THE PULMONARY VASCULAR RESPONSES TO UNILATERAL VAGAL OR SYMPATHETIC STIMULATIONS IN INTACT DOGS, CATS, AND RABBITS

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ABSTRACT

THE PULMONARY VASCULAR RESPONSES TO UNILATERAL VAGAL OR SYMPATHETIC STIMULATIONS IN INTACT DOGS, CATS, AND RABBITS

By

David Otto DeFouw

Regulation of the pulmonary circulation by neurohumoral mechanisms remains questionable. Assessment of remote pulmonary vasomotor control requires examination of pulmonary vascular architecture and innervation. Hyman (1966) reported a well-developed smooth muscle tunica media in pulmonary arteries (30-100μ diameter) while comparably sized veins contained relatively little smooth muscle. Hirsch and Kaiser (1969) described vagal and sympathetic motor nerve fibers forming a terminal reticulum within the smooth muscle vascular media.

Daly and Hebb (1966), employing isolated perfused lung preparations, recorded evidence of direct nervous control of pulmonary vascular perfusion. Pulmonary vascular resistance increased after sympathetic stimulation and decreased after vagal activation. In contrast, Krahl (1968) described vagally-induced arteriolar precapillary sphincter constriction. The in vivo lung observations also

demonstrated pulmonary arteriolar relaxation after sympathetic excitation.

The present investigation attempted to clarify the nervous component of pulmonary microvascular control. Unilateral stimulations of the left sympathetic or parasympathetic nerves in closed-chest dogs, cats, and rabbits were performed. Fluctuations in pulmonary vascular pressures were evaluated in conjunction with changes in cardiomotor and/or bronchomotor activity.

After general anesthesia, catheters, under fluoroscopic control, were positioned in the left ventricle and left pulmonary artery. Adaptation of a respirometer and volumetric-pressure transducer to the tracheal cannula enabled measurement of airway volume and pressure changes. After recording responses to unilateral stimulations, a final stimulation was performed with a concomitant intravenous Pelikan ink injection (volume approximated pulmonary blood volume). To prevent the heart from pumping ink past the pulmonary circuit, carbachol, a parasympathomimetic agent produced immediate cardiac arrest via left intraventricular injection. After pressure controlled formalin instillation into the airways, the lungs were removed and the extent of ink perfusion in stimulated (ipsilateral) and control (contralateral) lungs was recorded. In addition, a second group of non-stimulated animals was similarly infused with Pelikan ink. This enabled an additional

comparison between ink perfusion patterns in the stimulated and non-stimulated preparations. The lungs from both groups were then fixed under constant inflation pressure (25cm H_2O) for 24 hours. Serial, histologic sections (10μ) were taken from hilus to periphery of the middle lobes and mounted on 35 mm leader film. Stereologic evaluations (Weibel, 1963) determined the absolute volumes of the alveolar capillaries, larger pulmonary vessels and the conductive airways.

Correlations of pulmonary airway and vascular pressure changes with the ink perfusion patterns and stereologic determinations defined the nervous contribution toward pulmonary vasomotor control. Unilateral vagal stimulation produced bilateral decreases in pulmonary vascular perfusion in response to the vagally-induced decline in cardiac activity. This indirect vagal control of pulmonary vascular perfusion was most evident in the cat. Unilateral sympathetic stimulations produced ipsilateral decreases in pulmonary vascular compliance. Sympathetically-induced elevations in cardiac output failed to distend the pulmonary vascular tree while alveolar capillary perfusion tended to increase. Thus, the increased pulmonary inflow appeared to utilize the vascular reserve capacity rather than to passively dilate normally perfused channels. Vascular stiffening was more prominent in the rabbit than either the dog or cat.

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INTRODUCTION

Whether neurohumoral regulation of pulmonary blood vessels exists has long been a controversy. The mechanical hypothesis, an alternative to neurohumoral regulation, rests on two propositions. First, the pulmonary circuit, constructed as a short pathway between the right and left ventricles, adjusts automatically to changes in cardiac behavior without intervention of a special control system. Secondly, nervous and/or humoral regulation of the pulmonary vasculature would serve no useful purpose. Since all parts of the lung function similarly, selective changes in resistance producing regional differences in blood flow would be insignificant.

Daly and Hebb (1966a) appreciated the major role of passive or mechanical forces in controlling pulmonary perfusion. While such forces might be sufficient to obscure or overwhelm the active component of control, its physiologic significance should not be negated. The authors regarded nervous pulmonary vascular control as a modulating influence operating on a changing background of flow and pressure. Results from numerous isolated perfused lung preparations, provided these investigators with evidence of an active pulmonary vasomotor control system.

Aviado (1965a) reviewed evidence of autonomic nervous input to the pulmonary circulation. Electrical stimulation of parasympathetic or sympathetic pulmonary nerves and intravascular injections of neurohormones demonstrated direct pulmonary vasomotor activity. Failure to detect pulmonary response was also reported. The author hypothesized that nervous actions of bronchial smooth muscle and cardiac output complicated a simple pulmonary vasoconstriction or dilation.

Most investigations, depicting direct vasomotion as an important determinant of pulmonary blood flow, were obtained by measurements of pressure differences and flow rates across the lung. In addition, Krahl (1968), employing in vivo observations of rabbit lung, described local control of alveolar capillary perfusion on a lobular basis. Subsequent histologic examinations of ink perfused lungs demonstrated parasympathetically induced constriction of arteriolar precapillary sphincters.

With these studies in mind, the present investigations attempted to define pulmonary vascular reactivity to the autonomic nervous system. Using closed-chest dogs, cats, and rabbits, evaluations were based upon physiologic pressure responses to unilateral sympathetic or parasympathetic nerve stimulations.

The lungs, also ink-perfused during the final stimulations, were serially sectioned from hilus to periphery.

Stereologic principles, defined by Weiber (1963), enabled histologic evaluations of the 10μ serial sections. Unilateral nerve stimulations enabled the contralateral lung to serve as control during histologic examinations.

In conjunction with the preceding results, the following questions were asked:

1. Does the autonomic nervous system evoke active pulmonary vasomotion?
2. Do passive pulmonary responses, induced by bronchomotor and/or cardiomotor actions, dominate an active response?
3. If an active reaction predominates, what specific site(s) is responsible for the activity?
4. Are both passive and active pulmonary vascular responses nonexistent after autonomic nervous stimulation?

REVIEW OF LITERATURE

Subgross Pulmonary Anatomy

Variations in pulmonary structure among the mammalian species suggest comparable differences in physiologic behavior. Therefore, specific morphologic characteristics and relationships must be recognized in considering control of pulmonary vascular and airway mechanics.

Miller (1947) described connective tissue septae extending from pleural tissue into the lung parenchyma and marking the boundaries between adjacent secondary lobules. The primary lobule, consisting of a single alveolar duct and its accompanying alveolar sacs and alveoli, defined the basic unit of lung structure. Fifty or more primary lobules comprised a secondary lobule.

Tyler, McLaughlin, and Canada (1961) studied 3mm serial sections of latex-injected lungs from several mammalian species. Three principle subgross patterns were observed.

Type I was found in cow, sheep, and pig. This group displayed well-developed secondary lobules with marked interlobular septae and a thick visceral pleura. The distal airways consisted of terminal bronchioles leading directly into alveolar ducts without intervention of respiratory

bronchioles. The pulmonary vein closely followed the course of the bronchus and pulmonary artery from hilum to periphery. The bronchial artery, also paralleling the airways, terminated in a common capillary network with the pulmonary artery at the terminal bronchioles' distal end. A number of bronchial-pulmonary arterial anastomoses were found.

Type II was found in dog, cat, rabbit, and monkey. Contrary to type I, there was an absence of secondary lobules with haphazard intraparenchymal septae and an extremely thin visceral pleura. The canine visceral pleura, however, was intermediate in thickness relative to types I and II. The type II distal airways included well-developed respiratory bronchioles leading into large alveolar ducts. The bronchovascular tree resembled type I, however, the pulmonary vein maintained an independent course along connective tissue septae to the hilum and no bronchial-pulmonary arterial anastomoses were found.

Type III was identified in horse and man. Incompletely defined secondary lobules and a thick, highly vascular pleura characterized this group. Distal airway design paralleled that of type I. However, occasional, poorly developed respiratory bronchioles intervened between the terminal bronchioles and alveolar ducts. The bronchovascular tree was similar to type II with the exception of numerous proximally located (hilar region) bronchial-pulmonary arterial shunts.

Architecture of Pulmonary Circulation

According to Daly and Hebb (1966b), intrapulmonary arterial bronches accompanied the airways distally to the alveoli in mammalian lung. However, Reid (1968), working with formalin-fixed human lungs, reported a striking difference between branching patterns of the bronchial and arterial trees. While each bronchial branch was accompanied by a pulmonary arterial division, termed a conventional arterial branch, the pulmonary artery also generated additional side branches. These supernumerary arterial branches occurred throughout the length of an artery from hilum to the capillaries. Being most numerous at the periphery, the supernumerary also outnumbered the conventional at all pulmonary levels.

Ferenz (1969) reported a basic histologic structure of pulmonary vessels similar in all mammals including man. Four vessel types were defined:

		diameters
1.	elastic arteries	greater than 500 μ
2.	transitional arteries	100-500 μ
3.	muscular arteries	30-115 μ
4.	endothelial vessels (arterioles)	15-40 μ

A brief description of histologic design clarified this arterial classification. The large elastic arteries possessed numerous circumferentially arranged layers of elastic tissue, interspersed with collagen and sparse smooth muscle. The transitional vessels possessed a muscular wall

containing scattered elastic fibers not arranged in parallel laminae. The muscular arteries consited of a circular layer of smooth muscle bounded by two elastic laminae. The endothelial arterioles were thin-walled precapillary vessels of a single elastic lamina supporting an endothelium.

Hyman (1966) described muscular pulmonary arteries (100-1000μ diameter) accompanying respiratory bronchioles and alveolar ducts in mammalian lung. Nonmuscular arterioles arose at right angles from these arteries and fed the alveolar capillary networks. VonHayek (1960a) reported similar small pulmonary arteries in human lungs. These vessels had an incomplete muscular tunica media with distinct muscular rings surrounding right-angled origins of nonmuscular precapillary branches which abruptly divided into alveolar capillaries. Sobin (1966) failed to identify smooth muscular precapillary sphincters. However, the distributing artery (60-100μ diameter) was defined by the author as partially muscular in dog, cat, and rabbit.

Knisely (1960) and Reeves, Leathers, and Quigley (1965) defined the morphology of the rabbit pulmonary microcirculation. Small (30-150μ) rapidly tapering arteries (termed arterioles by these authors) branched at right angles in contrast to simple dichotomous systemic arteriolar branching. In addition, two types of right-angled branches arose from the arterioles: 1) precapillaries (15-20μ diameter) supplied alveoli contiguous to the arteriole; and

2) precapillary arterioles (up to 100μ diameter) supplied more distant alveoli. The alveolar capillaries (10-12μ diameter) were drained by short right-angled venules which fed into larger pulmonary veins.

Krahl (1964) likened this right-angled branching pattern to the teeth of an elastic comb. Stretching the parent artery (comb's base) simply moved the right-angled branches (comb's teeth) further apart. Upon relaxation, the vessels returned to their original position. Similarly, during the respiratory cycle undue traction was not placed on the arteriolar branches during shortening (expiration) or lengthening (inspiration) of the parent vessel.

VonHayek (1960b) described the histologic design of postcapillary vessels found in human lung. Union of alveolar capillaries formed amuscular 50μ diameter post-capillaries which led into sparsely muscularized pulmonary venules. Postcapillaries approached the venules at right angles and their entrance sites were marked by smooth muscular rings. Draining the venules were larger pulmonary veins which possessed generally thinner and less muscular walls than comparably sized arteries.

The presence of smooth muscle fibers within pulmonary veins of mammalian lung was reported by Hyman (1966). In contrast to the pulmonary arterial vessels, veins and venules less than 1000μ diameter contained relatively little muscle. The adventitia of large pulmonary veins

within one or two cm of the left atrium contained a tube-like extension of cardiac muscle. Hyman also described a second architectural contrast between the pulmonary arterial and venous systems. Whereas arterial branches were intimately associated with respiratory tissue and thus subjected to mechanical effects of breathing, the veins in most species coursed through connective tissue interlobular septa distant from the airways. Thus, respiratory movements would have considerably less mechanical effect on pulmonary venules and veins than on arterial vessels.

Innervention of Pulmonary Circulation

Histologic evidence reviewed by Daly and Hebb (1966c) indicated that efferent fibers belonging to both the parasympathetic and sympathetic divisions may innervate pulmonary blood vessels 30μ diameter or larger in mammalian lung. The authors also reported a predominately ipsilateral distribution of the autonomic nerve fibers. Unilateral pneumonectomy produced chromatolysis in cells of the ipsilateral middle cervical, stellate, and nodose ganglia in the dog. Additional chromatolysis was observed in the contralateral ganglia, however, the relative number of cells affected was minor. In cats, unilateral lung removal had no effect on the contralateral ganglia.

Hebb (1969) studied the distribution of cholinesterases and catecholamines in rabbit, dog, and cat lungs as means of identifying intrapulmonary nerve fibers. The following variations among species were reported:

Parasympathetics:

Rabbit: Cholinergic fibers appeared most evident in large and medium sized arteries. Fibers were distributed perimedially in the largest intrapulmonary arteries, while arteries 500μ or less showed intramedial fibers. The large veins also received perimedially distributed cholinergic fibers. No vessels less than 100μ appeared innervated.

Cat: Cholenergic fiber distribution was similar to the rabbit; however, arterial vessels down to 40μ diameter were innervated.

Dog: Both large pulmonary arteries and veins demonstrated cholinergic fibers running perimedially. Arteries down to 30μ diameter demonstrated cholinergic innervation, however, similarly sized veins lacked a nervous input.

Sympathetics:

Rabbit: Pulmonary arterial branches down to 70μ diameter were innervated. The highest density of fiber distribution appeared in the smaller arteries. Adrenergic fibers appeared in large and medium sized (greater than 500μ) veins.

Cat: The arterial innervation pattern was similar to the rabbits' adrenergic design except that vessels 30-40μ were more intensely innervated. A profuse pulmonary venous innervation was an outstanding feature of the feline lung.

Dog: The adrenergic innervation appeared identical to the cholinergic fiber distribution.

Spencer and Leof (1964) presented histologic evidence of pulmonary vascular innervation in humans. Intrapulmonary nerves accompanying the vascular tree, contained few thick and many thin fibers. In large arteries, fibers terminated

within the adventitia or at the media-adventitial interface. The remainder of the arterial tree, extending distally to the arterioles, was innervated by thin fibers positioned within the tunica media. Perivenous fibers, after supplying the large pulmonary veins, continued distally with a predominately thin fiber distribution. The pulmonary tissue was vitally stained with methylene blue. However, the authors did not differentiate between cholinergic and adrenergic fibers.

The most extensive investigation of mammalian lung innervation was performed by Hirsch and Kaiser (1969). Serial, paraffin-embedded sections routinely stained with Bielschowski, followed by Gomori trichrome were observed from normal lung tissue. Intrapulmonic vagal and sympathetic distributions were also traced by cervical vagal and thoracic sympathetic neurectomines. Histologic changes of the subsequent Wallerian degeneration demonstrated intrinsic nervous tissue designs.

Parasympathetic and sympathetic nerves entered at the ipsilateral hilus and extended in the arterial and airway adventitial tissue to the alveolar ducts. At this point, terminal branches arose and passed into the interalveolar septae. The structural complex of these nerves identified a parasympathetic predominance and their abundance implied a large pulmonary vagal supply. Both motor and sensory fibers were identified. The great number of

myelinated axons associated with clusters of ganglion cells suggested a predominance of vagal (mostly afferent) fibers. Less numerous sympathetic nerves consisted mainly of unmyelinated (motor) axons.

Fascicles of nerve fibers extended from the adventital vagal and sympathetic plexuses to supply both the bronchial and arterial trees. Thick, sensory fibers arose from the periarterial plexuses and terminated as small tight-meshed arborizations or glomerular spindles within the adventitia. Thin, motor (vagal and sympathetic) fibers, which also originated from the main plexuses, formed a terminal reticulum of wide-meshed arborizations within the smooth muscular media. The terminal branches extending into the interalveolar septae, consisted of curved argyrophilic segments composed of a thick, sinuous fiber and one or more thin fibers. Associated with these dense argyrophilic receptors were rich plexuses of tortuous fiber and fibril branches which curved around the alveolar capillaries.

