THE EFFECTS OF VAGAL STIMULATION ON THE PULMONARY CIRCULATION OF THE DOG, RABBIT, AND CAT AS SEEN IN 35 MM FILM MOUNTED SERIAL SECTIONS

THESIS FOR THE DEGREE OF M. S.

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BY

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INTRODUCTION

A primary clinical manifestation of many respiratory disorders is an abnormality in pulmonary vascular perfusion. Knowledge of the factors controlling this hypo-or hyper-perfused state would give the clinician a solid base on which sound corrective therapy could be built. Unfortunately, this controlling mechanism is an elusive one, and to date no single group of regulatory factors has been universally accepted.

The role of the autonomic nervous system in regulating the pulmonary circulation has been intensively studied for many years. The results of these investigations have often been contradictatory and inconclusive. Two camps of thought have been established. One, lead by Daly and Hebb (1966a), sought to isolate the lung and perfuse it with a regulatory pump. They measured neural effects by monitoring changes in pressure and flow rate. Elimination of the heart confined the observed response to the pulmonary circulation but left a rather poor physiologic preparation.

The other camp measured pressure and flow changes in intact animals. Their preparations were more physiologic but left some speculation as to whether the neural effects

acted directly on the pulmonary vascular system or were merely a passive reaction to changes in cardiac output.

Krahl (1968), an advocate of *in situ* techniques, has developed a method whereby Pelikan ink can be injected into the pulmonary vascular system. He concluded that mid-cervical stimulation of the distal end of either severed vagus nerve followed by ink injection would reveal the effects of that stimulation on pulmonary vascular perfusion.

The present study is a combination of the *in situ* techniques developed by Krahl and a much more expanded histologic evaluation. Its goal is to further define the parasympathetic nervous system's role in regulating the pulmonary circulation.

Electric mid-cervical stimulation of the distal end of the severed left vagus nerve of dogs, rabbits, and cats was followed by ink injection and *in situ* formalin fixation. The middle lobes from both stimulated (left) and control (right) lungs were evaluated grossly for the degree of peripheral ink perfusion, and histologically by serial sectioning from the hilus to the periphery. Histologic sections were mounted on 35 mm leader film utilizing a modification of a technique developed by Wilson and Pickett (1970).

With the aid of an ocular micrometer and the Weibel, Kistler, and Scherle (1966) method for stereologic measurements, the following were calculated for both the stimulated and control lobes: (1) the relative volume occupied by the

pulmonary arteries, arterioles, veins and venules (2) the relative volume occupied by the ink filled pulmonary capillaries (3) the relative volume occupied by the pulmonary airways ranging in size from secondary bronchi to terminal bronchioles and (4) the length per unit volume of the pulmonary arteries, arterioles, veins, and venules.

The average volumes of right and left middle lobes from three experimental animals of each species were determined by the water displacement technique. The calculated relative volumes were then multiplied by the average lobar volume to determine the absolute volumes of the pulmonary components being studied.

A representative animal from each species was studied for control data. The surgical, histologic, and microscopic evaluation procedures were identical to those used in the experimental animals. The only difference was that the control animals did not undergo vagal stimulation. The control data was used to determine whether the variations seen in the experimental animals were due to the vagal stimulation or to the normal variations in lung architecture.

REVIEW OF LITERATURE

Effects of Vagal Stimulation on Pulmonary Vasomotion

Pulmonary vasomotor studies generally involve chemical or electrical stimulation on either *in situ* or isolated perfused preparations. The inherent disadvantages of these techniques necessitates a cautious evaluation of their results. Secondary or masking effects are often seen in *in situ* studies where all bodily functions and inter-relationships must be considered. The lack of this physioligic setting in dealing with organs separate from the whole is the cause of concern in isolated perfusion studies. Fortunately these methods tend to complement each other and valid conclusions can be drawn when both are viewed in light of each other's weaknesses.

Isolated Perfused Technique

Daly and Hebb (1966b) summarized the technique most commonly used in the study of isolated perfused pulmonary tissue. This method employed two mechanical pumps which fed the bronchial and pulmonary circulations. Only the lungs and the extrapulmonary course of their nerves were kept viable throughout the procedure. The two pumps were fed by a single reservoir of perfusate (animal's own blood).

The first pump, replacing the right ventricle, was situated between the right heart and the main pulmonary artery. Its perfusate supplied the pulmonary circulation. The second pump, replacing the left ventricle, was situated at the base of the aortic arch and perfusion allowed down the thoracic aorta to T_7 or T_8. Its perfusate supplied the bronchial circulation. Blood returning from the pulmonary and bronchial circulations was drained into the single reservoir for later recycling. The free end of a cannula was then inserted into the pulmonary artery and its opposite end connected to a manometer.

This isolated perfused technique allowed perfusion of the pulmonary and bronchial circulations at either a constant volume or a constant pressure. When constant volume inflow was employed, vasomotor responses to electric or chemical stimuli were assesed according to changes in the volume outflow by monitoring the volume of blood draining into the reservoir. A decrease in the volume outflow would indicate a possible vasoconstriction, while an increase in volume outflow would suggest a vasodilation. When constant pressure was employed, vasomotor responses to stimuli were assesed according to changes in either the outflow volume or the pulmonary arterial pressure. An increase in pressure suggested vasoconstriction and a decrease in pressure indicated vasodilation.

According to Daly and Hebb (1966c) one of the first isolated perfused studies of the pulmonary circulation was

performed by Cavazzani in 1891. He studied blood flow in isolated perfused rabbit lungs before and after electric mid-cervical stimulation of the vagus nerve. His results suggested the presence of a vagally mediated pulmonary vasoconstriction.

Hunt (1918) perfused cat and rabbit lungs with a Ringer's solution containing acetylcholine and observed its effect on outflow volume. He noted that small doses (quantity not stated) of acetylcholine did not alter volume outflow. Larger doses added to the Ringer's solution produced a prolonged decrease in outflow volume which was not affected by comparably large doses of atropine.

Von Euler (1932) electrically stimulated (primary current of approximately 3 volts) the mid-cervical intact vagus nerve on an isolated perfused preparation of rabbit lung. Fifteen to thirty seconds of vagal stimulation resulted in a rise in pulmonary arterial pressure, an effect that was abolished by injection of atropine (5 mg). Injection of 0.5 mg of acetylcholine into the pulmonary artery also resulted in a significant rise in pulmonary arterial pressure accompanied by a diminished outflow volume. He concluded that the vagus nerve was at least partially responsible for vasopressor activity in the pulmonary circulation of the rabbit.

Daly and von Euler (1932), studying isolated perfused canine lungs, electrically stimulated (current from a DuBois-Reymond induction coil, coil distance = 7 cm) the vagus

nerve a distance of 6-10 cm above the inferior cervical ganglion. Vasomotor responses to electric stimuli were assessed according to changes in pulmonary arterial pressure. Simultaneous stimulation of both the left and right intact vagus nerves on three isolated perfused preparations resulted in an increase in pulmonary arterial pressure (vasoconstriction) in two animals and a slight decrease in pulmonary arterial pressure (vasodilation) in one animal. In similar preparations, a vasodilation response was observed after injection of 1.0 mg of acetylcholine into the pulmonary artery of five experimental animals. One preparation demonstrated a pulmonary vasoconstrictor response which was abolished after injection of atropine.

Gaddam and Holtz (1933) studied the effects of acetylcholine on isolated perfused dog and cat lungs. The responses, seen as changes in perfusion pressure, were dual in nature. In both the dog and the cat small doses of acetylcholine (1-10 ug) produced vasodilation while larger doses (over 20 ug) resulted in vasoconstriction.

Alcock, Berry, and Daly (1935) observed isolated canine lungs perfused with defibrinated blood and placed under negative pressure ventilation. Injection of acetylcholine (200 ug) resulted in a slight fall in pulmonary arterial pressure accompanied by inconsistent changes in pulmonary outflow. Larger doses (1.0 mg) produced a rise in pulmonary arterial pressure accompanied by a slight increase in outflow.

Borst, Berglund, and McGregor (1957) studied the effect of acetylcholine injection on isolated perfused canine lungs. Pressures in the left and right pulmonary arteries were measured by electromanometers and continuously recorded on an ocillograph. Responses to chemical stimuli were assessed according to changes in pulmonary arterial pressure. The lungs were ventilated with 100% oxygen and allowed to collapse immediately prior to the drug injection. Acetylcholine (3-9 ug per kg of body weight) was injected into the pulmonary artery of the non-ventilated lungs of six animals. Two animals exhibited vasoconstriction, another two vasodilation, one demonstrated vasoconstriction followed by prolonged vasodilation, and one animal gave no response. All responses were of a small magnitude. Borst et al. concluded that the lack of consistent pulmonary vasodilation after injection of acetylcholine may be due to the fact that the tone of the pulmonary vascular bed was low before the drug was injected.

Daly and Hebb (1966d) summarized their observations on the responses of isolated perfused canine lungs to injections of acetylcholine. They found that injection of 10-100 ug of acetylcholine resulted in pulmonary vascular dilation seen as a fall in pulmonary arterial pressure. Injection of acetylcholine in units ranging from 5-1000 ug resulted in pulmonary vascular constriction seen as an increase in pulmonary arterial pressure.

Hague, Lunde, and Waaler (1966) infused acetylcholine into the pulmonary artery of isolated perfused rabbit lungs. A marked increase in pulmonary arterial pressure was observed after injection of acetylcholine in doses down to 5.19 ug. They considered that this increase in pressure was due to a marked pulmonary vasoconstrictor response to acetylcholine injection.

In Situ Techniques

The majority of *in situ* studies employ one or a combination of three possible techniques; transillumination of pulmonary tissue, thoracic windows, and pulmonary arterial or venous pressure recordings. Common to all is the requirement that the animal's lungs, cardiovascular system, peripheral, and central nervous systems be kept intact. Preparations may involve either open or closed chested surgery.

Transillumination of Pulmonary Tissue

According to Wagner and Filley (1965) attempts to observe alveolar function date back to 1661 when Malphigi, using the open thorax technique, transilluminated frog lungs with candle light. More than two centuries passed before Hall (1925) expanded the Malphigian technique in his studies on the rabbit and cat. After light anesthesia the animals were pithed and their lungs aerated by a modification of the Meltzer's (1909) method of intratracheal inflation. The necessary portions of the rib cage were removed and the

inflated lobe elevated and its margins held in place by clips. The marginal areas of the lobe were transilluminated and the pattern of blood flow observed. Hall noted that flow in the larger arterioles and veins was pulsatory while flow in the smaller arterioles, venules, and capillary networks was continuous. He then painted the heart with nicotine to eliminate any changes in cardiac rate during subsequent vagal stimulation. Electric vagal stimulation, which on non-nicotine treated animals caused a slowing in heart rate, had no effect on cardiac rate, the caliber of any pulmonary vessel being observed or the velocity of blood flow within these vessels.

Melvin Knisely (1934, 1938, 1967) has developed and improved the technique of studying living tissue through the use of fused quartz rod transillumination. William Knisely (1969), in conjunction with the work of Irwin (1958), applied the technique of quartz rod transillumination in his studies on pulmonary tissue. His experimental procedures were as follows: After surgical anesthesia and tracheal cannulation a thoracotomy was performed allowing the edge of a lobe to be visualized. An oxygen catheter (diameter less than that of the cannula) was inserted into the tracheal cannula and the flow of oxygen regulated so that the lungs remained constantly inflated. Gases were allowed to escape around the inner edges of the tracheal cannula. The tip of a fused quartz rod was then placed under the edge of the lobe in question. A beam of light falling upon the

base of a clean, smooth, polished quartz rod is reflected inside the rod until the majority of it reaches the opposite end. Knisely has likened this property to the way a hose conducts water. The rod is broken near its light source to prevent heat from reaching the tissue by conduction or convection. To protect the tissue from heat delivered by the transformation of radiant energy a flowing wash solution of Ringer's lactate was applied to the exposed tissue by means of two glass tubes. The first tube brought the wash solution to the tip of the quartz rod so that the tissue being placed above it would never come into direct contact with the quartz. The second wash was delivered to the upper surface of the tissue. All microscopic observations were made from this upper surface. William Knisely has utilized this technique to further advance the anatomical knowledge of the pulmonary circulation. The results of his observations will be included in the review of the pulmonary anatomy.

Irwin and Burrage (1954) utilizing fused quartz rod transillumination of the rabbit lung observed that intermittent linear blood flow occurred in the pulmonary arterioles, capillaries, and venules. This flow was observed in vessels up to 160 u in diameter.

Wilson (1970) suggested replacing the fused quartz rod with a rod made of borosilicate glass. Borosilicate, while having similar light transmission properties, is cheaper and conducts less heat than quartz and may be pigmented to increase photographic contrast.

Thoracic Windows

In 1934 Wearn, Ernstene, Bromer, Barr, German, and Zachiesche developed a technique of directly observing the smaller superficial blood vessels and air sacs in the closed thorax of the cat. A circular layer of costal parietal pleura (0.6-1.2 cm in diameter) was exposed by stripping away the skin and muscle in the mid-axillary line between the eighth and ninth ribs. A similar window was made directly opposite the first by dissecting the muscle away from the abdominal surface of the diaphragm, exposing the disphragmatic parietal pleura. Pulmonary tissue was transilluminated through this diaphragmatic window with light from a fused quartz rod. All microscopic observations were made through the chest window.

Wearn et al. noted intermittent blood flow through pulmonary arterioles less than 100 u in diameter. They also found that the velocity of blood flow increased after atropine injection into the femoral vein.

Terry (1939) developed the technique of implanting a permanent artificial thoracic window. His device, consisting of a short hollow bronze cylinder one end of which was covered by a glass plate, was surgically installed in the right fifth intercostal space of the cat. Using a binocular microscope and an arc lamp, direct observations were made on the pulmonary tissue.

Krahl (1962) eliminated the irritating reactions of direct contact of metal and glass on the pulmonary visceral

pleura by jacketing a lucite window frame in transparent teflon. In this case, the transparent teflon served as the viewing port. By observing rabbit lungs through various areas of the costal parietal pleura he noted an area on the right side at the level of the third rib where the movement of the lungs was at a minimum. Installation of the thoracic window at this static area required prior surgical amputation of the right thoracic limb.

When Krahl observed the surface of the right lungs through the teflon window he noted polygonal areas light pink in color surrounded by wider areas having a deeper pink hue. He concluded that these lighter areas corresponded to the bases of the pyrimidal units of the lung referred to as secondary pulmonary lobules. By the transient nature of the lighter pink areas he suggested that a mechanism possibly exists which regulates pulmonary blood flow on a lobular basis.

According to Miller (1950), a primary lobule consists of one alveolar duct and the atria, alveolar sacs, and alveoli which arise from it. Approximately fifty primary lobules are grouped together to form a secondary lobule. This subgross anatomical division is surrounded by a layer of connective tissue which separates it from adjacent secondary lobules.

Krahl (1963) injected a mixture of India ink, saline, and KCl directly into the beating right heart. Post mortum examination of the lung surfaces revealed a

mosaic pattern of ink distribution. He concluded that the lighter mosaic areas were underperfused and the darker areas well perfused when functional cardiac pumping ceased. Subsequent histologic evaluation demonstrated ink perfusion in both the arterioles and capillaries of the darker areas. In the lighter areas ink perfusion ceased at the right angled branching precapillary arterioles leaving the capillary networks void of ink.

His next step (1965) was to electrically stimulate the distal end of the mid-cervically severed right vagus nerve while injecting a mixture of India ink and saline (1:1) through the external jugular vein. The animals were terminated by either increasing the current to produce cardiac arrest, or adding a few crystals of KCl to the India ink saline mixture. Examination of the surfaces of the lungs revealed that the vagally stimulated right lung was considerably lighter than the control left lung. Histologic sections of the lighter areas of the stimulated lung demonstrated a constriction of a circular layer of smooth muscle at the precapillary arteriolar site. This constriction stopped the flow of ink to the capillary network. Subsequent histologic evaluation of the black areas of the control (left) lung revealed that the sphincter mechanism was relaxed allowing ink to freely enter the pulmonary capillary networks.

Further experiments (1966) involved intracardiac injection of acetylcholine (0.01 mg/kg of body weight of

a 1:25,000 solution of acetylcholine) just prior to the injection of ink. The surfaces of these lungs appeared nearly as pink as the normal non-injected lungs.

From the results of both the electric vagal stimulations and injections of acetylcholine, Krahl concluded that blood flow to the pulmonary capillary networks was at least partially regulated by vagally induced constriction of the precapillary muscular sphincter.

Krahl (1969) discussed the clinical significance of this vagally mediated pulmonary vasoconstriction. He suggested that atropine (parasympathetic blocking agent) be used in treating infants with respiratory distress syndrome to counteract the clinically established pulmonary vasoconstriction. (Chu et al., 1965).

Wagner and Filley (1965) implanted a thoracic window (plexiglass and vinyl) on the right side of the canine lung at the level of the second and third intercostal spaces. The thickness of the lung at this static site did not permit transillumination and a Leitz Ultropack incident illuminator microscope was used for all observations. They observed a pulsatory flow of blood through the arterioles (up to 100 u), capillaries, and venules (up to 200 u). Vagal stimulation (20 volts/15 cycles per second) resulted in a significant decrease in the velocity of red blood cells in all vessels with no change in vessel lumen size. The stimulation effects were qualitatively evaluated by observing cinemicrographs taken during the stimulation.

In Situ Pressure Recordings

Rudolph, Kurland, Auld, and Paul (1959) injected acetylcholine and observed its effects on the pulmonary circulation of the dog. Responses were seen as changes in pulmonary arterial pressure. Injection of acetylcholine (40-50 mg/kg/min) resulted in a slight rise in pulmonary arterial pressure. Similar injections of acetylcholine in pulmonary vessels which were preconstricted by a prior injection of serotonin (500 ug) resulted in a significant decrease in pulmonary arterial pressure.

Rudolph et al. studied the effects of similar injections of acetylcholine on two open chested preparations in which left pulmonary venous and pulmonary arterial wedge pressures were recorded. Drug injection resulted in a mild increase in pulmonary arterial wedge and pulmonary venous pressure. Injection of acetylcholine while the pulmonary arterial pressure was in an elevated state due to continuous injection of 5-hydroxytryptamine creatinine sulfate (75-100 ug/kg/min) resulted in a decrease in pulmonary wedge and venous pressures.

Braun and Stern (1967) in their review of Rudolph et al.'s work (1959) concluded that the response of the pulmonary venous system to drugs may depend on pre-existing venous tone.

Shimomura, Pierson, Krstulovic, and Bell (1962) studied the effects of acetylcholine injection (0.5-10 ug) on the perfusion pressure of a catheter wedged in a small

pulmonary artery of the dog. The perfusate (heparinized autogenous venous blood) was delivered to the wedge segment at a constant flow. The catheter was designed to both perfuse and measure the wedge arterial pressure. A micro-catheter was inserted to the tip of the wedge catheter to permit direct injection of acetylcholine. Injection of a single bolus of acetylcholine (0.5-1.0 ug) resulted in a rise in perfusion pressure with no change in cardiac rate, left atrial or systemic blood pressures. When larger doses (2-10 ug) were injected, a rise in perfusion pressure was also noted before the drug was able to decrease systemic pressure. Shimomura et al. concluded that the rise in pressure seen after injection into the perfused wedged segment of the canine lung was ample proof for a direct vasoconstrictor effect of acetylcholine on the pulmonary vascular bed of the dog.

Rudolph and Scarpelli (1964) studied the effects of acetylcholine injection (1-25 ug/min) on the pulmonary arterial pressure of closed chested dogs. Electromagnetic flow transducers were placed around the main and left pulmonary arteries. Catheters were situated in the right ventricle, the main and left pulmonary arteries. Infusion of acetylcholine into the right ventricle or main pulmonary artery resulted in no significant changes in the total (main pulmonary artery) or left pulmonary artery blood flow. Similar infusions into the left pulmonary artery resulted in a significant decrease in left pulmonary flow and no

changes in total flow. Rudolph and Scarpelli interpreted these responses to mean that acetylcholine caused a direct pulmonary vasoconstriction in the dog.

