few scattered varicosities are found in the red pulp of the spleen (33). Thus, the splenic capsule and the trabecular, pulp and follicular arteries comprising the white pulp of the spleen are sympathetically innervated and may be adrenergically regulated.

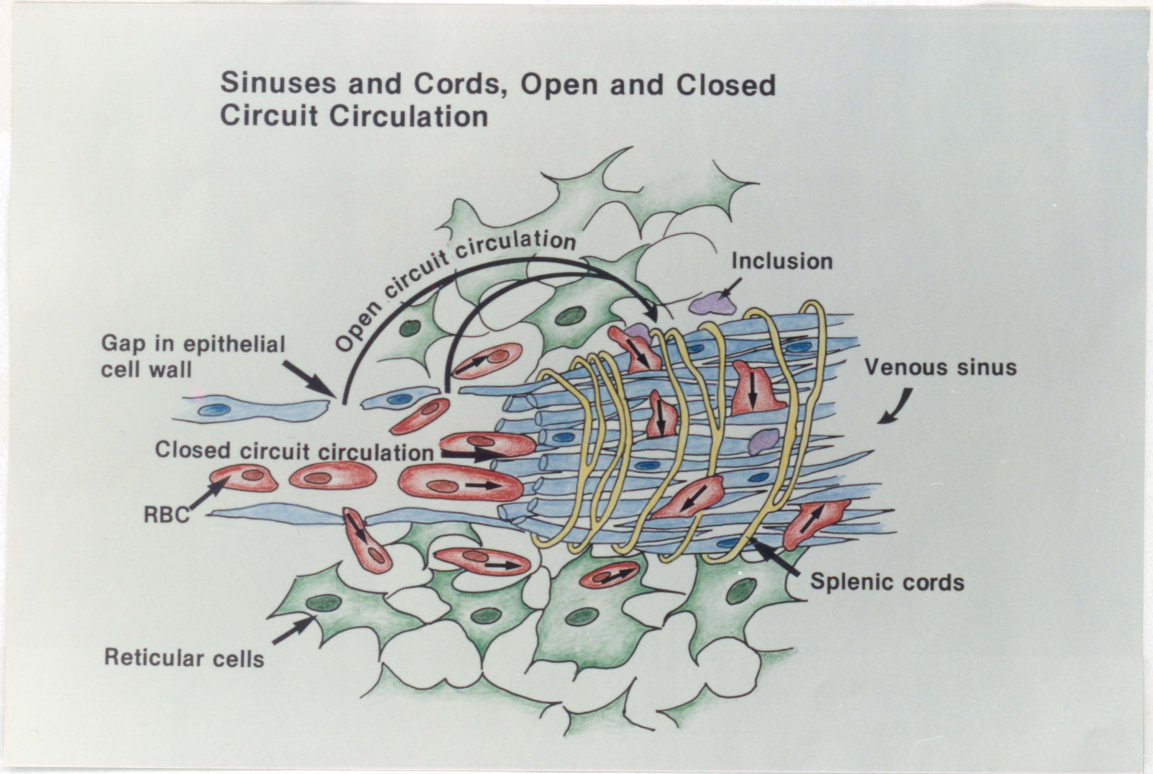

Figure 10 illustrates the routes of circulation through the venous sinuses in the red pulp of the spleen. In the closed circuit route of circulation the arterial blood leaves the husk capillaries by flowing directly into the end of a venous sinus. In the open circuit route of circulation, the arterial blood leaks out of the husk capillaries through gaps in the epithelial wall just prior to entering the sinus. The blood then squeezes through the trabecular meshwork of the splenic cords and gaps in the epithelial lining of the sinus. This illustration demonstrates one of three processes of filtration, pitting, where an inclusion (purple) is removed from a red blood cell as it squeezes through the splenic cords. (Modified from Leeson and Leeson. A Brief Atlas of Histology. W.B. Saunders Co., Philadelphia, 1979.)

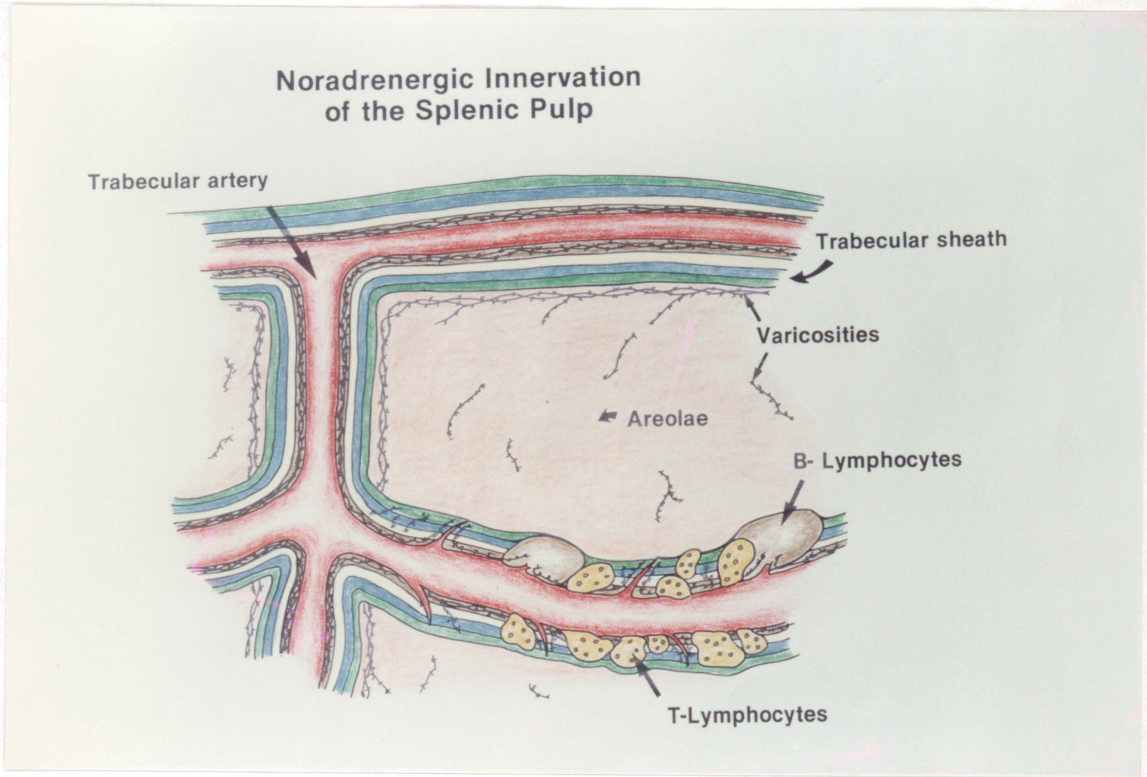

Figure 11 illustrates the sympathetic innevation of the splenic pulp. Sympathetic nerve fibers enter the splenic capsule with the trabecular arteries and continue into the pulp of the spleen. The varicosities follow the follicular arteries into the white pulp of the spleen and enter into the periarterial sheath where T-cells predominantly reside. These varicosities avoid the areas inhabited by B-cells (malpighian bodies) and terminate abruptly at the marginal zone. Only scattered varicosities are seen in the red pulp of the spleen. (Modified from Livnat et al., "Involvement of peripheral and central catecholamine systems in neural-immune interactions." *J. of Neuroimmunology*, Vol. 10, 1985.)

c. Functions of the spleen

The spleen is a very complex organ that it services many of the needs of the body in maintaining homeostasis, yet the body can function without it. The importance of the spleen's functions depends upon the species in question. In the fetus, the human spleen functions as a hemopoietic organ up until the fifth month of development. The spleen gradually begins to loose this capability as the fetus approaches birth (42). After parturition, the bone marrow assumes the responsibility of red blood cell manufacture. However, there are some instances where the spleen continues to produce erythrocytes after birth. Such diseases as erythroblastosis fetalis where abnormal antibodies in the plasma destroy red blood cells, result in the reinstatement of the spleens hemopoietic abilities (24). It is thought that the fetal spleen functions as a hemopoietic organ because of the presence of precursor normoblasts and megakaryotes detected through light microscopy (48). Wolf and Neiman (48) identified these cells through cytochemical and immunohistochemical techniques and found these cells to be merely reaching a late stage in maturation, incapable of red blood cell production. They concluded fetal hematopoiesis does not take place in situ. They suggest hematopoietic cells circulating through the blood filter into the spleen and thus it appears as if the fetal spleen is acting as a hemopoietic organ (48).

The spleen functions as a reservoir for red blood cells in many species whose splenic capsules are composed primarily of smooth muscle. Sympathetic stimulation causes the spleen to contract and the stored blood in the venous sinuses is subsequently expressed into the general circulation. An increase in the hematocrit of the systemic circulation results (24). Sympathetic inhibition, however, results in the relaxation of the splenic capsule and subsequent expansion of the spleen with an increase in sinus stores of blood (24). The large amount of smooth muscle in the splenic capsule of the swine coupled with its extensive mobility make the porcine spleen an ideal candidate for a reservoir organ. The porcine spleen is sinusoidal in type (possessing a large quantity of venous sinuses) with its primary function being that of a reservoir. The canine and equine spleens are of the sinusoidal type as well

and also function predominantly as reservoirs (43). The human, bovine and avian spleens, containing only a small amount of smooth muscle in their capsules, are non-sinusoidal in type and do not function primarily as a reservoir.

The adult mammalian spleen also functions as a filter for the circulating peripheral blood (9,24). The spleen removes both damaged and worn out erythrocytes in the red pulp. The arterial blood en route to the venous sinuses passes into the venous system in the spleen by two routes (fig. 10). The closed circuit route involves the passage of arterial blood from the end of a husk capillaries directly into the end of a venous sinus (2). This route is not the one usually taken by the arterial blood in the spleen, but is used predominantly in times of stress where an increase in blood volume and hematocrit are required as soon as possible. The open circuit route is the preferred route under normal physiological conditions. Here, the arterial blood may leak out of the porous husk capillaries just prior to entering the sinuses. These cells are then squeezed through the trabecular meshwork of the cords and through the gaps in the endothelial lining of the sinuses. Those red blood cells that are worn out, fragile, coated with particulate matter and antibody are less distensible and are ruptured as they pass through the cords (48). Their hemoglobin is released and ingested by the macrophages lining the cords. These macrophages also line the venous sinuses. Together they remove bacteria, abnormal cells, parasites and unwanted debris from the blood via phagocytosis. The hereditary disease hemolytic anemia results in an abnormally high loss of red blood cells in the spleen because of the inability of these abnormally shaped cells to be squeezed through the splenic cords and venous sinuses. Often seen with this disease is the engorgement of the cordal macrophages with iron from the hemoglobin. This results in the macrophages inability to return the iron to the bone marrow and a severe systemic iron deficiency results (48).

When the body is infected with unwanted foreign bodies, the human spleen will enlarge so as to ensure a more adequate cleaning function. As the spleen enlarges, the pulp cords widen and thus the circulating cells are exposed to the macrophages of the splenic cords for longer periods of time and are

subsequently destroyed at a faster rate than normal (48). In such cases, the bone marrow may fail to produce these cells at a rate comparable with their destruction, and cytopenia often results (24). Splenomegalic conditions naturally result in pooling of blood in the spleen. However, this apparent reservoir function of the human spleen is merely due to the presence of disease. Under normal physiological conditions, the human spleen does not store and release red blood cells as with some of the lower animal species.