Parasympathetic Mechanisms in Pulmonary Circulation

Original investigation of pulmonary vasomotor response to acetylcholine appeared in the 1930's. Subsequently, a number of investigators, utilizing various methods, have observed parasympathetic activity within the pulmonary vascular tree.

In 1932, VonEuler recorded pulmonary arterial pressure during vagal stimulation in the rabbit. The animal, with opened thorax, was sealed under negative pressure within a wooden box. A pump delivered constant inflow perfusion into the cannulated pulmonary artery. With vagal stimulation or direct injection of acetylcholine, pulmonary arterial pressure was elevated. This vasoconstrictor effect was abolished by atropine.

Gaddum and Holtz (1933) perfused isolated dog and cat lungs in a nearly deflated condition with heparinized autologous blood under constant inflow. In both species small doses of acetylcholine (1-3ug/kg) produced vasodilation followed by constriction with increased dosage (10-20 ug/kg). Initially, at constant inflow, arterial pressure dropped (vasodilation). A secondary rise in pressure (vasoconstriction) followed increased acetylcholine injection. Both responses were prevented by atropine.

Improvements of these isolated perfused lung preparations led to the technique described by Daly, Hebb, and Petrovskaia (1941). To insure viability of the pulmonary nervous input, the bronchial circulation was perfused in conjunction with the pulmonary vasculature. Heparinized blood was perfused at constant inflow into the cannulated pulmonary artery. Blood from the pulmonary veins was returned via a cannula from the left atrium to a venous reservoir. A second pump perfused the bronchial circulation.

The arterial cannula was inserted into the thoracic aorta from which all vessels had been ligated with exception of those supplying the bronchial tree. Blood from the bronchial veins was returned to the reservoir from a cannula inserted into the azygos vein. The entire preparation was placed in an air-tight chamber where the lungs were respired by application of rhythmic negative pressure or the lungs were artifically ventilated with positive pressure. Generally, the pulmonary and bronchial circulations were perfused at constant-volume inflow and changes in arterial inflow pressures were recorded.

Employing the perfused lung preparation, Daly and Hebb (1952) described atropine-sensitive vasodilator fibers within the cervical vagosympathetic trunk in dogs. Stimulation of these fibers decreased pulmonary vascular resistance without affecting ventilation volumes. However, the association of these vasodepressor fibers with atropine-resistant adrenergic pulmonary vasoconstrictor fibers prevented large vasodepressor responses to vagosympathetic trunk stimulation. Dirken and Heemstra (1948) perfused isolated rabbit lungs with constant inflow pressures before and after unilateral vagotomy. Inflow volume through the ipsilateral lung was unchanged after section of the vagus nerve. In addition, unilateral, hypoxia-induced pulmonary vasoconstriction was unaffected by vagotomy.

Suggestion of acetylcholine's pulmonary vasoconstrictor potential was presented by Bohr, Goulet, and Taquini (1961). Helical strips were cut from pulmonary arteries (rabbit and dog) ranging from 200-300μ in diameter. The strips, bathed in Kreb's solution, were mounted on a displacement transducer which recorded phasic changes in tension. Acetylcholine (30-100ug) produced arterial smooth muscular contraction.

According to Aviado (1965b) and Fishman (1961), it was most difficult to demonstrate effects of vagal stimulation or acetylcholine injection on pulmonary blood vessels. Most results demonstrated decreased pulmonary vascular resistance. However, an increased resistance or simply no response had also been revealed. For example, Borst, Berglund, and McGregor (1957) injected acetylcholine into isolated perfused dog lungs. The vasomotor changes were always of small magnitude and varied as dilation or constriction. The constrictor response appeared minimal in absence of increased airway pressure or bronchoconstriction. A passive arterial response was suggested. Failure to record consistant vasodilation was attributed to low basal or "control" tone in the pulmonary arteries. Similarly, Harris (1957) and Fritts, Harris, Clauss, and O'Dell (1958) failed to detect a fall in pulmonary arterial pressure following acetylcholine injection (0.5ug/kg) in normal humans. However, patients with mitral stenosis or other

hypoxic conditions (well-known pulmonary constrictors) responded with convincing pulmonary vasodilation. Pulmonary arterial pressure fell while cardiac output and left atrial pressure (measured as pulmonary wedge pressure) remained unchanged. The authors related pulmonary vascular responsiveness to acetylcholine with initial pulmonary arterial pressure or tone.

Alternate pulmonary vascular responses to acetylcholine have been recorded in isolated, perfused dog lungs. Bell (1961) and Shimomura, Pierson, Krstulovic, and Bell (1962) demonstrated vasoconstriction in wedged pulmonary segments. Perfusion of acetylcholine (0.5-50ug/kg) into a catheter wedged in a small pulmonary artery produced an immediate rise in perfusion pressure prior to change in left atrial pressure or heart rate. Niden, Burrow, and Barclay (1960) reported variable pulmonary arterial pressure responses to acetylcholine. Generally, arterial pressure rose slightly but occasionally a fall or diphasic response appeared. Coupled with these pressure variations was a decrease in systemic arterial oxygen saturation. Respiratory depression with occasional apnea closely paralleled the oxygen content changes. The major effect of acetylcholine appeared to be dimunition of overall ventilation. However, persistance of the drug response with constant ventilation and perfusion indicated either physiologic shunting (local changes in ventilation-perfusion

ratio) or anatomic shunting via arterio-venous anatomoses. Bean, Mayo, O'Donnell, and Gray (1951) observed decreased pulmonary outflow with constant perfusion pressure (25mmHg) following acetylcholine injection. Increased air expulsion from the lungs (bronchoconstriction) with an attendant increased total lung blood volume implied retention of blood within the lungs. Therefore, the reduced outflow was attributed to pulmonary arterial dilation coupled with venous constriction.

Pulmonary vasomotor responses to acetylcholine in unanesthetized dogs and rabbits were also reported. Rudolph and Scarpelli (1964), following initial anesthesia, placed electromagnetic flowmeters around the main pulmonary trunk and left pulmonary artery in dogs. After the cannulations, leads from the catheters were exteriorized in preparation of the animals' recovery from anesthesia. Acetylcholine, infused into the main pulmonary trunk did not change total or left pulmonary arterial flow. However, its injection into the left pulmonary artery produced marked decrease in ipsilateral flow without altering total pulmonary flow. Site of this local vasoconstriction was not predicted because pre and postcapillary changes were not differentiated.

Lehr, Tuller, Fishman, and Fisher (1963) recorded color patterns produced by India ink injections into the pulmonary vasculature of unanesthetized rabbits. Under

preliminary anesthesia, a catheter was introduced into the right ventricle via the external jugular vein. Following recovery from anesthesia, five ml of India ink immediately followed by a five to kn ml solution of sodium pentobarbital and potassium chloride, which produced cardiac arrest, were injected into the cannula. The lungs demonstrated a "patchy" appearance, that is, hilar portions were black while the periphery appeared pink or grayish-pink. Urokon, an angiographic tracer, caused hemorrhage or edema depending on its contact duration with capillary endothelium. The ink-Urokon-blood mixture had traversed more rapidly through pink areas (edematous) indicating an increased resistance to flow through the black (hemorrhagic) regions. This "patchy" lung was not prevented by atropine, vagotomy, or vagal stimulation. The authors hypothesized that right ventricular catheterization initiated a reflex culminating in pulmonary vasoconstriction which affected hilar portions more than the periphery.

Utilizing India ink injections, Krahl (1968) detected a regulatory mechanism which altered pulmonary capillary perfusion on a secondary lobular basis. Under anesthesia, an ink-saline-potassium chloride mixture was injected directly into the right ventricle of the closed-chest rabbit. Lung surface color patterns presented a mosaic of black, grayish-pink, and pink. Subsequent ink injections with simultaneous unilateral vagal stimulation

produced a predominately pink lung ipsilaterally while the contralateral lung displayed the mottled pattern of lobular ink distributions. Histologic sections from adjacent black and pink areas of the normal and vagally stimulated lungs were observed. Muscular arteries, which accompanied the respiratory bronchioles, contained similar quantities of ink in both black and pink regions. Ink was prevented from entering pulmonary capillary beds of pink areas by marked contraction of smooth muscular sphincter mechanisms located at origins of short, right-angled supply vessels of alveolar capillaries. In blackened areas this muscular apparatus was relaxed, permitting unobstructed entry of ink into alveolar capillary networks. Thus, vagally-mediated precapillary arteriolar sphincter contriction was demonstrated in rabbits.

Demonstration of active pulmonary vascular responses to parasympathetic stimuli demands exclusion of passive effects which may simulate or modify direct actions on the vessels. Fishman (1961) linked discordant results of actelycholine injections with difficulty of distinguishing between active and passive vascular responses. Passive responses were attributed to vagally induced cardiomotor and bronchomotor effects.

Daly and Hebb (1966d) reported bronchoconstrictor activity of acetylcholine in most mammalian species. Rodbard (1966) believed pulmonary blood flow was dependent

upon mechanisms extrinsic to the vasculature. Thus, flow was intimately associated with respiratory function via bronchiolar tone. Administration of acetylcholine caused decreased pulmonary blood flow with concomitant bronchoconstriction. The airway contriction increased alveolar pressure thus compressing alveolar capillaries with consequent decrease in vascular conductance. As previously mentioned, Borst, et al. (1957) suggested similar passive vasoconstriction in response to active bronchoconstriction.

Campbell and Haddy (1949) studied effects of vagally mediated bradycardia upon pulmonary vascular pressure in anesthetized dogs. Prompt increase in mean venous pressure accompanied by smaller decreases in mean arterial pressure were observed. With continuous vagal stimulation, pulmonary arterial pressure eventually rose above control levels, while venous pressure remained elevated. Similarly, Johnson, Hamilton, and Katz (1937) recorded lowered systemic and pulmonary arterial pressures following acetylcholine infusion into the cannulated pulmonary trunk. Cardiac slowing and subsequent decreased cardiac output passively induced a decreased pulmonary arterial pressure. Therefore, local pulmonary vasomotor activity was discounted.

Eliakim, Rosenberg, and Braun (1957) accorded a rise in pulmonary arterial pressure after acetylcholine injection (1-500ug/kg) to both bradycardia and bronchoconstriction. Working with anesthetized dogs, pulmonary

arterial pressure failed to rise until systemic arterial pressure had fallen. In addition, aminophylline (potent bronchodilator) appeared to diminish slightly the increased pulmonary arterial pressure.

Sympathetic Mechanisms in Pulmonary Circulation

Numerous isolated perfused lung preparations have established pulmonary vasomotor activity of epinephrine and norepinephrine. Additional evidence has evolved from *in vivo* observations of the pulmonary microcirculation and from interpretations of pulmonary pressure changes observed in intact animal preparations.

Daly, Duke, Hebb, and Weatherall (1948) demonstrated pulmonary vasopressor responses in perfused dog lungs to electrical stimulation of the upper thoraic sympathetic outflow from T_1-T_5, the stellate and middle cervical ganglia and their branches, and the main thoracic sympathetic chain. Nervous stimulation during constant inflow perfusion produced 10-15 percent elevations in pulmonary arterial pressure. More recently, Daly, Ramsay, and Waaler (1970) attributed the adrenergic vasopressor response to pulmonary venoconstriction. A greater response during reverse perfusion of isolated dog lungs suggested more extensive nervous control of the venous bed. However, abnormal direction of blood flow during reverse perfusion, may have initiated

turbulence within the blood stream and thus created greater resistance to flow. Eliakim and Aviado (1961) reported comparable increases in total pulmonary and extrapulmonary venous resistances following stimulation of postganglionic fibers from the stellate ganglion in isolated dog lungs. Since constant pulmonary perfusion was maintained, resistance changes were directly proportional to pressure gradient changes across the isolated vascular segments. Therefore, the pressure gradient between the pulmonary artery and left atrium and that between the pulmonary vein and left atrium were both elevated.

Duke and Stedeford (1960) stimulated efferent fibers from the stellate ganglion in isolated cat lungs and recorded an increased pulmonary vascular resistance. Conversely, stellectomy produced decreased pulmonary resistance. These resistance changes were calculated by subtracting left atrial pressure from pulmonary arterial pressure and dividing by the constant inflow volume.

Ingram, Szidon, Skalak, and Fishman (1968) employed an isolated lobe preparation to investigate effects of stellate ganglion stimulation in dogs. A pulsatile pump perfused the left caudal lobe at constant inflow while the remainder of the pulmonary circulation was perfused by the right ventricle. Venous effluent from the isolated lobe was collected and measured in a blood reservoir maintained at 37°C. A constant volume pump ventilated the lungs. The

ventilatory pump's outflow was submerged under three cm of water to maintain a constant expiratory pressure. To avoid complicating effects of positive pressure inflation or lung volume changes on pulmonary hemodynamics, all measurements were taken while the respiratory pump was stopped in expiration. Mean pulmonary arterial pressure in the isolated lobe remained virtually unchanged during the stimulation. Because stroke volume and rate were held constant by the pump, calculated resistance was unchanged. However, both amplitude and contour of the arterial pressure pulse were altered during the stimulation. This suggested a sympathetically induced change in large pulmonary arterial physical properties. Therefore, to evaluate compliance changes within the isolated lobar precapillary bed, pressure-volume relationships were examined by lobar flow loading. Doubling pump rate produced the flow load with associated increases in lobar blood volume and intravascular pressure. Blood volume increases during flow loading coupled with nerve stimulation were consistently less than during the control state. Therefore, a less distensible vascular bed, rather than calculated pulmonary vascular resistance, presented a more meaningful measure of heightened sympathetic activity. In addition, Szidon and Fishman (1971) reported that effects of hypothalamic (preoptic area) stimulation on the isolated lobar circulation were similar to the stellate ganglion stimulations.

Hauge, Lunde, and Waaler (1967) also recorded stiffening of capacitance vessels after norepinephrine or epinephrine injection into isolated rabbit lungs. Marked reductions in lung weight regardless of changes in pulmonary vascular resistance rapidly followed infusion of both substances. Localization of capacitance vessels within the pulmonary vascular bed reacting to the catecholamines was not determined. However, the lungs' vascular capacity was markedly reduced with a slight elevation in resistance to blood flow. Therefore, the authors suggested the venous compartment was primarily responsible for decreased pulmonary blood volumes. Similarly, Aarseth, Nicolaysen, and Waaler (1971) recorded lung weight reductions following vagosympathetic nerve stimulations in isolated dog lungs. The dependence of weight reduction on pulmonary outflow pressures (regulated by varying left atrial pressure) indicated a predominately venous response. That is, marked weight reduction after nerve stimulation occurred only when left atrial pressure remained above one cm of water. In addition, mean pulmonary arterial pressure remained unchanged after stimulation. Thus, reduced vascular compliance rather than increased resistance typified the response.

McGaff and Leight (1963), using fluoroscopy, catheterized the main pulmonary arterial trunk and the left atrium in dogs. Cardiac output and pulmonary blood volume

were measured via dye dilution by sampling from the femoral artery and left atrium respectively. Following sympathetic ganglionic blockage, cardiac output and pulmonary arterial and left atrial pressures were markedly decreased while pulmonary blood volume was elevated. Autonomic regulation of pulmonary vascular tone was suggested; however, the site of vascular relaxation after ganglionic blockage was not determined.

Bauman and Fletcher (1967) induced pulmonary vasoconstriction with hypoxia as evidenced by increased pulmonary arterial pressure in dogs. Femoral arterial blood was monitored for oxygen saturation and systemic pressure. After sympathetic blockage via segmental epidural anesthesia from T_1 to T_7, systemic arterial oxygen saturation in hypoxic dogs was increased and pulmonary arterial pressure was decreased. Improvement of arterial oxygen saturation was attributed to decreased pulmonary vascular resistance.