Harris (1957) studied the effects of acetylcholine injection (0.25-8.0 mg) on normal and diseased humans. Of the three normal subjects studied, no significant change in pulmonary arterial pressure was observed after drug injection. Of the forty-two patients suffering from various abnormalities, eighteen demonstrated a fall in pulmonary arterial pressure, two responded with a rise in pressure, and twenty-four gave no response. Harris noted that the decrease in pressure was found most frequently in patients who's normal pulmonary arterial pressure was elevated. He concluded that pre-existing vessel tone must be considered in evaluating the effects of acetylcholine on the pulmonary circulation.

Soderholm and Werko (1958) injected acetylcholine (3.0-14.5 mg/min) into the pulmonary artery of thirteen patients with mitral valve disease. Drug injection produced a statistically significant decrease in pulmonary arterial pressure and systemic oxygen saturation, accompanied by an increase in cardiac output and no significant differences in cardiac rate. They concluded that the decrease in systemic arterial saturation was due to the dilation of pulmonary arterioles in poorly ventilated areas of the lung.

Schlants, Tsagaris, Robertson, Winter, and Edwards (1962) observed the effects of acetylcholine on the human pulmonary circulation of twenty-one patients suffering from various abnormalities. Arterial blood gases and pressures from the brachial and main pulmonary arteries were recorded throughout the experiment. Cardiac output was calculated by the Fick principle and minute ventilation was recorded with Douglas bags. Infusion of acetylcholine into the pulmonary artery or right ventricular outflow (20-75 ug/kg/min) resulted in a decrease in mean arterial saturation, an increase in mean cardiac index, and a decrease in calculated mean total pulmonary resistance and total systemic resistance with no significant changes in pulmonary and brachial arterial pressures.

Schlants et al. (1962) reviewed the work of Chidsey (1960) in which acetylcholine (3 mg/min) was injected into the right atrium of patients with pulmonary emphysema. Chidsey observed that systemic arterial saturation decreased in nine out of thirteen patients. He concluded that acetylcholine dilated pulmonary vessels constricted by hypoxia thus increasing the perfusion of poorly ventilated areas of the lung.

Isolated Pulmonary Vessels

Franklin (1932) placed rings cut from isolated pulmonary blood vessels of the dog in Ringer's solution (37^0C) containing acetylcholine (up to 1:1,000,000). An

optical device was used to record changes in ring diameter. All vessels studied had a circumference larger than 4 mm. He observed relaxation of the extrapulmonary arteries, no response from intrapulmonary arteries, mostly contraction of intrapulmonary veins, and consistant contraction of the extrapulmonary veins. He concluded that parasympathetic stimulation may result in engorgement of the lungs by dilating the arteries and constricting the veins.

Bohr, Goulet, and Taquini (1961) studied the effects of acetylcholine on helical strips of rabbit and dog pulmonary arterial vessels (200-300 u in diameter). The helical strips were placed in Krebs solution and tension measured on a Grass displacement transducer. Significant constriction was observed in the pulmonary vessels of both the rabbit and the dog when placed in Krebs solution containing acetylcholine (30-100 ug/L).

Subgross Pulmonary Anatomy

McLaughlin, Tyler, and Canada (1961) injected latex into the pulmonary arteries, bronchial arteries, pulmonary veins, and pulmonary airways in their studies on the subgross anatomy of rabbit, dog, and horse lungs. Basic anatomical similarities were observed between the dog and the cat. The subgross anatomy of the horse lung appeared to be very similar to that of the human. Their observations were as follows:

Dog and Cat--- Secondary lobules and interlobular septa were absent. The membranous pleura was extremely thin, and its blood supplied via the pulmonary artery. No bronchial artery-pulmonary artery anastomoses were observed.

Horse--------- Secondary lobules were incompletely developed and interlobular septa were thick. The pleura was thick and its blood supplied via the bronchial artery. One bronchial arteriolar-pulmonary arteriolar anastomosis was observed.

Anatomy Of The Pulmonary Circulation

According to Wagner and Filley (1965) anatomical studies of the pulmonary circulation date back to the sixteenth century when Malphigi, observing transilluminated frog lungs, noted that the systems for blood and air were anatomically independent of each other. Malphigi was the first researcher to observe and describe pulmonary capillaries. Subsequent anatomical studies involving more elaborate equipment have allowed the researcher to examine all compartments of the pulmonary circulation. This expanded knowledge has been accompanied by an overlap in nomenclature. Thus, the researcher has learned to place more stock in a concise anatomical description of the vessel rather than relying on a term as misleading as pulmonary arteriole.

Pulmonary Arterial Supply

Brenner (1935) studied the post-mortum anatomy of the pulmonary circulation of fifteen human subjects. Through the use of histologic sections, he was able to

distinguish the microscopic components of the pulmonary arterial vessels. Brenner noted that the primary component of pulmonary arteries larger than 1000 u in diameter was elastic tissue. He also found that smooth muscle was the consistant feature in arterial vessels 100-1000 u in diameter while arterial vessels 33-66 u in diameter were mainly endothelial.

Ferencz (1969) expanded the work of Brenner (1935) by using thick serial sections (120-500 u) to study the pulmonary arterial supply of the human, rabbit, dog, and cat. She noted that the basic histologic structures of the pulmonary arterial vessels of all four were quite similar. Her evaluation was summarized as follows:

Elastic arteries	(>500 u)	Capacitance vessels consisting mainly of elastic fibers
Transitional arteries	(100-500 u)	Resistance vessels having an incomplete muscular wall with scattered elastic fibers
Muscular arteries	(30-115 u)	Resistance vessels having a complete smooth muscle wall covered by an elastic lamina
Endothelial arteries (arterioles)	(15-40 u)	Channeling vessels consisting of an endothelial lining supported by a single elastic lamina

Ferencz concluded that the pulmonary arterial supply of the dog and cat were quite similar to that of the human in that the vessels tapered very smoothly showing no abrupt gross or histologic alterations. The transition from one

vessel type to another in the rabbit lacked this uniform tapering quality. The rabbit also exhibited numerous right angled branching of the endothelial vessels and large irregular amounts of smooth muscle in the transitional and muscular arteries.

William Knisely (1960, 1969), utilizing the principle of fused quartz rod transillumination, observed the normal morphology of the surface alveoli and the small pulmonary blood vessels on open chested inflated lungs of dogs, rabbits, and cats via the still lung technique (Irwin, 1958). Direct observations of the pulmonary vasculature revealed a unique architectural pattern in which the arterioles gave off numerous branches and rapidly tapered ending in rounded, blunt tips. Exposing the pulmonary arterial vessels to a shower of blood emboli resulted in the majority of these emboli being trapped in the blunt ends of the pulmonary arterioles. Knisely postulated that these pulmonary arteriolar tips could act as a catch-trap mechanism which would contain pulmonary blood emboli until they were disolved. Knisely concluded that surface alveoli vary in size and shape from one species to another. A right angled pattern of branching was seen in pulmonary arterioles up to 100 u in diameter. He also observed that a single alveolar capillary network may be supplied by more than one arteriole, and one arteriole may supply many alveoli.

Von Hayek (1960a), in his review of human pulmonary circulation, stated that precapillary pulmonary vessels often arise at right angles to their parent artery. The length of these precapillary vessels appeared to be void of smooth muscle, but sphincter-like muscular bands were observed at their right angled origins. Von Hayek considered that these muscular sphincters played, at least a partial role, in the regulation of pulmonary circulation.

Reeves, Leathers, and Quigley (1965) injected a suspension of barium sulfate in gelatin (50 mm Hg pressure) into the pulmonary arteries of excised rabbit lungs. The lungs were fixed by intratracheal inflation of 10 percent formalin and 50 u thick sections radiographed and the plates viewed under a microscope. The pulmonary arterioles demonstrated regular right angle branching into vessels of progressively smaller size. The capillary networks were filled by short right angled branching arterioles (10-20 u long and 10-15 u in diameter).

Sobin, Intaglietta, Frasher, and Tremmer (1966) injected silicone rubber (25 mm Hg pressure) into the main pulmonary artery of dogs, rabbits, and cats. The lungs were fixed by intratracheal inflation of 10 percent formalin until their distension matched that at end inspiration. Frozen serial sections (47 u thick) were taken from various lobes. Arterial vessels large enough to be seen by the unaided eye down to precapillary alveolar branches showed a consistent pattern of right angled branching with minimal

changes in the direction of the parent vessel. An examination of the precapillary arteriole revealed that its average diameter was between 18-25 u. The lack of an increase in the amount of smooth muscle nor a specific orientation of the muscle cells at the right angled branching origin of the precapillary vessels lead Sobin et al. to negate the presence of a functional precapillary muscular sphincter. These observations were essentially consistent for all three species.

Pulmonary Capillary Network

Pulmonary capillaries are primarily thin-walled endothelial tubes surrounded by a basement membrane (von Hayek, 1960b). In man and most laboratory animals, they exhibit a diameter of 7-10 u which is sufficient to allow passage of red blood cells (Daly and Hebb, 1966e). They lack smooth muscle and it is generally agreed that their vasomotion is passively influenced by either upstream or downstream events.

The vast network of pulmonary capillaries serves in the transportation of nutrients and wastes across the blood air barrier. According to Wagenvort (1964a) this barrier consists of five anatomical layers:

1. Alveolar epithelium
2. Alveolar basement membrane
3. Tissue space containing occasional reticular fibers
4. Capillary basement membrane
5. Capillary endothelial layer

Clements (1957) and Pattle (1967) would amend the work of Wagenvort by adding a sixth anatomical layer to the blood air barrier. This physiologically vital component is the continuous film of surfactant that lines the alveolar epithelium. In a review of the pulmonary surfactant system, they discussed the functional significance of this alveolar lining. A simplified version is as follows: There is a direct relationship between the thickness of the surfactant film and the surface tension exerted on pulmonary alveoli. When the alveoli are partially deflated at end expiration, the film of surfactant is relatively thick and the resultant surface tension low. As the alveoli expands during inspiration the film of surfactant streaches with the alveoli. As the film thins out, alveolar surface tension increases to the point where partial alveolar collapse occurs and expiration ensues. The probable source of the surfactant is considered to be the type II alveolar epithelial cells.

Goldenberg, and Buckingham (1967) examined the pulmonary ultrastructure of rats after bilateral cervical vagotomy. The most consistent ultrastructure response was a decrease in the amount of osmiophilia in the inclusion bodies of the type II alveolar epithelial cells. This was accompanied by atelectasis, focal edema, and capillary congestion. Their results suggested that a lack of vagal tone would result in atelectasis by decreasing the production of surfactant thus increasing alveolar surface tension.

Krahl (1969), in studies on the rabbit, suggested that vagally mediated pulmonary vasoconstriction would give rise to atelectasis by decreasing the nutrients to the type II alveolar cells. He concluded that the decrease in nutrients would slow surfactant production resulting in an increase in alveolar surface tension.

Staub (1966) has presented data indicating that pulmonary blood flow is directly related to the cyclic changes in respiration. He concluded that the increase in alveolar surface tension seen during inspiration would result in an increase in the diameter (volume) of the larger (elastic) pulmonary arteries, and veins, due to the increase in transpulmonary pressure. Inspiration will also result in the partial collapse of the capillaries on the flat surface of the alveolar walls. Capillaries and larger vessels located at the curved junction of the alveolar walls are protected from the increase in alveolar pressure by the increased surface tension across the curvature. Staub concluded that surface tension and thus the factors which control the production and release of surfactant can have a definite influence on the patency of pulmonary vasculature.

Pulmonary Venous Return

Most authors will agree that the anatomical differences between pulmonary venous and arterial vessels less than 100 u in diameter are slight and functionally insignificant (Harris, 1962). Franklin (1937) and

Wagenvort (1964b) in their extensive studies of the pulmonary venous system observed the following species variations: Human pulmonary veins larger than 80 u in diameter possess an irregular circular muscular layer, while the corresponding veins in both the dog and the cat demonstrate even distribution of muscular fibers. Pulmonary veins often lack the external elastic layer seen in the arteries. In general, the media of the veins contains more elastic and fibrous tissue and less muscle than arteries of comparable size. The elastic tissue in the larger intrapulmonary arteries of man, dog, and cat was found to exceed that in the larger veins. Pulmonary veins lie in the interlobar septa and, unlike the arteries, are associated with the bronchial tree only at the hilus. Ferencz (1969) reported that the veins of the rabbit were uniformly thin, a rather striking difference from the thick walled muscular arteries.

If one uses the premise that smooth muscle is a prerequisite for vasoconstriction, then it becomes rather easy to speculate that pulmonary veins (larger than 100 u) have considerably less vasomotor control over pulmonary circulation than do their corresponding arteries. The work of Franklin (1932), previously reviewed, questions the validity of such speculation by his observation that inter- as well as extrapulmonary venous rings responded with significant constriction when placed in a Ringer's solution containing acetylcholine.

Vagal Innervation of Pulmonary Airways and Blood Vessels

The efferent pathway of the vagus to the lungs is structured with an upper and lower motor neuron complex. The cell bodies of the upper neurons are located in the rostral third of the dorsal motor nucleus of the vagus (Getz, 1949). Efferent preganglionic vagal fibers exit the skull through the jugular foramen, transverse either side of the trachea, and synapse with their lower motor neurons in the peribronchial tissue, within the walls of the bronchi, and alongside the larger veins in the pulmonary septal tissue (Spencer, 1964). The resultant postganglionic fibers are extremely short. A smaller portion of the parasympathetic preganglionic fibers synapse with postganglionic cell bodies in the perineural tissue or within the vagus nerve as it courses toward the lungs (Hirsch and Kaiser, 1969). These vagal efferents demonstrate long postganglionic fibers.

Larsell (1921) and Larsell and Dow (1933) using methylene blue and silver staining techniques evaluated human and rabbit pulmonary innervation. Unmyelinated fibers were assumed to be postganglionic efferents. The smaller of the unmyelinated fibers were considered to be sympathetic. Vagal efferent fibers were observed leaving their postganglionic cell bodies to innervate the smooth muscle and epithelial cells of the bronchioles. Innervation of the pulmonary blood vessels, including capillaries, was primarily by very thin unmyelinated fibers

which were considered to be sympathetic. Numerous myelinated afferent fibers were distributed throughout the pulmonary tissue down to the level of the atria.

Spencer and Leof (1964) using vital methylene blue and silver impregnation were able to demonstrate the general distribution of autonomic nerves to pulmonary airways and blood vessels in the human. Pulmonary nerves entering the hilus branched several times and accompanied either the bronchi, the pulmonary arteries, or the pulmonary veins in their course toward the periphery. Parasympathetic motor fibers leaving the peribronchial ganglia were observed penetrating smooth muscle in airways down to respiratory bronchioles. The innervation of the pulmonary vasculature was evaluated by the presence of either thick or thin fibers. The results were as follows:

1. Elastic arteries were poorly supplied with both thick and thin nerve fibers lying between the media and adventitia.

2. Transitional and muscular arteries, down to the arterioles, were invested only with thin fibers.

3. Large pulmonary veins were richly innervated with thin fibers, some of which ended just below the endothelium. From their position with its close proximity to blood flow they were thought to be chemoreceptive.

4. Smaller veins were innervated by thin fibers.

Hirsch and Kaiser (1969) utilizing the principle of Wallerian degeneration and histologic silver staining techniques compiled an extensive review of the innervation of the mammalian lung. Vagal nerves, seen entering the hilar portion of the lungs, continued toward the periphery

in the adventitia surrounding the airways and associated blood vessels to the level of the terminal bronchioles. The majority of nerves penetrating the pulmonary tissue were associated with ganglionic cells, thus linking them to the parasympathetic nervous system. Using the affinity of myelin for silver stains they were able to determine that most of the nerve fibers were enclosed within a thick myelin sheath. This suggested the presence of a large number of vagal afferents. Fasicles of axons (motor and sensory) left the adventitia to supply the airways and blood vessels. These fibers were evaluated by noting the anatomical site of their termination and the structural complex at their endpoints. Fibers terminating in the smooth muscle of the airways and blood vessels were assumed to be both motor and sensory. Fibers terminating in the endothelium of the blood vessels were thought to be afferent chemoreceptors because of their close association with blood flow. Fibers terminating in the subepithelial tissue of the bronchioles, the alveolar ducts, and the alveolar sacs were thought to be afferent stretch receptors reacting to distension of the pulmonary airways. From their studies on the innervation of the canine lung, Hirsch and Kaiser concluded that vagal innervation was substantially ipsilateral.

The most definitive study on parasympathetic innervation of the pulmonary airways and vasculature was done by Hebb (1969). These histochemical studies utilized

the presence of acetylcholinesterase to determine the distribution of vagal fibers. They concluded that:

1. The dog, rabbit, and cat demonstrated more numerous cholinergic fibers in the bronchial tree than in the arteries or veins.

2. Cholinergic fibers extend down to arterial vessels 30-40 u in diameter in the dog and the cat, while the rabbit exhibits cholinergic fibers only in arterial vessels larger than 100 u.

3. The cholinergic fibers of the rabbit and cat appear more extensive in the larger vessels.

4. The pulmonary veins of all animals appears to be moderately innervated.

MATERIALS AND METHODS

Experimental studies were performed on dogs, rabbits, and cats, in groups of five. In each case, the distal end of the severed left vagus nerve was mid-cervically stimulated followed by ink injection and *in situ* formalin fixation. Left vagal fibers were chosen for stimulation because they inhibit cardiac activity to a lesser degree than do right vagal fibers (Truex, 1955). The vagal effect on pulmonary circulation was evaluated grossly by the degree of peripheral ink perfusion and histologically with serial sections. In all cases, the right or contralateral lung, served as a control. All experimental animals utilized in this study had undergone cardiac catheterization and three one minute vagal stimulations just prior to the initation of ink injection procedures. The parameters for the three vagal stimulations were within the ranges of 2-10 volts/ 2.5-6.0 msec/10-30 cycles per second and had a duration of one minute.

A control animal for each species was studied in a similar manner. These animals did not undergo vagal stimulation and the data obtained was compared to that from the stimulated animals.

Experimental Dogs

Five mongrel dogs, each weighing between seven and eleven kg, composed the first study group. The anatomical orientation in the dog is such that a mid-cervical stimulation of only vagal fibers would be extremely difficult due to the fact that both sympathetic and parasympathetic nerves are intertwined in one common truck. Stimulation of this vagosympathetic nerve trunk would thus result in the activation of both sympathetic and parasympathetic nerve endings. To remove the undesired sympathetic effect, phenoxybenzamine hydrochloride (alpha-adrenergic blocker) and propranolol hydrochloride (beta-adrenergic blocker) were administered prior to surgery. Used in the proper dosage, these two drugs have been reported to be sufficient to block all sympathetic activity (Goodman and Gilman, 1970a).

Each dog was anesthetized with sodium pentobarbital (32 mg/kg of body weight) via the left cephalic vein. The ventral cervical and thoracic regions were then shaved with electric clippers. A mixture of propranolol hydrochloride (1 mg/kg) and forty ml of normal saline was infused through intravenous (I.V.) drip via the left cephalic vein over a period of forty-five minutes. Upon completion, the I.V. drip was replaced with a solution of phenoxybenzamine hydrochloride (2 mg/kg) and forty ml of normal saline which was also infused over a forty-five minute duration.

The animal was then restrained in a supine position on the surgical table and a ventral cervical incision made

through the skin and superficial fascia. The incision began at the larnyx and was extended caudad a distance of approximately 7.5 cm. Using a blunt hemostat, the sternohyoid and sternothyroid muscles were separated at their midventral lines thus exposing the trachea. The left vagosympathetic trunk and right common carotid artery were separated from their respective carotid sheaths and a loop of umbilical tape placed around each structure. The trachea was then cleaned of any excess fascia and looped in a similar manner. Blunt dissection was continued to expose and loop the right external jugular vein.