There are three processes by which the human spleen filters the blood: culling, pitting and erythroclasis (48). Culling is the manner in which a physiologically normal spleen clears the blood of bacteria, and other cells that have been damaged or worn out (9,48). The splenic cords contain numerous macrophages enriched with lysosomes whose hydrolytic contents are capable of destroying the aged, weakened red blood cells. The process of pitting involves the removal of inclusions, such as denatured hemoglobin and intracellular parasites, from red blood cells without the destruction of the cell itself (fig. 10). The inclusions are pinched off of the cell as it squeezes through the cords and the viable portion of the cell enters into the venous sinuses. Erythroclasis describes the fragmented destruction process of red blood cells. Here, the red blood cells are fragmented and then returned to the general circulation before they are sequestered. Unlike the process of culling, these fragments are not removed immediately but are removed with the subsequent passage through the spleen (48). Certain hemolytic anemias and sickle cell anemias involve the destruction of red blood cells through the process of erythroclasis (24).

The spleen's role in immune response is not yet fully understood. The white pulp is the the largest lymphoid organ in the body and is capable of trapping and processing antigens, and producing and activating antibodies and lymphokines (48). The active role of the spleen is due to its vast supply of phagocytizing cells and immune competent cells (42). The spleen is the only lymphoid organ capable of responding to disseminated blood-borne antigens, such as bacteria or septicemia, not requiring the local antigenic stimulation necessitated by the lymph nodes and mucosal-associated lymphoid tissue (48).

The stationary macrophages found in the cords and marginal zones are capable of phagocytizing blood bourn antigens either directly entering the blood stream or antigens that have left behind only a low titer of specific antibody from an earlier contact (fig.9). The macrophages pass the antigenic information on to the B and T-cells via mRNA and humoral factors so that a mobile defense against the invader may be launched (42). Without the interaction between these foreign bodies and stationary phagocytes, the response of the lymphocytes to an initial blood bourn invader would be inadequate. In the spleen, antigens have a prolonged exposure to antigen-processing cells in the marginal zones and to the macrophages in the red pulp which facilitates their phagocytosis (2,48). When the level of specific antibody is low, such as with an initial encounter of an antigen, the spleen actually becomes more efficient in clearing the antigen-antibody complexes than the liver (42). The long contact time of the blood with the lymphoid cells in the spleen due to the slower rate of blood flow and higher concentration of macrophages than the liver facilitate this occurrence (42).

The lymphocytes in the splenic white pulp are constantly moving. The mean time of passage for a T-lymphocyte through the spleen is 3 to 4 hours while it is much longer for the B-cells (48). Those T-cells capable of proliferating are found in the periarterial sheath while those found near the marginal zones do not appear to under go mitosis and are therefore thought to be mature cells involved in antigen processing (48). The arterial terminations in the marginal zones allow for the entrapment of antigens and the vast supply of macrophages situated there allow for their processing (fig.10). If an antigen is present, these cells become activated and proliferate to from germinal centers, antibodies and lymphokines. Once the antigens have entered the marginal zones, they then penetrate the germinal centers where the presence of T-helper cells facilitate the interaction between the B-cells and the antigen (48). If no antigens are present, the antibodies return to the general circulation.

The spleen is believed to be more essential in maintaining body homeostasis in children than in adults. The foundation for this belief is in the 1952 reported findings of King and Schumaker (27) that a higher rate of

postsplenectomy sepsis occurred in children than in adults. In a study of 686 patients whose spleens had been removed, the occurrence of severe postsplenectomy sepsis in children was 55 times greater than in the general population. Furthermore, the mortality rate from the sepsis in these children was 200 times greater than in children whose spleens had not been removed. The majority of cases of postsplenectomy sepsis are due to respiratory tract-directed encapsulated microrganisms including Streptococcus pneumoniae and Haemophilus influenzae (32). Most children between the ages of 2 and 3 lack circulating antibodies against the polysaccharide capsular antigens of these organisms (38) and consequently these children are more susceptible to postsplenectomy sepsis.

The spleen is necessary for the rapid antibody production that occurs following the challenge of a previous encountered antigen (42). Those subjects whose spleens have been removed do not show this rapid rise in titer following challenge with a previously encountered antigenic strain (48). There are two theories as to why infection occurs following a splenectomy. The first theory attributes postsplenectomy sepsis to the loss of the filtering capacity of the spleen (8). The reduced contact time between the blood and the macrophages in the liver results in a decreased phagocytic efficiency and hence, sepsis is more likely to occur following a splenectomy. The second theory attributes postsplenectomy sepsis to an altered antibody response. A reduced T-helper cell activity without a change in suppressor T-cell activity has been observed following the loss of the spleen (8). Postsplenectomy patients have been reported to respond poorly to intravenous immunization with particulate antigens (8), probably due to the loss of spleen-mediated opsinization of antibodies to polysaccharide encapsulated organisms. The opsonizing antibodies produced by the spleen facilitate phagocytosis and the destruction of these organisms.

2. ADRENERGIC REGULATION OF SPLENIC CIRCULATION AND OXYGEN METABOLISM

The effects of adrenergic control of lymphocyte activity in the spleen have been extensively investigated (12,15,16,26,29). However, the physiology of autonomic control of hemodynamic and functions and oxygen metabolism remains unclear. Nervous control of the splenic circulation is achieved by sympathetic noradrenergic outflow in the splanchnic nerve branches accompanying the splenic artery into the spleen. The smooth muscle of the splenic capsule and trabeculae also are innervated heavily with these fibers. The contraction of the smooth musculature of the spleen results in the release of blood from the spleen with a concomitant decrease in splenic blood volume and increase in splenic resistance (47). The response of the capsular smooth muscle and the vascular smooth muscle to sympathetic stimulation varies in intensity with the species. The capsular and vascular smooth muscle of the feline spleen are equally sensitive to sympathetic stimulation, but the capsular smooth muscle in the dog is more sensitive to noradrenergic outflow than the smooth muscle of the splenic vasculature (7). Stimulation of the splenic nerve of the dog at frequencies of 1 HZ and below resulted in extensive contraction of the splenic capsule while contraction of the splenic vessels was only minimal at these frequencies. Since intra-arterial injections of epinephrine and norepinephrine into the splenic artery mimicked the responses to nerve stimulation, they concluded that the splenic capsule of the dog is more sensitive to noradrenergic outflow than the splenic vasculature of this species.

Electrical stimulation of the splenic nerve also results in the release of large volumes of blood from the spleen in dogs and cats with hematocrit levels ranging between 70 and 80 %, proportional to the intensity of stimulation (4,10,19,20). Splenic nerve stimulation decreases splenic arterial inflow and increases splenic venous outflow and pressure in dogs and cats. The mobilization of blood from the spleen has been demonstrated to be an active process independent of arterial inflow (3,10). Donald and Aarhus (10) investigated the participation of splenic arterial inflow and venous outflow pressure on the active and passive release of blood from the spleen. They

measured changes in the weight of an isolated perfused spleen in anesthetized dogs during supramaximal nerve stimulation and during mechanical restriction of inflow matched to that observed during nerve stimulation. They found expulsion of blood from the spleen to be almost an entirely active process. At splenic venous pressures equivalent to those found in intact dogs (10 cmH_2O) supramaximal nerve stimulation resulted in the expulsion of a mean of 148 ml of blood from the spleen with hematocrit levels ranging between 70 and 90%. Mechanical occlusion of the splenic arterial inflow resulted in a passive release of blood from the spleen that was only 14% of that released during nerve stimulation. Little passive release of blood was observed unless venous outflow pressure was reduced to zero. The splenic venous hematocrit was unchanged during mechanical restriction of arterial inflow but increased to more than 80% during nerve stimulation. Thus, it was concluded that the mobilization of blood from the isolated perfused spleen in anesthetized dogs was an active process that was not affected by alterations in arterial inflow to the spleen. Similar results also were observed by Brooksby and colleagues (3).

Webb-Peploe (47) found stimulation of the splenic nerves in anesthetized dogs, after the temporary arrest of splenic circulation, caused an increase in the smooth muscle tension of the capsule and veins. This resulted in an increase in venous pressure that was graded to the frequency of stimulation. Hypoxia and carotid hypotension also produced a significant rise in splenic venous pressure. The splenic circulation in the anesthetized dogs was capable of being arrested for at least 10 minutes without changing the sensitivity of the splenic capsular and venous smooth muscle to a standard stimulus. From these results, Webb-Peploe concluded changes in the capacitance function of the spleen, as measured by changes in the venous pressure after temporary arrest of the circulation, representative of capacitance vessel changes throughout the splanchnic circulatory bed.

Greenway and Lawson (21) examined the splenic arterial flow and weight responses to splenic nerve stimulation in anesthetized dogs at various frequencies. Stimulation of the splenic nerves resulted in a decrease in splenic arterial flow and splenic weight that was graded to the frequency of stimulation.

Intravenous administration of atropine or propranolol did not effect the responses to nerve stimulation but these responses were blocked by intravenous administration of phenoxybenzamine prior to splenic nerve stimulation. Since the responses of splenic weight and arterial flow are completely blocked by phenoxybenzamine but unaffected by propranolol, Greenway and Lawson concluded that the smooth muscle controlling splenic weight changes during nerve stimulation are mediated by the alpha-adrenoceptors but not the beta-adrenoceptors of the spleen.

Malik (34) has found that stimulation of the periarterial nerve plexus and injections of norepinephrine into the splenic artery enhanced the efflux of a prostaglandin E-like substance from the rat spleen. The administration of prostaglandin synthetase inhibitor, indomethacin, potentiated the vasoconstrictor responses to nerve stimulation and injected norepinephrine. Thus, Malik concluded that prostaglandins of the E series may modulate the vascular responses of the rat spleen to adrenergic stimuli by diminishing the release of norepinephrine from the nerve terminals innervating the spleen.

It is clear that changes in splenic flow are generally due to the activity of the sympathetic nerves innervating the spleen. Reflex stimuli such as baroreceptor activation have been shown to alter blood flow through the spleen as well. Sympathetic out flow to the spleen is highly responsive to change in activity of carotid and aortic baroreceptors. Ninomiya and colleagues (36) studied the effects of baroreceptor reflex on sympathetic nerve activity to the spleen, kidney and heart in anesthetized cats. They found the baroreceptor-splenic sympathetic reflex was greater than that of the baroreceptor-renal and baroreceptor-cardiac sympathetic systems. They observed grouped discharges synchronous with the cardiac cycle and its respiratory modulation in the splenic, renal and cardiac nerve activities simultaneously before and during occlusion of the descending aorta. Occlusion of the descending aorta resulted in an increase in aortic pressure which in turn inhibited the sympathetic nerve activity in all three nerves. At low aortic pressure, activity became continuous with the cardiac cycle. Stimulation of the left aortic nerve and increases in pressure within the isolated carotid sinus mimicked these responses. Aortic pressure was

reduced by bleeding through a catheter inserted in to the femoral artery. With acute hemorrhage, aortic pressure fell rapidly and induced an increase in sympathetic nerve activity to the spleen, kidney, and heart. Following hemorrhage, aortic pressure recovered while, sympathetic nerve activity decreased slowly to a level higher than the initial value (36). Therefore, it is apparent that changes in the activity of the carotid and aortic baroreceptors may alter splenic blood flow by alleviating sympathetic outflow to the spleen. In a similar study Fan and colleagues (13) examined the the hemodynamic responses of the spleen to sodium nitroprusside (SNP). They found splenic resistance increased while splenic arterial inflow decreased to 50% of control values.