In vivo observations of the pulmonary microcirculation with the fused quartz rod illuminator (Knisely, 1936) also depicted adrenergic vasomotor activity. After incising an intercostal space and parietal pleura (visceral pleura remained intact), the ribs were retracted and a marginal portion of the lung, permanently distended by positive pressure with 100 percent oxygen, was transilluminated with light conducted down the quartz rod. The

peripheral pulmonary microcirculation was then observed microscopically. Wearn (1934), using the quartz rod illuminator, also observed the cats' pulmonary vasculature. Spontaneous opening and closing of alveolar capillary beds was noted. The intermittent flow pattern was completely independent of changes in systemic blood pressure. Therefore, arteriolar contractility was hypothesized as the governing factor. Furthermore, arteriolar tone was altered after epinephrine injection. An arteriole, that previous to injection was allowing only single erthyrocyte passage, dilated sufficiently to allow passage of eight or more red blood cells abreast. Irwin and Burrage (1958), also employing the quartz rod transilluminator, observed similar intermittent alveolar capillary flow in rabbits. After epinephrine injection, initial pulmonary arteriolar and venular constriction was rapidly replaced by dilation. Wagner and Filley (1965) combined the quartz rod with insertion of a thoracic window, consisting of teflon stretched across a lucite frame, at the second intercostal space. After epinephrine injection, erthyrocyte velocity through the dogs' pulmonary microcirculation was visually increased. There was, however, no evident change in vessel lumen diameter. The increased flow velocity was attributed to epinephrine's positive inotropic effect in contrast to direct pulmonary vasomotor activity.

Hirschman and Boucek (1963) employed pulmonary angiography in dogs to examine pulmonary vasomotion. A pulmonary catheter, under fluoroscopic control, was advanced into an arterial segment of the caudal lobe. Injection of opacifying dye during early systole enabled maximum visualization of small muscular pulmonary arteries. Angiograms obtained after epinephrine injection (.01mg) depicted obvious alterations in the precapillary bed. Bands or collars of constriction appeared at bifurcation sites of 1-2mm arteries. Beading occurred along arteries of 0.5-1.5mm size and spiralling developed along visible arteries down to 0.1mm. Patel and Burton (1957) reported gnarling and bands of constriction within pulmonary arterial vessels (25-100μ diameter) after norepinephrine (2-8mg) injection in rabbits. After thoracotomy and initiation of positive pressure respiration, a needle was inserted into the main pulmonary trunk for vinyl acetate injection at 100mmHg perfusion pressure. After hardening, plastic casts of the pulmonary arterial tree depicted the pressor response to norepinephrine.

Further confirmation of cathecholamines' pulmonary vasoconstrictor potential was presented by Somlyo and Somlyo (1964). Main pulmonary arteries, removed from anesthetized dogs, were mounted on displacement transducers resting in chamber bathes containing 37°C Kreb's solution. Both epinephrine (2.5ug) and norepinephrine (5ug), when

added to the baths, induced arterial smooth muscular contraction. Bevan and Nedergaard (1968) observed contractile responses of isolated rabbit pulmonary arterial segments with attached sympathetic nerve supply. Stimulations were applied to the nerve and contractile responses recorded with an isometric strain gauge and pen recorder. Consistent vasoconstriction was displayed. Conversely, Bohr, et al. (1961) observing isolated pulmonary arterial vessels with 200-300μ diameter from dogs and rabbits, found no response to epinephrine or norepinephrine.

Pulmonary vasomotor effects of intravascularly injected norepinephrine and epinephrine have also been examined in isolated, blood-perfused lung preparations. Daly, Foggie, and Hebb (1940) injected epinephrine (10-25ug) into the bronchial arterial system of isolated dog lungs. It was assumed the drug would reach the pulmonary vessels only through confluence of the bronchial and pulmonary capillary beds. Therefore, during normal perfusion epinephrine, injected into the bronchial artery, would reach only the pulmonary capillaries, venules, and veins. During reverse perfusion, only the capillaries, arterioles, and arteries would be effected. An apparent constriction of post and precapillary vascular beds was recorded after epinephrine infusion during normal and reverse perfusion respectively.

Gilbert, Hinshaw, Kuida, and Visscher (1958) reported increased pulmonary venous pressure (venoconstriction) after epinephrine infusion in isolated dog lungs. Rose, Kot, Cohn, Fries, and Eckert (1961) demonstrated direct pulmonary vasoconstriction with a rise in pulmonary arterial pressure and decrease in pulmonary venous outflow after norepinephrine infusion (10ug/kg) into isolated dog lungs. Takaski and Ahlquist (1963) recorded identical results with epinephrine injection (10ug/kg) into isolated, perfused lobes of dog lungs. Patel, Mallos, and deFreitas (1961) reported a 27 percent increase in pulmonary vascular resistance after norpinephrine injection (0.5-4.5ug/kg) into isolated dog lungs. Passive bronchomotor effects were eliminated by momentarily halting artifical ventilation at three cmH_2O pressure during data collection.

Fenyvesi and Kallay (1966) intravenously administered physiologic doses of norpinephrine (0.20-0.47ug/kg) into the pulmonary circulation of intact dogs. The authors concluded that the sympathetic nervous system played a minor role in direct regulation of the pulmonary vasculature. Feely, Lee, and Milor (1963) also observed the actions of norepinephrine and epinephrine in intact dog lungs. Two effects were recorded: 1) increased contraction of pulmonary vessels thus increasing vasomotor tone (measured as an increased pulmonary vascular resistance) and 2) increased transmural pressure, resulting from

actions on the heart, which tended to distend the pulmonary vessels. Therefore, the end result depended on relative magnitude of each effect. Generally, vascular distention predominated and thus, pulmonary volume rose and resistance fell.

Witham and Fleming (1951) catheterized the pulmonary artery of lightly anesthetized human subjects for recording pressure responses to epinephrine injections. Pulmonary arterial pressure increased within eight to ten seconds after the injections, while pulse rate and cardiac output increased immediately. Therefore, passive pulmonary arterial response was defined. Fowler (1951) reported increased pulmonary arterial pressure in humans after norepinephrine injection. However, it was attributed to increased cardiac output rather than pulmonary arterial constriction. Bousvaros (1962) observed a rise in pulmonary vascular resistance, calculated from measurements of cardiac output and pressure gradient between the main artery and tip of a wedged catheter, after norepinephrine (20-40ug) injections in humans. The author noted, however, increased resistance was soon obscured by passive pulmonary vascular dilation arising from increased left atrial pressure. Dollery and Glazier (1966) recorded similar increases in pulmonary vascular resistance after norepinephrine (20-40ug) infusion, however, secondary passive responses were not observed.

Summary

Neurohumoral activity within the pulmonary circulation has been viewed with a variety of experimental methods. Direct autonomic nerve stimulations and intravascular neurohormone injections in isolated perfused lungs and intact animal preparations have been reported. After reviewing the results from these investigations, an obvious controversy concerning nervous control of pulmonary perfusion was established. Evidence of pulmonary vasodilation after acetylcholine or norepinephrine infusions has been presented. However, equal support has evolved suggesting that these humoral agents elicit a primary pulmonary vasoconstrictor response. Thus, after viewing pulmonary vascular function and its regulation by a nervous input, further assessment of structure-function relationships was required. The present study, coupling physiologic measurements and morphometric evaluations, provides an essential step in further investigation of this problem.

MATERIALS AND METHODS

Pulmonary vascular responses to unilateral stimulation of left vagal or sympathetic nerves were observed in closed-chest dogs, cats, and rabbits. The contralateral lung served as control and afforded immediate comparison to the experimental side.

Dogs

Ten mongrel dogs weighing approximately 6.75-9.0kg, were studied. Five sympathetic and five parasympathetic evaluations were made. Specific technical differences are presented at appropriate places below.

The animals were anesthetized with sodium pentobarbital (32mg/kg) injected intravenously (cephalic vein) and secured in the supine portion. The skin and superficial fascia were incised and the right external jugular vein was exposed. After removing the surrounding fascia, a loop of umbilical tape was loosely placed around the vessel. A second loop of surgical suture, positioned three to four cm cephalad to the umbilical tape, was tightly secured to occlude venous blood flow. A blunt probe was placed immediately behind the umbilical tape and elevated slightly to position the vein for cannulation. An incision, extending

through three-quarters of the vessel's circumference, was made. Fine forceps were applied on the incision's edge to facilitate entrance of the catheter. A 50cm, 6F radio-opaque Lehman-Ventriculographic cardiac catheter (U.S. Catheter Co., New York) was inserted into the vessel and secured by gently tightening the umbilical tape. The catheter remained undisturbed until later positioning in the left pulmonary artery. The catheter's free end was connected to a P23AC Statham strain gauge which recorded blood pressures on a Grass ink writing oscillograph (5D polygraph). To insure catheter patency, periodic flushing with heparized saline (64mg/l) was maintained at approximate two minute intervals throughout the procedure. Zero reference level for all pressures was five cm above the surgical table. This approximated the midpoint of the right atrium.

The sternohyoid and sternothyroid muscles were separated at the midline to expose the trachea. An umbilical tape loop served to isolate and to prepare the trachea for later cannulation. Lateral retraction of the right sternohyoid and sternothyoid muscles exposed the right common carotid artery. Following identical cannulation procedures employed for the vien, the artery was catheterized with a 50cm, 5F, radio-opaque Lehman-Ventriculographic cardiac catheter and left undisturbed until later positioning in the left ventricle.

After preliminary cannulation procedures, the animals were placed in the left lateral recumbent position. Fluoroscopy was essential in controlling advancement and location of the catheters. The intravenous catheter was carefully guided into the right ventricle. Its flexible design permitted a 180° bend necessary to reach the pulmonic valve. Slight force advanced the catheter through the valve into the left pulmonary artery. The intra-arterial catheter bent slightly at the aortic valve and then snapped into the left ventricle.

Following catheter placement and animal replacement in the supine position, trachetomy enabled intratracheal insertion of a five-sixteenth inch diameter glass cannula. It was secured within the tracheal lumen by tightening the previously placed umbilical tape. The cannula's open end was adapted to a glass T-tube. One outlet was connected to a Grass PT5 volumetric-pressure transducer which recorded intratracheal pressures during respiratory movements. The other outlet was connected to a Wright Respirometer (Harris Calorific Co., Ohio) which directly measured tidal volumes during expiration. A one-way valve enabled continuous recording during expiration without measuring accompanying inspiratory volumes. After securing the tracheal cannula and its attachments, subcutaneous electrodes (20 gauge needles) were applied to each forelimb and hindlimb. From these limb leads, standard EKG's were recorded on the Grass

oscillograph. Generally, lead II presented the best measuring source of cardiac activity.

Prior to isolation of the vagosympathetic trunk and subsequent nerve stimulation, three, one minute control periods were recorded for the following measurements:

left pulmonary arterial pressure
left ventricular pressure
intratracheal pressure
minute volume and frequency of respiration

After EKG control data had been collected, the left vagosympathetic trunk was isolated and severed mid-cervically. Electrical stimulations of either the sympathetic or parasympathetic component during one minute experimental periods were subsequently performed.

Parasympathetic Stimulations

Specific parasympathetic stimulations required pharmacologic sympathetic blockage. Phenoxybenzamine (Smith, Kline, and French), an alpha-receptor blocker, and propranolol (Ayerst), a beta-receptor blocker, inhibited sympathetic response to vagosympathetic stimulation. Doses of 2mg/kg and 1mg/kg were given respectively. Each blocking agent, in solution with 40cc saline, was administered via intravenous drip through the cephalic vein. The I.V. perfusion unit, inserted immediately following anesthesia, delivered each agent over a 45-50 minute duration.

The severed left vagosympathetic trunk's distal stump was positioned around a silver wire stimulating electrode. The electrode was connected to a Grass S-8 stimulator which generated monophasic square wave stimuli with parameters of 2-3 volts/2.5 msec/10 cps. Pressure and volume measurements during three, one minute stimulations were recorded. Bradycardia, demonstrated on the EKG tracings, verified the parasympathetic responses.

After three stimulations, the glass T-tube was detached from the tracheal cannula and thus eliminated the respirometer and volumetric-pressure transducer. The pulmonary arterial catheter was removed from the external jugular vein and replaced with a short segment of PE 190 polyethylene tubing. The tubing's free end was attached to a syringe containing biologic Pelikan ink (No. C11-1431A J. Herschel Co., New York). To insure physiologic pH, the ink was buffered with 1.0 N HCl to pH of 7.4. The ink's volume approximated the animal's pulmonary blood volume which represented one-tenth of the total blood volume. The animal's total blood volume (cc) was calculated as eight percent of body weight measured in grams (Schmer, 1967).

The stimulating electrode was repositioned and activated. After 15 seconds, the ink syringe was rapidly emptied (approximately 10 seconds) during continuous stimulation. After three-quarters of the ink infusion, one cc of 50 percent carbachol solution (Mereck, Sharpe, and Dohme)

was injected directly into the left ventricle via the intraventricular catheter. Carbachol, a parasympathomimetic drug, caused immediate cardiac arrest upon entering the coronary circulation. This arrest was presumably due to extensive hyperpolarization of cardiac muscle fibers associated with selective permeability increase to potassium (Koelle, 1970a). The prompt cessation of cardiac output prevented the heart from pumping ink past the pulmonary circuit. Thus, ink perfusion patterns within the pulmonary vasculature, coupled with changes in recorded intrapulmonary pressures demonstrated parasympathetic effects on the pulmonary vasculature.

Immediately following ink injection, a 25cm segment of rubber tubing (five-sixteenth inch external diameter) was connected to the tracheal cannula's free end. The tubing led to the sidearm of a 500ml pyrex aspirator bottle containing approximately 250cc of 20 percent buffered formalin. The bottle, elevated 25cm above the trachea, established lung fixation at 25cm H_2O pressure which approximated the physiologically inflated state (Heard, 1962).

After a 30-60 second period of formalin instillation into the airways, the lungs were carefully removed from the thorax. First, the abdominal cavity was incised. The sternum's xiphoid process was clamped with a hemostat and slightly elevated to allow for an incision through the

ventral thoracic wall. The costochondral junction of each rib was severed and the lungs exposed by retraction of the cut edges. The visceral contents of the thoracic cavity were then removed by careful dissection.

Sympathetic Stimulations

Evoking sympathetic activity from vagosympathetic nerve stimulations necessitated parasympathetic blockage. Therefore, atropine (National Biochemical Corp., Cleveland, Ohio) was delivered intravenously (2mg/kg) directly into the cannulated external jugular vein.

To activate pulmonary sympathetic nerves, the left middle cervical ganglion was electrically stimulated during one minute periods and fluctuations in pulmonary pressures and volumes were assessed relative to control valves. The stimulation parameters, intravascular catheterizations, Pelikan ink infusions, and lung fixation were identical to those in the parasympathetic studies.

Lung Photography and Gross Analysis

After lung removal, the ink's distribution as displayed by lung surface color patterns, was evaluated. Each lobe was assessed according to percent black (ink perfused) versus percent pink (non-ink perfused). Duplicate blind examinations were conducted on each specimen to test

consistency of this subjective judgement. The lungs were photographed after color evaluations for permanent records of surface perfusion patterns. A Nikon F. camera with a 55mm automicro Nikkor lens was used for all photography. High speed Ektachrome B film (Kodak) was used for color photographs and Panatomic-X film for black and white photography.

Microscopic Analysis

Gross lung evaluations depicted only ink distribution within the peripheral pulmonary circulation. To augment evaluations of the lungs' surface color patterns, histologic serial sections were prepared from hilus to periphery of experimental and control lungs.

After photography, the lungs underwent 24 hour fixation as described by Weiss and Tweeddale (1966). The lungs were placed in a plastic pan containing approximately six liters of 20 percent buffered formalin (Fig. 1). The tracheal connula's free end was attached to polyethylene outflow tubing (five-sixteenth inch diameter) extending from a 2000ml pyrex aspirator bottle. The bottle was supported by a ringstand at 30cm above the lungs. A small aquarium pump (Little Giant Pump Co., Oklahoma City, Okla.), placed within the formalin-filled pan, continuously filled the aspirator bottle. A double hose system was employed by threading a 40cm segment of polyetheylene tubing

(five-sixteenth inch diameter) through a penrose tubing drain. The plastic tubing, attached to the outflow port of the pump, rose to and entered the top of the bottle. The surrounding penrose drain, used as an overflow system, automatically regulated pressure at 30cm H_2O. It was securely attached to the neck of the aspirator bottle and was allowed to fall unobstructively back into the formalin-filled pan. For safety purposes, the inflation-fixation procedure was performed within a well-ventilated hood system.

After 24 hour inflation-fixation, the lungs were removed from the pump and stored in formalin for at least three days. Following proper fixation, the right and left middle lobes were excised at the hilus. The tertiary bronchus and artery were located and centered within the hilar surface of the lobar boundaries. With these structures centered, the lobes were trimmed along their borders into approximate two cm squares.