Cannulation of the right external jugular vein was initiated by placing a loop of 2-0 silk around the vessel and ligating it as far cranial as the surgical exposure would permit. A probe was then placed under the caudal extent of the vessel, just caudad to the loop of umbilical tape. Using the probe and one end of the 2-0 silk, tension was applied to the vessel. A cut extending through approximately three fourths of the vessel's diameter was made and the tip of a 25 cm piece of polyethylene (P.E.) 190 tubing maneuvered a distance of approximately 5 cm down the vessel. The tubing was then secured by tying the umbilical tape around both the vessel and catheter approximately 1 cm caudal to the catheter's insertion. The free end of the P.E. 190 tubing was connected to a plastic syringe which

contained physiologically buffered Pelikan ink[1] (volume approximated animal's total pulmonary blood volume, Schermer, 1967).

A similar procedure was used in the cannulation of the right common carotid artery. The only differences were that a Lehman 5F ventriculography cardiac catheter[2] was used and that this catheter was maneuvered into the left ventricle before being secured. The free end of this catheter was connected to a Stathem P23AC transducer which recorded on a model 5D direct writing Grass polygraph. Left ventricular pressure recordings taken throughout the experiment were used to determine the degree of effectiveness of the vagal stimulation. The left ventricular catheter was periodically flushed with heparinized saline (8 units/cc) to maintain the integrity of the pressure recordings. A syringe containing one cc of a 50% carbamylcholine chloride (carbachol) solution was then connected to the left ventricular catheter via a two-way stopcock on the Statham transducer.

A tracheotomy was performed by elevating the trachea with the previously placed umbilical loop, placing a blunt hemostat under it for stability, and incising its ventral aspect carrying the incision up its lateral walls. A glass cannula was then placed in the tracheal lumen and secured by tying the umbilical tape.

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[1]John Henschel Co., 141 Albertson Ave., Albertson, N.Y. 11507

[2]United States Catheter Corp., Glenn Falls, N.Y.

The left vagosympathetic trunk was then elevated with the umbilical loop, clamped with a blunt hemostat, and severed just cranial to the hemostat. The distal end of the nerve was then stimulated with a monophasic square wave current produced by a Grass S-8 stimulator. All stimulations throughout the experiments were within the parameters of 2-10 volts/2.5-6.0 msec/10-30 cycles per second, and had a duration of one minute. The criteria for parameter selection was that range which decreased left ventricular rate by one third of the control readings. After fifteen seconds of stimulation, the ink was injected, a process taking another fifteen seconds. As the last few cc of ink were being infused, the carbachol was injected into the left ventricle followed by a five cc saline flush. Carbachol's prolonged parasympathomemetic action is more resistant to the enzymatic breakdown by cholinesterase than is acetylcholine chloride. Its high concentration and durability caused immediate cardiac arrest by hyperpolarizing the cardiac muscle fibers (Goodman and Gilman, 1970b). This procedure insured that the greater majority of ink remained in the lungs. Vagal stimulation continued for a duration of one minute. The glass tracheal cannula was then immediately connected, via polyethylene tubing, to a reservoir of 20% phosphate buffered formalin (Carlton, 1967) held at 25 cm above the animal. The formalin used throughout all subsequent experimental procedures had the same concentration and buffer system. The gravitational pull on formalin held

at this height was sufficient to allow infusion into the trachea at a pressure of 25 cm of water which is considered to be the optimum pressure for fixation of the lungs in a physiologically inflated state (Heard, 1962). The formalin was allowed to infuse into the lungs for five minutes, after which the trachea was clamped and the lungs removed.

Lung removal was accomplished by stripping the skin and muscle away from the thorax and severing the costo-chrondral junctions just lateral to the sternum. The ribs and diaphragm were then manually retracted and the remaining fascia and vessels severed thus freeing the lungs and heart. The external surface of the lungs was then gently washed with tap water and evaluated visually for areas of ink penetration.

Eash lobe was carefully studied for the percent of ink perfusion. Pink areas were considered non-perfused, and black areas perfused. These rather subjective measurements were taken by two people at different times and the inter-observer percentages averaged.

The lungs were then photographed from the dorsal, ventral, left lateral, and right lateral surfaces and the film stored for later reference.

Histologic Preparation

Preservation of normal lung architecture was accomplished by utilization of the Weiss and Tweeddale (1966) method of infusing formalin down the trachea at a constant

pressure. This insured the integrity of the tissue by keeping the alveoli in a physiologically inflated state.

The lungs were placed in a plastic pan (Figure 1) containing six liters of formalin. The glass tracheal cannula was connected to an outflow valve at the base of a two liter pyrex aspirator bottle by means of a 30 cm piece of 5/16 inch P.E. tubing. The aspirator bottle was then supported in a manner such that when filled its upper level of fluid was 25 cm above the level of fluid in the plastic pan. A 40 cm piece of 5/16 inch P.E. tubing was connected to the outflow valve at the neck of the aspirator bottle and allowed to rest in the bottom of the plastic pan. A small aquarium pump[3] was then placed with the lungs in the plastic pan. One end of a 45 cm 5/16 inch piece of P.E. tubing was connected to the pump outflow valve and the other end was allowed to enter the top of the aspirator bottle coming to rest about 5 cm from the bottom of the bottle. This tubing was enclosed in a double layer of #1 penrose tubing; one end of which rested freely in the formalin pan at the level of the pump valve, the other end being secured around the neck of the aspirator bottle. The penrose tubing acted as a second overflow valve. The aquarium pump was then activated thus inflating the lungs. The lungs remained under constant pressure (25 cm of water) for twenty-four hours after which they were removed and stored in formalin for a period of at least three days.

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[3]Little Giant Pump Co., Oklahoma City, Oklahoma.

The above procedures were repeated until five canine lungs rested in formalin storage. The left ventricular pressure recordings were then evaluated for the degree of effectiveness of the vagal stimulation and the speed with which carbachol arrested the heart. One animal was chosen which demonstrated the most rapid cardiac arrest, and a uniform decrease of left ventricular rate by one third of the control readings. This set of lungs was then removed from the formalin and the right and left middle lobes severed at the hilus. The peripheral edges of each lobe were then trimed to a rectangle having sides of approximately two and two and one half cm respectively. The main pulmonary artery and bronchus supplying each lobe was oriented in the center of their respective rectangle.

The tissue was then cleared and infiltrated as follows:

70% alcohol	overnight
80% alcohol	one hour under 15 ins. Hg. vacuum
95% alcohol	" " " " " " "
95% alcohol	" " " " " " "
100% alcohol	" " " " " " "
100% alcohol	" " " " " " "
100% alcohol	" " " " " " "
Toluene	" " " " " " "
Toluene	" " " " " " "
50% paraffin & 50% toluene	" " " " " " "
Paraffin	" " " " " " "

Paraffin _____ one hour under 15 ins. Hg. vacuum

Paraffin _____ " " " " " " "

Embed

The paraffin embedded tissue was then serially sectioned and mounted in a manner similar to that described by Wilson and Pickett (1970). The paraffin blocks were first mounted on a Spencer rotary microtome. Extending down from the microtome blade was a rigid strip of paper which aided in guiding the serial sections (Figure 2). The microtome, set to cut at 10 microns, was engaged and a strip of paraffin encased tissue allowed to slide down the paper. Cutting continued until approximately thirty sections had passed onto the paper guide at which time the paraffin strip was cut leaving only five sections attached to the knife. This process was repeated until all of the tissue had been sectioned. The ribbons of tissue were stored sequentially on a nearby table. Using a scalpel, the strips of tissue were then subdivided into units each containing approximately six sections.

In order to mount the tissue, a film transport device was constructed in our laboratory using a pattern similar to that developed by Wilson and Pickett (1970). The unit was made of inexpensive plexiglass, and a Kinderman guide and developing reel[4] (Figure 3). The transport device

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[4]Ehrenreich Photo-Optical Industries Inc., Executive Office, 623 Sterart Avenue, Garden City, N.Y. 11530.

was lowered into a 7 1/2 inch lo-boy water bath kept at a constant 48°C. A reel of 100 feet of P40b Cronar motion picture leader film[5] was placed on the free end of the transport device. The exposed end of the film was then slightly rounded and guided under the rollers of the transporter, through the Kinderman film guide and secured in the center of the developing reel. The film was continually coated with egg albumen before entering the water bath to insure proper adherence of the tissue.

The first strip of tissue was then placed in the water bath (Figure 4). It was aligned directly above the roller guides with the tip of the first section just touching the center of the film as it emerged from the water. The reel was then slowly turned and the paraffin strip gently guided onto the film until all sections were resting on a film base. This process was repeated until the reel held its maximum of five feet of film. The film was then cut and the reel removed and stored in a dry place for twenty-four hours. The mounting procedure was continued until all sections had been placed on the film.

After twenty-four hours had elapsed, each reel was stained in hematoxylin and eosin and coated with RC905 methacrylate plastic.[6] A blunt hemostat was attached to the center of each reel and used in elevating them in and

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[5]Available from E.I. duPont De Nemours & Co., Wilmington, Delaware.

[6]Ibid.

out of the staining dishes (Figure 5). The method of staining and plastic coating was as listed below:

Xylene	_____	2 1/2 minutes
Xylene	_____	2 1/2 minutes
Absolute alcohol	_____	2 1/2 minutes
Absolute alcohol	_____	2 1/2 minutes
95% alcohol	_____	few dips (in and out)
80% alcohol	_____	few dips (in and out)
Tap water	_____	two changes (one minute each)
Hematoxylin	_____	five minutes
Tap water	_____	three changes (one minute each)
Acid alcohol	_____	one to three rapid dips
Tap water	_____	two changes (one minute each)
Eosin	_____	one minute
95% alcohol	_____	several dips
95% alcohol	_____	several dips
Absolute alcohol	_____	one minute
Absolute alcohol	_____	one minute
Absolute alcohol	_____	one minute
Xylene	_____	two minutes
Xylene	_____	two minutes
Xylene	_____	two minutes
Drying period	_____	one minute on a paper towel
Plastic coating	_____	quantity sufficient to cover the lower 7/8 of film

The reels were removed from the liquid plastic and supported over the staining dish for a period of one minute to allow any excess plastic to fall back into the dish.

The reels were then placed on paper towels for five minutes and any additional plastic allowed to drain. They were then placed in a staining dish containing 20 cc of xylene which cleared the plastic from the lower 1/8 of the film. This allowed the film to be maneuvered freely during subsequent microscopic evaluation.

The film strips were then removed from their respective reels and one end attached to the laboratory ceiling while the other end was allowed to hang freely. After a twenty-four hour drying period, the film strips were trimmed and spliced together sequentially in preparation for microscopic evaluation.

Microscopic Evaluation

Before proceeding with the microscopic evaluation, it was necessary to develop a method of stabilizing the film on the microscope stage. A film holder (Figure 6) was constructed out of 1/8 inch glass and two strips of etched 1/8 inch plexiglass. The film holder is a modification of one developed by Wilson and Pickett (1970). This device kept the film secure as it passed under the objective. The holder was firmly adhered to the movable stage on the microscope and thus was easily maneuvered in horizontal and vertical directions.

In order to make stereologic measurements, a viewing plate was constructed in accordance with specifications developed by Weibel et al. (1966) (Figure 7). The plate consisted of a round piece of 1/16 inch plexiglass

having a diameter of 3 1/2 inches. A metal etcher was used to groove the plate at the proper points. The grooves were then filled with black wax and the excess wiped off. The circumference of the plate was covered with a strip of tape to protect it from scratches.

The microscopic equipment used in section evaluation was then assembled in the following manner (Figure 8). A Nikon vertical monocular phototube (cat. no. 77745) was secured atop a Nikon S-KE microscope stand equipped with Koehler illumination and a movable stage. A 10X Nikon high eyepoint compensating widefield eyepiece (cat. no. 77857) was then placed in the photo tube. A projection head with a 3 1/2 inch screen (cat. no. 76865) was attached, through a universal adapter (cat. no. 77111), to the photo tube through which all subsequent measurements were taken. The equipment was assembled in such a manner that the micrometer was projected onto the screen at all times and could be rotated to measure any vessel in the field. The microscope was equipped with 1.2X, 4X, 10X, and 42X objectives. The Weibel viewing plate was then fixed to the front of the viewing screen by means of small strips of tape placed on its upper and lower surfaces. The complete unit (Figure 9) allowed one to see the Weibel counting plate superimposed over an ocular micrometer which was superimposed over the tissue being evaluated.

Stereologic Measurements

Utilization of stereologic techniques enables the researcher to obtain three-dimensional information from two-dimensional random section sampling. Sections were evaluated by superimposing a stereologic grid on each sample and noting the relationship of structures in the section to points and lines on the grid. The present investigation is concerned with length and volume measurements. According to Weibel et al. (1966), length measurements require a specific test area and volume measurements require a uniform lattice of test points. A grid (Figure 7) was designed to meet these specifications.

Volume Measurements

Weibel et al. (1966) proposed a method for determining relative and absolute volumes of structures enclosed within histologically sectioned tissue. He suggested that a grid (Figure 7) be placed over the serially sectioned tissue at regular intervals. This grid contained forty-two evenly spaced crossbars. Each time one of these crossbars fell partially or wholly within the outer walls of the structure being measured it was counted as a hit. This process was repeated on all subsequent tissue samples. The relative volume was equal to the number of hits recorded divided by the possible number of hits. Thus:

$$\text{Relative volume} = \frac{\text{Number of hits recorded}}{\text{Number of possible hits}}$$

The relative volume indicated the percent of the total volume of the sample occupied by the structure being measured.

The absolute volume of the structure could be determined by taking the actual volume of the tissue sample and multiplying it by the relative volume of the structure. Thus:

$$\text{Absolute volume} = \frac{\text{Actual volume of the tissue sample}}{\text{Relative volume of the structure}}$$

The Relative Volume Occupied By The Pulmonary Arteries, Arterioles, Veins, and Venules

In order to insure an unbiased evaluation, it was necessary to develop a method whereby all areas of the tissue could be equally sampled. This was accomplished by subjectively dividing each side of a section into three equal parts and placing imaginary dots at these divisions. Imaginary lines were then drawn to connect opposite dots. The resultant figure was that of a tissue section divided into nine units numbered as seen in Figure 10. The sections were then sampled according to unit areas in the following manner:

The first hilar section was brought into focus at 100X and the tissue maneuvered to unit area 1. The section being observed had superimposed on it a movable ocular micrometer and the Weibel stereologic viewing plate. Counting proceeded as follows: When any portion of one of the forty-two crossbars of the Weibel plate fell partially or

wholly within the lumen of an artery, arteriole, vein, or venule, it was counted as a hit and the number recorded. The same section was subsequently maneuvered to unit areas two and three and counts again taken. The film strip was then advanced six sections and the process repeated; this time, counting the number of hits in unit areas four, five, and six. The film strip was then advanced another six sections and counts taken in unit areas seven, eight, and nine. This method of sampling every sixth section with rotating unit areas continued in the manner described above until the stimulated and control lobes had been counted. The data was placed in the previously stated relative volumetric formula and the percent of the total volume of the lobe occupied by the pulmonary arteries, arterioles, veins, and venules calculated.

The Relative Volume Occupied By The Ink Filled Pulmonary Capillaries

At this point, a few comments on the rationale of taking ink perfusion measurements should be included. Krahl (1968) stated that the point of constriction during vagal stimulation is the precapillary arteriolar site. One would thus expect that during vagal stimulation, ink penetration would be terminated, or significantly reduced, at this constricted site. A comparison of the volume of ink reaching the capillary beds of both stimulated and control lobes would thus indicate the degree of effectiveness of vagal stimulation on the precapillary anteriolar smooth

muscle. With this in mind, a measurement of the relative volume occupied by the ink filled capillary beds was undertaken.

The first hilar section was brought into focus at 420X and maneuvered to unit area 1. At this magnification, pulmonary capillaries were easily seen and those with and without ink penetration could readily be identified. Counting proceeded as follows: An ink filled capillary falling on any portion of one of the forty-two crossbars of the Weibel plate was counted as a hit and that number recorded. The same section was then moved to unit areas two and three and the process repeated. The method of choosing sample areas for capillary ink perfusion was the same as that described earlier for the relative volume occupied by the larger vessels. Again, every sixth section was sampled using rotating unit areas until the stimulated and control lobes had been counted. The data was then placed in the relative volumetric formula and the percent of the total volume of the lobe occupied by the ink filled pulmonary capillaries calculated.

The Relative Volume Occupied By The Pulmonary Airways Ranging From Secondary Bronchi To Terminal Bronchioles

A study of the vagal effects on pulmonary circulation would be incomplete without some mention of the vagal effects on pulmonary airways. An increase or decrease in the patency of these airways would obviously have a secondary effect on

the nearby vessels. With this in mind, a study was undertaken to determine the relative volume occupied by the pulmonary airways ranging in size from secondary bronchi to terminal bronchioles.

The first hilar section was brought into focus at 40X and maneuvered to unit area 1. Counting proceeded as follows: Whenever one of the forty-two crossbars of the Weibel viewing plate fell partially or wholly within the lumen of the airway it was counted as a hit and the number recorded. The same section was then maneuvered to unit areas two and three and the process repeated. The method for choosing sample areas for measuring the relative volume occupied by the airways was the same as that described earlier for the relative volume occupied by the larger vessels. As before, every sixth section was sampled using rotating unit areas until the stimulated and control lobes had been counted. The data was then placed in the relative volumetric formula and the percent of the total volume of the lobe occupied by airways ranging in size from secondary bronchi to terminal bronchioles calculated.

Absolute Volume

The absolute volume of a tissue component can be determined by multiplying the relative volume by the measured volume of the tissue sample. The volume of the right and left middle lobes of three experimental dogs was determined by the water displacement technique. The method was carried out as follows: A 500 cc wide mouthed flask

was fitted with a double holed rubber stopper. A graduated pipet was inserted through one hole and allowed to penetrate the flask a distance of five cm. The other hole was fitted with a glass tube which penetrated the flask a distance of one cm. The outer end of the glass tubing was connected to a 50 cc plastic syringe. A zero point was noted on the pipet a distance of one cm above the rubber stopper. Using the syringe the flask was filled with water until the water level reached the zero point on the pipet. The volume of water was then recorded and the flask emptied and dried. The middle lobe in question was placed in the flask and the rubber stopper secured. Again, the flask was filled with water until the zero point was reached. The volume of water was then recorded and the flask emptied. The differences between the first and second volumes equaled the volume of the lobe in question. This process was repeated for all subsequent volumetric analyses.

The Length of Pulmonary: Arteries, Arterioles, Veins, and Venules

Evaluation of vascular constriction or dilation in histologic sections most often concerns itself solely with changes in vessel diameter. This would seem to be only part of the picture for Patel (1956) has shown that vessels can and will change in their total length. His corrosion cast studies of the rabbit pulmonary arterial tree revealed that injection of norepinephrine (doses greater than 10 ug) resulted in extensive knarling and twisting of the arterial

vessels. Rabbits who were not pre-injected with norepinephrine demonstrated smooth and straight arterial vessels. Measuring the stimulated and control lobes for changes in length of the pulmonary vessels would thus give a third dimension to the evaluation of the stimulation effects.

Elias and Henning (1967) proposed that the length of specific structures per unit volume could be determined by stereologic evaluation of histologic sections. Their suggested techniques were incorporated in the present investigation to measure the combined lobar lengths of all pulmonary vessels (larger than capillaries).

The measurements proceeded as follows: The first hilar section was brought into focus at 100X and maneuvered to unit area 1. At this magnification the superimposed Weibel square covered an area of tissue .81 mm^2. The screen was then scanned counting any pulmonary vessel which fell within the outer limits of the Weibel square. Vessels which were intersected by the left and upper edges of the square were counted. Vessels which were intersected by the right and lower edges of the square were not counted. The section was then maneuvered to unit areas two and three and the process repeated. The method for choosing sample areas for measuring the length of pulmonary vessels was the same as that described for measuring the relative volume occupied by the larger vessels. As before, every sixth section was sampled using rotating unit areas until the stimulated and control lobes had been counted.