Exercise, hypoxia and fright also have been shown to alter the blood flow through the spleen (23,40,45,). Sanders and colleagues (40) measured the splenic arterial inflow in conscious dogs during moderate and exhaustive exercise. They found splenic inflow significantly decreased with exhaustive exercise but decreased only slightly with moderate exercise. They concluded the effect of exercise on the arterial inflow into the spleen in conscious dogs is dependent upon the intensity of the exercise. In a similar study, Vatner and Higgens (45) found the arterial hematocrit increased by 9 % during running in conscious dogs with intact spleens, and remained unchanged during running following splenectomy. Guntheroth and colleagues (23) found the splenic blood volume decreased in conscious dogs during moderate exercise as well. However, the splenic blood volume in these dogs was reduced to an even greater degree in response to hemorrhage and hypoxia. They concluded the effect of hypoxia and hemorrhage on the blood volume of the spleen is more intense than the effect of moderate exercise.

Alterations in splenic blood flow have been shown to affect cardiac output and oxygen consumption in anesthetized dogs. Lieng and Huckabee (31) postulated that stimulation of the splenic nerve releases a cardiotonic substance termed splenitransin. Splenitransin is thought to be responsible for the increase in cardiac output and oxygen consumption associated with sympathetic stimulation of the splenic nerve. They found sympathetic

stimulation of the spleen produced by electrical stimulation of the splenic nerves or by infusion of norepinephrine into the splenic artery increased the cardiac output and systemic mean arterial blood pressure in anesthetized dogs. However, this treatment did not result in a change in heart rate and right ventricular diastolic pressure. Since pretreatment with intraportal infusion of norepinephrine or arterial femoral venous blood (5 ml/kg/min, the amount typically released from the canine spleen upon contraction) did not alter cardiac output, they concluded hemodynamic changes produced by sympathetic stimulation of the spleen were not due to the amount of blood discharged into the general circulation during splenic contraction nor to the actions of catecholamines released by the spleen. The infusion of 5 ml/kg of splenic venous blood obtained from a dog during a period of splenic nerve stimulation into a second dog increased the recipients cardiac output. Therefore they concluded the observed increase in cardiac output was produced by the cardiostimulatory action of an unknown substance (later termed splenitransin) which was released from the spleen following sympathetic stimulation.

There is little information available concerning oxygen metabolism in the spleen. Changes in splenic oxygen consumption are generally due to changes in blood flow to the spleen. Wantanabe (46) reported a significant decrease in splenic oxygen consumption in patients with Banti's Syndrome, a splenomegalic condition secondary to portal hypertension as well as a predominant venous sinus hyperplasia. Using histometrical methods to determine the compositions of the Banti spleen, he found a 5.1 % increase in the red pulp, a 5.4 % decrease in white pulp and a 0.3 % increase in trabecular composition over the normal human spleen (46). Additionally, he found the endothelial cells of the venous sinuses increased markedly. Supporting his findings was the work of Sedi (41) who found the total surface of the venous sinuses to increase in the spleens of Banti's syndrome patients. Wantanabe (46) and Sedi (41) suggest the increase in endothelial cells in the red pulp of the Banti spleen is responsible for the decrease in splenic oxygen consumption rather than the decrease in splenic flow associated with this disease.

CHAPTER II.

METHODS AND MATERIALS

A. SURGICAL PREPARATION AND EXPERIMENTAL DESIGN

The studies were carried out on 17 neonatal pigs of both sexes (mixed breed). They ranged in age from 4 to 8 weks and in weight from 6.5 kg to 26.5 kg. All were anesthetized with intraperitoneal injections of pentobarbitol, 30 mg/kg body weight, and placed in dorsal recumbancy for the surgical procedures. The detailed preparations are shown in figure 12. A tracheotomy was performed for mechanical ventilation with a Harvard respirator. The femoral artery was cannulated with a polyethylene catheter (PE 50-90) for measurements of arterial blood pressure and heart rate with the use of a Statham transducer (P23ID) and Grass polygraph system (model 7). The jugular vein was cannulated for pentobarbitol and norepinephrine infusion. Following laparotomy, the spleen was exposed and isolated. The gastroepiploic vessels and gastrosplenic ligament were ligated. Total splenic venous flow was measured by retrograde cannulation of the greater splenic vein. The flow from the splenic vein catheter was diverted through a polyethylene tubing to a femoral vein catheter. A T-connector was placed in the tubing to permit temporary diversion of splenic flow into a graduated cylinder for timed collections and sampling.

After all cannulation and surgical procedures were completed, 1000 units heparin was administered intravenously to prevent clotting in the catheters. Simultaneous arterial and venous samples were withdrawn from the femoral artery and splenic vein for determination of oxygen content, (Lex-O2-Con-K). Splenic oxygen consumption is calculated as the product of splenic venous flow and splenic arteriovenous (A-V) oxygen difference and expressed as ml/min/100gm splenic tissue (wet weight). The coefficient of splenic oxygen extraction [(A-V)/A] was also estimated. For study of leukocyte and erythrocyte migration and activity, white blood cell differentials from the femoral artery and the splenic vein were measured with an automated coulter counter (ZBI series).

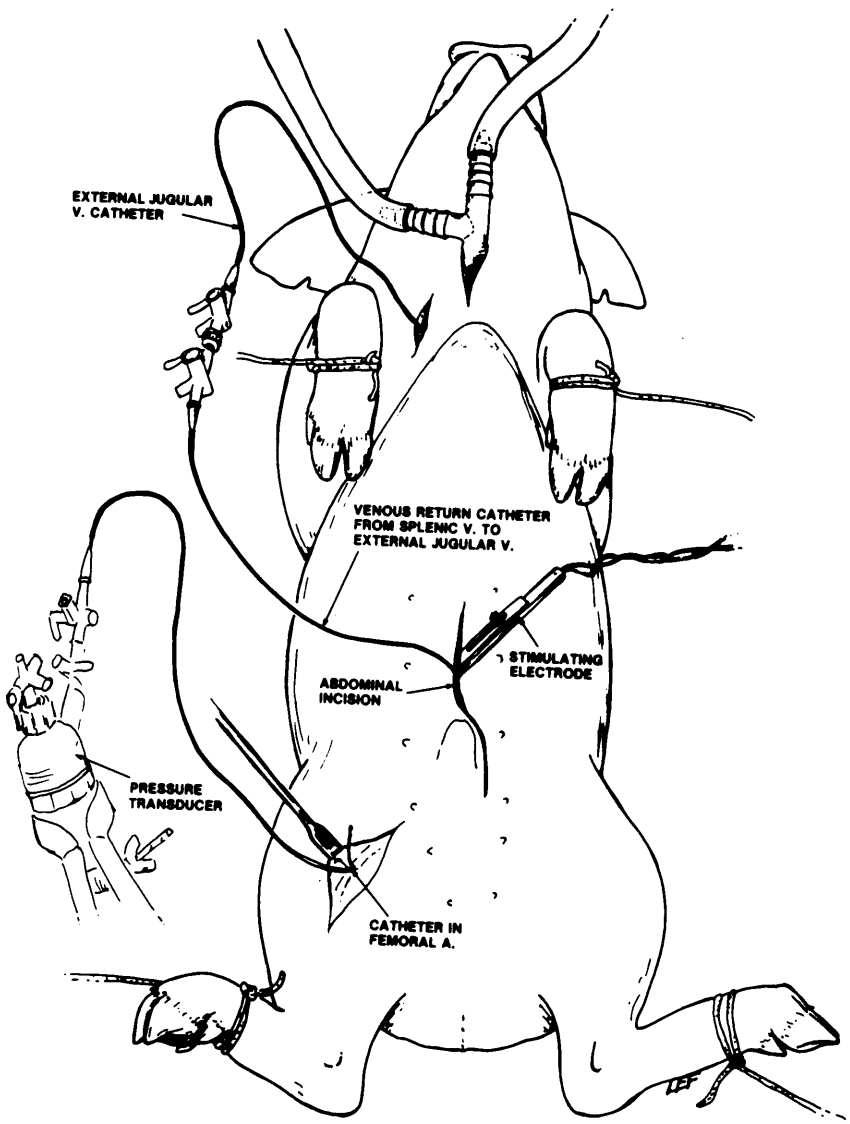

Figure 12 illustrates the surgical preparation of the piglets. A tracheotomy was performed for mechanical ventilation. The femoral artery was cannulated with a polyethylene catheter and connected to a pressure transducer. The splenic vein was cannulated with a polyethylene catheter and flow was diverted into a similar catheter in the external jugular vein. The stimulating electrode was placed on the distal ends of the splenic nerve.

Hematocrit was determined by centrifuging blood samples from the femoral artery and splenic vein in heparinized microhematocrit tubes in an Autocrit Ultra 3 centrifuge. In the norepinephrine infusion study, norepinephrine was infused through the jugular vein with a Harvard infusion pump at a rate of 2μg/kg/ min for 10 minutes. Splenic flow was calculated 5 minutes post infusion for 5 minutes. Splenic vascular resistance was determined by dividing mean arterial blood pressure by splenic flow. Blood samples were obtained from the femoral artery and splenic vein 10 minutes post infusion.

In the study of splenic nerve stimulation, the splenic nerve was isolated and sectioned and distal ends placed on platinum electrodes (fig. 13). Supramaximal stimulation was employed. Electrical stimulation was accomplished using Grass square-wave stimulator and isolation unit with a frequency from 5-15 Hz, 5 millisecond duration and 5-20 volts. The splenic nerve was stimulated for 5 minutes. Splenic flow and metabolic changes were studied during stimulation. Responses to nerve stimulation also were studied before and after the administration of adrenergic receptor blockers.

Adrenergic blockade was achieved by administering beta-adrenoceptor blocker propranolol (Sigma) and alpha-adrenoceptor blocker phentolamine (Sigma) at 1 mg/kg body weight, respectively, through the femoral vein. The effects of splenic nerve stimulation were compared with those results from norepinephrine (2μg/kg/min) infused intravenously.