In preparation for paraffin impregnation, the lobar tissue was dehydrated with ethyl alcohol and cleared with toluene:

1. 70% alcohol----------overnight
2. 80% alcohol----------one hour under 375mmHg vacuum
3. 95% alcohol----------two, one hour changes under vacuum
4. 100% alcohol----------three, one hour changes under vacuum
5. toluene----------two, one hour changes under vacuum

6. 50% toluene, 50% paraffin--one hour under vacuum
7. paraffin----------three, one hour changes under vacuum
8. embedded with paraffin and kept overnight at 0°C.

The paraffin-embedded lobar segments were positioned on a Spencer rotary microtome for cutting. Serial sectioning at 10μ thickness produced continuous paraffin ribbons. A paper trough extending from the microtome blade, supported the paraffin strips (Fig. 2). After collecting 25-30 sections, the ribbon was severed several sections from the blade and removed to an adjacent table for subsequent mounting procedures. The serial sectioning was repeated across the lobes from hilus to periphery.

To mount the tissue sections, a film transport device, modified after Wilson and Pickett (1970), was employed (Fig. 3). The plexiglass unit supported a kinderman guide and developing reel (Ehrenreich Photo-optical Industries Inc., Garden City, New York). The transport system was lowered into a seven and one-half inch water bath maintained at 45-50°C. Five feet segments of 35mm P40B Cronar motion picture leader film (E.T. duPont de Nemours and Co., Wilmington, Delaware) were guided under the roller of the plexiglass transporter,through the Kinderman film guide, and secured in the developing reel. The film was continually coated with albumin-glycerol before entering the water bath to insure tissue adherence.

Figure 1
Lung inflation-fixation apparatus. The aspirator bottle was continuously pump-filled with formalin. Thus, the lung airways were steadily perfused with the fixative.

Figure 2
Serial sections of the ink-perfused middle lobar segments. The continuous paraffin ribbons are displayed.

Figure 3
The plexiglass film transport device used to mount the serial histologic sections on plastic leader film.

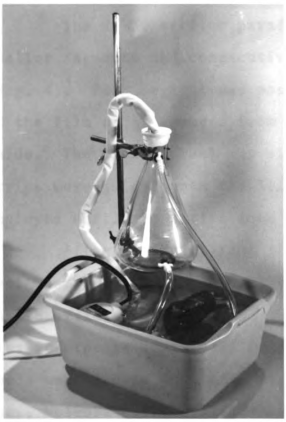


Figure 1

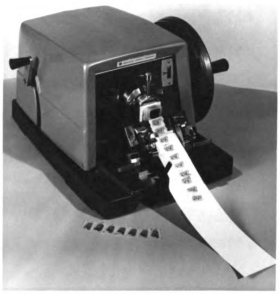


Figure 2

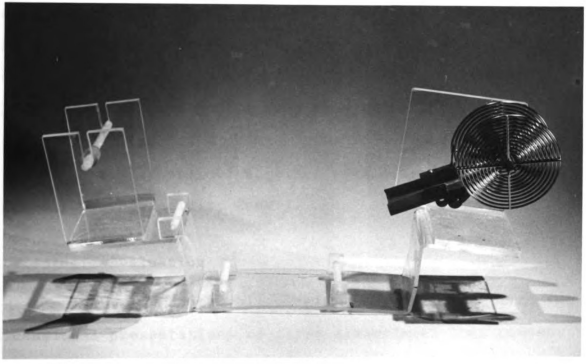


Figure 3

The 25-30 section paraffin ribbons were divided into smaller segments and consecutively placed in the water bath (Fig. 4). Each segment was positioned to join the center of the film as it emerged from the water onto the Kinderman guide. The pick-up reel was slowly turned as the paraffin strips were guided onto the film. Consecutive reels were employed until the entire lobe had been mounted.

After drying at room temperature for 24 hours, each reel was routinely stained with hematoxylin and eosin. To enable efficient staining of the reels, ordinary round plastic storage containers were used (Fig. 5). Next, the reels were dipped in liquid plastic (Lab-line Instruments, Inc., Melrose Park, Illinois) for two minutes. After allowing excess plastic to drain, the reels were placed for 30-60 seconds in staining dishes containing 20cc xylene which removed plastic from the films' edge and facilitated subsequent stereologic evaluations. Prior to evaluation, the film strips were hung freely from the laboratory ceiling and dried for 24 hours. The film segments were then sequentially spliced together with liquid film cement (Craig Inc., Plainville, Conn.).

Stereologic Evaluation

The film-mounted histologic sections represented two dimensional presentations of three-dimensional lung components. Physiologic evaluations of these preparations

necessitated application of sterologic principles (Elias, Henning, and Schwartz, 1971).

First, to insure film strip stabilization upon the microscope stage, a film holding device (Fig. 6) was modified after Wilson and Pickett (1970). Two strips of etched one-eighth inch plexiglass, glued upon a one-eighth inch glass base, served to guide the film beneath the microscope objectives. The device was taped to the microscope stage and thereby followed all horizontal and vertical movements of the stage.

Next, a stereologic test system based upon an eyepiece grid, which presented test points within a test area (Weibel, Kistler, and Scherle, 1966) was constructed. The guide lines and points were etched upon a rounded one-sixteenth inch plexiglass plate with a three and one-half inch diameter. The etched grooves were filled with black wax and the excess wiped off (Fig. 7). The square test area contained 21 lines of constant length, Z (10mm), arranged on seven equidistant and parallel rows, whereby distance between the 42 end-points was Z in every direction.

The circular viewing plate was taped on the screen of a three and one-half inch Nikon projection head (cat. no. 76865). A Nikon universal eyepiece adapter (cat. no. 77111) joined the projection head with a vertical monocular photo tube (cat. no. 77745) which contained a high eyepoint

Figure 4

The mounting device, containing the plastic leader film, is shown lowered into the water bath. Note the short segment of paraffin ribbon positioned on the water surface to join the emerging film strip.

Figure 5

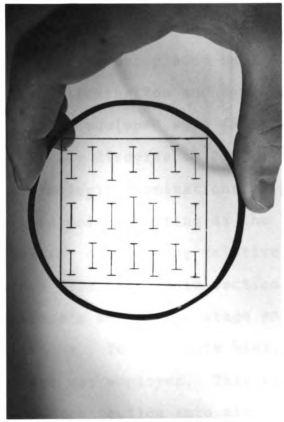
Plastic dishes used in staining the mounted histologic sections. Consecutive reels were used to stain the entire lober segments.

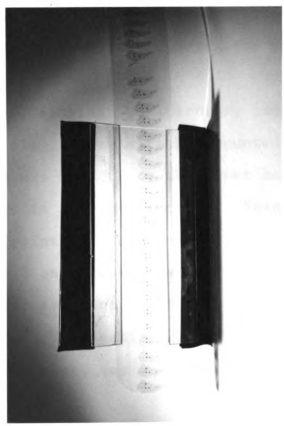
Figure 6

The plexiglass film holding device helped to insure rapid and efficient microscopic evaluation of the serial sections.

Figure 7

The stereologic test system consisted of a square test area and 42 test points.





compensating widefield 10x eyepiece (cat. no. 77857) and ocular micrometer. The photo tube was placed on a Nikon S-KE microscope with Koehler illumination and moveable stage. The microscope was also equipped with 4x, 10x, and 40x objectives (Fig. 8). Easily discernable counts were obtained for subsequent stereologic examinations (Fig. 9).

Weibel (1963) defined point counting as the simplest stereologic method for determining relative volumes of tissue components from histologic sections. Displacement of the microscope's mechanical stage enabled sampling of individual sections. To eliminate bias, a systematic sampling procedure was employed. This was accomplished by subdividing each section into nine units, numbered as in Fig. 10. Each unit was further divided into equal upper and lower halves. Such segmentation avoided overlapping of fields evaluated during the point counting procedures.

Pulmonary airways, ranging from tertiary bronchi through terminal bronchioles, were initially counted. The first peripheral section was moved into the upper half of unit area one and focused in the 4x objective. When any portion of a single end-point (42 total end-points) fell partially or wholly within an airway lumen, a "hit" was recorded. Next, pulmonary arteries, arterioles, veins, and venules were recorded. The section, remaining within the upper half of unit area one, was refocused in the 10x

Figure 8

Complete microscopic system employed in the stereologic analysis of the serial histologic sections.

Figure 10

Arbitrary division was made of each histologic section into nine unit areas.

Figure 9

Close-up view of the projection head and stereologic test system. Note the test point "hits" within the lumen of the centrally positioned airway.

Figure 8

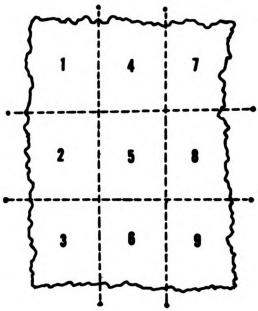


Figure 10

Figure 9

objective and luminal "hits" were counted. In addition, the length of these pulmonary vessels was recorded. The screen was scanned and any vessel lying within the test area was included. Vessels intersected by the left and upper edges of the squared test area were counted. Those intersected by the right and lower edges were not counted. Finally, the relative volume of ink-perfused capillaries was recorded. The section was refocused in the 40x objective and the number of ink-filled capillary "hits" was counted. The same section was manuvered to the upper halves of unit areas two and three and subsequent counts recorded.

After recording counts from the first peripheral section, the film strip was advanced six sections and the counting process repeated. The number of "hits" from the upper halves of unit areas four, five, and six were recorded. After advancing another six sections, the upper halves of unit areas seven, eight, and nine were counted. Sampling every sixth section was continued by positioning within the lower half of unit area one and sequentially evaluating the lower halves of unit areas two and three. After advancing another six sections, the lower halves of unit areas four, five, and six were evaluated. The lower halves of unit areas seven, eight, and nine were finally recorded on the next sixth section. After another six section advancement, the repetitive evaluations were

continued by returning to the upper half of unit area one. This sampling of every sixth section with rotating unit areas continued until stimulated and control lobes had been counted.

The point counting procedure readily determined relative volumes of the organ's tissue components. Relative volumes were defined as number of test points falling on the respective components (airways, vessels, and capillaries). For example, a test system, containing a total of P_T test points, yielded P_K test points counted as falling within component K. The quotient of P_K and P_T defined the relative volume V of that component within the specimen: $V_{vk} = P_{K/P_T}$. Therefore, the volume fraction of a component, contained in a unit volume of an organ, equalled the number of test points falling on profiles of that component divided by the total number of test points.

Thus, the volume fractions of pulmonary airways, blood and/or ink perfused pulmonary arteries, arterioles, veins, and venules and ink perfused alveolar capillaries were determined. Respective volume fractions were evaluated for both experimental and control lobes. In addition, the length of all pre- and postcapillary vessels was evaluated. Counts of vessel profiles were made within each test area observed. Vessel length (L) was defined as two times the average profile counts (P) divided by the

area of the test square $L = \frac{2P}{A}$. The length dimensions were recorded as cm/cm^3.

Further evaluation of the volume fractions required stereologic examinations of ink perfused, non-stimulated (control) animals. Following previously described surgical preparation, biologic Pelikan ink (volume approximated pulmonary blood volume) was delivered into the external jugular vein. After carbachol-induced cardiac arrest and lung formalin-fixation, the lungs' surface color patterns were assessed. After serial sectioning the left and right middle lobes from hilus to periphery, the volume fractions of vessels, capillaries, and airways were recorded. Pre- and postcapillary vessel lengths were also determined.

Final assessment of the control and stimulated preparations demanded conversion of the volume fractions to absolute values. Therefore, total middle lobe volumes were then measured by water displacement. The middle lobes of the remaining control and stimulated lungs (Tenney and Remmers, 1963, reported equal lung volumes in animals of similar weights) were excised at the hilus and individually placed in a 500ml volumetric flask. The flask was sealed with a two-hole rubber stopper. One inlet contained a one milliteter pipette and the other a hollow glass rod. Polyethylene tubing connected the rod to a syringe containing 20 percent formalin. The flask was initially filled with formalin until the fluid reached the 0.5 mark on the

pipette. The procedure was then repeated after placing each middle lobe into the flask. Differences between the initial formalin volume and that required with each middle lobe represented the lobar volumes. Mean volumes of the left and right middle lobes from the stimulated and control groups were then determined. Linking the respective volume and length fractions to the lobar volumes yielded absolute volumes and lengths.

Cats

Ten cats, weighing approximately 3.6-4.0kg were studied. Six parasympathetic and four sympathetic evaluations were made. Surgical procedures employed and pressure and volume measurements recorded were similar to those previously described. Therefore, only technical differences are presented below to elucidate specific procedures used.

Initial sodium pentobarbital anesthesis (32mg/kg) was administered intraparitoneally rather than intravenously. However, maintenance of proper anesthetic dosage often required secondary injection via 26 gauge needle within the cephalic vein. The right external jugular vein was catheterized with a 50cmm 4F radio-opaque Lehman-ventriculo-graphic cardiac catheter. Smaller vessel diameters relative to the dog and potential vasospasm (peculiar to cats during catheterizations)

necessitated use of the smaller catheter. Left ventricular cannulation also required the smaller 4F cardiac catheter. To maintain small catheter patency the animals were heparinized immediately following insertion of the intravenous catheter. The heparin solution (2.5-4.0mg/kg) was directly infused into the venous catheter. In addition, during sympathetic stimulations, atropine (2mg/kg) was delivered intravenously to insure pure sympathetic responses and to eliminate excessive salivation (frequent response to sodium pentobarbital).

Tracheal cannulation was performed with a three-sixteenth inch diameter polyethylene catheter. Its free end was adapted to a glass T-tube which presented, in series, the volumetric-pressure transducer and Wright respirometer.

Parasympathetic Stimulations

As with dogs, the cervical portion of the feline vagus nerve was bound with the sympathetic chain within a common connective tissue sheath. Contrary to the dog, it was feasible to grossly separate the two nerve trunks allowing stimulation of one component independent of the other.

Using fine scissors, the common nerve trunk sheath was incised and the fascial connection between the nerves was carefully severed. Vagus nerve identification was

readily made for its diameter always exceeded that of the sympathetic chain. Final verification was determined by the bradycardia induced by subsequent nerve stimulation. After isolating a five to six cm segment, the left vagus nerve was severed. The distal stump was positioned around the electrode for subsequent electrical stimulations. During three, one minute left vagal stimulations (2-10v/ 2.5-5msec/10-20cps), pressure and volume measurements were recorded. Subsequent Pelikan ink infusion and lung fixation paralleled previously defined methods.

Sympathetic Stimulations

According to Daly and Hebb (1972), mammalian sympathetic outflow originated from T_1-T_5 ganglia, the stellate ganglion, and the middle cervical ganglion. Duke and Stedeford (1960) described pulmonary sympathetic fibers after stellate ganglion stimulation in cats.

Thus, after grossly isolating the left cervical sympathetic trunk, additional isolation of either the left middle cervical ganglion (sometimes termed posterior cervical ganglion) or stellate ganglion was necessary. Since the former proved inconsistent in size and location, stellate ganglion stimulation was performed. Lying immediately caudal to the first rib, ganglion stimulation in closed-chest cats demanded additional dissection.

Initially, the sternohyoid and sternothyroid muscles had been midcervically separated along the midline for tracheal and intravascular cannulations. The separation was extended to the sternal manubrium. Surgical retractors, applied to the muscles' medial surfaces, enabled lateral retraction of the cervical muscle mass. In addition, two six and one-half inch straight hemostats, interspaced slightly, were secured along the cranial third of the origins of the left pectoantibrachialis and pectoralis major muscles. After muscular incision between the hemostats, the retractor was positioned to include the severed thoracic muscles. Brief electrical stimulation of the isolated left sympathetic nerve trunk always produced dilation of the left pupil. Thus, sympathetic nerve fibers were conclusively identified. Following midcervical sectioning, the left sympathetic chain was traced caudally to its union with the stellate ganglion. The ganglion, lying dorsal to the subclavian artery, was readily identified and subsequent tachycardia following electrical stimulation (2-8v/2.5msec/18-30cps) verified sympathetic motor activation.

Gross and Microscopic Evaluations

Surface color patterns, determined by ink perfusion into the pulmonary circulation, were subjectively assessed

as previously described. Serial, histologic sectioning from hilus to periphery again enabled stereologic evaluations.

Rabbits

Eight, white, New Zealand rabbits, weighing approximately 3.0-3.5kg were studied. Five parasympathetic and three sympathetic evaluations were made. Specific variations from previously defined surgical procedures are discussed below.