Elias and Henning concluded that the length (L) of linear structures within a unit volume (V) equals twice the number of mean profiles (P) counted divided by the area (A) of the test square. Thus:

$$L_v = \frac{2P}{A}$$

For example, if a sampling of 10 histologic sections resulted in a total profile count of 20 vessels then:

$$L_v = \frac{(2)\ 20/10}{0.81\ mm^2}$$

$$= 4.9\ mm/mm^3$$

Thus in every cubic milimeter of pulmonary tissue vessels larger than capillaries had a total length of 4.9 mm. In order to compute the combined length of these vessels in an entire lobe it was necessary to make the following conversions:

$4.9\ mm/mm^3 = 4900\ mm/cm^3 = 490\ cm/cm^3$

If the actual volume of the lobe in question was 10 cc then the combined lengths of all the measured pulmonary vessels in that lobe was equal to:

$(10\ cc)\ (490\ cm/cm^3) = 4900\ cm = 49\ m$

Statistical Analysis

Control and experimental data was evaluated statistically by an analysis of covariance (Ostie, 1970), and Stapelton's (1972) procedure for comparing individual means.

Each lobe was divided into three equal parts, a hilar, a middle, and a peripheral portion. The absolute

lobar values were then statistically analyzed as stated above. If statistically significant differences at the 95 percent confidence level were observed between the lobar values, the three pulmonary compartments were examined to determine the specific anatomical regions most responsive to vagal stimulation.

Control Dog

The surgical, histologic, microscopic, and statistical evaluation procedures just described were repeated on a dog whose specifications met those of the experimental group. The animal did not undergo vagal stimulation and therefore it was not necessary to administer alpha and beta blocking agents prior to surgery. All other procedures were identical to those previously described. The volumes of right and left middle lobes of the control dog were determined by the water displacement method and the data used to compute the absolute volumes of the tissue components being studied.

Experimental Rabbits

Five New Zealand white rabbits, each weighing between three and five kg, were used in the second animal group. The animals were intravenously anesthetized with sodium pentobarbatal via the left marginal ear vein, and the ventral cervical and thoracic regions shaved with electric clippers. The animals were then restrained in a supine position on the surgical table.

The anatomical orientation in the rabbit is such that a mid-cervical stimulation of only vagal fibers is quite feasible because, unlike the dog, the rabbit has a separate cervical vagus nerve and sympathetic trunk. Both are enclosed within the carotid sheath but can easily be separated and identified since the vagus is consistently larger than the sympathetic trunk. Therefore, it was not necessary to administer sympathetic blocking agents prior to the initiation of the surgical procedure.

The surgery was carried out in a manner similar to that performed on the dog. The cannulations were also similar save for the fact that a size 4F Lehman ventriculography cardiac catheter was used for the left ventricle catheterization.

The parameters for vagal stimulation were within the ranges of 2-10 volts/ 2.5-6.0 msec/10-30 cycles per second and had a duration of one minute. Ink injection, formalin fixation, histologic preparation, microscopic and statistical evaluation were all performed in a manner similar to that described for the dog.

The volumes of the right and left middle lobes of three experimental rabbits were determined by the water displacement method. The average volumes were then used to compute the absolute volumes of the tissue components being studied.

Control Rabbits

The surgical, histologic, microscopic and statistical evaluation procedures just described were repeated on a rabbit whose specifications met those of the experimental group. The animal did not undergo vagal stimulation. The volumes of the right and left middle lobes of the control rabbit were determined by the water displacement method.

Experimental Cats

Five mongrel cats, each weighing between three and five kg, were used in the third group. The experimental procedures followed the pattern described for the rabbit with a few exceptions.

The vasculature of the cat is subject to intense reflex vasoconstriction in vessels where catheters are placed. This vasoconstriction often sealed the open end of the size 4F cardiac catheter as it was being maneuvered down the right common carotid artery. Blood trapped in the catheter readily clotted. The subsequent decrease in the catheter's patency impaired the successful recording of left ventricular pressure. To counteract the clotting mechanism the animals were heparinized prior to surgery. This was accomplished by injecting heparin (350-500 units/ kg) via the cephalic vein just prior to the initial incision.

The method of anesthesia differed from the rabbit in that the dosage was administered intraperitoneally. The vagal and sympathetic fibers are in closer association than in the rabbit and careful dissection was required to separate

the two without damage to the nerve tissue. The cervical vagus nerve, as in the rabbit, is of larger diameter than the cervical sympathetic trunk.

The stimulation parameters were within the ranges described for the dog and were employed for a duration of one minute. Ink injection, formalin fixation, lung removal, histologic preparation, microscopic and statistical evaluation were all performed in a manner similar to that described for the dog.

The volumes of the right and left middle lobes of three experimental cats were determined by the water displacement technique. The average volumes were then used to compute the absolute volumes of the tissue components being studied.

Control Cat

The surgical, histologic, microscopic, and statistical evaluation procedures just described were repeated on a cat whose specifications met those of the experimental group. The animal did not undergo vagal stimulation. The volumes of the right and left middle lobes of the control cat were determined by the water displacement technique.

Figure 1. Formalin inflation apparatus

Figure 2. Microtome with tissue strip

Figure 3. Film transport device with Kinderman guide and developing reel

Figure 1

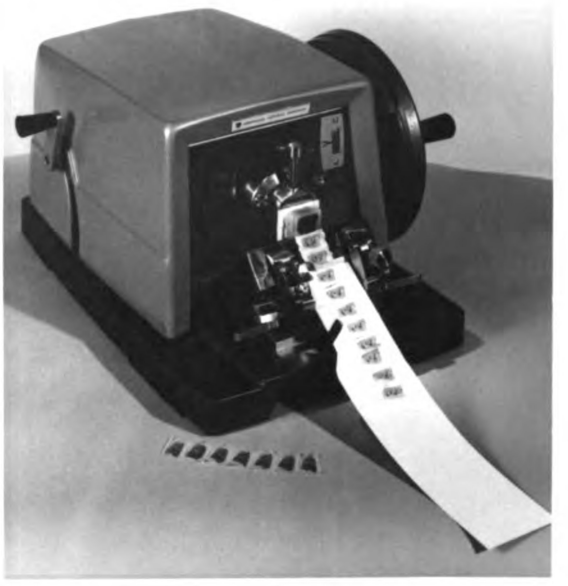


Figure 2

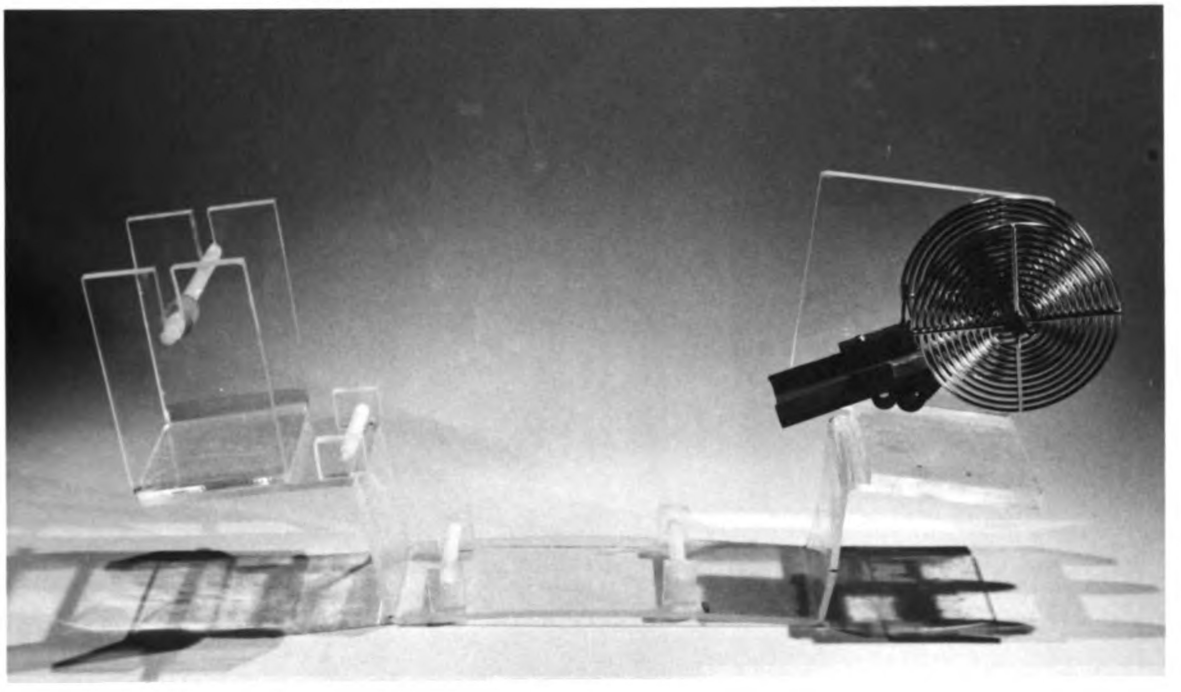


Figure 3

Figure 4

Tissue being placed on film strip.

Figure 5

Tissue loaded reel with staining dishes.

Figure 6

Film holder with film strip.

Figure 7

Modified Weibel stereologic viewing plate.

Figure 4

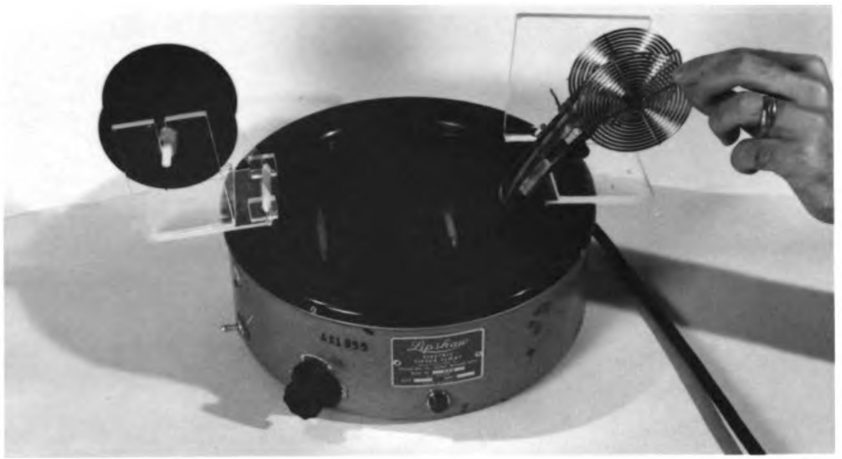
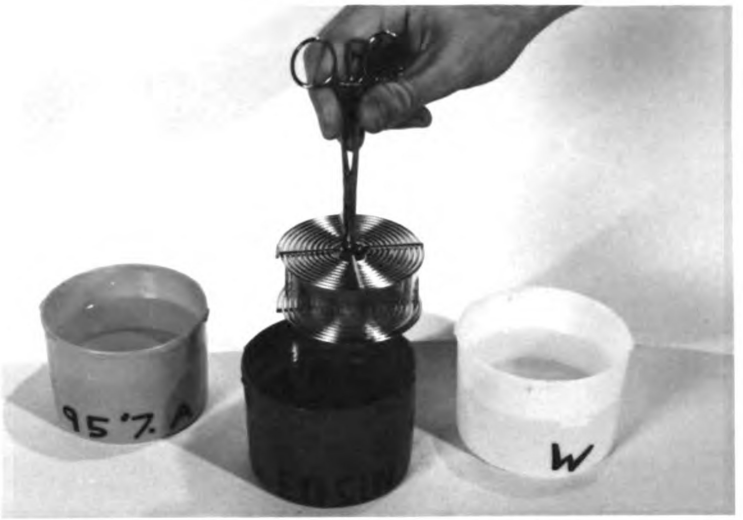
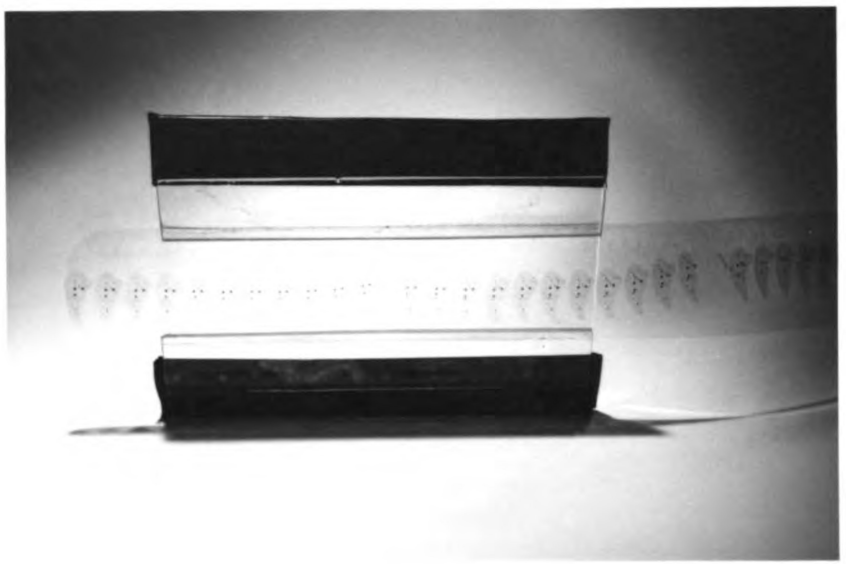


Figure 5





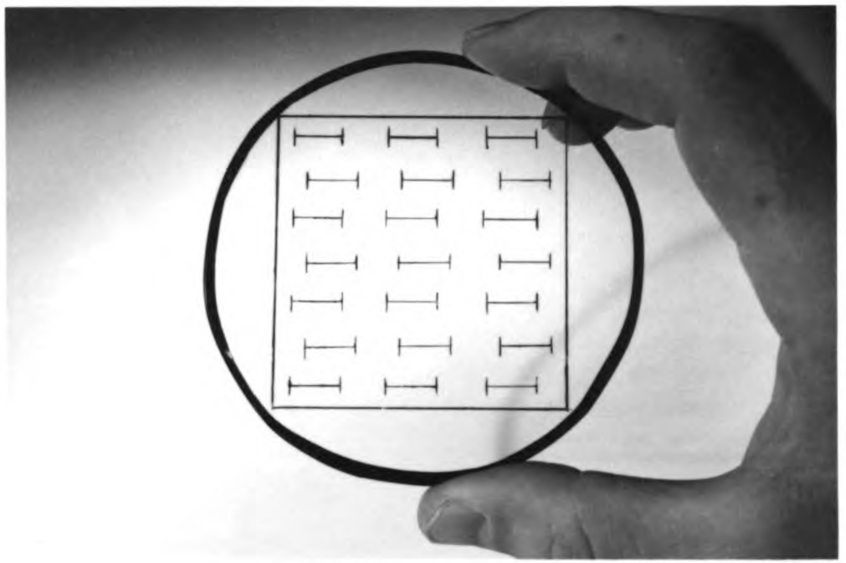


Figure 6

Figure 7

Figure 8. Assembled microscopic equipment

Figure 9. Viewing screen with Weibel plate and ocular micrometer superimposed over tissue sample at 100X magnification

Figure 10. Tissue sample divided into unit areas

Figure 8

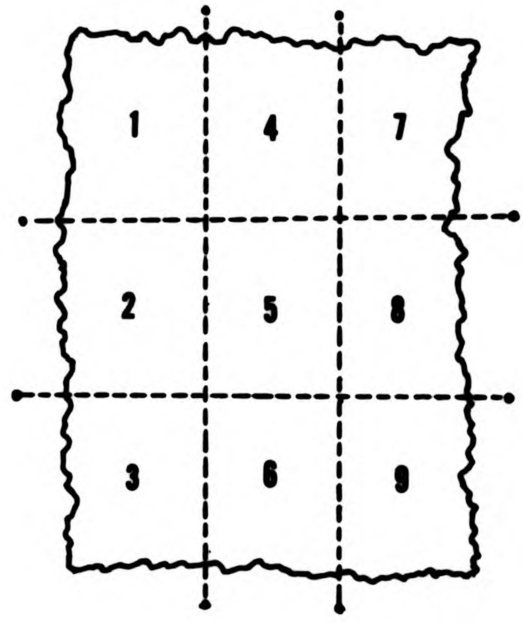


Figure 10

Figure 9

RESULTS AND DISCUSSION

Perfusion-Percentage Results

Tables 1, 2, and 3 contain the interobserver percentage averages of the distribution of ink on the external surface of the lungs following left vagal stimulation. The tables are listed by dog, rabbit, and cat respectively. Black areas are considered ink-perfused and pink areas non-perfused. Figures 11, 12, and 13 graphically demonstrate the percentage of ink distribution on the external surface of the lungs following left vagal stimulation on a lobe basis. Table 4 contains the lobar perfusion-percentages for the control animals. These animals did not undergo vagal stimulation. As before, black areas are considered ink-perfused.

Photographic Results

Figures 14, 15, and 16 contain photographic reproductions of a representative experimental animal from each species. They are listed by dog, rabbit, and cat respectively. Pictures were taken of the right lateral, left lateral, dorsal, and ventral surfaces of the lungs.

Stereologic And Volumetric Results

Table 5 contains the mean lobar volumes and the vessel lengths of the experimental and control dogs,

rabbits, and cats. An asterisk was placed to the left of the appropriate data when statistically significant differences, at the 95 percent confidence level, were observed in comparing lobes from the experimental animals, or lobes from experimental to control animals.

Each lobe underwent dual analyzation. The mean lobar values were statistically analyzed to determine any significant lobar differences. The data from each lobe was then divided into a hilar, middle, and peripheral portion. If statistically significant differences were observed in the mean lobar values, the data from the three pulmonary compartments was examined to determine the anatomical site most responsible for the observed differences. The original data used to compute actual volume, relative volume, absolute volume, and length per unit volume on a lobar basis and in the pulmonary compartments is contained within Appendix B.

Table 1.--Lobar perfusion-percentages for the dog.
Percent presented: black - pink

Lobe Studied	Dog Number		
Left Vagal Stimulation			
Left lung	Dog-1	Dog-2	Dog-3
cranial lobe	98%- 2%	92%- 8%	100%- 0%
middle lobe	100 - 0	88 - 12	100 - 0
caudal lobe	98 - 2	85 - 15	98 - 2
Right lung			
cranial lobe	100 - 0	43 - 57	95 - 5
middle lobe	95 - 5	28 - 72	95 - 5
caudal lobe	100 - 0	95 - 5	98 - 5
intermed. lobe	100 - 0	45 - 55	100 - 0
Left lung	Dog-4	Dog-5	
cranial lobe	98 - 2	100 - 0	
middle lobe	100 - 0	95 - 5	
caudal lobe	100 - 0	100 - 0	
Right lung			
cranial lobe	100 - 0	100 - 0	
middle lobe	100 - 0	95 - 5	
caudal lobe	100 - 0	100 - 0	
intermed. lobe	98 - 2	100 - 0	

Table 2.--Lobar perfusion-percentages for the rabbit.
Percent presented: black - pink

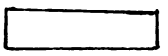
Lobe Studied	Rabbit Number		
Left Vagal Stimulation			
Left lung	Rabbit-1	Rabbit-2	Rabbit-3
cranial lobe	60%- 40%	93%- 7%	100%- 0%
middle lobe	55 - 45	67 - 33	70 - 30
caudal lobe	93 - 7	75 - 25	100 - 0
Right lung			
cranial lobe	48 - 52	78 - 22	100 - 0
middle lobe	10 - 90	75 - 25	98 - 2
caudal lobe	48 - 52	88 - 12	98 - 2
intermed. lobe	30 - 70	88 - 12	93 - 7
Left lung	Rabbit-4	Rabbit-5	
cranial lobe	80 - 20	95 - 5	
middle lobe	70 - 30	75 - 25	
caudal lobe	90 - 10	95 - 5	
Right lung			
cranial lobe	95 - 5	90 - 10	
middle lobe	70 - 30	75 - 25	
caudal lobe	60 - 40	80 - 20	
intermed. lobe	85 - 15	80 - 20	

Table 3.--Lobar perfusion-percentages for the cat.
Percent presented: black - pink

Lobe Studied	Cat Number		
Left Vagal Stimulation			
Left lung	Cat-1	Cat-2	Cat-3
cranial lobe	100%- 0%	100%- 0%	100%- 0%
middle lobe	75 - 25	100 - 0	100 - 0
caudal lobe	100 - 0	100 - 0	100 - 0
Right lung			
cranial lobe	100 - 0	100 - 0	100 - 0
middle lobe	75 - 25	100 - 0	100 - 0
caudal lobe	100 - 0	100 - 0	100 - 0
intermed. lobe	100 - 0	100 - 0	100 - 0
Left lung	Cat-4	Cat-5	
cranial lobe	100 - 0	95 - 5	
middle lobe	100 - 0	98 - 2	
caudal lobe	100 - 0	90 - 10	
Right lung			
cranial lobe	100 - 0	95 - 5	
middle lobe	100 - 0	93 - 7	
caudal lobe	100 - 0	75 - 25	
intermed. lobe	100 - 0	67 - 33	

Figure 11.--Average lobar perfusion-percentages for the dog.