B. FLUORESCENCE HISTOCHEMISTRY

Preliminary studies of fluorescence histochemistry of the spleen with glyoxylic acid were made to confirm the existence of adrenergic innervation in the neonatal piglet. The histochemical technique has been described in detail by Furness and Costa (17). In brief, after the spleen was removed from the animal, it was placed in a 2% glyoxylic acid solution made up of 0.1 M phosphate buffer (initially pH 7.0 and readjusted to pH 7.0 with 10 M NaOH). The tissue was incubated for 30 minutes at room temperature, 18^{o}C, and then sectioned

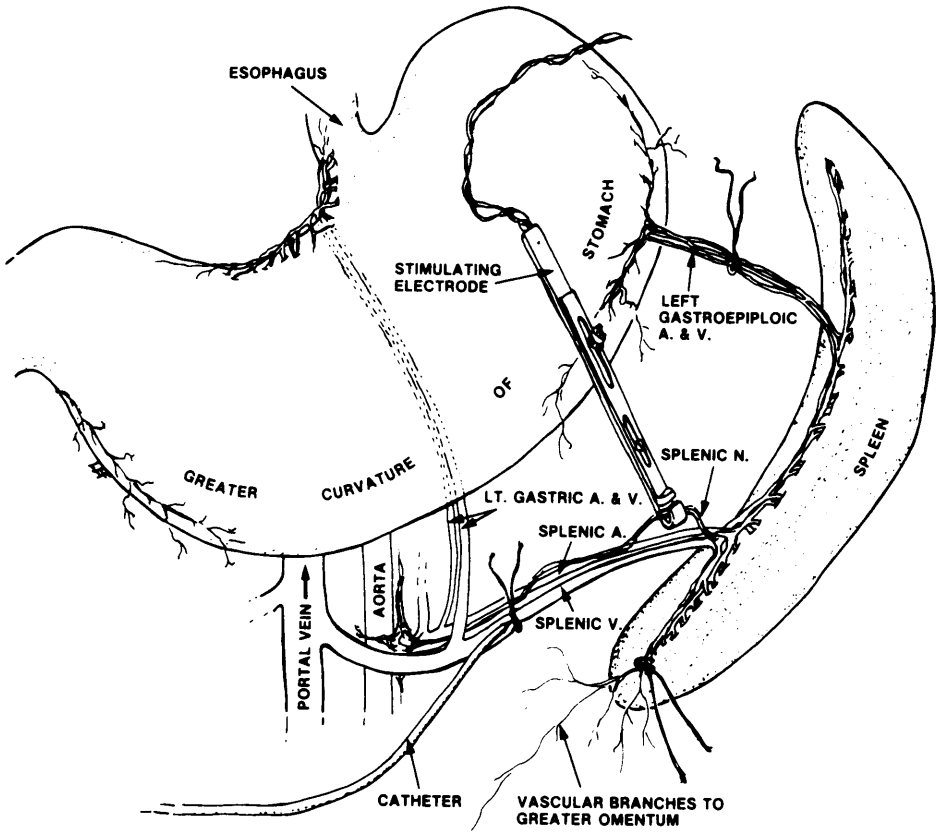

Figure 13 illustrates the electrical stimulation of the splenic nerve. The splenic nerve is isolated at the basal extremity of the spleen and its distal ends placed on the electrode.

longitudinally while still in solution. The sections were mounted on labeled cork blocks with Tissue Tek O.C.T. (optimum cutting temperature) embedding compound and transferred to propane cooled by liquid nitrogen for about 30 seconds. Sections of the frozen tissue were prepared on a Damon/EC Division minotome and stretched on clean glass slides to which they adhered as they dried. The slides were left on a lab bench for three minutes under a hair dryer and then placed in an oven set at 100 C for four minutes. The slides were then covered with paraffin oil and a coverslip. Fluorescence was examined under a microscope using an XBO 75 amps light source and a BA470 excitation and emission filter from Image Systems. Microphotographs were taken with an automatic exposure system UFX-II (Nikon) on Kodak ektachrome EES 800/1600 slide film. The noradrenaline containing fibers were distinguished from the 5-hydroxytryptamine containing fibers by the color of the florescence produced. The fluorescence of the 5-hydroxytryptamine is bright yellow and the noradrenaline is green under these conditions. A section of splenic artery taken from the hilus of the spleen of an 8 week old piglet showed extensive green fluorescence which demonstrates the presence of noradrenergic fibers in the arterial vasculature.

C. SPLENIC VENOUS FLOW DRAINAGE IN THE PIGLET

Four spleens from piglets 1-8 weeks old were studied by retrograde injection of x-ray contrast medium into either the catheter of the splenic vein or left gastroepiloic vein to identify the pattern of distribution of splenic venous drainage. Radiographs of the spleen were taken in the anterioposterior planes with an x-ray unit (Siemen's Polyphos 50). In general, the radiographic factor 6 MAS, 44 KV for exposure were set, with a focal distance of 100 cm, appropriate variations in factors were made for various sizes of spleens. The figure 14 shows a clear appearance of branching in the venous system throughout the entire spleen; providing evidence that the spleen venous drainage in these preparations were derived from the entire spleen.

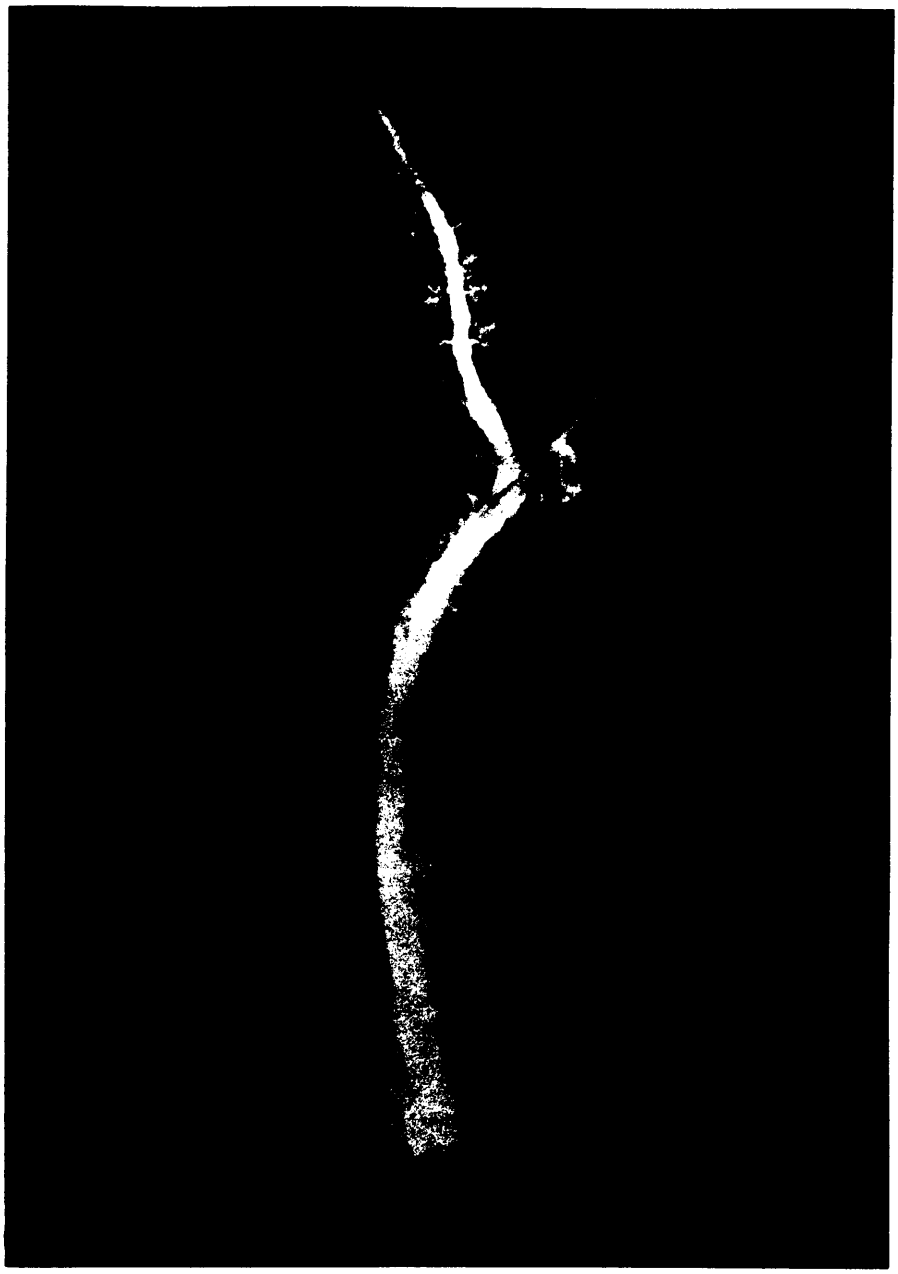

Figure 14 shows a representation sample of a postmortem spleen venous angiogram from a 4 week old piglet. Note the venous filling and clear appearance of large and small branches of the venous system throughout the entire spleen. The pattern of venous drainage vessels are readily visualized. These observations provide clear evidence that the spleen venous drainage in these preparations were derived from the entire spleen.

C. STATISTICAL ANALYSIS

All results are presented as mean values ± SE. Mean control values of splenic flow, splenic resistance, arterial and venous hematocrit, total white blood cells and the lymphocyte percentiles were compared with mean values during norepinephrine infusion. Comparisons of mean values of splenic flow, splenic resistance, mean arterial blood pressure, heart rate, arterial and venous hematocrit, oxygen consumption and extraction before and during nerve stimulation and following beta-and alpha-adrenergic blockade also were made. The statistical significance of these parameters was determined with a paired Student's t test (51). Results were considered statistically significant at $P < 0.05$ for all tests.

CHAPTER III.

RESULTS

A. RESPONSES TO NOREPINEPHRINE INFUSION

The effect of norepinephrine (NE) on splenic flow and splenic resistance were studied in 7 neonatal piglets. (The effect of NE on white blood cell differentials and lymphocyte percentiles in the spleen also were studied in these 7 piglets). Mean data, showing norepinephrine infusion significantly decreased splenic flow piglets, are illustrated in figure 15. The average value of splenic flow fell from 1.7 $\pm$ 0.14 (SE) to 0.89 $\pm$ 0.13 (SE) ml/min ($P< 0.01$). Correspondingly, the splenic vascular resistance rose significantly to an average of 290% during NE infusion ($P<0.005$). (Fig. 24). The splenic venous hematocrit increased in all 7 piglets (before NE 32.0 $\pm$ 1.05 (SE), after NE 41.6 $\pm$ 1.25 (SE) %; $P< 0.001$), but the arterial hematocrit was unchanged in these animals (before NE 31.1 $\pm$ 1.9 (SE), after NE 31.7 $\pm$ 2.2 (SE), %). (Fig. 16). Table 1 shows changes in splenic white blood cell differentials and lymphocyte percentiles during NE infusion. Furthermore, norepinephrine infusion had no significant effect on the migration and proliferation of leukocytes in the spleen (Fig. 17).

B. RESPONSES TO NERVE STIMULATION

The effects of electrical stimulation of the splenic nerve (NS) on splenic flow, splenic vascular resistance, and splenic oxygen metabolism were studied in a separate group of piglets (n=7). The average value for splenic flow fell from 13.2 $\pm$ 1.8 (SE) to 6.9 $\pm$ 1.8 (SE) ml/min ($P< 0.05$). (Fig. 18). The splenic vascular resistance rose significantly to an average of 75% during NS in 5 of the 7 NP ($P<0.05$). (Fig. 24). The splenic venous hematocrit increased in all 7 piglets (before NS 31.4 $\pm$ 1.9 (SE), during NS 38.9 $\pm$ 2.4 (SE); $P< 0.05$), but the changes in arterial hematocrit in these piglets were not significant (before NS 31.0 $\pm$ 1.8 (SE), during NS 31.1 $\pm$ 0.35 (SE) %;). (Fig.19). Table 2 shows

propranolol and phentolamine had no significant effect on splenic oxygen metabolism. The presence of beta-and-alpha adrenergic receptor blockade also had no significant effect on the response of splenic oxygen metabolism to NS.