Sodium pentobarbital (32mg/kg) was intravenously delivered via the marginal ear vein. Contrary to the cat, the external jugular vein was catheterized with a 50cm, 5F Lehman-ventriculo-graphic cardiac catheter. Absence of inherent vasospasm enabled use of the slightly larger catheter. The left ventricle was again catheterized with the 4F cardiac catheter, which again required animal heparinization (2.5-4.0mg/kg). Atropine (2mg/kg) was also administered intravenously during sympathetic stimulations.

Parasympathetic Stimulations

In rabbits, the cervical vagus nerve was independent of the sympathetic chain, lying immediately lateral to the carotid artery. After mid-cervical transsection, the left vagal distal stump was electrically stimulated (2v/2.5msec/30cps). Pelikan ink infusion and lung fixation followed the pressure and volume measurements.

Sympathetic Stimulations

Stimulation of pulmonary sympathetic fibers required activation of the inferior cervical ganglion (rabbits lack stellate ganglia). The left ganglion, positioned immediately caudal to the first rib and dorsal to the subslavian artery, was reached by employing the previously described dissection techniques. Again, subsequent tachycardia and increased left ventricular systolic pressure after electrical stimulation (5v/2.5msec/18cps) verified sympathetic activity.

Gross and Microscopic Evaluations

Surface color patterns were subjectively assessed and stereologic evaluations were performed as previously described.

RESULTS AND DISCUSSION

Fluctuations in pulmonary arterial pressures initially depicted the pulmonary vascular response to unilateral sympathetic or vagal stimulations. Active versus passive vascular reactivity was assessed by concomitant measurements of left ventricular pressure and the minute ventilation and tracheal pressure responses to the stimulations. That is, the sources of passive pulmonary vascular activity, namely cardiomotor and bronchomotor tone, were monitored. Secondary, intravascular injections of biologic Pelikan ink served to isolate the site(s) within the pulmonary circulation responsible for any changes in pulmonary vascular pressures. Observations of the gross lungs established surface ink perfusion patterns. Stereologic evaluations made from the serial-sectioned middle lobes enabled final correlation between the ink's distributions and pulmonary vascular pressures in accordance with the nerve stimulations. For clarification, presentations of these results will be presented according to species.

Dog

Table 1 presents average values for the pressure and volume parameters in the control, parasympathetic, and

Table 1. Effects of autonomic nerve stimulations on pressure and volume parameters in the dog.

	Control	LVS	Control	LMCGS
PAP (mmHg)	25/12 (20)*	22/10 (16)$^{++}$	25/13 (16)*	25/14 (16)
LVP (mmHg)	119/9 (64)	94/9 (52)$^{++}$	126/11 (69)	141/14 (78)$^{+}$
HR (beats/min)	85	53^{++}	143	172^{+}
TP (cmH_2O)	12	10	13	16^{+}
$\dot{V}_E$ (l/min)	2.00	2.10	4.10	4.40
f (breaths/min)	11	13	36	36
V_T (ml/breath)	182	162^{+}	114	122

*Systolic pressure/diastolic pressure and the electronically determined mean.

$^{+}P < .05$

$^{++}P < .01$

PAP = left pulmonary arterial pressure; LVP = left ventricular pressure; HR = heart rate; TP = tracheal pressure; $\dot{V}_E$ = minute ventilation; f = respiratory frequency; V_T = tidal volume; and LVS = left vagal stimulation; LMCGS = left middle cervical ganglion stimulation.

sympathetic trials. Each animal served as its own control and stimulation induced changes were assessed for statistical significance by the paired t-test (Lewis, 1966).

The lungs' surface color patterns, determined by the ink's intravascular distribution are represented in Fig. 11 and 14. Average perfusion percentages for the five vagal and five sympathetic preparations are graphically presented in Figs. 12 and 13. Black, ink-perfused areas and light, non-perfused regions were contrasted. Representative photomicrographs from the serial-sectioned middle lobes are also shown in Figs. 15 and 16. Table 2 presents the stereologically determined absolute length and volumetric data from control and stimulated animals. In addition to the mean values, each lobar evaluation was divided into hilar, middle, and peripheral portions. These approximately equal segments consisted of composite samples from the respective lobar regions. For example, the hilar evaluation represented the mean lobar value obtained when sampling only from the hilar segment. Thus, contrasting these segmentally determined lobar means served to elucidate any differences observed between the grand lobar mean values. The control and experimental mean values were statistically analyzed (Appendix D) by an analysis of covariance (Ostle, 1970) and Stapelton's (1972) procedure for comparing individual means.

Table 2. Stereologic evaluations in the dog lung.

	Control (Non-Stimulated)		Left Vagal Stimulation		Left Sympathetic Stimulation	
	Left (25cc)+	Right (35cc)	Left (23cc)	Right (33cc)	Left (18cc)	Right (28cc)
Airway Volume	1.20cc*	1.86cc	0.71cc	0.76cc	0.74cc	1.09cc
H	1.10	2.35	0.67	0.79	1.22	1.74
M	1.88	2.10	0.83	0.96	0.61	0.78
P	0.75	1.12	0.51	0.56	0.38	0.76
Vessel Length	163.70m	240.59m	156.29m	188.54m	141.73m	157.90m
H	110.0	234.54	177.01	190.42	129.02	156.66
M	159.56	247.33	150.26	193.43	143.69	167.03
P	223.51	237.31	145.66	174.78	152.22	152.09
Vessel Volume	1.65cc	3.92cc	1.96cc	3.20cc	1.39cc	1.74cc
H	1.15	6.34	2.55	5.31	1.67	2.04
M	1.98	2.91	2.14	2.54	1.53	2.18
P	1.80	2.52	1.22	1.65	0.94	1.01
Capillary Volume	7.58cc	9.17cc	5.69cc	7.72cc	6.03cc	9.41cc
H	6.40	6.90	5.31	6.30	5.36	8.23
M	6.93	10.01	5.61	8.55	6.46	9.55
P	9.38	10.61	6.14	7.92	6.13	10.30

+Total lobar volumes in parentheses.

*Mean lobar values underlined.

H = hilar portion; M = middle portion; P = peripheral portion.

Figure 11

Representative dog lungs after unilateral vagal stimulation. Note the complete bilateral ink distribution into both left and right sides. The figure's right column shows the left and right lateral surfaces while the left column shows the ventral and dorsal surfaces respectively.

Figure 11

Figure 12

Average lobar perfusion percentages after left vagal stimulation in the dog.

percentage of lungs' surface showing ink perfusion (black color)

Cr cranial lobe Ca caudal lobe

M middle lobe I intermediate lobe

Figure 13

Average lobar perfusion percentages after left middle cervical ganglion stimulation in the dog.

percentage black

As in the vagal preparations, each lobe appears predominately black.

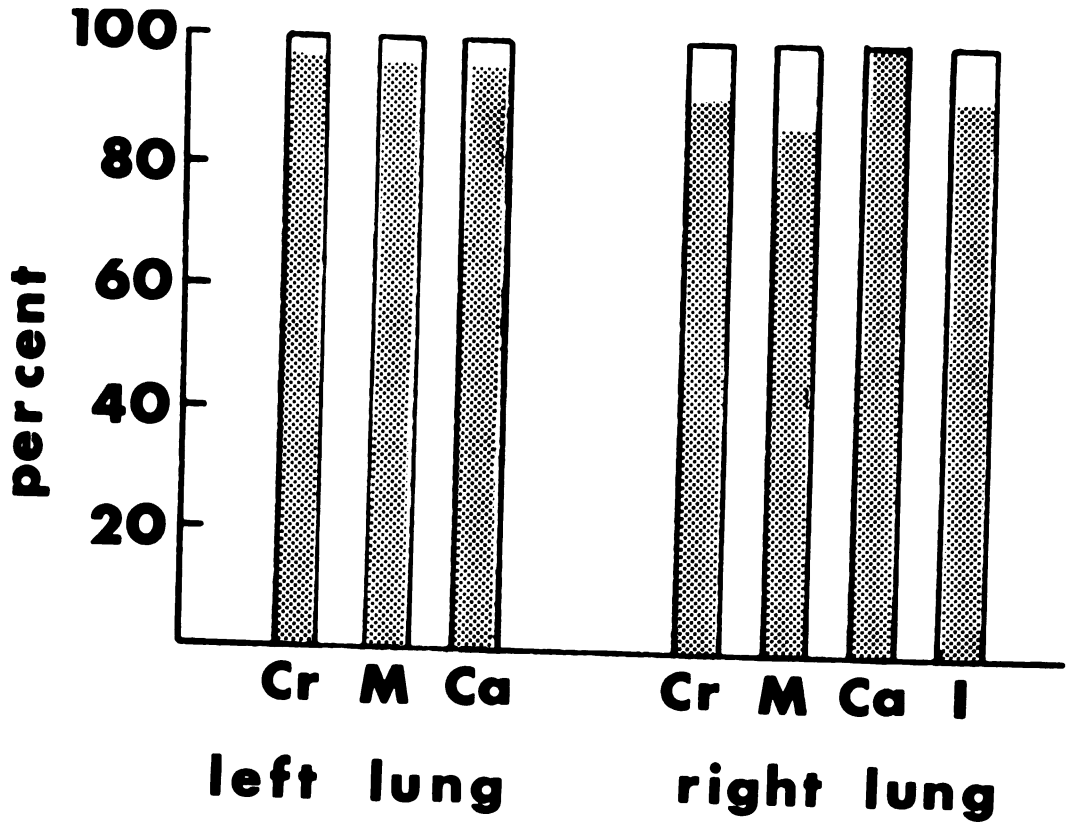


Figure 12

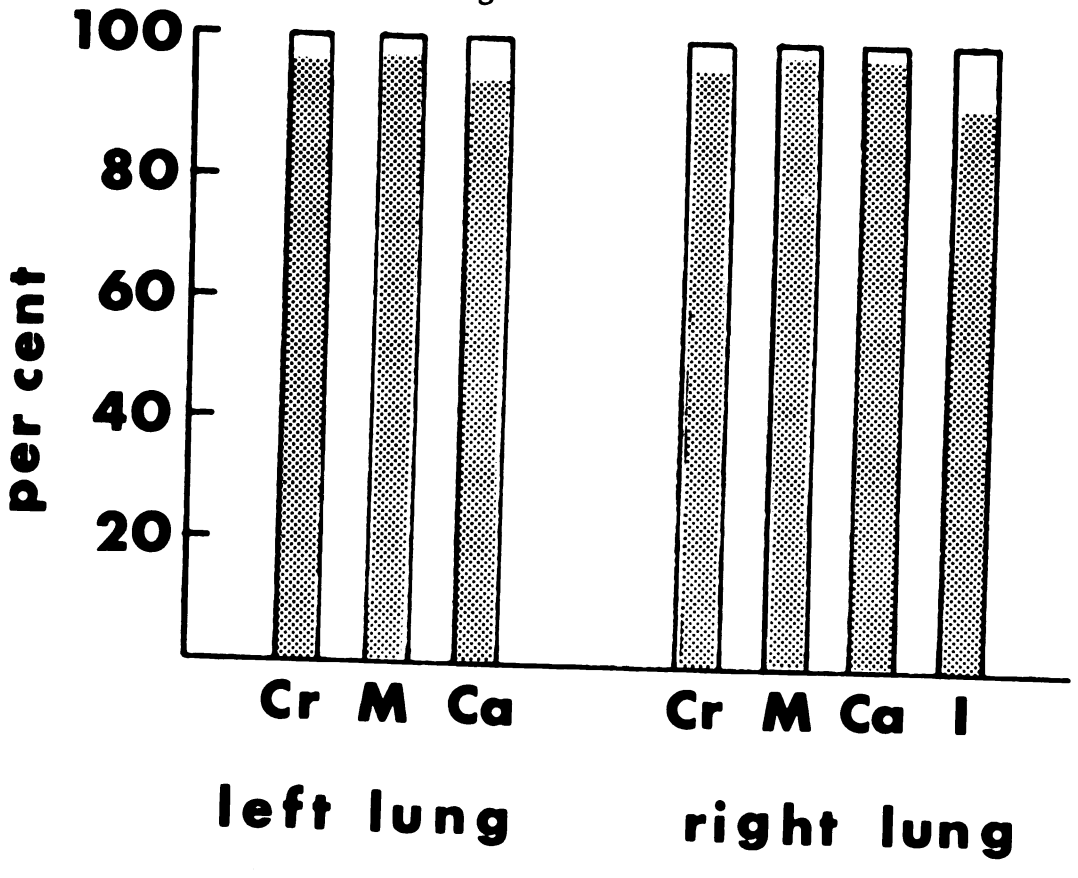


Figure 13

Individual control and experimental pressure and volume measurements are listed in Appendix A. Perfusion percentages for each of the stimulated and control animals are also presented in Appendix A. In addition, the stereologic count totals, which determined the relative length and volumetric fractions for the experimental and control animals are listed in Appendix A.

Parasympathetic Studies

Mean pulmonary arterial and left ventricular pressures decreased significantly after left vagal stimulation (Table 1). As shown (Fig. 17), the pressure deflections occurred concomitantly. This suggested a passive pulmonary vascular response to decreased cardiac output induced by the vagally-mediated bradycardia (Table 1). Active pulmonary vasodilation and/or opening of arteriovenous shunts, the alternate explanations of decreased pulmonary arterial pressure, were not supported. Such responses under conditions of constant pulmonary inflow, would generate a secondary increase in return to the left heart causing a rise in left ventricular pressure. However, pulmonary flow was diminished as cardiac output fell, thus, the secondary activity was not expected.

The virtually even bilateral ink perfusions (Fig. 11 and 12) also failed to establish unilateral vagally-induced vasodilation. In addition, the stereologic

measurements defined comparable volumes in the left and right middle lobes occuppied by the vessels and ink-filled alveolar capillaries (Table 2). Contrasting the control and stimulated animal vascular measurements presented further evidence of a passive pulmonary vasculature. After vagal stimulation, the total left and right middle lobar volumes (determined by water displacement) maintained the 10cc difference observed in the control animals. Thus, the differences, shown in Table 2, were attributed to this size differential only as evidenced by the statistical insignificance.

Rushmer (1961) reported decreased heart rate and atrial contractility with diminishing cardiac output, after vagal stimulation in mammals. During the present investigation, the ink injection created a proportionately similar venous return in the control and stimulated animals. Thus, the vagally-induced bradycardia suggested a reduced cardiac output relative to the control preparation. Accordingly, the ink-filled capillaries demonstrated a slight bilateral volume decline, especially in the peripheral segments, of the stimulated animals (Table 2 and Appendix A). However, the decrease was not significant which suggested a small reduction in cardiac output after the stimulation.

Nadel, Colebatch, and Olsen (1964) recorded constriction of terminal bronchioles after vagal stimulation in dogs and cats. Bronchiolar constriction caused increases

in airway resistance and alveolar volumes (attributed to greater difficulty in expiration). Respiratory bronchiole and alveolar duct smooth muscle was unaffected by vagal activation. In addition, Karczewski and Widdicombe (1968) recorded only slight decreases in lung compliance after vagal stimulation in rabbits. Thus, vagal efferent fibers allegedly acted on large conduction airways and not the smaller divisions responsible for lung compliance.

Staub (1966) defined extra-and intra-alveolar vessels in relating airway mechanics and pulmonary vascular resistance. The larger extra-alveolar vessels experienced tractional forces from the surrounding lung parenchyma during normal respiratory movements. With lung expansion (inspiration) the vessels were elongated and widened while expiration passively reduced the vessel dimensions. The intra-alveolar vessels (muscular arteries, arterioles, capillaries, and venules), associated directly with the alveolar septal framework, were flattened or "squeezed" during rises in alveolar pressure. In addition, Simon, Linde, Miller, and O"Reilly (1961) recorded rises in pulmonary vascular resistance after increased or decreased lung volumes. During volume increases, intra-alveolar vessel caliber was reduced, while decreased lung volumes tended to collapse the extra-alveolar vessels.