% black



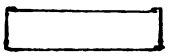
% pink

Cr --- cranial lobe Ca --- caudal lobe

M --- middle lobe I --- intermediate lobe

Figure 12.--Average lobar perfusion-percentages for the rabbit.

% black



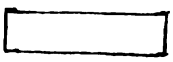
% pink

Cr --- cranial lobe Ca --- caudal lobe

M --- middle lobe I --- intermediate lobe

Figure 13.--Average lobar perfusion-percentages for the cat.

% black



% pink

Cr --- cranial lobe Ca --- caudal lobe

M --- middle lobe I --- intermediate lobe

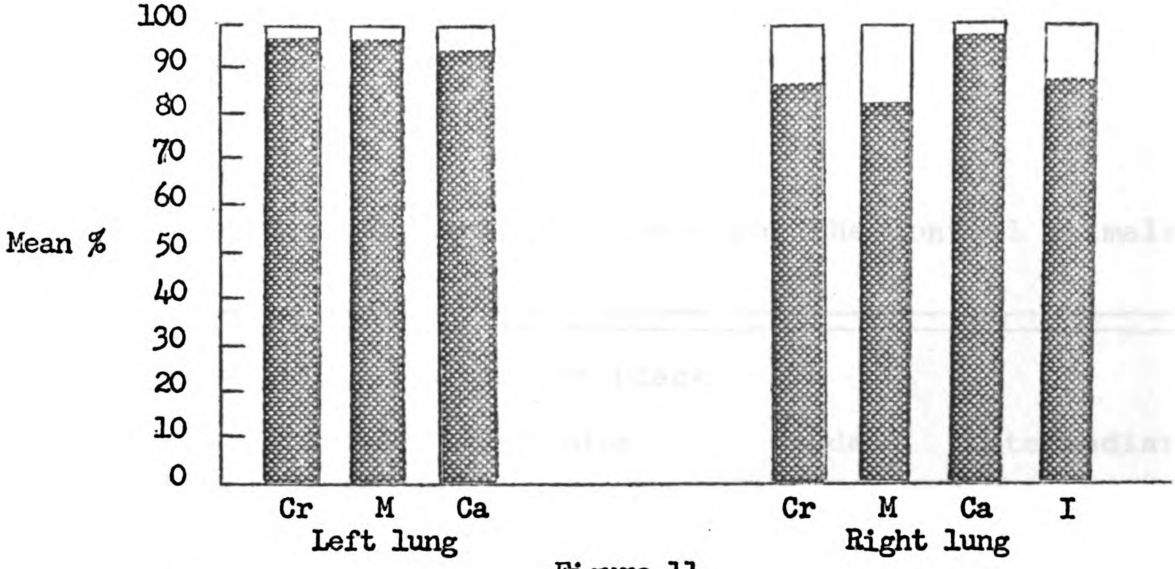


Figure 11.

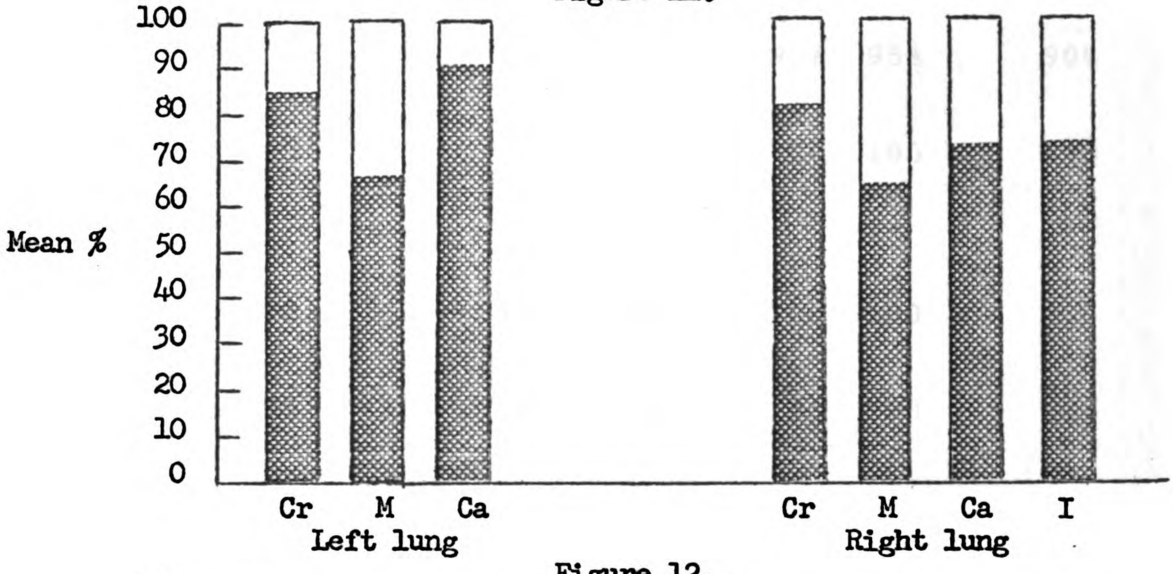


Figure 12.

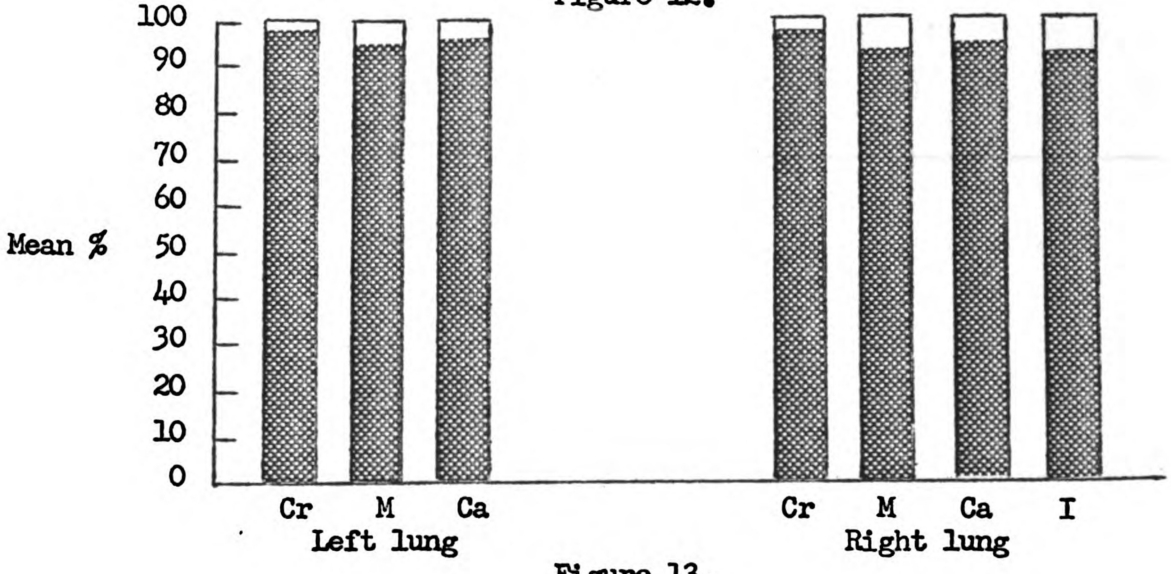


Figure 13.

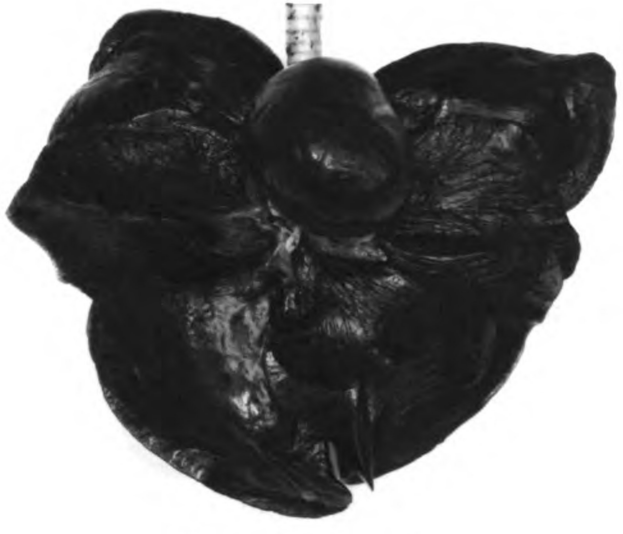
Table 4.--Lobar perfusion-percentages for the control animals

	Percent black						
	Cranial		Middle		Caudal		Intermediate
	L	R	L	R	L	R	R
Dog # 1	100%	95%	100%	100%	95%	95%	90%
Dog # 2	95	95	95	95	100	100	100
Rabbit # 1	100	100	100	100	100	100	100
Rabbit # 2	95	90	90	90	90	90	80
Cat # 1	95	100	95	95	90	100	90
Cat # 2	80	100	95	95	95	90	90

Right Lateral View

Left Lateral View

Dorsal View



Ventral View

Figure 14 Dog

Right Lateral View

Left Lateral View

Dorsal View

Ventral View

Figure 15 Rabbit

Right Lateral View

Left Lateral View

Dorsal View

Ventral View

Figure 16 Cat

Table 5.--Absolute lobar volumes and total vessel lengths

		Experimental		Control	
		Left	Right	Left	Right
		(23cc)	(33cc)	(25cc)	(35cc)
Dog	Large vessel volume	1.96cc	3.20cc	1.65cc	3.92cc
	Capillary volume	5.69	7.72	7.85	9.17
	Airway volume	0.71	0.76	1.20	1.86
	Vessel length	156m	189m	164m	241m
		(9cc)	(12cc)	(9cc)	(11cc)
Rabbit	Large vessel volume	0.62cc	1.01cc	0.93cc	1.17cc
	*Capillary volume	2.44	3.49	2.96	3.92
	Airway volume	0.39	0.55	0.36	0.47
	*Vessel length	74m	97m	96m	129m
		(8cc)	(10cc)	(9cc)	(11cc)
Cat	Large vessel volume	0.53cc	0.56cc	0.75cc	0.99cc
	*Capillary volume	1.31	1.77	2.30	2.67
	Airway volume	0.21	0.16	0.36	0.48
	*Vessel length	49m	60m	83m	109m

* = Differences significant--P is less than 0.05

Actual lobar volumes shown in parenthesis

Subjective evaluation of the stereologic data from right and left middle lobes of both experimental and control animals revealed the following:

(1) In all species, the calculated absolute volume occupied by pulmonary vessels (larger than capillaries) was greater in the right middle lobes than in the left.

(2) In all species, the calculated absolute volume occupied by ink filled pulmonary capillaries was greater in the right middle lobes than in the left.

(3) In all animals, with the exception of the experimental cat, the calculated absolute volume occupied by airways (larger than respiratory bronchioles) was greater in the right middle lobes than in the left.

(4) In all species, the calculated total length of the pulmonary vessels (larger than capillaries) in meters per lobe was greater in the right middle lobes than in the left.

Actual lobar volumes determined by the water displacement technique revealed that the right middle lobes of all species were larger than their left counterparts. Rahn and Ross (1957) determined the percent weights of the total canine lung for its individual lobes. The right middle lobe proved to be 8.9 percent and the left middle lobe 5.8 percent of the total lung weight. An increase in the actual volume and weight of pulmonary tissue would logically be accompanied by a greater number of blood vessels and airways. This numerical increase accounts for the consistantly larger stereological values observed in the right middle lobes.

Weibel (1964) stereologically determined the percent volume of the lung occupied by the alveoli, the alveolar capillary networks, alveolar ducts and sacs, and respiratory

bronchioles. He concluded that these components occupied 90 percent of the total lung volume. The remaining 10 percent was occupied by the larger blood vessels and airways. The stereologically determined percent volumes and vessel lengths in the present investigation (Tables 21-26) closely coincide with those determined by Weibel. Morphometric analysis revealed that 7 to 13 percent of the pulmonary tissue was occupied by blood vessels larger than capillaries and airways larger than respiratory bronchioles. The remaining 87 to 93 percent of the pulmonary tissue was occupied by the alveoli, the alveolar capillary networks, alveolar ducts and sacs, and respiratory bronchioles.

The combined lengths of pulmonary vessels (larger than capillaries) were calculated in meters per lobe. In all instances the total vessel length in the right middle lobe was greater than that in the left. The combined lengths in the control rabbit left middle lobe was approximately 96 m. According to Rahn and Ross (1957) this lobe occupies 5.8 percent of the total canine lung weight. Thus the projected vessel lengths for the entire canine lung is:

$$\frac{96 \text{ m}}{5.8\%} = \frac{X \text{ m}}{100\%}$$

$$X = 1{,}655 \text{ m}$$

A review of the literature has revealed no other similar stereologic studies of intrapulmonary vessel lengths other than Weibel's (1964) determination of pulmonary capillary lengths.

According to Daly and Hebb (1966f), the pulmonary artery accompanies the bronchi as far as the respiratory bronchioles. At this point an artery is given off to each alveolar duct which in turn gives off a number of small arteries supplying the alveolar capillary networks.

Weibel (1964) has calculated the combined length of the branches of the adult human respiratory tree down to the level of the terminal bronchiole. This length approaches 257 m. The fact that the present investigation measured both pulmonary arteries and veins down to the alveolar capillary level accounts for the greater vessel length.

Weibel (1964) has also determined the combined length of the pulmonary capillary networks. He concluded that:

The total number of capillary segments per alveolus = 1800

The mean length of each capillary segment = $10.3 \cdot 10^{-4}$ cm

The total number of alveoli = $300 \cdot 10^{6}$

Therefore the combined lengths of all pulmonary capillary segments is equal to:

$(1800)(10.3 \cdot 10^{-4}\ \text{cm})(300 \cdot 10^{6}) = 5{,}562{,}000$ m

Thus the calculated length of pulmonary vessels larger than capillaries appears to be realistic.

The stereologic data was then statistically evaluated by an analysis of covariance (Ostie, 1970), and Stapelton's (1972) procedure for comparing individual means. This technique eliminated the inherent anatomical variations between the right and left lobes and gave an accurate

evaluation of the stimulation effects. The results of that analysis are incorporated in the following discussion.

Essentially, there are three primary avenues through which vagal stimulation may influence pulmonary circulation: (1) indirectly through vagally induced bronchomotion and its mechanical effects on nearby vessels, (2) indirectly through a vagally induced decrease in cardiac output, and (3) directly by either constricting or relaxing smooth muscle in the pulmonary blood vessels.

Neither isolated perfused nor in situ techniques have been able to eliminate the simultaneous bronchomotor and vasomotor responses to electrical or chemical stimuli. Aviado (1965) discussed the interrelationship of the pulmonary airway and arterial vascular trees. He suggested that, because of their close anatomical relationship, some of the vascular responses observed during parasympathetic stimulation may be due to purely mechanical effects on the vessels from associated changes in the caliber of pulmonary airways. Constriction of the airways would increase the extra-vascular tension on nearby vessels resulting in an increase in pulmonary vascular resistance. Relaxation of the airways would have the opposite effect.

Daly and Hebb (1966g) summarized the passive effects of acetylcholine on the pulmonary circulation. They noted that in the majority of studies changes in pulmonary arterial pressure (vasomotion) were accompanied by changes in tidal volume (bronchomotion). The likelihood of these

secondary effects made it difficult to authoritatively state that variations in pulmonary arterial pressure seen during parasympathetic stimulation were solely due to direct pulmonary vasomotion.

Several researchers have been able to demonstrate significant vasomotor responses without concomittant bronchomotor activity. Dale and Narayana (1935) studied isolated perfused guinea-pig lungs under positive pressure intratracheal inflation. Injection of acetylcholine (5-50 ug) into the pulmonary artery resulted in a decrease in outflow volume (vasoconstriction). The majority of animals also demonstrated a decrease in tidal volume (bronchoconstriction). In three preparations, a significant vasoconstriction was observed unaccompanied by bronchomotor activity. Dale and Narayana concluded that the increase in pulmonary vascular resistance seen during parasympathetic stimulation was a result of the combination of two factors: (1) the direct vasomotor activity of the pulmonary blood vessels, and (2) the mechanical force exerted on the pulmonary vessels due to changes in airway caliber.

Alcock, Berry, and Daly (1935) studied the effects of acetylcholine injection (200 ug-1.0 mg) on isolated perfused canine lungs in which volume inflow and tidal air was recorded. Smaller doses of acetylcholine resulted in an increase in volume inflow (fall in pulmonary arterial pressure) while larger doses resulted in a decrease in volume inflow (rise in pulmonary arterial pressure). Both

these responses were accompanied by a decrease in tidal air (bronchoconstriction). They concluded that a situation could exist where pulmonary vasomotion would be partially or solely independent of bronchomotor secondary effects.

In the present investigation, stereologically determined absolute lobar volumes occupied by pulmonary airways larger than respiratory bronchioles (Tables 21-26) were statistically unaltered during the vagal stimulation. The observed differences were due solely to the normal variations in actual lobar volumes. The fact that active bronchomotion was absent during vagal stimulation negates the possibility of airway constriction secondarily influencing pulmonary vasomotion.

With the elimination of secondary bronchomotor effects on pulmonary vasculature we now concern ourselves with the possibility of cardiac output passively influencing pulmonary circulation.

According to Folkow and Neil (1971), vagal stimulation results in a decrease in cardiac rate, a decrease in the contractility of atrial muscle, and no significant alterations in the contractility of ventricular musculature. The ultimate outcome of the combination of these three effects is a decrease in cardiac output proportionate to the degree of vagal stimulation.

Bell (1956) stated that the decrease in cardiac output during vagal stimulation would be partially compensated for by an increased stroke volume. He concluded

that the prolonged diastolic ventricular filling period and its resultant increase in ventricular volume would stimulate ventricular musculature thus increasing stroke volume.

The present investigation approached the evaluation of passive cardiac output influences on pulmonary circulation through an examination of the perfusion percentage results. If variations in ink perfusion patterns were solely due to changes in cardiac output then one would expect these changes to be observed bilaterally on the surface of the lungs.

Krahl (1968) studying the rabbit noted a marked difference in ink perfusion between vagally stimulated and unstimulated lobes. He found that vagally stimulated lobes were extremely underperfused while the non-stimulated lobes demonstrated the usual mosaic pattern of ink perfusion. Krahl concluded that parasympathetic stimulation results in active pulmonary vasoconstriction.

DeFouw (1970) in similar studies on the dog, rabbit, and cat was unable to demonstrate extreme differences in ink perfusion following either right or left vagal stimulation. Contrary to Krahl, DeFouw concluded that vagal stimulation results in a mild active pulmonary vasodilation in the rabbit and cat, and no significant changes in the perfusion of the canine lung.

The present study is in partial agreement with the work of DeFouw. Comparison of the perfusion-percentages (Tables 1-4) of the stimulated and unstimulated lobes from

the experimental animals demonstrates no significant alterations in all three species. Examination of the photographs (Figures 14-16) taken of the right and left lungs of the experimental animals readily reveals that vagal stimulation did not produce the marked differences in ink perfusion as earlier reported by Krahl. Comparison of the perfusion-percentages of the experimental to the control animals demonstrates no significant variations in the dog and the cat but a bilateral decrease in perfusion in the experimental rabbit. The bilateral nature of this decrease indicates that it was most probably due to a passive reaction to the vagally induced decrease in cardiac output. All other perfusion-percentage variations were extremely small and when one takes into consideration the subjective nature of the data collection these variations become functionally insignificant.