The effect of NS on mean arterial blood pressure (MABP) and heart rate (HR) were measured in all piglets. Table 3 shows the changes in MABP and HR during NS were not significant. Propranolol and phentolamine also had no significant effect on the cardiovascular parameters measured during NS.

C. RESPONSE TO ADRENERGIC BLOCKADE

Figure 20 illustrates the effects of beta-adrenoceptor blockade on the response of splenic flow to splenic nerve stimulation. The average value of splenic flow fell from 10.5 $\pm$ 1.9 (SE) to 5.4 $\pm$ 1.7 (SE) ml/min ($P< 0.05$). (Fig 20). The splenic vascular resistance rose significantly to an average of 280% during NS ($P<0.05$). (Fig. 24). Beta-adrenergic receptor blockade with propranolol had no significant effect on all these measurements. The splenic venous hematocrit increased in all 5 piglets (before NS 30.2 $\pm$ 2.5 (SE), during NS 36.6 $\pm$ 2.2 (SE) %; $P< 0.001$), and the arterial hematocrit in these piglets was again unchanged (before NS 33.8 $\pm$ 0.7 (SE), during NS 33.2 $\pm$ 0.9 (SE) %). (Fig. 21).

Figure 22 illustrates the effects of beta-and-alpha adrenoceptor blockade on the response of splenic flow to NS. Phentolamine abolished the effect of NS on splenic flow in 5 of the 7 piglets (before NS 6.4 $\pm$ 1.5 (SE), during NS 5.7 $\pm$ 0.4 (SE) ml/min). The splenic vascular resistance rose only to an average of 17%. (Fig. 24). On the other hand, alpha-adrenergic blockade had no significant effect on the responses of splenic venous and arterial hematocrit to NS. The average value for splenic venous hematocrit increased significantly from 32.4 $\pm$ 3.3 (SE) to 43.4 $\pm$ 3.2 (SE) % ($P< 0.05$), and the arterial hematocrit was unchanged (before NS 30.6 $\pm$ 1.86 (SE), during NS 30.4 $\pm$ 2.5 (SE) %). (Fig. 23).

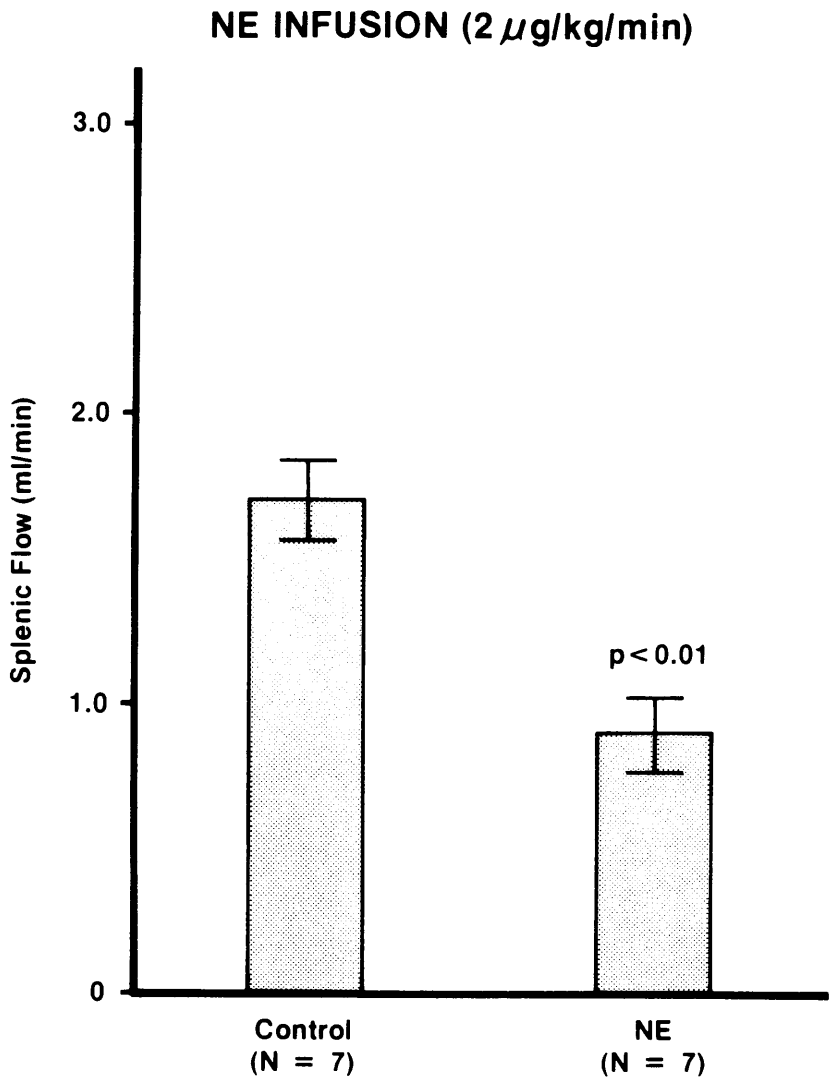

Figure 15. Response of splenic flow to norepinephrine infusion. Mean values of splenic blood flow (ml/min) before and during norepinephrine infusion (NE) in 7 NP.

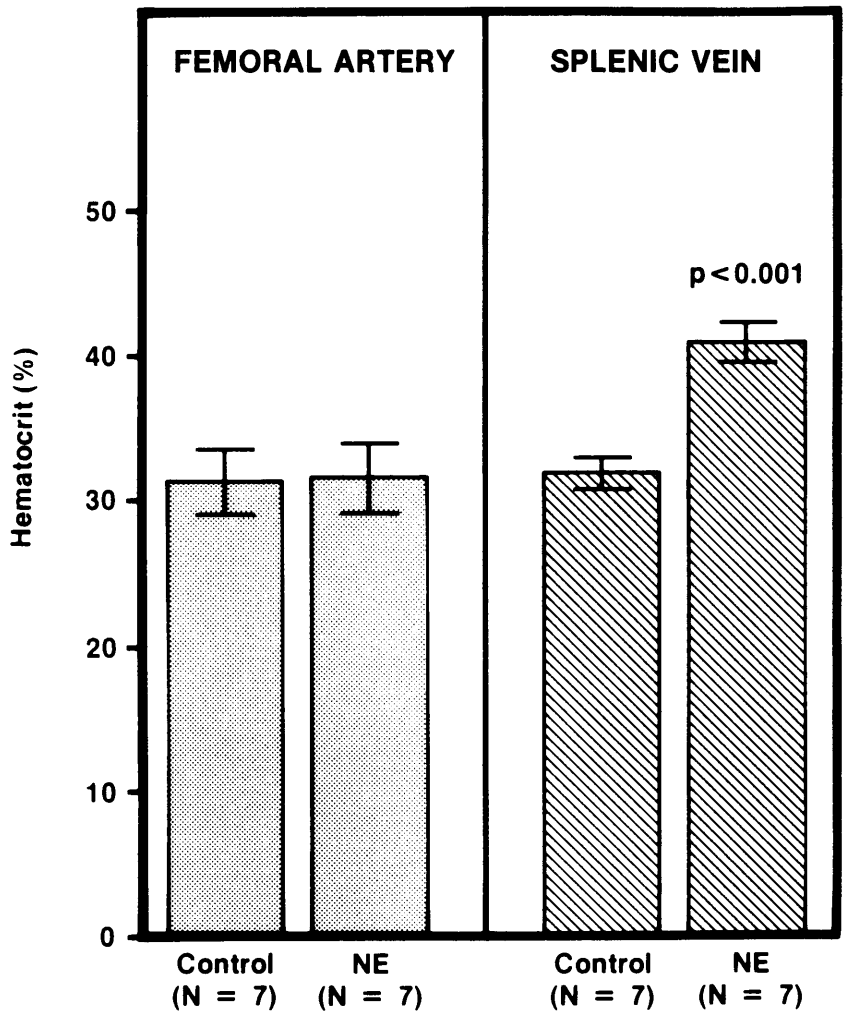

Figure 16. Response of arterial and splenic venous hematocrit to norepinephrine infusion. Mean values of arterial and splenic venous Hct (%) before and during NE infusion in 7 NP.

TABLE 1. Response of lymphocyte percentiles and white blood cell differentials to norepinephrine infusion.

WHITE BLOOD CELL DIFFERENTIALS

	WBC	LYMPHOCYTE
C	12,279 ± 2,022	73.1 ± 6.0
NE	10,358 ± 1,928	66.7 ± 6.2

Values are mean ± SE. C = pre-nervous stimulation. NS = nervous stimulation. WBC = total white blood cell. Lymphocyte in (%).

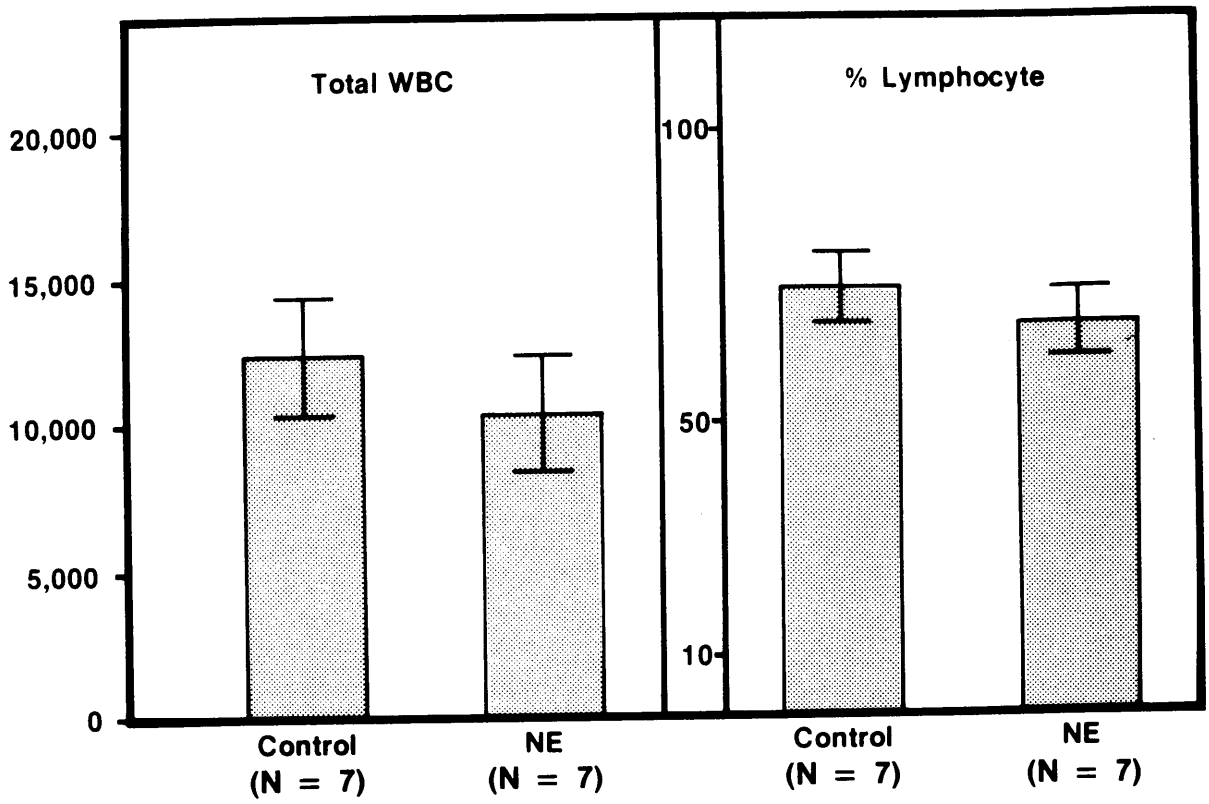

Figure 17. Response of lymphocyte percentiles and total white blood cell volume to norepinephrine infusion. Mean values of lymphocyte percentiles (%) and total WBC volume before and during NE infusion in 7 NP.