Rodbard (1966) suggested an increased resistance to capillary flow during vagal stimulation. Bronchiolar

constriction created an increased alveolar pressure which passively compressed the contiguous alveolar capillaries. Staub, however, suggested that alveolar surface tension would reduce capillary compression with increased alveolar pressures. The alveolar walls were defined as flat surfaces with a sharp curvature at the corners or wall junctions. When alveolar pressure exceeded capillary pressure, capillaries within the flat walls were compressed while those at the wall junctions were protected by surface tension acting across the curved corners. Earlier, Mead, Whittenberger, and Radford (1957) reported surfactant induced fluctuations in alveolar surface tension during the respiratory cycle. Surface tension progressively rose during inspiration as surfactant activity concomintantly declined. At end-inspiration peak surface tension was reached, which coupled with elastic fiber recoil, activated the expiratory phase. During expiration, the surfactant molecules demonstrated increased activity which lead to the minimal surface tension at end-expiration. Thus, surface tension may be triggered to counteract vagally-induced alveolar expansion thereby limiting passive capillary compression.

During the present unilateral vagal stimulations, lobar volumes occupied by the conductive airways (tertiary bronchi through terminal bronchioles) were not significantly decreased in the ipsilateral lung (Table 2). Tracheal

pressure and minute ventilation also failed to substantially change after stimulation (Table 1). This data, however, may have masked an ipsilateral bronchiolar constriction. An increased downstream resistance would tend to expand volumes of the bronchi and primary bronchioles in the stimulated lung, thereby offsetting the diminished terminal bronchiolar volumes. That is, the stereologically measured volume would be unchanged. Nadel, et al. also reported a shifting of ventilation away from the constricted lung into the contralateral side. Increased ventilation would create airway volume expansion to approximate the volume of the ipsilateral lung. The unaltered tracheal pressure and minute volumes suggested that a ventilation shift toward the non-stimulated lung would adequately compensate for any active bronchomotor response to vagal stimulation.

DuBois (1964) described normal bronchiolar smooth muscle tone in mammalian lung maintained by tonic vagal discharge. Such tone eliminated undue airway expansion which prevented an excessively large anatomic dead space and its attending increase in tidal volume and work of breathing. Therefore, the significant decrease in tidal volume after vagal stimulation (Table 1) suggested a decreased anatomic dead space. A unilateral airway constriction would account for the decreased dead space, or a possible vagal reflex response may have been initiated by increased flow through the contralateral airways.

The alternate hypothesis of essentially unaltered bronchomotor tone after vagal stimulation also merits discussion. The unchanged airway volumes and ventilation parameters coupled with the decreased tidal volume may have suggested moderate ipsilateral bronchiolar contraction. That is, dead space within the stimulated lung was diminished without causing major shifts in air flow resistance or distribution.

Whatever the airway response was to vagal stimulation, it ultimately failed to elicit substantial changes in pulmonary vascular perfusion. That is, minimal vagal control passively exhibited via decreased cardiac output, was recorded in the canine pulmonary vasculature.

Sympathetic Studies

Left middle cervical ganglion stimulation yielded an increased mean left ventricular pressure while mean left pulmonary arterial pressure was unchanged (Table 1 and Fig. 17). Sarnoff, Gilmore, and Wallace (1965) reported an increased heart rate and force of ventricular contraction after sympathetic stimulation. However, pulmonary arterial pressure remained nearly unchanged after threefold increases in cardiac output. In addition, Pace, Keefe, Armour, and Randal (1969) demonstrated two to five fold increases in pulmonary arterial flow after stellate ganglion stimulation in dogs. Pulmonary arterial pressure remained

Figure 14

The dog lungs after left middle cervical ganglion stimulation. As shown in the vagal preparations, thorough bilateral ink distribution is demonstrated. The lateral surfaces (figure's right column) and the lung's ventral and dorsal aspects (left column) display the ink's surface perfusion patterns.

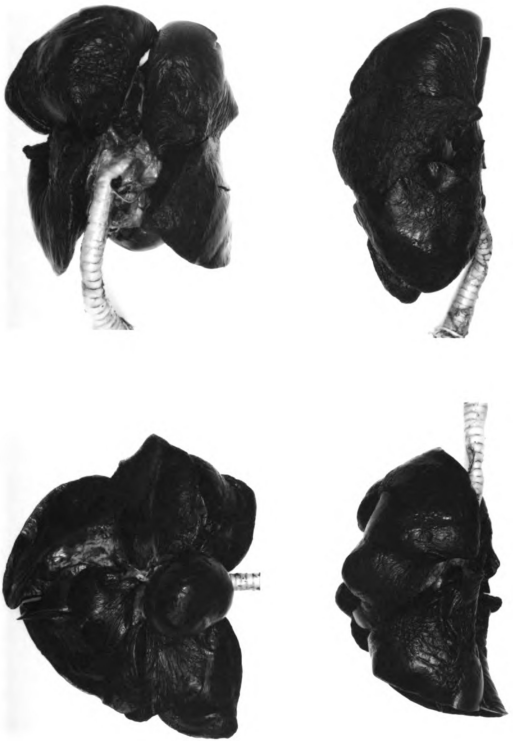


Figure 14

Figure 15

Histologic section from dog lung after vagal stimulation. Note the right angled branching pattern of the arterial and precapillary-sized vessels. The thorough ink perfusion, noted previously on the lung's surface, is depicted in the pulmonary microvasculature. 225X.

Figure 16

Section from the hilar region of left middle lobe after left sympathetic stimulation in the dog. A bronchus (lower right corner) is shown branching into the successive bronchiolar tree. Note the ink-filled pulmonary vessels adjacent to the air-ways. The bronchial vessels, located within the bronchial adventitia also appear ink perfused. 90X.

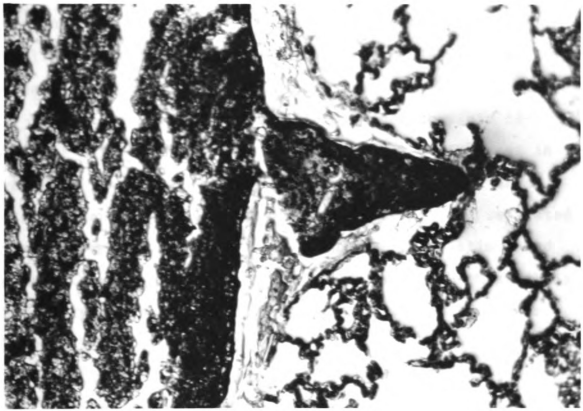


Figure 15

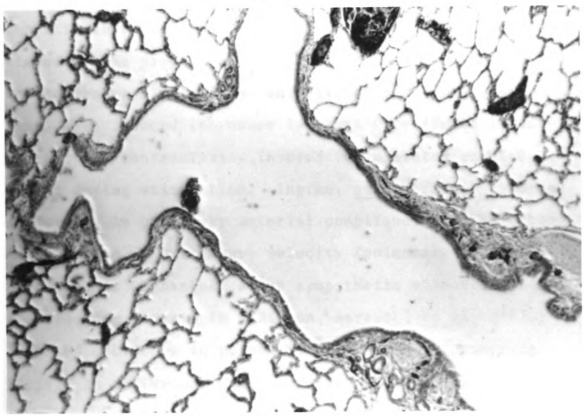


Figure 16

unchanged or rose slightly. The authors attributed the unaltered pressure to ample pulmonary vascular reserve capacity and/or vessel compliance.

Figures 13 and 14 and Table 2 show complete bilateral ink distribution and insignificant differences in vascular length and volumes after unilateral sympathetic stimulation. Again, the consistent 10cc interval separated the left and right lungs. The sympathetically stimulated animals possessed smaller total lobar volumes because their body weights were less, i.e. 16 lbs. versus 20 lbs. mean body weights (Appendix A).

If vessel compliance had enabled accommodation of the increased flow during stimulation, greater filling would have been expected in both left and right lungs of the stimulated animals. Comparable venous return, established by the proportionally similar ink injections in the control and stimulated animals, coupled with sympathetically induced increases in heart rate (Table 1) and ventricular contractility insured the elevated cardiac output during stimulation. Ingram, et al. (1968) recorded a decrease in pulmonary arterial compliance with an attendant increase in pulse wave velocity (pulmonary arterial pressure was unchanged) after sympathetic stimulation in isolated dog lungs. In addition, Aarseth, et al. (1971) reported decreases in pulmonary blood volume (occurring largely in the venous compartment) after sympathetic

stimulation in isolated dog lungs. Active decreases in venous compliance were suggested. Such alterations in the arterial and venous beds would account for similar vascular compartment volumes in the control and stimulated animals. However, the comparable volumes in left and right lungs of the stimulated animals requires further discussion. Normal pulmonary arterial compliance would tend to create slight "pooling" with an increased flow in the right (control) lung. The less compliant, stimulated lung also accepted an increased flow. However, it appeared to rapidly pass across its conductive vascular bed without greatly increasing vessel volume.

Previously, Franklin, VanCitters, and Rushmer (1962) demonstrated precise phasic balance between outputs of the left and right ventricles. For example, an increase in right ventricular stroke volume increased stroke volume of the subsequent left ventricular beat. In addition, Morkin, Collins, Goldman, and Fishman (1965) reported that the flow pulse generated by the right ventricle was transmitted across the pulmonary circuit in approximately 0.10 seconds and was propelled into the left atrium at the precise moment of rapid ventricular filling. Thus, during sympathetic stimulation the ventricular synchrony would be upset without the reported increase in pulse wave transmission rate (induced by pulmonary arterial stiffening) across the pulmonary circulation. The stiffening of the

Figure 17

Physiologic pressure measurements during control and stimulation periods in the dog. The accompanying abbreviations represented the following:

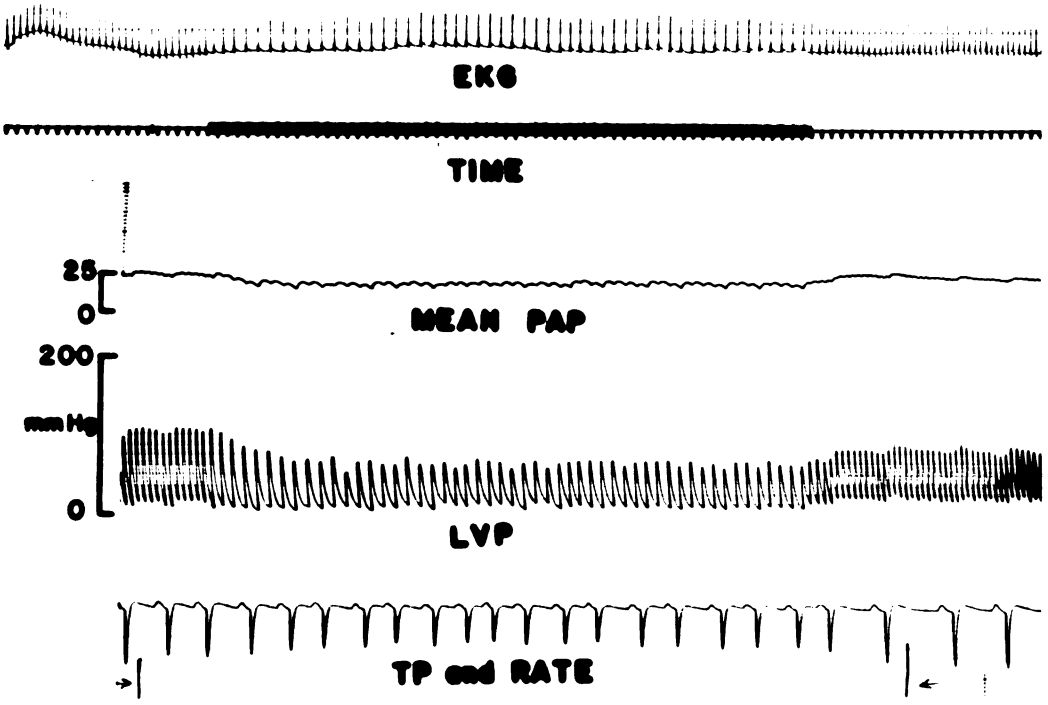
MEAN PAP: mean pulmonary arterial pressure

LVP: left ventricular pressure

TP and RATE: tracheal pressure and respiration frequency

Upper record depicts changes during left vagal stimulation (darker portion of time line). Note the bradycardia and decreased pulmonary arterial and left ventricular pressures. Tracheal pressure and respiratory frequency were virtually unchanged.

Lower record represents the pressure responses to left middle cervical ganglion stimulation. Mean pulmonary arterial pressure remained steady while left ventricular pressure was increased. Again, tracheal pressure and respiratory frequency were unchanged.


EKG
TIME
25
0
MEAN PAP
200
mm Hg
0
LVP
TP and RATE

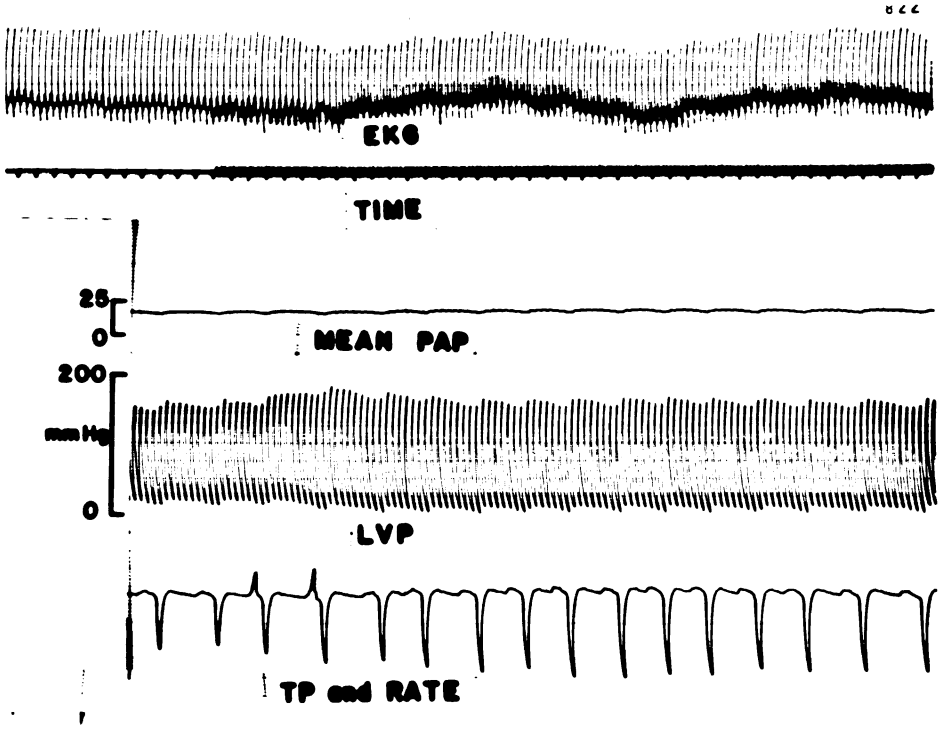

EKG
TIME
25
0
MEAN PAP
200
mm Hg
0
LVP
TP and RATE

Figure 17

large conductive vessels in the stimulated lung, coupled with the right lung's normal distensibility, would also enable greater filling of the intra-alveolar vessels on the stimulated side. That is, the increased pulmonary flow would distend the larger extra-alveolar vessels in the control (right) lung, while the stimulated side's decreased compliance would induce greater filling of the smaller intra-alveolar vessels. Thus, the increased flow would "pool" in the larger pulmonary vessels of the control lung while diminished compliance on the stimulated side would prevent such pooling in its extra-alveolar vasculature. Hence, a greater proportion of right ventricular output would traverse the stimulated side with a tendency to increase intra-alveolar vessel perfusion.

Further assessment of sympathetically-induced decreases in vascular compliance requires evaluation of pulmonary flow distribtuion during normal and altered right ventricular outputs. West, Glazier, Hughes, and Maloney (1968), employing oxygen labelled gas inhalation methods, demonstrated that blood flow decreased steadily from the base to the apex of the normal upright human lung. This distribution was accounted for by the relative magnitude of pulmonary arterial, venous, and alveolar pressures across the lung. Thus, the lung was divided into apical, middle, and basal zones. In the apical zone, arterial pressure was less than alveolar pressure and there was no

flow. That is, the alveolar capillaries collapsed when alveolar pressure exceeded perfusion pressure. Arterial pressure steadily increased down the middle zone as more vascular units were opened coupled with distension of those already perfused. Therefore, the intra-alveolar vessels behaved as Starling resistors, that is, collapsible tubes surrounded by a pressure chamber with flow determined by arterial minus alveolar pressures. In the bottom zone, venous pressure exceeded alveolar pressure and flow was determined by the arterial-venous pressure difference. The increase in flow down the zone was attributed to gravitational forces which created a rise in transmural pressure leading to vessel distension and the opening of additional channels. Additional observations of dogs in the supine position demonstrated approximately equal apical and basal flows while flow within the dependent (dorsal) channels exceeded that through the ventral lung aspect.