Stereologically determined absolute lobar volumes occupied by pulmonary vessels (larger than capillaries) were statistically unaltered during the vagal stimulation and subsequent ink injection. The analysis of covariance revealed that the differences observed between left and right middle lobes were in accordance with that expected due to the normal variations in lobe size. These results negate the possibility of direct parasympathetic vasomotor activity influencing the volume occupied by the larger pulmonary vessels. They also suggest that cardiac output was not significantly altered during the vagal stimulation and subsequent ink injection.

Statistical evaluation of the volume occupied by ink filled pulmonary capillary beds, and the length of larger pulmonary vessels gave negative results in the dog and statistically significant differences in the rabbit and cat. These differences are discussed according to species. In each case four comparisons were made.

Left control lobe to right control lobe

Left stimulated lobe to right stimulated lobe

Left control lobe to left stimulated lobe

Right control lobe to right stimulated lobe

Interpretations of the results of these comparisons are incorporated in the following discussion.

Rabbit

Statistical evaluation of the capillary ink perfusion in the rabbit middle lobes revealed the following:

Left control volume was less than right control volume

Left stimulated volume was less than right stimulated volume

In both the control and stimulated left lobes, the volume occupied by ink filled pulmonary capillaries was less than in their right counterparts. The decrease in volume was over and above that which was expected due to the normal variations in total lobar volume. The fact that both the stimulated and control lobes were affected indicates that the differences were not due to vagal stimulation. The data suggests the presence of an inherent variation in the pulmonary capillary perfusion of the rabbit allowing greater perfusion to the right middle lobe.

Left control volume was not different than left stimulated volume. Right control volume was not different than right stimulated volume.

The fact that no differences were observed between unilateral stimulated and control lobes suggests that vagal stimulation had neither a passive nor an active effect on the rabbit pulmonary capillary ink perfusion.

Statistical evaluation of the length of pulmonary vessels in the rabbit revealed the following:

Left control vessels were shorter than right control vessels Left stimulated vessels were shorter than right stimulated vessels

Again, in the rabbit, a situation exists in which both the stimulated and control vessels of the left lobes were shorter than their right counterparts. This was over and above that expected due to the normal variations in total lobar volume. It suggests the presence of an inherent variation in the rabbit pulmonary tissue in which vessels in the left middle lobe are significantly shorter than those in the right.

Left control vessels were longer than left stimulated vessels. Right control vessels were longer than right stimulated vessels.

This data indicates that there was a bilateral decrease in the length of the pulmonary vessels in the stimulated rabbit. The decrease in vessel length cannot be directly attributed to an active pulmonary vasomotor response because

it was observed in both the right and left lobes of the stimulated animals. A vagally induced decrease in cardiac output could result in a decrease in vessel length but the volume occupied by these vessels was not significantly altered during the stimulation and subsequent ink injection.

Cat

Statistical evaluation of the volume occupied by ink filled capillaries in the cat middle lobes revealed the following:

Left control was not different than right control

Left stimulated was not different than right stimulated

Thus, the numerical differences in the volume occupied by ink filled pulmonary capillaries between left and right lobes of both experimental and control cats were due to the normal variations in total lobar volume.

Left control volume was greater than left stimulated volume

Right control volume was greater than right stimulated volume

The bilateral nature of the increase suggests that it was passively mediated. The fact that large vessel volume was unaltered during the stimulation indicates that pulmonary capillaries may be the first vessels affected by a decrease in right ventricular outflow.

Statistical evaluation of the length of pulmonary vessels in the cat revealed the following:

Left control was not different than right control

Left stimulated was not different than right stimulated

The numerical differences observed in the length of pulmonary vessels between the right and left stimulated and control lobes were due solely to the normal variations in total lobar volume.

Left control vessels were not different than left stimulated vessels. Right control vessels were longer than right stimulated vessels.

A reevaluation of the statistical data showed that the vessels in both the left and right stimulated lobes were shorter than those in their control counterparts. The differences between left stimulated and left control were not large enough to be statistically significant. The observed decrease in vessel length could be attributed to a decrease in cardiac output, but the volume occupied by the same vessels was not significantly altered during the stimulation.

By far, the most perplexing results were obtained in the stereologic data from the rabbit. Both the volume occupied by the ink filled capillaries and the lobar vessel lengths were significantly less in the left middle lobes than in the right. These differences were observed in both the stimulated and control animals and were larger than that expected due to the normal variations in total lobar volume. One can only conclude that there exists an inherent difference in the pulmonary tissue of the rabbit which accounts for the observed variations.

The present investigation has found no evidence indicating that parasympathetic stimulation results in a direct vasomotor response by the pulmonary circulation.

Evaluation Of The Experimental Technique

As with any experiment the more complicated the technique the greater the probability of error. It is therefore advantageous to discuss the areas most likely subject to variability. Examination of the present technique reveals several areas which require further clarification.

Does the effect of vagal stimulation under sodium pentobarbital anesthesia differ from that in a physiologically normal state? One must concede that any anesthesia alters the physiologic norm. The researcher must therefore choose the type of anesthesia which produces the least significant adverse effects. The barbiturate sodium pentobarbital, was the anesthetic of choice in the present investigation. Unfortunately there have been very few definitive studies on the effects of pentobarbital on the pulmonary circulation. Goldberg (1968) studying the effects of barbiturates on the dog concluded that sodium pentobarbital insignificantly altered the pulmonary circulation. It did produce tachycardia and decreased cardiac stroke volume.

Carbachol, used in terminating the animal, is an extremely potent parasympathomemitic drug. Should it reach the lungs via the bronchial circulation would its effect

overshadow that of the vagal stimulation? If immediate cardiac arrest does not follow the left ventricular carbachol injection there is every likelihood that a small percentage of the drug will reach the pulmonary circulation via bronchial-pulmonary anastomoses. Carbachol entering the lungs in this manner would be distributed equally to both the stimulated and unstimulated lobes, making evaluation of the vagal effects extremely difficult. The animals chosen for histologic study were those which demonstrated the most rapid cardiac arrest following injection of carbachol. The drug's effect on the pulmonary circulation of these animals was assumed to be minimal. Evaluation of the ink perfusion-percentages revealed no significant differences between animals who demonstrated immediate cardiac arrest and those where the heart gave several functional beats after left ventricular carbachol injection.

Is the vagal effect on pulmonary airways disrupted by the constant pressure formalin inflation technique used during the histologic preparation? The possibility does exist that the constant pressure formalin inflation technique employed for a twenty-four hour period may partially overshadow a vagally induced bronchoconstriction. Further studies are required to accurately interpret the significance of this proposed variability.

The technique of arranging the main artery and bronchus in the center of each lobe sample was the method employed to subjectively decrease sample variability.

Future attempts to stereologically evaluate pulmonary tissue on a lobe basis would be enhanced by utilization of the 70 mm film strip technique developed by Wilson and Pickett (1970). This would allow the entire lobe to be histologically prepared and mounted on the larger film strip. It would thus eliminate all questions concerning the reliability of samples on a lobe basis.

The reliability of stereologic data could also be increased by premeasuring the actual volumes of the right and left histologically sectioned lobes by the water displacement technique. Thus the volume of each lobe would be directly related to the tissue being sectioned.

Section evaluation need not be carried out at the sixth section interval. Data in Appendix A indicates that sampling every twenty-first section would yield reliable results. Only every twenty-first section from the strip of serial sections need be mounted on the film. Thus, for example, the number of mounted sections in the rabbit experimental left middle lobe would decrease from 844 to 40. This would enable the researcher to increase the number of lobes being sampled and thus increase the data reliability.

SUMMARY AND CONCLUSIONS

Electric mid-cervical stimulation of the distal end of the severed left vagus nerve of dogs, rabbits, and cats was followed by ink injection and *in situ* formalin fixation. The middle lobes from both stimulated (left) and control (right) lungs were evaluated grossly for the degree of peripheral ink perfusion, and histologically by sectioning from the hilus to the periphery.

Histologic serial sections were stereologically evaluated and the following calculated for both stimulated and control lobes: (1) the relative volume occupied by the pulmonary arteries, arterioles, veins, and venules, (2) the relative volume occupied by the ink filled pulmonary capillaries, (3) the relative volume occupied by the pulmonary airways ranging in size from secondary bronchi to terminal bronchioles, and (4) the length per unit volume of the pulmonary arteries, arterioles, veins, and venules. The above procedures were repeated on control animals which did not undergo vagal stimulation.

Statistical analysis of the stereologic data as well as subjective analysis of ink perfusion-percentages revealed the following:

Dog. Data from ink perfusion-percentages and stereologic section evaluation revealed that the vagal stimulation and subsequent ink injection had neither a passive nor an active effect on the canine pulmonary circulation and airway volume.

Rabbit. Data from ink perfusion percentages and total vessel length suggested the presence of a passively induced pulmonary vasoconstriction during vagal stimulation.

The vessel length and volume occupied by ink filled capillaries of both the stimulated and control left lobes were significantly smaller than their right counterparts. These observations suggest the presence of inherent variations in rabbit pulmonary tissue.

The remainder of the data indicated neither an active nor a passive effect.

Cat. Data from the volume occupied by the ink filled capillaries and vessel lengths suggests the presence of a passively induced pulmonary vasoconstriction during the vagal stimulation.

The remainder of the data indicated neither an active nor a passive effect.

Statistical and subjective evaluations of the experimental data, in all species, revealed no evidence indicating that parasympathetic stimulation results in active pulmonary vasomotion.

The possibility exists that during the vagal stimulation small muscular pulmonary arteries constricted and larger elastic arteries dilated. In such a situation, the net effect on the total volume occupied by the larger vessels would remain unaltered. This could account for the concomitant bilateral decrease in capillary ink volume in the stimulated rabbit and cat and the unaltered large vessel volume.

Vagal stimulation did not result in the massive constriction of precapillary muscular sphincters as earlier reported by Krahl (1968). The evidence suggests that the primary function of pulmonary vagal fibers is to carry afferent signals from the pulmonary tissue to the central nervous system. This is in agreement with Hirsch's (1969) conclusions that the majority of pulmonary vagal fibers are structurally and functionally afferent.

The close correlation between the present stereologic data and that retrieved by Weibel (1964) attests to its validity. The inherent variations observed in the rabbit pulmonary tissue demonstrates the need for further quantitative morphologic studies of the pulmonary circulation.

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APPENDICES

APPENDIX A

APPENDIX A. Original data collected from the rabbit left middle lobe.

Tables 6-9 contain the original data collected during the histologic evaluation of the rabbit left middle lobe. A survey of literature revealed that the optimum sample size for this type of experiment had not been established. An assumption was made that sampling every third section would give extremely reliable data. The results of sampling every third section were then tested for reliability at sixth, twelfth, fifteenth, twenty-first, and thirtieth section intervals. The data from the reliability tests were applied to all subsequent section sampling.

Section sampling proceeded according to unit areas. Three unit areas were sampled in each section large enough to accomodate them without overlap. Obviously sections close to the hilus and the periphery were too small to allow three unit area samples. These sections necessitated sampling of only one or two unit areas and are listed individually on the tables. When section size permitted three unit area samples, the counts of all three areas were totaled and appear in the table as a sum.

Table 6.--Rabbit--Left Middle Lobe

The original data indicating the number of times any portion of one of the forty-two cross bars of the Weibel viewing plate fell partially or wholly within the lumen of an artery, arteriole, vein, or venule.

Sampling every third section beginning with section one at the periphery and proceeding to the hilus.

A = Unit Area

Section #	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9
1.	0								
4.		0							
7.			1						
10.				1					
13.					0				
16.						2			
19.							4		
22.								4	
25.									2
28.	0								
31.		0							
34.			3						
37.				0					
40.					4				
43.						2			
46.							4		
49.								3	
52.									1
55.	1								
58.		3							
61.			0						
64.				3					
67.					5				
70.						5			
73.							3		
76.								3	
79.									2
82.	1								
85.		3							
88.			1						
91.				3					
94.					1	6			
97.							1	2	
100.	4								4
103.		1	2						
106.				1	0				
109.						0	1		
112.								1	5
115.	2	1							
118.			2	4					

Table 6.--(cont'd.)

Section #	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9
121.					1	4			
124.							0	2	
127.	1								2
130.		0	3						
133.				1	1				
136.						1	6		
139.								1	3
142.	10	4							
145.			1	1					
148.					0	1			
151.							2	2	
154.	3								1
157.		2	0						
160.				0	0				
163.						0	3		
166.								0	3
169.	4	2							
172.			5	1					
175.					7	1			
178.							0	3	
181.	4								1
184.		1	1						
187.				2	0				

Section #	A 1-2-3	Section #	A 4-5-6	Section #	A 7-8-9
193.	2	196.	6	190.	10
202.	20	205.	3	199.	8
211.	4	214.	8	208.	7
220.	5	223.	4	217.	6
229.	6	232.	8	226.	2
238.	4	241.	6	235.	11
247.	8	250.	3	244.	12
256.	3	259.	7	253.	8
265.	8	268.	7	262.	5
274.	18	277.	9	271.	7
283.	17	286.	17	280.	15
292.	12	295.	16	289.	7
301.	14	304.	17	298.	14
310.	7	313.	4	307.	6
319.	24	322.	5	316.	9
328.	4	331.	11	325.	7
337.	19	340.	12	334.	13
346.	8	349.	5	343.	3
355.	15	358.	9	352.	7
364.	17	367.	5	361.	15
373.	5	376.	7	370.	17

Table 6.--(cont'd.)

Section #	A 1-2-3	Section #	A 4-5-6	Section #	A 7-8-9
382.	9	385.	8	379.	3
391.	10	394.	10	388.	12
400.	6	403.	8	397.	9
409.	5	412.	10	406.	2
418.	3	421.	19	415.	9
427.	9	430.	10	424.	5
436.	21	439.	13	433.	5
445.	5	448.	14	442.	12
454.	12	457.	5	451.	11
463.	11	466.	11	460.	9
472.	6	475.	9	469.	8
481.	5	484.	10	478.	7
490.	7	493.	13	487.	3
499.	5	502.	17	496.	15
508.	15	511.	25	505.	8
517.	6	520.	13	514.	9
526.	4	529.	13	523.	3
535.	5	538.	10	532.	9
544.	8	547.	12	541.	7
553.	28	556.	7	550.	7
562.	10	565.	9	559.	3
571.	16	574.	8	568.	6
580.	2	583.	7	577.	12
589.	8	592.	10	586.	5
598.	7	601.	7	595.	8
607.	39	610.	6	604.	8
616.	6	619.	11	613.	12
625.	13	628.	11	622.	5
634.	6	637.	19	631.	3
643.	5	646.	8	640.	8
652.	3	655.	10	649.	4
661.	5	664.	5	658.	4
670.	9	673.	23	667.	8
679.	5	682.	5	676.	3
688.	8	691.	21	685.	13
697.	6	700.	7	694.	18
706.	8	709.	28	703.	11
715.	7	718.	5	712.	3

Section #	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9
721.							0	2	
724.	12								0
727.		3	2						
730.				1	1				
733.						2	1		
736.								1	2

Table 6.--(Cont'd.)

Section #	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9
739.	1	2							
742.			1	2					
745.					6	8			
748.							1	0	
751.	3								0
754.		1	1						
757.				3	2				
760.						0	3		
763.								0	9
766.	1	2							
769.			4	2					
772.					2	14			
775.							1	3	
778.	1								0
781.		2	2						
784.				2	4				
787.						2	0		
790.								1	2
793.	0	17							
796.			1	8					
799.					0	1			
802.							0	0	
805.	2								2
808.		0	12						
811.				1	2				
814.						1	3		
817.								0	4
820.	7	3							
823.			2	3					
826.					12	2			
829.							1	0	
832.	1								9
835.		2	0						
838.				0	2				
841.						0	4		
844.								2	4

Table 7.--Rabbit--Left Middle Lobe

The original data indicating the number of times any portion of one of the forty-two cross bars of the Weibel viewing plate fell on an ink filled capillary.

Sampling every third section beginning with section one at the periphery and proceeding to the hilus.

A = Unit Area

Section #	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9
1.	13								
4.		0							
7.			0						
10.				1					
13.					13				
16.						15			
19.							16		
22.								15	
25.									12
28.	7								
31.		7							
34.			16						
37.				1					
40.					18				
43.						0			
46.							17		
49.								2	
52.									13
55.	16								
58.		18							
61.			5						
64.				18					
67.					14				
70.						19			
73.							10		
76.								12	
79.									0
82.	13								
85.		16							
88.			15						
91.				17					
94.					13	9			
97.							12	10	
100.	19								20
103.		8	11						
106.				12	6				
109.						3	12		
112.								14	11
115.	4	17							
118.			8	12					
121.					6	13			
124.							6	2	

Table 7.--(Cont'd.)

Section #	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9
127.	8								16
130.		11	18						
133.				13	13				
136.						3	14		
139.								15	11
142.	3	9							
145.			19	11					
148.					13	14			
151.							0	12	
154.	18								16
157.		19	17						
160.				4	10				
163.						22	21		
166.								10	0
169.	0	7							
172.			18	14					
175.					12	15			
178.							0	4	
181.	0								17
184.		21	12						
187.				11	12				

Section #	A 1-2-3	Section #	A 4-5-6	Section #	A 7-8-9
193.	51	196.	12	190.	38
202.	35	205.	14	199.	25
211.	46	214.	30	208.	43
220.	30	223.	17	217.	34
229.	44	232.	10	226.	37
238.	43	241.	26	235.	42
247.	21	250.	15	244.	48
256.	36	259.	16	253.	42
265.	16	268.	11	262.	51
274.	36	277.	15	271.	26
283.	24	286.	24	280.	30
292.	27	295.	30	289.	52
301.	44	304.	31	298.	40
310.	38	313.	23	307.	35
319.	30	322.	30	316.	32
328.	50	331.	21	325.	42
337.	22	340.	37	334.	36
346.	55	349.	19	343.	47
355.	49	358.	40	352.	58
364.	32	367.	23	361.	26
373.	33	376.	32	370.	45
382.	30	385.	25	379.	43
391.	47	394.	40	388.	35

Table 7.--(cont'd.)

Section #	A 1-2-3	Section #	A 4-5-6	Section #	A 7-8-9
400.	52	403.	23	397.	52
409.	26	412.	23	406.	48
418.	21	421.	22	415.	58
427.	43	430.	22	424.	48
436.	30	439.	14	433.	55
445.	53	448.	35	442.	26
454.	45	457.	35	451.	48
463.	44	466.	27	460.	43
472.	31	475.	42	469.	40
481.	52	484.	33	478.	48
490.	41	493.	48	487.	47
499.	46	502.	24	496.	38
508.	37	511.	32	505.	33
517.	15	520.	27	514.	42
526.	32	529.	36	523.	19
535.	38	538.	46	532.	47
544.	32	547.	23	541.	46
553.	17	556.	30	550.	31
562.	46	565.	23	559.	46
571.	16	574.	35	568.	51
580.	41	583.	57	577.	48
589.	28	592.	26	586.	45
598.	39	601.	19	595.	55
607.	30	610.	35	604.	37
616.	25	619.	22	613.	39
625.	31	628.	30	622.	42
634.	25	637.	32	631.	45
643.	48	646.	46	640.	47
652.	39	655.	33	649.	47
661.	46	664.	37	658.	43
670.	27	673.	24	667.	44
679.	24	682.	37	676.	40
688.	26	691.	24	685.	36
697.	37	700.	28	694.	37
706.	22	709.	19	703.	47
715.	35	718.	17	712.	36

Section #	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9
721.							12	13	
724.	3								9
727.		19	0						
730.				20	13				
733.						14	12		
736.								15	17
739	11	11							
742.			17	10					

Table 7.--(cont'd.)