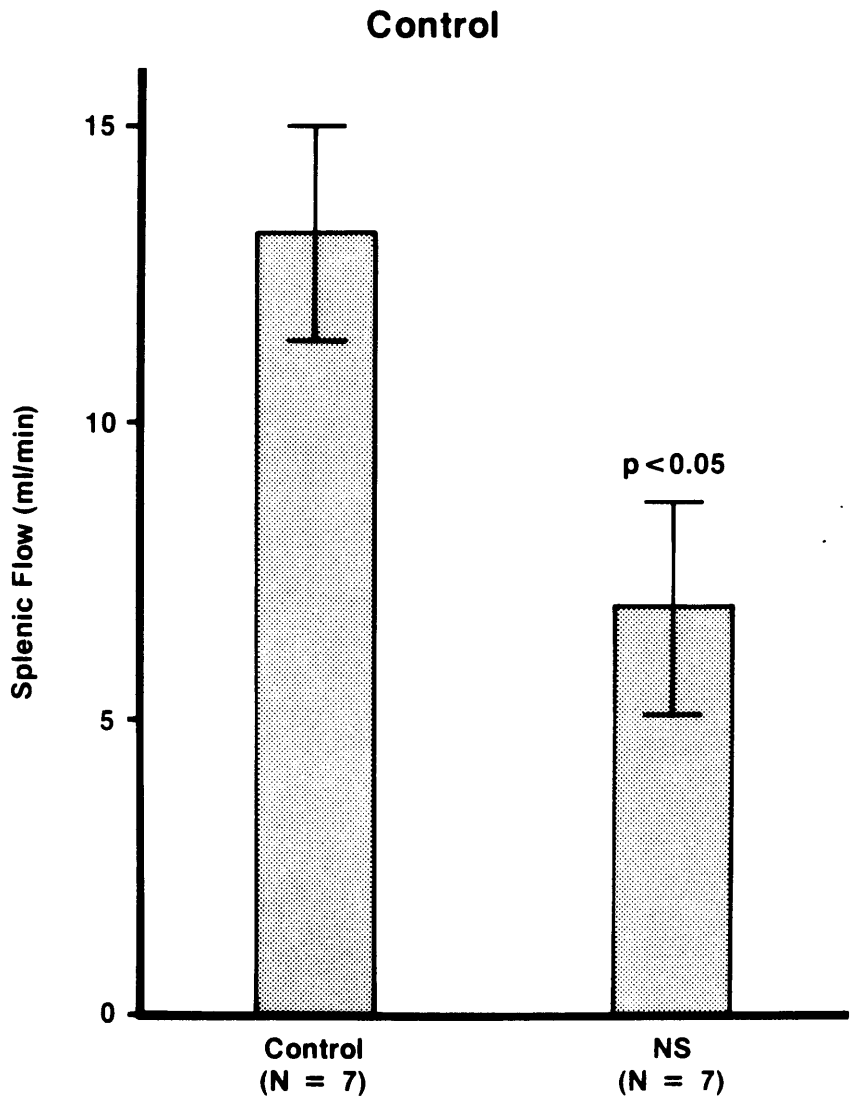

Figure 18. Response of splenic flow to nerve stimulation. Mean values of splenic blood flow (ml/min) before and during nervous stimulation (NS) in 7 NP.

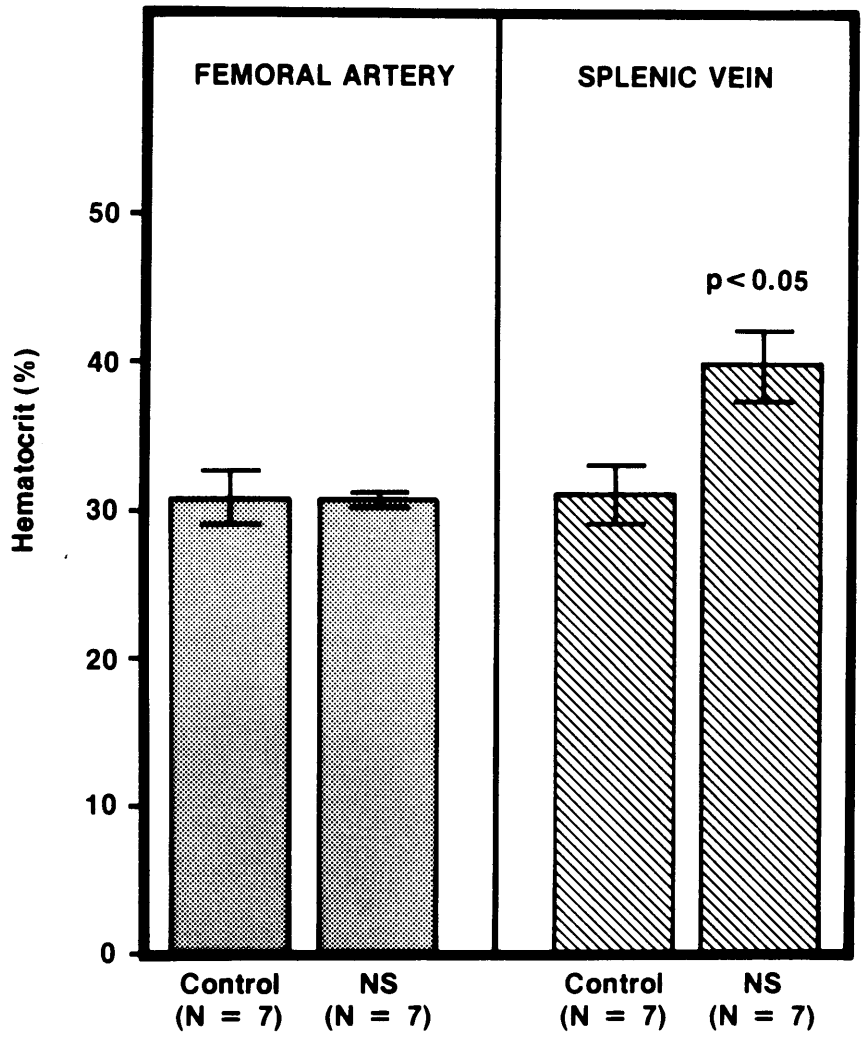

Figure 19. Response of arterial and splenic venous hematocrit to nerve stimulation. Mean values of arterial and splenic venous Hct (%) before and during NS in 7 NP.

TABLE 2. Response of oxygen extraction and consumption to nerve stimulation and adrenergic blockade.

	OXYGEN			
	EXTRACTION		CONSUMPTION	
GROUP	BEFORE	DURING	BEFORE	DURING
NS	19.3 ± 12.7	43.2 ± 13.1	27.8 ± 25.0	21.2 ± 3.7
β-BLOCKADE + NS	48.0 ± 11.1	50.5 ± 11.3	64.7 ± 18.2	38.1 ± 15.8
$\beta + \alpha$ BLOCKADE + NS	64.7 ± 18.2	38.1 ± 15.8	17.8 ± 11.0	10.0 ± 10.5

Values are mean ± SE. NS = Nervous stimulation. β-blockade = Beta adrenergic receptor blockade (propranolol, 1 mg/kg). α blockade = Alpha adrenergic receptor blockade (phentolamine, 1 mg/kg). Extraction in (%). Utilization (μl/min). Before = pre-nervous stimulation. During = during nervous stimulation.

TABLE 3. Response of mean arterial blood pressure and heart rate to nerve stimulation and adrenergic blockade.

HEMODYNAMIC DATA

	CONTROL		β-BLOCKADE		$\beta+\alpha$ -BLOCKADE	
	C	NS	C	NS	C	NS
MABP	65.1 ± 2.9	66.3 ± 3.9	71.8 ± 2.1	72.8 ± 3.4	61.3 ± 4.0	62.0 ± 4.2
HR	271.0 ± 25.3	221.0 ± 24.2	186.1 ± 24.6	144.0 ± 22.5	228.0 ± 21.3	254.0 ± 16.3

Values are mean ± SE. β-blockade = Beta adrenergic receptor blockade (propranolol, 1 mg/kg). α-blockade = Alpha adrenergic receptor blockade (phentolamine, 1 mg/kg). C = pre-nervous stimulation. NS = nervous stimulation. MABP = mean arterial blood pressure (mmHg). HR = heart rate (BPM).

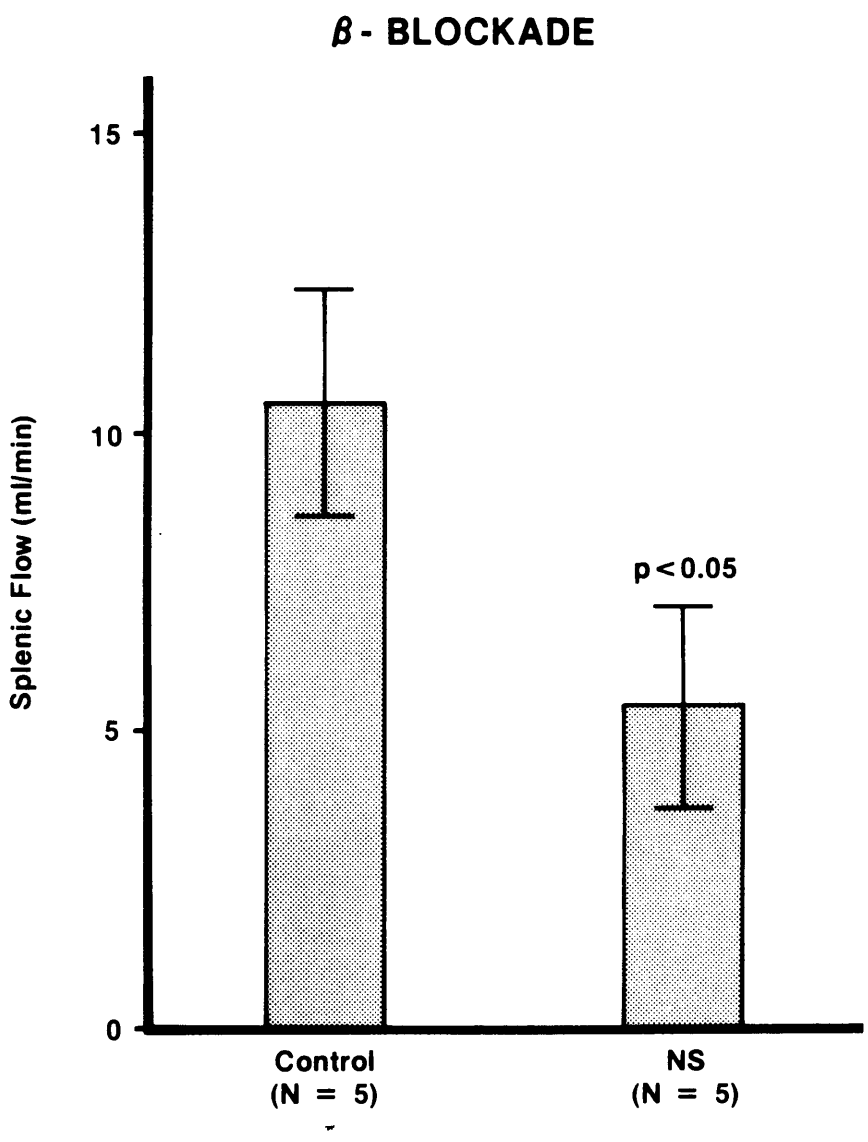

Figure 20. Response of splenic flow to nerve stimulation and beta-adrenergic blockade. Mean values of splenic blood flow (ml/min) before and during NS in 5 NP pretreated with ß-adrenergic receptor blocker propranolol (1 mg/kg).