Increases in right ventricular output, e.g. via exercise, without concomitant pulmonary arterial pressure elevations were previously described. West et al. reported virtually equal apical and basal flow distribution during exercise in upright subjects. Permutt, Caldini, Maseri, Palmer, Sasamori, and Zierler (1969) reported that changes in pulmonary blood volume produced by alterations in pressure or flow were due to recruitment or derecruitment of parallel units differing only in their critical opening

pressures. Using isolated dog lungs, end-tidal P_{co_2} was measured while left atrial pressure was elevated and pulmonary blood flow and alveolar pressure were held constant. As left atrial pressure rose, both pulmonary arterial pressure and end-tidal P_{co_2} were elevated. Thus, alveolar dead space was decreased which suggested recruitment of previously unperfused alveolar capillaries rather than distension of channels already open. The same results were also obtained when left atrial and alveolar pressures were held constant and pulmonary arterial pressure was elevated by increasing flow into the isolated lungs. The authors concluded that the small intra-alveolar vessels were essentially indistensible and the number of parallel units perfused was determined by inflow pressures.

In addition, Engelberg and DuBois (1959) reported greater compliance of the arterial and venous beds than of the capillaries in isolated rabbit lungs. The lungs were suspended in a plethysmograph and passively ventilated with 100 percent N_2O. A motor-driven pump delivered a constant volume inflow of Kreb-Ringer's solution into the cannulated pulmonary artery. Equilibration of the N_2O gas with the perfusate occurred in the capillaries which afforded removal of gas molecules from the lung. Hence, lung volume decreased which in turn decreased pressure within the plethysmograph. Prior calibration of plethysmograph pressure changes with known volume flow rates enabled

calculation of capillary flow from the measured decreases in plethysmograph pressure. Thus, pulmonary arterial compliance was determined by measuring flow rates entering and leaving the arterial tree. Pulmonary venous compliance was similarly recorded by retrograde perfusion through the cannulated left atrium. Thus, increased flow into the pulmonary vasculature appeared to distend the arterial and venous beds while the less compliant intra-alveolar vessels prompted perfusion of previously closed capillary networks. Accordingly, Engelberg and DuBois reported that 60-70 percent of increased pulmonary blood volume with elevated cardiac output was accommodated in the small intra-alveolar vessels.

Contrasting the stereologic capillary compartment measurements in the control and stimulated animals (Table 2 and Appendix A) demonstrated bilateral increases in the volumes occuppied by ink-filled capillaries in the stimulated animals. Since the volume increase was not statistically significant, this suggested thorough pulmonary perfusion in the control preparations (ink volumes were proportionally similar in the control and experimental trials). The lung volumes occupied by the arteries and veins were virtually equal in the control and stimulated animals. Therefore, the ultimate unilateral sympathetic effect appeared to be a stiffening of the larger vessels allowing greater capillary perfusion and more rapid transit across the entire vascular bed.

A second alternative, namely entrance of ink into the pulmonary circulation via the bronchial arteries must be considered. Daly and Hebb (1966e) failed to establish anatomic evidence of bronchial-pulmonary artery (precapillary) anastomoses in rabbit, dog, and cat. Evidence was presented, however, of capillary communications between the bronchial and pulmonary vascular systems chiefly in the respiratory bronchiole region. The extent of blood flow (maximal bronchial arterial flow represented one to two percent of cardiac output) through these connections remains unknown. However, the speculated direction of flow suggested a bronchial to pulmonary direction in conjunction with normal pressure gradients. Drainage of bronchial flow was accomplished by three potential channels. Bronchial veins drained the primary arterial divisions, bronchopulmonary veins drained smaller order divisions into the pulmonary veins, and the pulmonary veins also drained bronchial blood that had reached the alveolar capillaries. The relative flow magnitudes within the channels remains unknown.

Aviado (1965c) reported a direct relation between aortic pressure and bronchial arterial flow, that is, increased transmural pressure passively reduced arterial resistance allowing an increased flow in isolated dog lungs. Sympathetic stimulation appeared to elicit bronchial venous constriction as evidenced by decreased venous flow after stimulation. However, the author emphasized further

evidence was required to establish the extent of bronchial flow and its control.

In the present investigation, ink may have entered the pulmonary capillary bed via the bronchial circulation. However, as suggested by Daly and Hebb, the low average bronchial arterial flow would not be expected to significantly alter pulmonary vascular resistance and hence pulmonary perfusion. The rapid cessation of cardiac activity via carbachol administration also suggested a limitation of bronchopulmonary ink flow.

Intravascular injections of epinephrine characteristically promoted bronchodilation in mammalian lung (Koelle, 1970b). Konzett and Hebb (1949), however, reported the bronchodilator potential of norepinephrine as one-tenth to one-fifth that of epinephrine. In addition, the bronchodilator effects were dependent upon a pre-existent state of tonus usually established by prior injections of histamine or acetylcholine. According to Koelle (1970b) the neurohumoral transmitter released at the postganglionic sympathetic terminal was norepinephrine. Thus, bronchodilator response to the present sympathetic stimulations was not anticipated, that is, the atropine administration blocked vagal tone which further diminished the slight dilator potential of norepinephrine.

Table 1 shows essentially unchanged minute ventilation and tidal volumes after left middle cervical ganglion

stimulation. In addition, lung volumes occupied by the conductive airways of the left (stimulated) lung were unaltered relative to the right (control) side (Table 2). However, tracheal pressure substantially increased, and thus, coupled with unchanged minute volumes, suggested an increased work of breathing. The lack of increased tidal volumes reflected a consistent anatomic dead space. Conductive airway volumes in the non-stimulated animals were also virtually equal to those in the stimulated preparations. Thus, tracheal pressure rose without altering airway volumes or flow rates.

As discussed above, intra-alveolar vessel volume, namely capillary volume, tended to increase bilaterally after stimulation. The increased tracheal pressure might denote a passive response to decreased transmural pressures within the alveoli. Obviously, tracheal pressure elevation failed to diminish pulmonary vascular perfusion. Therefore, the reverse phenomenon of vascular perfusion affecting tracheal pressure is a possibility. However, alveolar pressure was not monitored, thus, conclusive evidence of the purported passive activity was not obtained.

Cat

Average values of pressure and volume measurements in the control, vagal, and sympathetic preparations are listed in Table 3. A sequential review of the Pelikan ink injections is again presented by Figs. 18-23. Table 4

Table 3. Effects of autonomic nerve stimulations on pressure and volume parameters in the cat.

	Control	LVS	Control	SGS
PAP (mmHg)	21/12 (17)*	21/10 (14)$^{++}$	20/12 (18)	24/16 (20)$^{+}$
LVP (mmHg)	134/21 (78)	111/10 (60)$^{++}$	121/21 (71)	146/26 (86)$^{+}$
HR (beats/min)	123	87^{+}	154	176^{++}
TP (cmH_2O)	11	10	8	10
$\dot{V}_E$ (1/min)	0.96	0.85	1.54	1.55
f (breaths/min)	17	18	26	29
V_T (ml/breath)	56	47	59	54

*Systolic pressure/diastolic pressure and the electronically determined mean.

$^{+}P < .05$

$^{++}P < .01$

SGS = stellate ganglion stimulation.

LVS = left vagal stimulation.

Table 4. Stereologic evaluations in the cat lungs.

	Control (Non-Stimulated)		Left Vagal Stimulation		Left Sympathetic Stimulation	
	Left (9cc)	Right (11cc)+	Left (8cc)	Right (10cc)	Left (7cc)	Right (10cc)
Airway Volume	0.36cc	0.48cc*	0.21cc	0.16cc	0.32cc	0.55cc
H	0.52	0.72	0.13	0.17	0.43	0.84
M	0.42	0.43	0.31	0.22	0.39	0.43
P	0.14	0.32	0.16	0.10	0.13	0.37
Vessel Length	83.11m	108.86m^{S}	48.69m^{S}	60.49m^{S}	68.81m	91.73m
H	68.16	99.41	49.15	73.18	64.66	82.79
M	80.27	100.93	46.50	56.25	67.22	89.06
P	100.91	126.30	50.49	49.53	74.53	103.36
Vessel Volume	0.75cc	0.99cc	0.53cc	0.56cc	0.39cc	0.58cc
H	0.77	0.79	0.54	0.54	0.44	0.47
M	0.95	1.34	0.70	0.82	0.39	0.80
P	0.53	0.74	0.34	0.30	0.33	0.47
Capillary Volume	2.30cc^{S}	2.67cc^{S}	1.31cc^{S}	1.77cc^{S}	1.79cc	2.20cc
H	2.00	2.39	1.46	1.95	1.73	2.29
M	2.27	2.74	1.25	1.64	1.78	2.04
P	2.45	2.88	1.21	1.69	1.83	2.26

+Total lobar volumes in parentheses.

*Mean values are underlined

$^{S}P < .05$ (see text).

H = hilar portion; M = middle portion; P = peripheral portion.

includes the stereologic measurements of absolute pulmonary vascular lengths and volumes. Statistical analysis of the recorded data again involved the paired t-test and an analysis of covariance (Appendix D). The individual physiologic measurements, perfusion percentages, and stereologic count totals are listed in Appendix B.

Parasympathetic Studies

Table 3 and Fig. 24 define the characteristic cardiac and pulmonary vascular response to left vagal stimulation. Decreased pulmonary arterial pressure again reflected the passive response to a reduced cardiac output (pulmonary flow).

The even bilateral ink distributions (Figs. 18 and 19) also negated active pulmonary vasomotor response to unilateral vagal activation. Stereologic evaluations of the middle lobar vascular compartments also indicated a passive pulmonary vascular bed (Table 4). After vagal stimulation, pulmonary arterial and venous lengths in the stimulated animals were less than their counterparts in the control preparations. The control animals' right lung demonstrated significantly greater vessel lengths than either lung of the stimulated animals. In addition, the right control vessel lengths were slightly greater (not significantly) than those of the left control side. Consequently, vessels of the left control lung were longer, but not

Figure 18

The cat lungs after left vagal stimulation. As demonstrated in the dog, complete bilateral ink distribution is depicted on the lungs' lateral surfaces (figure's right column) and its ventral and dorsal aspects (left column).

Figure 18

Figure 19

Average lobar perfusion percentages after left vagal stimulation in the cat.

percentage of lungs' surface showing ink perfusion (black color)

Cr cranial lobe

Ca caudal lobe

M middle lobe

I intermediate lobe

Figure 20

Average lobar perfusion percentages after left stellate ganglion stimulation in the cat.

percentage black

As in the vagal preparation, each lobe appears predominately black.

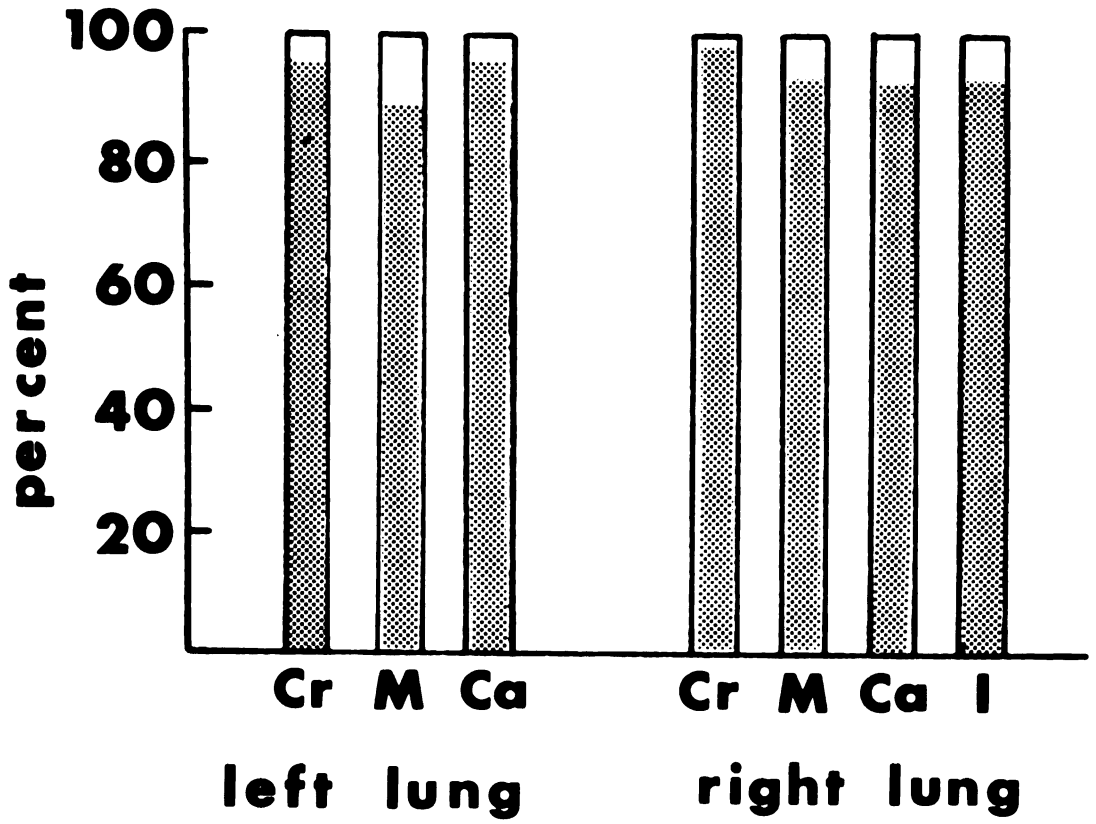


Figure 19

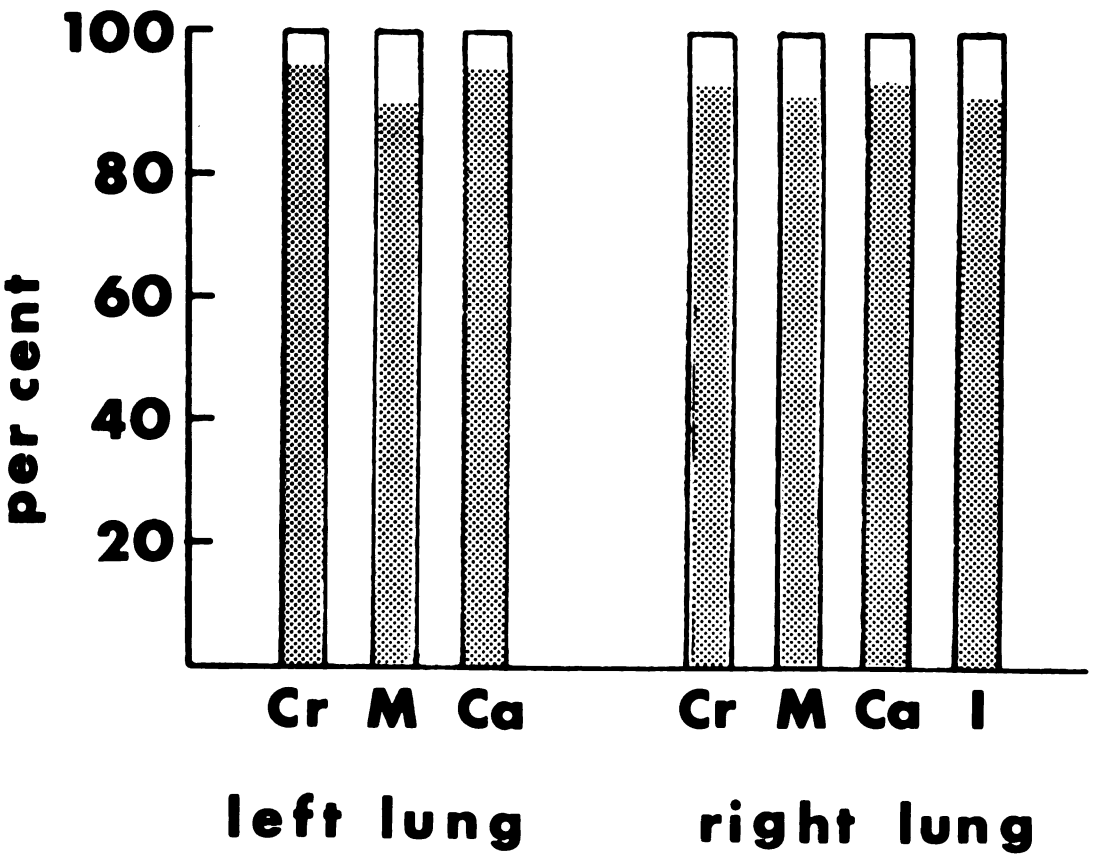


Figure 20

significantly, than those of either side in the stimulated preparations. Thus, an uneven flow distribution was suggested in the control animals. That is, greater flow through the right lung tended to lengthen its conductive vessels. The volumes occupied by the arteries and veins were also slightly greater in the right lung. However, both the length and volume measurements in the right side were not significantly greater than those on the left. In addition, the lobar volumes occupied by ink-filled capillaries were virtually equal in the control animals' left and right sides. Therefore, this suggested tendency toward greater perfusion through the right control lung was not substantiated.