Section #	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9
745.					9	7			
748.							10	10	
751.	11								0
754.		11	9						
757.				17	16				
760.						0	16		
763.								26	11
766.	20	18							
769.			17	15					
772.					19	18			
775.							13	16	
778.	3								0
781.		17	6						
784.				11	7				
787.						14	19		
790.								15	17
793.	0	12							
796.			12	19					
799.					16	12			
802.							12	0	
805.	17								20
808.		16	19						
811.				9	7				
814.						10	14		
817.								10	19
820.	6	11							
823.			14	17					
826.					14	14			
829.							16	22	
832.	19								18
835.		21	14						
838.				13	21				
841.						0	15		
844.								18	19

Table 8.--Rabbit--Left Middle Lobe

The original data indicating the number of times pulmonary arteries, arterioles, veins, and venules fell within the outer limits of the Weibel square. Vessels intersected by the left and upper edges of the square were counted while those intersected by the right and lower edges were not.

Sampling every third section beginning with section one at the periphery and proceeding to the hilus.

A = Unit Area

Section #	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9
1.	4								
4.		1							
7.			6						
10.				7					
13.					3				
16.						3			
19.							7		
22.								6	
25.									3
28.	3								
31.		1							
34.			8						
37.				2					
40.					6				
43.						3			
46.							8		
49.								5	
52.									5
55.	2								
58.		4							
61.			1						
64.				5					
67.					6				
70.						4			
73.							3		
76.								4	
79.									1
82.	5								
85.		5							
88.			2						
91.				5					
94.					1	3			
97.							1	5	
100.	7								2
103.		3	6						
106.				2	0				
109.						2	4		
112.								2	7
115.	5	5							

Table 8.--(cont'd.)

Section #	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9
118.			4	4					
121.					2	7			
124.							1	2	
127.	1								4
130.		1	4						
133.				2	5				
136.						2	2		
139.								3	9
142.	2	4							
145.			1	4					
148.					1	2			
151.							4	6	
154.	3								2
157.		1	1						
160.				1	1				
163.						1	3		
166.								1	1
169.	2	5							
172.			2	3					
175.					2	1			
178.							0	1	
181.	5								1
184.		1	3						
187.				5	6				

Section #	A 1-2-3	Section #	A 4-5-6	Section #	A 7-8-9
193.	6	196.	8	190.	8
202.	5	205.	5	199.	6
211.	10	214.	10	208.	11
220.	10	223.	8	217.	11
229.	13	232.	11	226.	4
238.	10	241.	11	235.	7
247.	12	250.	7	244.	9
256.	10	259.	14	253.	9
265.	9	268.	13	262.	9
274.	8	277.	8	271.	9
283.	8	286.	9	280.	4
292.	6	295.	10	289.	8
301.	11	304.	6	298.	10
310.	9	313.	4	307.	14
319.	10	322.	5	316.	13
328.	8	331.	12	325.	6
337.	11	340.	9	334.	10
346.	5	349.	8	343.	6
355.	11	358.	18	352.	12
364.	8	367.	10	361.	7

Table 8.--(cont'd.)

Section #	A 1-2-3	Section #	A 4-5-6	Section #	A 7-8-9
373.	7	376.	12	370.	7
382.	8	385.	12	379.	9
391.	8	394.	19	388.	17
400.	12	403.	12	397.	7
409.	9	412.	12	406.	10
418.	10	421.	13	415.	9
427.	10	430.	8	424.	11
436.	7	439.	9	433.	10
445.	9	448.	14	442.	10
454.	11	457.	13	451.	9
463.	13	466.	16	460.	9
472.	7	475.	12	469.	11
481.	12	484.	11	478.	14
490.	16	493.	8	487.	7
499.	11	502.	6	496.	13
508.	11	511.	10	505.	15
517.	11	520.	11	514.	12
526.	10	529.	19	523.	4
535.	11	538.	13	532.	6
544.	14	547.	9	541.	11
553.	8	556.	17	550.	12
562.	15	565.	14	559.	10
571.	12	574.	13	568.	14
580.	6	583.	12	577.	18
589.	7	592.	12	586.	17
598.	10	601.	16	595.	14
607.	8	610.	13	604.	20
616.	9	619.	11	613.	14
625.	7	628.	10	622.	17
634.	9	637.	11	631.	11
643.	10	646.	13	640.	14
652.	8	655.	8	649.	14
661.	11	664.	12	658.	10
670.	11	673.	6	667.	13
679.	10	682.	7	676.	15
688.	7	691.	11	685.	11
697.	9	700.	11	694.	17
706.	6	709.	8	703.	14
715.	14	718.	18	712.	16

Section #	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9
721.							3	3	
724.	2								1
727.		3	3						
730.				4	3				
733.						3	3		
736.								5	4
739.	5	5							

Table 8.--(cont'd.)

Section #	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9
742.			4	3					
745.					2	3			
748.							3	2	
751.	2								0
754.		6	1						
757.				5	2				
760.						0	8		
763.								2	2
766.	7	4							
769.			3	4					
772.					4	2			
775.							3	5	
778.	0								4
781.		2	5						
784.				1	5				
787.						3	0		
790.								2	2
793.	0	2							
796.			2	3					
799.					3	3			
802.							1	2	
805.	4								2
808.		1	3						
811.				1	3				
814.						4	4		
817.								0	2
820.	4	2							
823.			2	3					
826.					3	5			
829.							1	2	
832.	2								2
835.		7	3						
838.				5	6				
841.						0	5		
844.								4	8

Table 9.--Rabbit--Left Middle Lobe

The original data indicating the number of times any portion of one of the forty-two cross bars of the Weibel viewing plate fell partially or wholly within the lumen of an airway ranging in size from secondary bronchi to terminal bronchioles.

Sampling every third section beginning with section one at the periphery and proceeding to the hilus.

A = Unit Area

Section #	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9
1.	0								
4.		0							
7.			0						
10.				0					
13.					0				
16.						0			
19.							0		
22.								0	
25.									0
28.	0								
31.		0							
34.			0						
37.				0					
40.					0				
43.						0			
46.							0		
49.								0	
52.									0
55.	0								
58.		0							
61.			1						
64.				0					
67.					0				
70.						0			
73.							0		
76.								1	
79.									0
82.	0								
85.		1							
88.			0						
91.				0					
94.					0	1			
97.							1	0	
100.	3								0
103.		0	0						
106.				2	1				
109.						0	0		
112.								1	0
115.	0	1							
118.			1	2					

Table 9.--(cont'd.)

Section #	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9
121.					1	1			
124.							3	1	
127.	0								0
130.		3	2						
133.				1	0				
136.						0	5		
139.								1	2
142.	0	1							
145.			0	3					
148.					2	2			
151.							0	1	
154.	5								2
157.		1	1						
160.				0	0				
163.						1	0		
166.								1	4
169.	0	1							
172.			2	4					
175.					0	3			
178.							0	0	
181.	4								1
184.		0	0						
187.				0	2				

Section #	A 1-2-3	Section #	A 4-5-6	Section #	A 7-8-9
193.	2	196.	3	190.	10
202.	2	205.	6	199.	7
211.	10	214.	3	208.	9
220.	3	223.	2	217.	2
229.	2	232.	3	226.	4
238.	1	241.	1	235.	5
247.	5	250.	3	244.	10
256.	2	259.	1	253.	2
265.	4	268.	3	262.	12
274.	2	277.	4	271.	11
283.	6	286.	7	280.	7
292.	4	295.	2	289.	3
301.	8	304.	11	298.	6
310.	3	313.	6	307.	1
319.	4	322.	11	316.	3
328.	0	331.	2	325.	3
337.	10	340.	8	334.	10
346.	7	349.	0	343.	2
355.	1	358.	4	352.	3
364.	7	367.	1	361.	1
373.	9	376.	3	370.	0

Table 9.--(cont'd.)

Section #	A 1-2-3	Section #	A 4-5-6	Section #	A 7-8-9
382.	11	385.	2	379.	1
391.	11	394.	1	388.	1
400.	14	403.	0	397.	2
409.	8	412.	5	406.	2
418.	2	421.	2	415.	9
427.	6	430.	9	424.	8
436.	8	439.	3	433.	0
445.	16	448.	5	442.	7
454.	3	457.	1	451.	5
463.	5	466.	2	460.	9
472.	2	475.	2	469.	2
481.	9	484.	1	478.	5
490.	3	493.	14	487.	6
499.	1	502.	20	496.	1
508.	4	511.	1	505.	0
517.	3	520.	1	514.	2
526.	2	529.	3	523.	27
535.	1	538.	4	532.	22
544.	3	547.	1	541.	2
553.	29	556.	1	550.	0
562.	15	565.	1	559.	2
571.	26	574.	4	568.	11
580.	14	583.	13	577.	3
589.	15	592.	3	586.	4
598.	21	601.	4	595.	0
607.	14	610.	7	604.	5
616.	24	619.	1	613.	4
625.	8	628.	2	622.	3
634.	17	637.	2	631.	4
643.	7	646.	4	640.	1
652.	10	655.	6	649.	2
661.	5	664.	0	658.	5
670.	16	673.	4	667.	4
679.	12	682.	1	676.	5
688.	13	691.	2	685.	2
697.	9	700.	4	694.	6
706.	4	709.	1	703.	1
715.	11	718.	2	712.	1

Section #	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9
721.							0	0	
724.	0								2
727.		2	0						
730.				0	0				
733.						13	0		
736.								1	1
739.	0	0							

Table 9.--(cont'd.)

Section #	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9
742.			1	1					
745.					0	0			
748.							0	5	
751.	1								12
754.		1	0						
757.				1	2				
760.						13	0		
763.								0	0
766.	0	6							
769.			0	2					
772.					0	1			
775.							1	0	
778.	12								0
781.		0	0						
784.				1	1				
787.						0	6		
790.								0	4
793.	14	0							
796.			1	1					
799.					2	3			
802.							11	3	
805.	3								2
808.		1	1						
811.				2	3				
814.						1	2		
817.								7	4
820.	2	1							
823.			3	1					
826.					0	0			
829.							1	1	
832.	1								10
835.		0	0						
838.				0	4				
841.						13	0		
844.								1	0

1. Totals of the original data indicating the number of times any portion of one of the forty-two cross bars of the Weibel viewing plate fell partially or wholly within the lumen of an artery, arteriole, vein, or venule.

2. Totals of the original data indicating the number of times one of the forty-two cross bars of the Weibel viewing plate fell on an ink filled capillary.

3. Totals of the original data indicating the number of times pulmonary arteries, arterioles, veins, and venules fell within the outer limits of the Weibel square. Vessels intersected by the left and upper edges of the square were counted while those intersected by the right and lower edges were not.

4. Totals of the original data indicating the number of times any portion of one of the forty-two cross bars of the Weibel viewing plate fell partially or wholly within the lumen of an airway ranging in size from secondary bronchi to terminal bronchioles.

Table 10.--Rabbit--Left Middle Lobe

Section Intervals	3	6	12	15	21	30
1.						
Total hits	2054	1010	528	407	307	219
Unit areas sampled	710	354	178	142	103	71
Mean value	2.89	2.85	2.96	2.86	2.98	3.08
2.						
Total hits	8288	4069	2016	1607	1164	768
Unit areas sampled	710	354	178	142	103	71
Mean value	11.67	11.49	11.32	11.31	11.30	10.81
3.						
Total hits	2418	1180	578	493	367	244
Unit areas sampled	710	354	178	142	103	71
Mean value	3.40	3.30	3.80	3.47	3.56	3.43
4.						
Total hits	1248	585	296	256	194	144
Unit areas sampled	710	354	178	142	103	71
Mean value	1.75	1.65	1.66	1.80	1.88	2.02

APPENDIX B

APPENDIX B. Original data from lobar volumetric analyses and stereologic data measurements.

Tables 11, 12, and 13 contain the original data from the volumetric analyses. In each case, three experimental animals of each species were used to determine the mean lobar volume. The previously described water displacement technique was employed for all measurements. Table 14 contains the original data from the volumetric analyses of the control animals. Only one representative animal of each species was used in this study.

The extremely large quantity of numerical data prohibited individual lobar evaluations similar to that seen in Appendix A. The original data was summarized by evaluating each lobe as a whole and then dividing the data into three anatomical portions; a hilar, a middle, and a peripheral. Tables 15-20 contain the summarized results. Tables 21-26 contain the calculated absolute volumes and lobar vessel lengths for both the experimental and control animals. The abbreviations appearing in Tables 15-26 are listed as follows:

(1) = Volume occupied by pulmonary vessels larger than capillaries.

(2) = Volume occupied by ink filled pulmonary capillaries.

(3) = Volume occupied by pulmonary airways larger than respiratory bronchioles.

(4) = Length of pulmonary vessels larger than capillaries.

T. = Total number of hits

P. = Possible number of hits

R.V. = Relative volume

P.C. = Total number of profiles counted

U.A. = Total number of unit areas sampled

M. = Mean value

S.A. = Area of Weibel square

A.V. = Actual volume

Ab.V. = Absolute volume

Table 11.--Volumetric analysis by water displacement technique

Dog

Experimental animals-Middle lobes

		Left	Right
Dog # 1	Flask volume	2120 cc	2120 cc
	Flask volume with lobe	2095	2085
	Lobe volume	25	35
Dog # 2	Flask volume	2120	2120
	Flask volume with lobe	2095	2085
	Lobe volume	25	35
Dog # 3	Flask volume	2120	2120
	Flask volume with lobe	2100	2090
	Lobe volume	20	30
	Mean volume	23	33

Table 12.--Volumetric analysis by water displacement technique

Rabbit

Experimental animals-Middle lobes

		Left	Right
Rabbit # 1	Flask volume	510 cc	510 cc
	Flask volume with lobe	501	498
	Lobe volume	9	12
Rabbit # 2	Flask volume	510	510
	Flask volume with lobe	501	499
	Lobe volume	9	11
Rabbit #3	Flask volume	510	510
	Flask volume with lobe	501	498
	Lobe volume	9	12
	Mean volume	9	12

Table 13.--Volumetric analysis by water displacement technique

Cat

Experimental animals-Middle lobes

		Left	Right
Cat # 1	Flask volume	485 cc	485 cc
	Flask volume with lobe	477	474
	Lobe volume	8	11
Cat # 2	Flask volume	485	485
	Flask volume with lobe	478	475
	Lobe volume	7	10
Cat # 3	Flask volume	485	485
	Flask volume with lobe	477	475
	Lobe volume	8	10
	Mean volume	8	10

Table 14.--Volumetric analysis by water displacement technique

Control animals---Middle lobes

		Left	Right
Dog # 1	Flask volume	2130 cc	2130 cc
	Flask volume with lobe	2105	2095
	Lobe volume	25	35
Rabbit # 1	Flask volume	530	530
	Flask volume with lobe	521	519
	Lobe volume	9	11
Cat # 1	Flask volume	530	530
	Flask volume with lobe	521	519
	Lobe volume	9	11

Table 15.--Experimental Dog

		Left			Right		
		T.	P.	R.V.	T.	P.	R.V.
(1)	Hilar	907	8,190	.1107	1639	10,206	.1605
	Middle	1164	12,474	.0933	872	11,340	.0768
	Periph	474	8,988	.0527	471	9,492	.0496
	Mean	2545	29,652	.0856	2982	31,038	.0956
(2)	Hilar	1959	8,190	.2391	1951	10,206	.1911
	Middle	3040	12,474	.2437	2941	11,340	.2593
	Periph	2396	8,988	.2665	2281	9,492	.2403
	Mean	7395	29,652	.2498	7173	31,038	.2302
(3)	Hilar	237	8,190	.0289	557	10,206	.0238
	Middle	443	12,474	.0355	324	11,340	.0285
	Periph	200	8,988	.0222	164	9,492	.0172
	Mean	880	29,652	.0289	1045	31,038	.0232

		P.C.	U.A.	M.	S.A.	P.C.	U.A.	M.	S.A.
(4)	Hilar	608	195	3.117	.81mm^2	568	243	2.337	.81mm^2
	Middle	786	297	2.646	.81mm^2	641	270	2.374	.81mm^2
	Periph	549	214	2.565	.81mm^2	485	226	2.146	.81mm^2
	Mean	1943	706	2.776	.81mm^2	1694	739	2.286	.81mm^2

Table 16.--Control Dog

		Left			Right		
		T.	P.	R.V.	T.	P.	R.V.
(1)	Hilar	405	8,862	.0457	1480	8,526	.1811
	Middle	733	9,324	.0786	715	8,568	.0833
	Periph	665	9,198	.0723	606	8,442	.0719
	Mean	1803	27,384	.0657	2801	25,284	.1107
(2)	Hilar	2270	8,862	.2562	1610	8,526	.1967
	Middle	2582	9,324	.2769	2453	8,568	.2863
	Periph	3450	9,198	.3750	2554	8,442	.3025
	Mean	8302	27,384	.3027	6617	25,284	.2618
(3)	Hilar	395	8,862	.0443	547	8,526	.0669
	Middle	694	9,324	.0745	516	8,568	.0602
	Periph	279	9,198	.0303	268	8,442	.0317
	Mean	1368	27,384	.0476	1331	25,284	.0529

		P.C.	U.A.	M.	S.A.	P.C.	U.A.	M.	S.A.
(4)	Hilar	376	211	1.782	.81mm^2	551	212	2.714	.81mm^2
	Middle	574	222	2.585	.81mm^2	584	204	2.714	.81mm^2
	Periph	793	219	3.621	.81mm^2	552	186	2.746	.81mm^2
	Mean	1743	652	2.673	.81mm^2	1684	602	2.784	.81mm^2

Table 17.--Experimental Rabbit

		Left			Right		
		T.	P.	R.V.	T.	P.	R.V.
(1)	Hilar	411	5,040	.0815	663	7,434	.0892
	Middle	474	5,922	.0800	868	8,316	.1044
	Periph	182	3,906	.0466	275	6,174	.0445
	Mean	1067	14,868	.0693	1806	21,924	.0793
(2)	Hilar	1446	5,040	.2869	2109	7,434	.2836
	Middle	1688	5,922	.2850	2581	8,316	.3103
	Periph	946	3,906	.2422	1837	6,174	.2971
	Mean	4080	14,868	.2713	6527	21,924	.2971
(3)	Hilar	260	5,040	.0515	529	7,434	.0711
	Middle	233	5,922	.0393	377	8,316	.0453
	Periph	92	3,906	.0235	126	6,174	.0204
	Mean	585	14,868	.0381	1032	21,924	.0463

		P.C.	U.A.	M.	S.A.	P.C.	U.A.	M.	S.A.
(4)	Hilar	408	120	3.400	.81mm^2	624	177	3.525	.81mm^2
	Middle	464	141	3.291	.81mm^2	632	198	3.192	.81mm^2
	Periph	307	93	3.236	.81mm^2	453	147	3.081	.81mm^2
	Mean	1179	354	3.309	.81mm^2	1709	522	3.266	.81mm^2

Table 18.--Control Rabbit

		Left			Right		
		T.	P.	R.V.	T.	P.	R.V.
(1)	Hilar	590	4,494	.1312	471	5,124	.0919
	Middle	480	4,914	.0956	687	5,166	.1329
	Periph	392	4,788	.0818	474	5,166	.0917
	Mean	1462	14,196	.1029	1632	15,456	.1054
(2)	Hilar	1451	4,494	.3228	1711	5,124	.3339
	Middle	1506	4,914	.3064	1864	5,166	.3564
	Periph	1708	4,788	.3567	1953	5,166	.3780
	Mean	4685	14,196	.3286	5528	15,456	.3563
(3)	Hilar	590	4,494	.0518	471	5,124	.0772
	Middle	480	4,914	.0403	687	5,166	.0230
	Periph	392	4,788	.0273	474	5,166	.0284
	Mean	572	14,196	.0403	662	15,456	.0429

		P.C.	U.A.	M.	S.A.	P.C.	U.A.	M.	S.A.
(4)	Hilar	438	107	4.093	.81mm^2	585	122	4.795	.81mm^2
	Middle	467	117	3.991	.81mm^2	583	123	4.739	.81mm^2
	Periph	557	114	4.886	.81mm^2	586	123	4.763	.81mm^2
	Mean	1462	338	4.323	.81mm^2	1754	368	4.765	.81mm^2