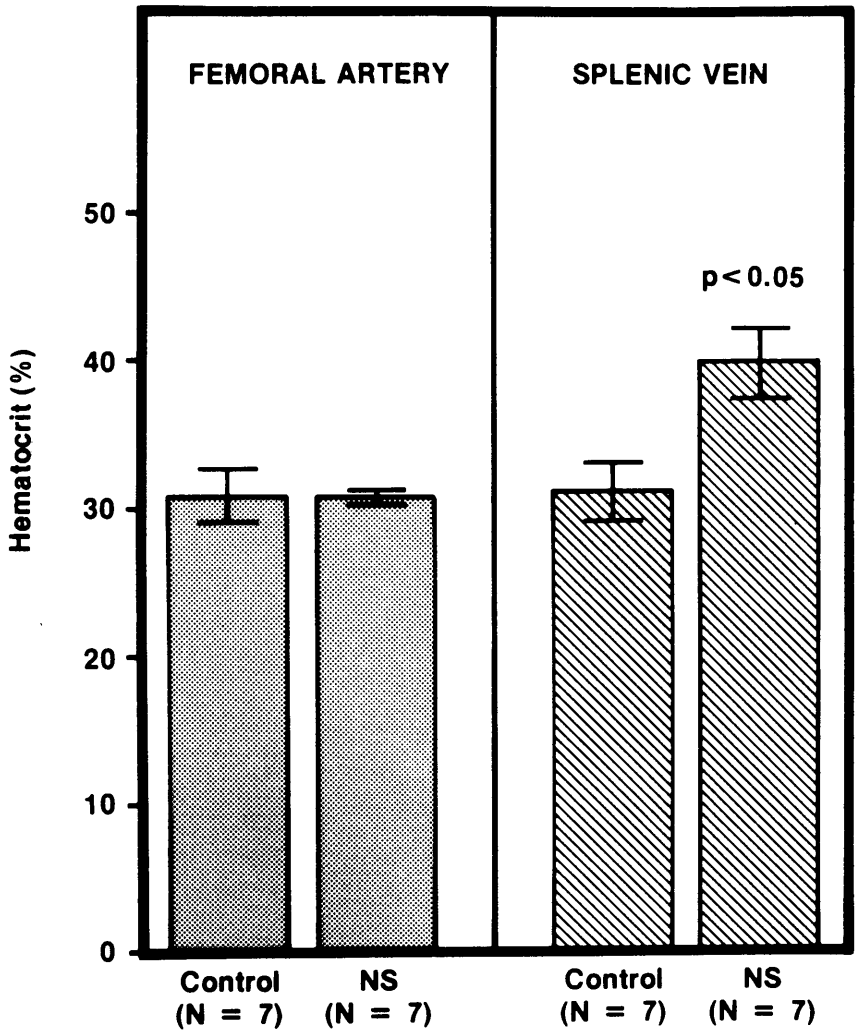

Figure 21. Response of arterial and splenic venous hematocrit to nerve stimulation and adrenergic blockade. Mean values of splenic venous Hct (%) before and during NS in 5 NP pretreated with ß-adrenergic receptor blocker propranolol (1 mg/kg).

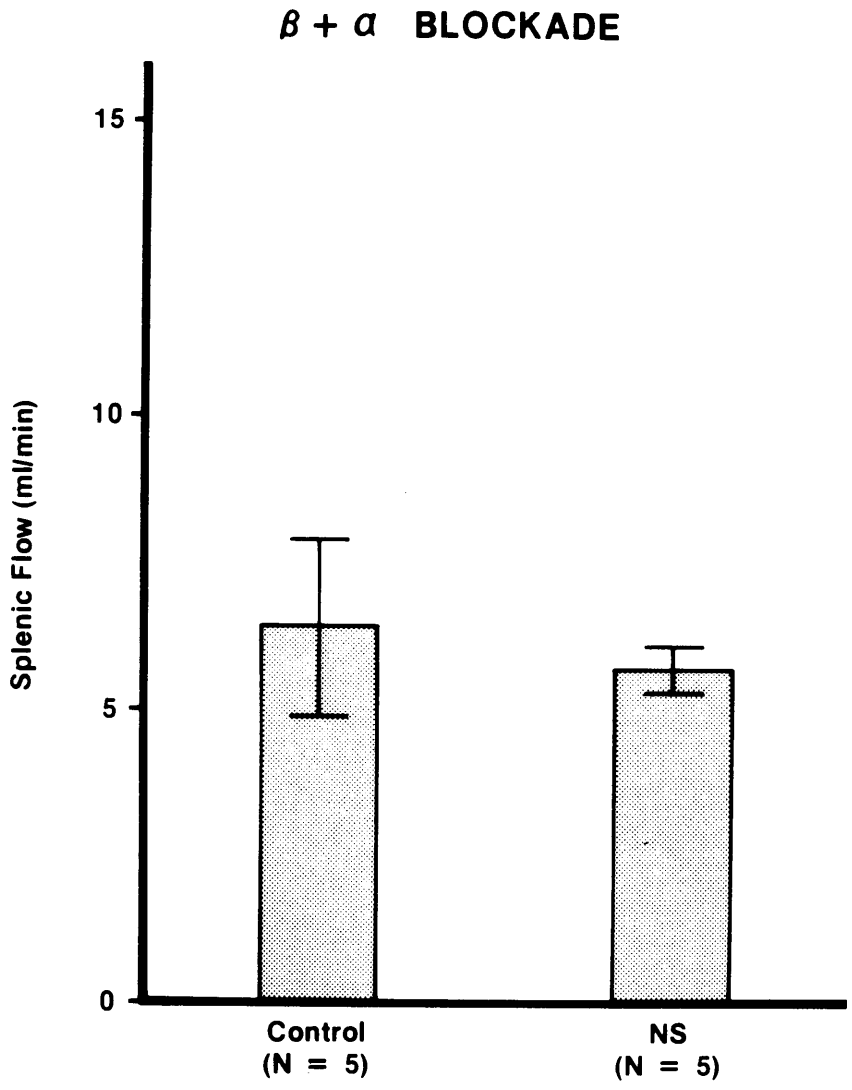

Figure 22. Response of splenic flow to nerve stimulation and beta plus alpha adrenergic blockade. Mean values of splenic blood flow (ml/min) before and during NS in 5 NP pretreated with ß-adrenergic receptor blocker propranolol (1 mg.kg) and a-adrenergic receptor blocker phentolamine (1 mg/kg).

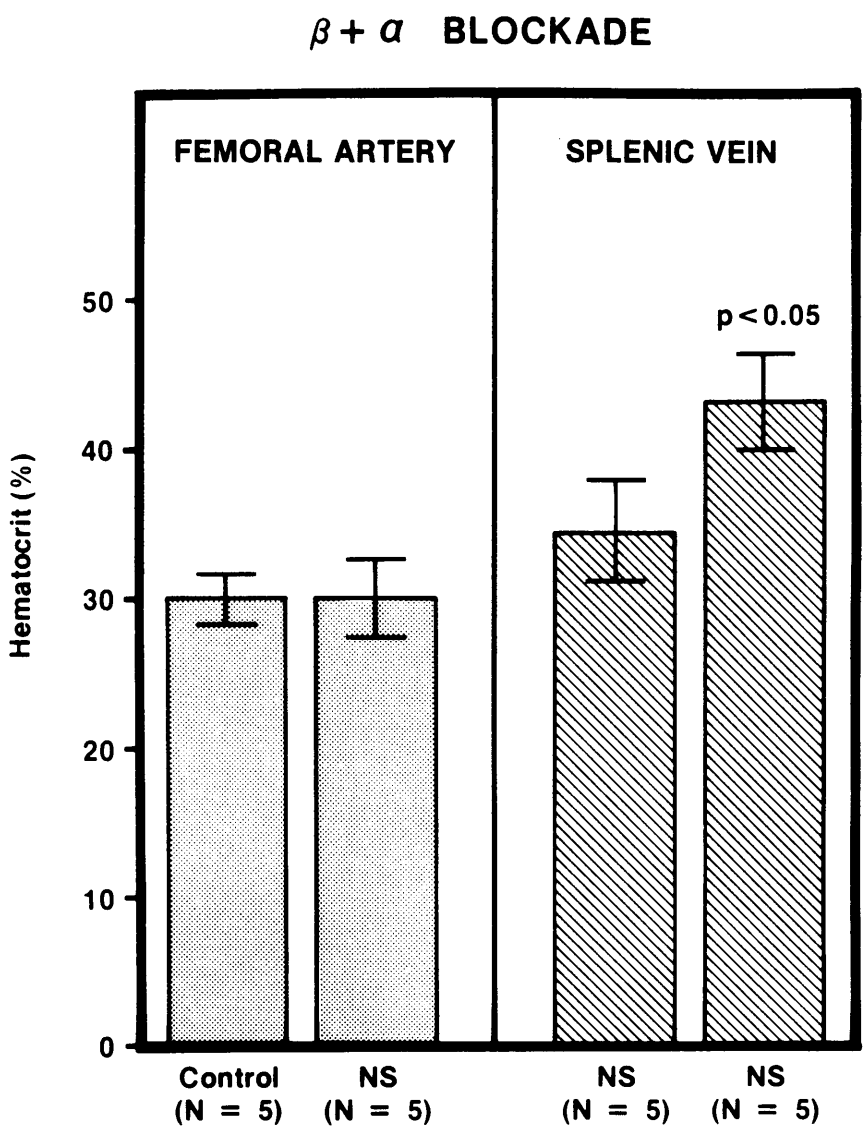

Figure 23. Response of arterial and splenic venous hematocrit to nerve stimulation and adrenergic blockade. Mean values of splenic venous Hct (%) before and during NS in 5 NP pretreated with ß-adrenergic receptor blocker propranolol (1 mg/kg) and a-adrenergic receptor blocker phentolamine (1 mg/kg).