Table 19.--Experimental Cat

		Left			Right		
		T.	P.	R.V.	T.	P.	R.V.
(1)	Hilar	368	5,418	.0679	372	6,930	.0536
	Middle	525	6,048	.0868	629	7,686	.0818
	Periph	202	4,830	.0418	195	6,468	.0301
	Mean	1095	16,296	.0655	1196	21,084	.0552
(2)	Hilar	986	5,418	.1819	1350	6,930	.1948
	Middle	941	6,048	.1555	1262	7,686	.1641
	Periph	727	4,830	.1505	1090	6,468	.1685
	Mean	2654	16,296	.1626	3702	21,084	.1758
(3)	Hilar	88	5,418	.0162	121	6,930	.0174
	Middle	238	6,048	.0393	166	7,686	.0215
	Periph	97	4,830	.0200	65	6,468	.0100
	Mean	423	16,296	.0252	352	21,084	.0163

		P.C.	U.A.	M.	S.A.	P.C.	U.A.	M.	S.A.
(4)	Hilar	321	129	2,488	.81mm^2	489	165	2.964	.81mm^2
	Middle	339	144	2.354	.81mm^2	437	183	2.278	.81mm^2
	Periph	294	115	2.556	.81mm^2	309	154	2.006	.81mm^2
	Mean	954	388	2.466	.81mm^2	1235	502	2.416	.81mm^2

Table 20.--Control Cat

		Left			Right		
		T.	P.	R.V.	T.	P.	R.V.
(1)	Hilar	532	6,216	.0855	563	7,812	.0721
	Middle	649	6,174	.1051	1010	7,686	.1314
	Periph	355	6,048	.0587	525	7,812	.0672
	Mean	1536	18,438	.0831	2098	23,310	.0902
(2)	Hilar	1480	6,216	.2218	1699	7,812	.2174
	Middle	1588	6,174	.2572	1916	7,686	.2492
	Periph	1645	6,048	.2719	2046	7,812	.2619
	Mean	4713	18,438	.2556	5661	23,310	.2428
(3)	Hilar	361	6,216	.0581	510	7,812	.0653
	Middle	292	6,174	.0473	296	7,686	.0385
	Periph	91	6,048	.0148	223	7,812	.0285
	Mean	744	18,438	.0403	1029	23,310	.0441

		P.C.	U.A.	M.	S.A.	P.C.	U.A.	M.	S.A.
(4)	Hilar	454	148	3.067	.81mm^2	640	186	3.660	.81mm^2
	Middle	521	147	3.612	.81mm^2	681	183	3.716	.81mm^2
	Periph	654	144	4.541	.81mm^2	865	186	4.650	.81mm^2
	Mean	1629	439	3.740	.81mm^2	2186	555	4.008	.81mm^2

Table 21.--Experimental Dog

Left Lobe				Right Lobe		
Large vessel volume						
R.V.	A.V.	Ab.V.		Ab.V.	A.V.	R.V.
.1107	(23cc) =	2.55cc	Hilar	5.31cc =	(33cc)	.1605
.0933	(23cc) =	2.14cc	Middle	2.54cc =	(33cc)	.0768
.0527	(23cc) =	1.22cc	Periph	1.65cc =	(33cc)	.0496
.0848	(23cc) =	1.96cc	Mean	3.20cc =	(33cc)	.0972
Capillary volume						
.2391	(23cc) =	5.31cc	Hilar	6.30cc =	(33cc)	.1911
.2437	(23cc) =	5.61cc	Middle	8.55cc =	(33cc)	.2593
.2665	(23cc) =	6.14cc	Periph	7.92cc =	(33cc)	.2403
.2585	(23cc) =	5.69cc	Mean	7.72cc =	(33cc)	.2403
Airway volume						
.0289	(23cc) =	0.67cc	Hilar	0.79cc =	(33cc)	.0238
.0355	(23cc) =	0.83cc	Middle	0.96cc =	(33cc)	.0285
.0222	(23cc) =	0.51cc	Periph	0.56cc =	(33cc)	.0172
.0306	(23cc) =	0.71cc	Mean	0.76cc =	(33cc)	.0232
Large vessel length						
M.	A.V.	S.A.		M.	A.V.	S.A.
2(3.117)	(23cc)/.81mm^2	=177m	Hilar	190m=2(2.337)	(33cc)/.81mm^2	
2(2.646)	(23cc)/.81mm^2	=150m	Middle	193m=2(2.374)	(33cc)/.81mm^2	
2(2.565)	(23cc)/.81mm^2	=146m	Periph	175m=2(2.146)	(33cc)/.81mm^2	
2(2.752)	(23cc)/.81mm^2	=156m	Mean	189m=2(2.314)	(33cc)/.81mm^2	

Table 22.--Control Dog

Left Lobe				Right Lobe		
R.V.	A.V.	Ab.V.		Ab.V.	A.V.	R.V.
Large vessel volume						
.0457	(25cc)	1.15cc	Hilar	6.34cc	(35cc)	.1811
.0786	(25cc)	1.98cc	Middle	2.91cc	(35cc)	.0833
.0723	(25cc)	1.80cc	Periph	2.52cc	(35cc)	.0719
.0655	(25cc)	1.65cc	Mean	3.92cc	(35cc)	.1121
Capillary volume						
.2562	(25cc)	6.40cc	Hilar	6.90cc	(35cc)	.1967
.2769	(25cc)	6.93cc	Middle	10.01cc	(35cc)	.2863
.3750	(25cc)	9.38cc	Periph	10.61cc	(35cc)	.3025
.3027	(25cc)	7.58cc	Mean	9.17cc	(35cc)	.2618
Airway volume						
.0443	(25cc)	1.10cc	Hilar	2.35cc	(35cc)	.0669
.0745	(25cc)	1.88cc	Middle	2.10cc	(35cc)	.0602
.0303	(25cc)	0.75cc	Periph	1.12cc	(35cc)	.0317
.0476	(25cc)	1.20cc	Mean	1.86cc	(35cc)	.0529

Large vessel length

M.	A.V.	S.A.		M.	A.V.	S.A.
2(1.782)	(25cc)	/.81mm^2=110m	Hilar	235m=2(2.714)	(35cc)	/.81mm^2
2(2.585)	(25cc)	/.81mm^2=160m	Middle	247m=2(2.862)	(35cc)	/.81mm^2
2(3.612)	(25cc)	/.81mm^2=224m	Periph	237m=2(2.746)	(35cc)	/.81mm^2
2(2.652)	(25cc)	/.81mm^2=164m	Mean	241m=2(2.784)	(35cc)	/.81mm^2

Table 23.--Experimental Rabbit

Left Lobe				Right Lobe		
Large vessel volume						
R.V.	A.V.	Ab.V.		Ab.V.	A.V.	R.V.
.0815(9cc)	=	0.74cc	Hilar	1.07cc	= (12cc)	.0892
.0800(9cc)	=	0.72cc	Middle	1.25cc	= (12cc)	.1044
.0466(9cc)	=	0.42cc	Periph	0.54cc	= (12cc)	.0445
.0686(9cc)	=	0.62cc	Mean	1.10cc	= (12cc)	.0841
Capillary volume						
.2896(9cc)	=	2.58cc	Hilar	3.41cc	= (12cc)	.2836
.2850(9cc)	=	2.57cc	Middle	3.72cc	= (12cc)	.3103
.2422(9cc)	=	2.18cc	Periph	3.68cc	= (12cc)	.2975
.2815(9cc)	=	2.44cc	Mean	3.49cc	= (12cc)	.2908
Airway volume						
.0515(9cc)	=	0.47cc	Hilar	0.85cc	= (12cc)	.0711
.0393(9cc)	=	0.35cc	Middle	0.54cc	= (12cc)	.0453
.0235(9cc)	=	0.22cc	Periph	0.24cc	= (12cc)	.0204
.0428(9cc)	=	0.39cc	Mean	0.55cc	= (12cc)	.0456
Large vessel length						
M.	A.V.	S.A.		M.	A.V.	S.A.
2(3.400)	(9cc)/.81mm^2	= 76m	Hilar 104m	= 2(3.525)	(12cc)/.81mm^2	
2(3.292)	(9cc)/.81mm^2	= 73m	Middle 95m	= 2(3.192)	(12cc)/.81mm^2	
2(3.236)	(9cc)/.81mm^2	= 72m	Periph 91m	= 2(3.081)	(12cc)/.81mm^2	
2(3.309)	(9cc)/.81mm^2	= 74m	Mean 97m	= 2(3.266)	(12cc)/.81mm^2	

Table 24.--Control Rabbit

Left Lobe				Right Lobe		
Large vessel volume						
R.V.	A.V.	Ab.V.		Ab.V.	A.V.	R.V.
.1312(9cc)	=	1.18cc	Hilar	1.01cc	=	(11cc).0919
.0956(9cc)	=	0.86cc	Middle	1.46cc	=	(11cc).1329
.0818(9cc)	=	0.74cc	Periph	1.01cc	=	(11cc).0917
.1029(9cc)	=	0.93cc	Mean	1.17cc	=	(11cc).1055
Capillary volume						
.3228(9cc)	=	2.19cc	Hilar	3.67cc	=	(11cc).3339
.3064(9cc)	=	2.75cc	Middle	3.93cc	=	(11cc).3569
.3567(9cc)	=	3.21cc	Periph	4.16cc	=	(11cc).3780
.3286(9cc)	=	2.96cc	Mean	3.92cc	=	(11cc).0426
Airway volume						
.0518(9cc)	=	0.47cc	Hilar	0.85cc	=	(11cc).0772
.0403(9cc)	=	0.36cc	Middle	0.25cc	=	(11cc).0230
.0273(9cc)	=	0.24cc	Periph	0.31cc	=	(11cc).0284
.0389(9cc)	=	0.36cc	Mean	0.47cc	=	(11cc).0426
Large vessel length						
M.	A.V.	S.A.		M.	A.V.	S.A.
2(4.093)	(9cc)	/.81mm^2 =	91m--Hilar---	130m=	2(4.795)	(11cc)/.81mm^2
2(3.991)	(9cc)	/.81mm^2 =	89m--Middle--	129m=	2(4.739)	(11cc)/.81mm^2
2(4.886)	(9cc)	/.81mm^2 =	108m--Periph--	129m=	2(4.763)	(11cc)/.81mm^2
2(4.323)	(9cc)	/.81mm^2 =	96m--Mean----	129m=	2(4.765)	(11cc)/.81mm^2

Table 25.--Experimental Cat

Left Lobe				Right Lobe		
Large vessel volume						
R.V.	A.V.	Ab.V.		Ab.V.	A.V.	R.V.
.0679	(8cc) =	0.54cc	Hilar	0.54cc =	(10cc)	.0536
.0868	(8cc) =	0.70cc	Middle	0.82cc =	(10cc)	.0818
.0418	(8cc) =	0.34cc	Periph	0.30cc =	(10cc)	.0301
.0664	(8cc) =	0.53cc	Mean	0.56cc =	(10cc)	.0564
Capillary volume						
.1819	(8cc) =	1.46cc	Hilar	1.95cc =	(10cc)	.1948
.1555	(8cc) =	1.25cc	Middle	1.64cc =	(10cc)	.1641
.1505	(8cc) =	1.21cc	Periph	1.69cc =	(10cc)	.1655
.1642	(8cc) =	1.31cc	Mean	1.77cc =	(10cc)	.1772
Airway volume						
.0162	(8cc) =	0.13cc	Hilar	0.17cc =	(10cc)	.0174
.0393	(8cc) =	0.31cc	Middle	0.22cc =	(10cc)	.0215
.0200	(8cc) =	0.16cc	Periph	0.10cc =	(10cc)	.0100
.0261	(8cc) =	0.21cc	Mean	0.16cc =	(10cc)	.0163
Large vessel length						
M.	A.V.	S.A.		M.	A.V.	S.A.
2(2.488)	(8cc)/.81mm^2 =	49m	Hilar	73m = 2(2.964)	(10cc)/.81mm^2	
2(2.354)	(8cc)/.81mm^2 =	46m	Middle	56m = 2(2.278)	(10cc)/.81mm^2	
2(2.556)	(8cc)/.81mm^2 =	50m	Periph	50m = 2(2.006)	(10cc)/.81mm^2	
2(2.465)	(8cc)/.81mm^2 =	49m	Mean	60m = 2(2.450)	(10cc)/.81mm^2	

Table 26.--Control Cat

Left Lobe				Right Lobe		
R.V.	A.V.	Ab.V.		Ab.V.	A.V.	R.V.
Large vessel volume						
.0855(9cc)	=	0.77cc	Hilar	0.79cc	= (11cc)	.0721
.1051(9cc)	=	0.95cc	Middle	1.34cc	= (11cc)	.1314
.0587(9cc)	=	0.53cc	Periph	0.74cc	= (11cc)	.0672
.0831(9cc)	=	0.75cc	Mean	0.99cc	= (11cc)	.0902
Capillary volume						
.2218(9cc)	=	2.00cc	Hilar	2.39cc	= (11cc)	.2174
.2572(9cc)	=	2.27cc	Middle	2.74cc	= (11cc)	.2492
.2719(9cc)	=	2.45cc	Periph	2.88cc	= (11cc)	.2619
.2553(9cc)	=	2.30cc	Mean	2.67cc	= (11cc)	.2428
Airway volume						
.0581(9cc)	=	0.52cc	Hilar	0.72cc	= (11cc)	.0653
.0473(9cc)	=	0.42cc	Middle	0.43cc	= (11cc)	.0385
.0148(9cc)	=	0.14cc	Periph	0.32cc	= (11cc)	.0285
.0401(9cc)	=	0.36cc	Mean	0.48cc	= (11cc)	.0441

M.	A.V.	S.A.		M.	A.V.	S.A.
Large vessel length						
2(3.067)	(9cc)/.81mm^2	= 68m	Hilar 99m	= 2(3.660)	(11cc)/.81mm^2	
2(3.612)	(9cc)/.81mm^2	= 80m	Middle 101m	= 2(3.716)	(11cc)/.81mm^2	
2(4.541)	(9cc)/.81mm^2	=101m	Periph 126m	= 2(4.650)	(11cc)/.81mm^2	
2(3.740)	(9cc)/.81mm^2	= 83m	Mean 109m	= 2(4.008)	(11cc)/.81mm^2	

APPENDIX C

Table 27.--Dog Parasympathetic Trials--Analysis of Covariance Tables

Airway volumes:

Source of variation	Sxx	Sxy	Syy	$Syy - \frac{(Sxy)^2}{Sxx}$	df	MS	F
Among treatments	312	18.48	2.62	1.53	3	0.51	0.35
Within treatments	0	0	10.26	10.26	7	1.47	
Total	312	18.48	12.88				

Vessel length:

Source of variation	Sxx	Sxy	Syy	$Syy - \frac{(Sxy)^2}{Sxx}$	df	MS	F
Among treatments	312	39.44	6.40	1.41	3	.47	0.88
Within treatments	0	0	3.76	3.76	7	.52	
Total	312	39.44	10.16				

Vessel volume:

Source of variation	Sxx	Sxy	Syy	$Syy - \frac{(Sxy)^2}{Sxx}$	df	MS	F
Among treatments	312	53.44	10.09	0.94	3	0.31	0.12
Within treatments	0	0	17.43	17.43	7	2.49	
Total	312	53.44	27.52				

Table 27.--(Cont'd.)

Capillary volume:

Source of variation	Sxx	Sxy	Syy	$Syy - \frac{(Sxy)^2}{Sxx}$	df	MS	F
Among treatments	312	63	18.30	5.58	3	1.86	0.81
Within treatments	0	0	16.04	16.04	7	2.29	
Total	312	63	34.34				

Table 28.--Rabbit Parasympathetic Trials--Analysis of Covariance Tables

Airway volumes:

Source of variation	Sxx	Sxy	Syy	$Syy - \frac{(Sxy)^2}{Sxx}$	df	MS	F
Among treatments	20.25	1.27	.08	.03	3	.010	0.17
Within treatments	0	0	.46	.46	7	.066	
Total	20.25	1.27	.54				

Vessel length:

Source of variation							
Among treatments	20.25	3.63	2.42	1.77	3	.590	24.38*
Within treatments	0	0	.17	.17	7	.024	
Total	20.25	3.63	2.59				

Vessel volumes:

Source of variation							
Among treatments	20.25	1.79	.44	.28	3	.093	1.15
Within treatments	0	0	.57	.57	7	.081	
Total	20.25	1.79	1.01				

Table 28.--(Cont'd.)

Capillary volumes:

Source of variation	Sxx	Sxy	Syy	$Syy - \frac{(Sxy)^2}{Sxx}$	df	MS	F
Among treatments	20.25	7.49	3.93	1.16	3	.387	6.94*
Within treatments	0	0	.39	.39	7	.056	
Total	20.25	7.49	4.32				

Scheffe confidence intervals were established to compare differences between individual means.

u_1 = left lung of control animal u_2 = right lung of control animal

u_3 = left lung of stimulated animal u_4 = right lung of stimulated animal

I am 95% confident that the following hold simultaneously:

Vessel length	Capillary volume
$-1.24 \leq u_1-u_2 \leq -.30$	$-1.67 \leq u_1-u_2 \leq -.25$
$-.03 \leq u_1-u_3 \leq .97$	$-.19 \leq u_1-u_3 \leq -1.23$
$-.50 \leq u_1-u_4 \leq .44$	$-1.24 \leq u_1-u_4 \leq .18$
$.80 \leq u_2-u_3 \leq 1.74$	$.77 \leq u_2-u_3 \leq 2.19$
$.27 \leq u_2-u_4 \leq 1.21$	$-.28 \leq u_2-u_4 \leq 1.14$
$-1.00 \leq u_3-u_4 \leq -.06$	$-1.76 \leq u_3-u_4 \leq -.34$

Table 29.--Cat Parasympathetic Trials--Analysis of Covariance Tables

Airway volumes:

Source of variation	Sxx	Sxy	Syy	$Syy - \frac{(Sxy)^2}{Sxx}$	df	MS	F
Among treatments	15	1.01	.21	.14	3	.047	1.72
Within treatments	0	0	.19	.19	7	.027	
Total	15	1.01	.40				

Vessel length:

Source of variation							
Among treatments	15	5.33	3.29	1.40	3	.467	4.67*
Within treatments	0	0	.70	.70	7	.100	
Total	15	5.33	3.99				

Vessel volume:

Source of variation							
Among treatments	15	1.64	.36	.18	3	.060	0.82
Within treatments	0	0	.51	.51	7	.073	
Total	15	1.64	.87				

Table 29.--(Cont'd.)

Capillary volume:

Source of variation	Sxx	Sxy	Syy	$Syy - \frac{(Sxy)^2}{Sxx}$	df	MS	F
Among treatments	15	5.41	3.13	1.18	3	.393	8.35*
Within treatments	0	0	.33	.33	7	.047	
Total	15	5.41	3.46				

Scheffe' confidence intervals were established to compare differences between individual means.

u_1 = left lung of control animal u_2 = right lung of control animal

u_3 = left lung of stimulated animal u_4 = right lung of stimulated animal

I am 95% confident that the following hold simultaneously:

Vessel length	Capillary volume
$-1.47 \leq u_1-u_2 \leq .37$	$-1.02 \leq u_1-u_2 \leq .28$
$-.11 \leq u_1-u_3 \leq 1.73$	$.34 \leq u_1-u_3 \leq 1.64$
$-.38 \leq u_1-u_4 \leq 1.46$	$-.12 \leq u_1-u_4 \leq 1.18$
$.44 \leq u_2-u_3 \leq 2.28$	$.71 \leq u_2-u_3 \leq 2.01$
$.17 \leq u_2-u_4 \leq 2.01$	$.25 \leq u_2-u_4 \leq 1.55$
$-1.19 \leq u_3-u_4 \leq .65$	$-1.11 \leq u_3-u_4 \leq .19$