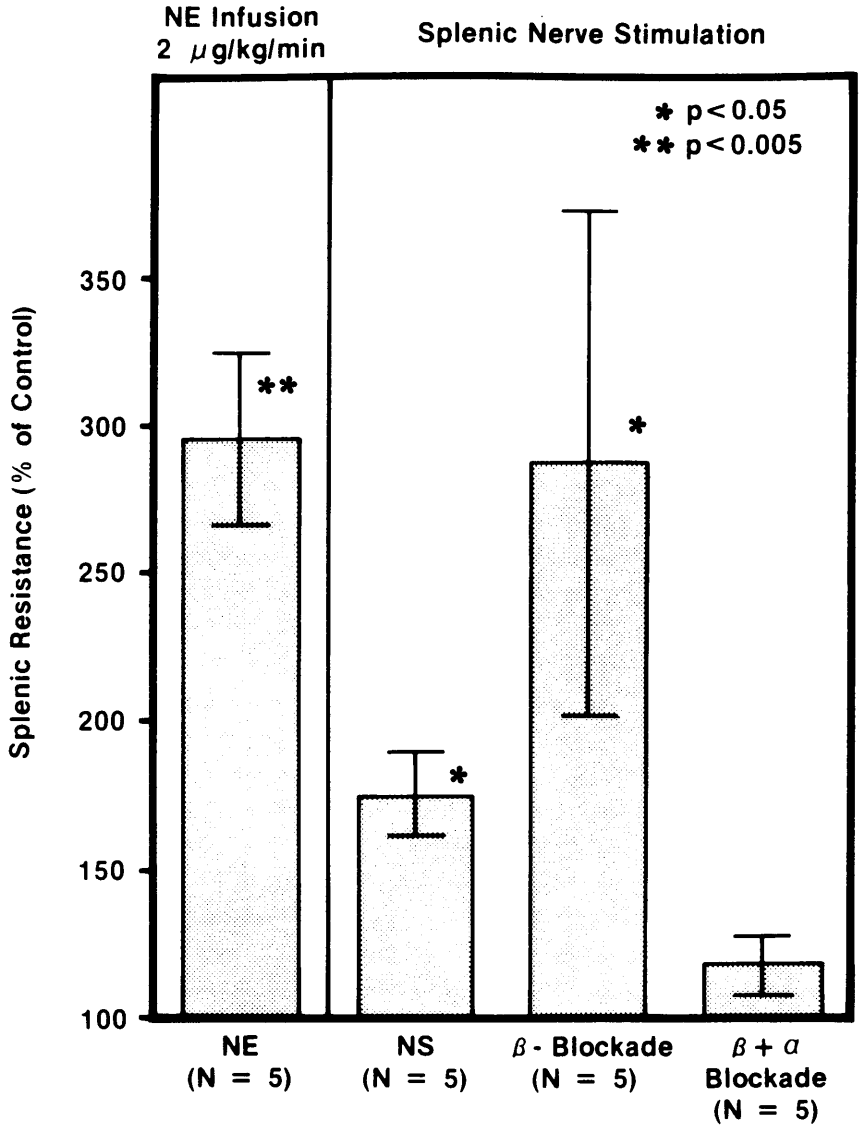

Figure 24. Response of splenic resistance to norepinephrine infusion and nerve stimulation and adrenergic blockade. Mean values of percentage increase in splenic resistance (Rsp) in NP during NS. NS= nerve stimulation, NE= norepinephrine infusion (2 g/kg/min), ß-blockade= ß-adrenergic receptor blocker, propranolol (1 mg/kg), å-blockade= å-adrenergic receptor blocker, phentolamine (1 mg/kg).

CHAPTER V.

DISCUSSION

The spleen has long been known to play a central role in many physiological functions such as blood filtration, hematopoiesis, immune response and blood reservoir functions as well. The details have been reviewed extensively in the chapter introduction. In many animals, such as the dog and cat, the spleen functions as an important blood reservoir in addition to its functions as part of the reticuloendothelial and leukocyte producing system. The splenic vasculature of these species contains extensive amounts of smooth muscle, and the capsule and trabeculae of the spleen are all heavily innervated with adrenergic fibers. Stimulation of the sympathetic fibers to the spleen produces capsular and trabecular contraction, which reduces the vascular capacity of the spleen and expels blood into the general circulation (47). On the other hand, in man, the splenic capsule contains little smooth muscle and its role as a blood reservoir is therefore negligible. It is recognized that important structural and biochemical differences in the spleen among various animal species presumably represents the various physiological responses that occur during stress. The postnatal changes of the physiological functions of the spleen also have been documented (48). Thus, extrapolation of findings in one animal species to another, or mature subjects to neonates, may be less than satisfactory. Furthermore, there is little information available for the farm animals. With this in mind, the present investigation was undertaken to obtain information on adrenergic control of hemodynamic functions and oxygen metabolism of the spleen in neonatal pigs. Adrenergic control of hemodynamic function of the spleen was assessed by direct splenic nerve stimulation and by intravenous infusion of norepinephrine.

Concurring with the results obtained in other species, this study shows that the activation of the adrenergic nervous system of the spleen causes a significant increase in splenic vascular resistance (fig. 24). In addition, this study further demonstrates that this effect is largely related to the activation of the alpha-adrenergic receptor system.

Earlier workers found electrical stimulation of the splenic nerve to result in the release of large volumes of blood from the spleen in the dog and cat with decreasing arterial inflow and increasing venous outflow and pressure (4,19,21). Similar findings were reported by Webb-Peploe (47) who further demonstrated that capsular contraction in response to splenic nerve stimulation is a major mechanism for increasing venous outflow in the canine spleen. These findings appear somewhat at variance with the results of the present investigation; but differences in preparation, species, and age must be considered. Splenic venous outflow may be varied by a) splenic arterial vasoconstriction, b) splenic venous vasoconstriction and c) contraction of the splenic capsule and trabeculae. Davies and coworkers (7) have demonstrated that the sensitivity of capsular smooth muscle and vascular smooth muscle to sympathetic stimulation varies with the species. It is possible that the smooth muscle of the splenic vasculature in the neonatal pig is more sensitive to noradrenergic stimulation than the smooth muscle of the splenic capsule. A second possibility that deserves attention is that adrenergic innervation of the splenic capsule and trabeculae in neonatal pigs may be incomplete. Comparable data from adult pigs, or neonatal animals of other species, are not available. Whether this specifically reflects incomplete sympathetic innervation of the spleen, or is part of a general immaturity of the adrenergic development in neonatal pigs in unknown.

In the present study, we further delineated the contribution of adrenergic influence on splenic hemodynamics. Results demonstrate that a major fraction of the adrenergic responses to splenic nerve stimulation in neonatal pigs can be attributed to the activation of the alpha-adrenoceptor system. As shown in figure 20, the extent of the vasoconstricting effect of splenic venous outflow to splenic nerve stimulation was not significantly altered by the beta-adrenergic blocker, propranolol, pretreatment. In contrast, addition of the alpha-adrenergic blocker, phentolamine, readily abolished the responses (fig.22). These findings are consistant with those of Greenway et al (21).

It is well known that a concentrated mass of red blood cells is released into the general circulation from the spleen upon contraction (4,24). Sympathetic stimulation resulting from fear, exercise, hypoxia and hemorrhage have been shown to increase splenic venous hematocrits in dogs (23,40,45). A significant rise in venous hematocrit levels in the dog and cat in response to splenic nerve stimulation also have been demonstrated (4,10,19,20). Donald and Aarhus (10) found the mobilization of blood from the spleen to be an active process independent of arterial inflow. Similar findings were reported by Brooksby and colleagues (3) where splenic venous hematocrits and splenic venous flow in dogs were unchanged during restriction of the splenic artery but increased to more than 80% during nerve stimulation. Chacalos and Moore (5) found constant infusions of epinephrine resulted in a 4 % increase in the systemic hematocrit. In a similar study, Guntheroth and coworkers (25) found infusions of epinephrine increased splenic venous hematocrits and splenic venous outflow in dogs. In another study, Opdyke (37) found infusions of epinephrine increased splenic outflow and hematocrits in dogs, but infusions of isoproterenol resulted in an increase in flow through the spleen without modifying the levels of splenic venous hematocrit.

The results of our investigations are compatible with those of Donald and Aarhus (10) and Brooksby and colleagues (3). Norepinephrine infusion and electrical stimulation of the splenic nerve resulted in significant increase in splenic venous hematocrits, but it had no significant effect on the arterial hematocrits (fig. 18 and 20). Since the arterial hematocrit values were unchanged, this suggested that the observed increase in splenic venous hematocrits must be a result of an active release of blood stores from the spleen. The administration of alpha-and beta-adrenoceptor blockers, however, had no significant effect on these responses. These findings may bare on previous observations by Opdyke (37) who indicated that the release of blood stores from the spleen may be mediated through some other mechanism independent of alpha- and beta-adrenoceptor activation. The possibility of this hypothesis being true will require more detailed studies at the receptor level.

It is well known that under normal conditions an increase of sympathetic activity in many organ systems is likely accompanied by a substantial augmentation of metabolic demands and oxygen consumption. Any change in the blood flow of an organ, as a result of change in vascular resistance or mean arterial blood pressure, can markedly alter oxygen extraction and consumption. However, prolonged restriction of blood flow may depress the oxygen utilization. This study demonstrated that electrical stimulation of the splenic nerve had no significant effect on either oxygen extraction or consumption (table 2). One possible explanation for these findings is that the spleen has a vey large oxygen availability because of the high levels of red blood cell storage in the spleen. In this circumstance, additional red blood cells might be anticipated to provide sufficient amounts of oxygen to the splenic tissue. Futhermore, the spleen is a blood conditioning organ which normally requires only minimal amounts of oxygen as compared to a metabolically active organ, such as the heart.

Yu and Clements (50) and Ernstrom and Sodor (12) have shown a significant increase in splenic leukocyte activity in response to sympathetic stimulation. In this study, we have found that norepinephrine infusion had no significant effect on the leukocyte migration activity of the spleen in neonatal pigs (table 1 and fig. 17). Whether this reflects a difference in species or age remains to be determined. Unfortunately, no data were obtained during nerve stimulation.

In conclusion, this study appears to be the first to investigate the adrenergic control of splenic hemodynamic function and oxygen metabolism in neonatal pigs. It may be concluded from the present investigation that in the neonatal pig, stimulation of the adrenergic nervous system of the spleen resulted in a significant decrease in splenic venous outflow with a concomitant increase in splenic vascular resistance. This is largely mediated by the activation of the alpha-adrenoceptor system in the spleen. Stimulation of the splenic nerves resulted in a significant increase in splenic venous hematocrit values but did not alter arterial hematocrit values.

Similar changes were observed with norepinephrine infusion. In contrast, adrenergic stimulation of the spleen does not importantly influence splenic oxygen metabolism in piglets. These responses were not altered by the adrenergic blockade. This may relate to the high red blood cell storage and low oxygen metabolism of the spleen. No significant changes in leukocyte migration and proliferation were detected during norepinephrine infusion. Currently, little is known about the neuro-immune interactions in farm animals, particularly in neonates. There is a wealth of evidence showing that splenic lymphocyte proliferation and migration are controlled through beta-adrenoceptor activation (1,6,12,14,28,29,49,50). The nervous system can influence the immune system and modulate the cellular function of lymphoid tissues (26,33,39). Obviated sympathetic innervation in the spleen suppresses immune response to T-dependent antigens in various lab animal species (15,16,25). The complex splenic leukocyte activity in response to splenic stimulation and stress in neonatal pigs remains to be established.

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