The bilaterally equal ink-filled capillary compartments in the control animals were also significantly greater than their counterparts in the stimulated preparations. Thus, capillary perfusion was markedly diminished through both lungs after unilateral vagal stimulation. Analysis of the hilar, middle, and peripheral lobar portions suggested ink congregation within the hilar capillary compartments of both lungs. The bilaterally equal capillary compartmental volumes and the attendant hilar ink concentration conclusively demonstrated a passive pulmonary vascular response to the reduced pulmonary flow (cardiac output).

Table 3 also depicts insignificant variations in tracheal pressure, minute ventilation, and tidal volumes

after the unilateral stimulations. The stereologically measured airway compartments were also bilaterally similar after left vagal activation. The slight decline in tidal volume failed to establish a substantially reduced anatomic dead space after stimulation. Therefore, a bronchoconstrictor response was minimal or nonexistent. Thus, the possible passive pulmonary vascular response to an altered bronchomotor tone was not observed in the cat.

Sympathetic Studies

Left stellate ganglion stimulation created increases in mean left ventricular and pulmonary arterial pressures (Table 3). The increased pulmonary arterial pressure appeared to reflect an increased flow rather than increased resistance. That is, the even bilateral ink distribution (Figs. 20 and 21) after unilateral sympathetic activation established essentially equal pulmonary vascular perfusion.

Evaluation of the absolute vascular compartmental volumes and lengths (Table 4) and the respective volume fractions (Appendix B) again suggested probable sympathetic moderation of pulmonary vascular compliance. Virtually equal volumes occupied by the vessels and ink-filled capillaries, found bilaterally in the control and stimulated animals, established the unilateral decrease in vessel compliance after stimulation. As discussed for the dog, the increased pulmonary flow, rather than distending the

Figure 21

Thorough ink perfusion into the left and right lungs is shown after left stellate ganglion stimulation in the cat. The dorsal and ventral surfaces (upper half of figure) and the lateral aspects (figure's lower half) display the predominately black colorization. Again, this pattern parallels the vagal preparations.

Figure 21

Figure 22

Histologic section from cat lung after vagal stimulation. In the center of the figure a longitudinally sectioned pulmonary artery and its right-angled precapillary vessels are shown. Note the thorough ink distribution within the pulmonary vasculature. 225X.

Figure 23

This figure depicts a higher magnification of the ink-filled feline pulmonary microcirculation. At the left a pulmonary arteriole feeds its right-angled precapillary vessels which, in turn, perfuse the alveolar capillary network. The extensiveness of this network is shown in the center of the figure. 900X.

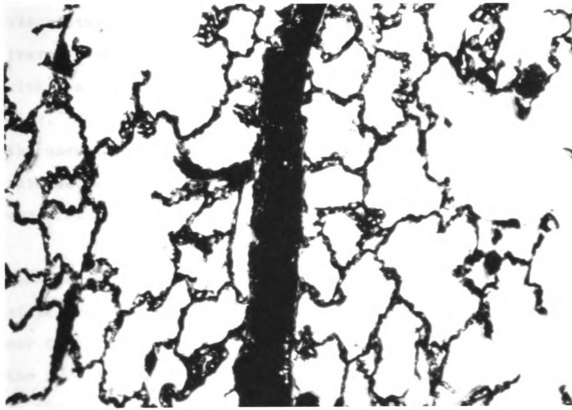


Figure 22

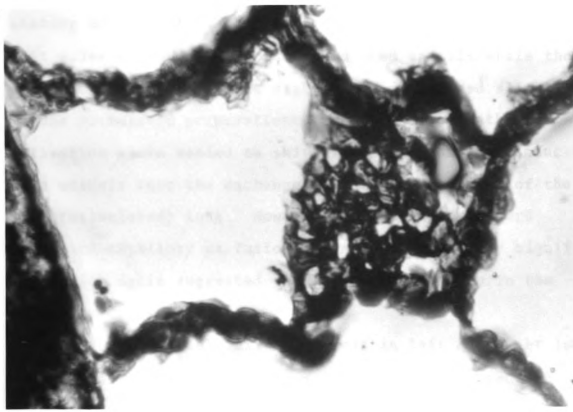


Figure 23

vasculature of the stimulated animals, tended to more rapidly traverse the left lung's pulmonary circuit in accordance with the diminished compliance after left sympathetic activation. Concurrently, less ink was permitted to collect in the normally distensible right lung as the left sides' flow rate accounted for greater proportions of the increased right ventricular output.

The normal (control) two to three cm size variation between the left and right middle lobes appeared consistently after sympathetic stimulation. Thus, the volume fractions may furnish additional evidence of vascular stiffening in the left or stimulated lung (Appendix B). Volumes occupied by the large vessels tended to decrease in both lungs of the stimulated animals. Portions of the lobar volumes consisting of ink-filled capillaries appeared constant in the left sides of the control and stimulated animals while the capillary component of the right sides diminished slightly in the stimulated preparations. Therefore, sympathetic activation again tended to shift ink away from the conduction vessels into the exchange vascular compartment of the left (stimulated) lung. However, this tendency toward increased capillary perfusion was not statistically significant which again suggested thorough ink perfusion in the control preparations.

The comparable airway volumes in left and right lungs of the control and stimulated animals (Table 4), coupled

Figure 24

Physiologic pressure measurements during control and stimulation periods in the cat. The accompanying abbreviations represented the following:

MEAN PAP: mean pulmonary arterial pressure

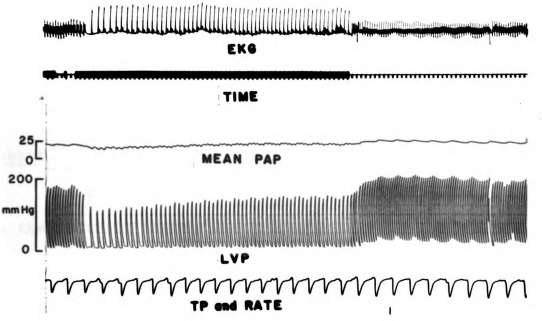
PAP: pulmonary arterial pressure

LVP: left ventricular pressure

TP and RATE: tracheal pressure and respiration frequency

Upper record represents changes during left vagal stimulation (darker portion of time line). Note the bradycardia and decreased pulmonary arterial and left ventricular pressures. Tracheal pressure and respiratory frequency were virtually unchanged.

Lower record depicts the pressure responses to left stellate ganglion stimulation. Pulmonary arterial pressure was steady while left ventricular pressure rose during the stimulation. The aberrant vascular pressure and EKG tracings represent extra-ventricular systole, resulting from ventricular cannulation. Again, respiratory parameters were unchanged.


EKG
TIME
25
0
MEAN PAP
200
mm Hg
0
LVP
TP and RATE

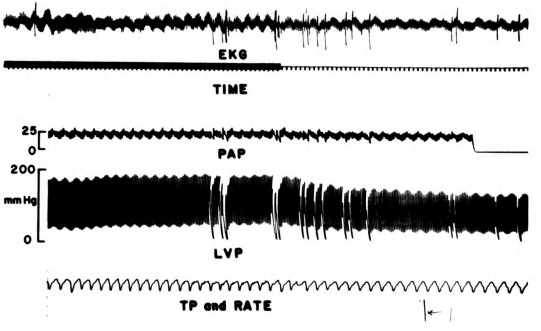

EKG
TIME
25
0
PAP
200
mm Hg
0
LVP
TP and RATE

Figure 24

with the unaltered ventilation patterns (Table 3) after stimulation, negated changes in bronchomotor tone. Hence, any consequent passive pulmonary vascular response was also eliminated.

Rabbit

Average values of pressure and volume measurements in the control, vagal, and sympathetic trials are listed in Table 5. Figures 25-30 present a review of the Pelikan ink injections. Table 6 includes the stereologic measurements of absolute pulmonary vascular lengths and volumes. Statistical analysis of the recorded data was again performed with the paired t-test and an analysis of covariance (Appendix D). The individual physiologic measurements, perfusion percentages, and stereologic count totals are listed in Appendix C.

Parasympathetic Studies

The characteristic decline in mean left ventricular and pulmonary arterial pressures after vagal stimulation is shown in Table 5 and Fig. 31. Passive pulmonary vascular reactivity to decreased cardiac output was again suggested. That is, the essentially even bilateral ink distribution (Figs. 25 and 26) negated an active pulmonary vasomotor response. In addition, the stereologically determined vascular lengths and volumes (Table 6) indicated cardiodynamic control of pulmonary perfusion.

Table 5. Effects of autonomic nerve stimulations on pressure and volume parameters in the rabbit.

	Control	LVS	Control	ICGS
PAP (mmHg)	25/12 (16)*	22/8 (15)$^{+}$	26/11 (22)	29/15 (23)
LVP (mmHg)	93/27 (60)	88/16 (52)$^{+}$	78/32 (56)	87/40 (64)$^{+}$
HR (beats/min)	192	138^{++}	210	258
TP (cmH_2O)	16	13	7	8
$\dot{V}_E$ (l/min)	2.70	2.46	1.83	1.85
f (breaths/min)	59	60	61	53
V_T (ml/breath)	46	41^{+}	30	35

*Systolic pressure/diastolic pressure and the electronically determined mean.

$^{+}P < .05$

$^{++}P < .01$

ICGS = inferior cervical ganglion stimulation.

LVS = left vagal stimulation.

Table 6. Stereologic evaluations in the rabbit lung.

	Control (Non-Stimulated)		Left Vagal Stimulation		Left Sympathetic Stimulation	
	Left (9cc)[+]	Right (11cc)	Left (9cc)	Right (12cc)	Left (7cc)	Right (10cc)
Airway Volume	0.36cc*	0.47cc	0.39cc	0.55cc	0.43cc	0.55cc
H	0.47	0.85	0.47	0.85	0.50	0.74
M	0.36	0.25	0.35	0.54	0.55	0.63
P	0.24	0.31	0.22	0.24	0.25	0.28
Vessel Length	96.07m[S]	129.42m[S]	73.53m[S]	96.77m[S]	64.30m	88.47m
H	90.85	130.23	75.56	104.44	55.72	93.48
M	88.69	128.71	73.13	94.58	65.30	99.21
P	108.58	128.63	71.91	91.29	72.47	72.34
Vessel Volume	0.93cc	1.17cc	0.62cc	1.01cc	0.48cc	0.59cc
H	1.18	1.01	0.74	1.07	0.59	0.85
M	0.86	1.46	0.72	1.25	0.43	0.59
P	0.74	1.01	0.42	0.54	0.42	0.32
Capillary Volume	2.96cc[S]	3.92cc[S]	2.44cc[S]	3.49cc[S]	2.55cc	3.27cc
H	2.91	3.67	2.58	3.41	2.36	3.46
M	2.75	3.93	2.57	3.72	2.77	3.47
P	3.21	4.16	2.18	3.68	2.53	2.92

[+]Total lobar volumes in parentheses.

*Mean values are underlined.

[S]$P < .05$ (see text).

H = hilar portion; M = middle portion; P = peripheral portion.

According to Weibel's (1964) stereologic measurements in the human lung, the total capillary length is 5.4 x 10^6 meters. Length measurements of the larger pulmonary vessels have not been recorded in the human nor any other species. Therefore, the present length measurements must be evaluated in respect to Weibel's capillary data. Such comparison yielded an acceptable correlation between pulmonary blood vessel length measurements in the human and those in the proportionally smaller rabbit.

Vessel lengths of the left and right control lungs were significantly greater than their counterparts in the stimulated preparations. Thus, the bilateral response suggested a passive vessel shortening in response to the decreased cardiac output. However, anatomic volumes of the ink-filled capillaries demonstrated only slight bilateral diminution (greatest in the peripheral segments) after vagal activation. The left and right pulmonary arterial and venous compartmental volumes were also slightly reduced after stimulation. As in the capillary compartment, the peripheral portions displayed the greatest volumetric reductions. Therefore, the vagally induced decrease in cardiac output was evident but not sufficient in magnitude to significantly diminish mean lobar vessel or capillary filling.

The changes in lung ventilation and airway capacities after stimulation paralleled those described in the canine

Figure 25

The rabbit lungs after left vagal stimulation. As shown in the dog and cat, thorough ink perfusion is demonstrated on the surfaces of both left and right lungs. The figure's left column displays the dorsal and ventral surfaces while the lateral lung aspects are shown in the right column.

Figure 25

Figure 26

Average lobar perfusion percentages after left vagal stimulation in the rabbit.

percentages of lungs' surface showing ink perfusion (black color)

Cr cranial lobe

Ca cadual lobe

M middle lobe

I intermediate lobe

Figure 27

Average lobar perfusion percentages after left inferior cervical ganglion stimulation in the rabbit.

percentage black

As in the vagal preparations, each lobe appears predominately black.

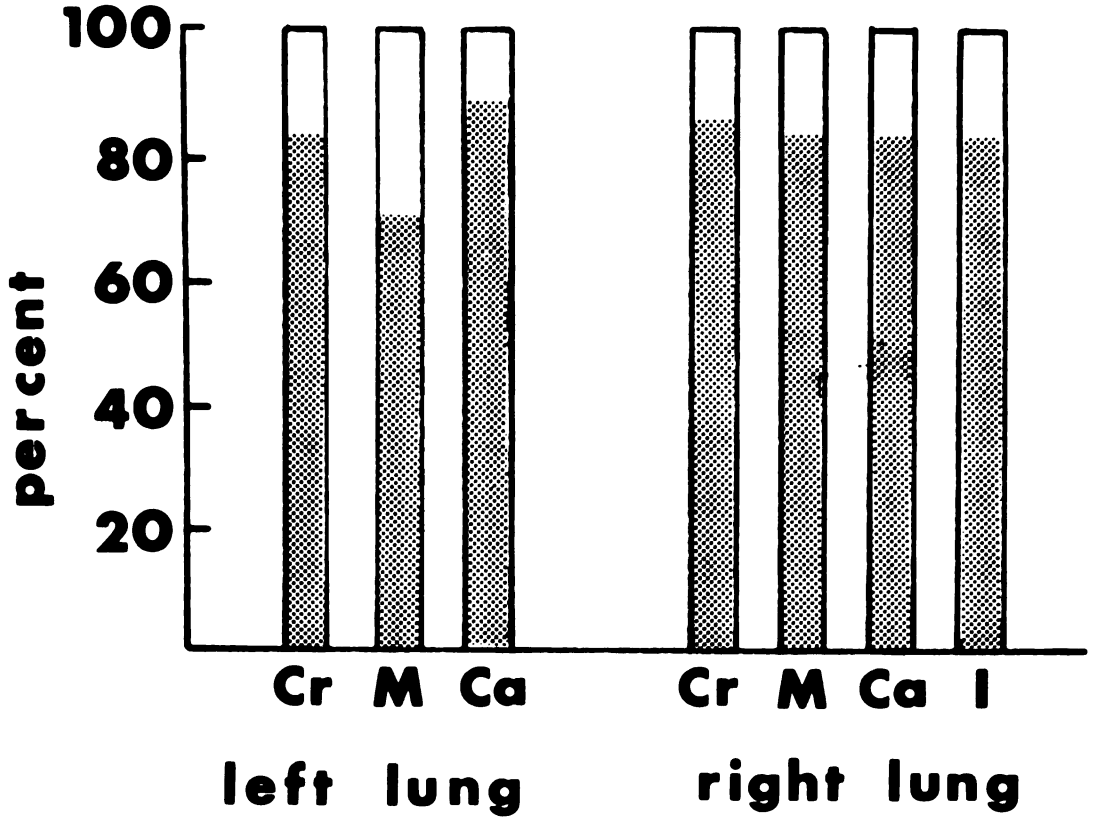


Figure 26

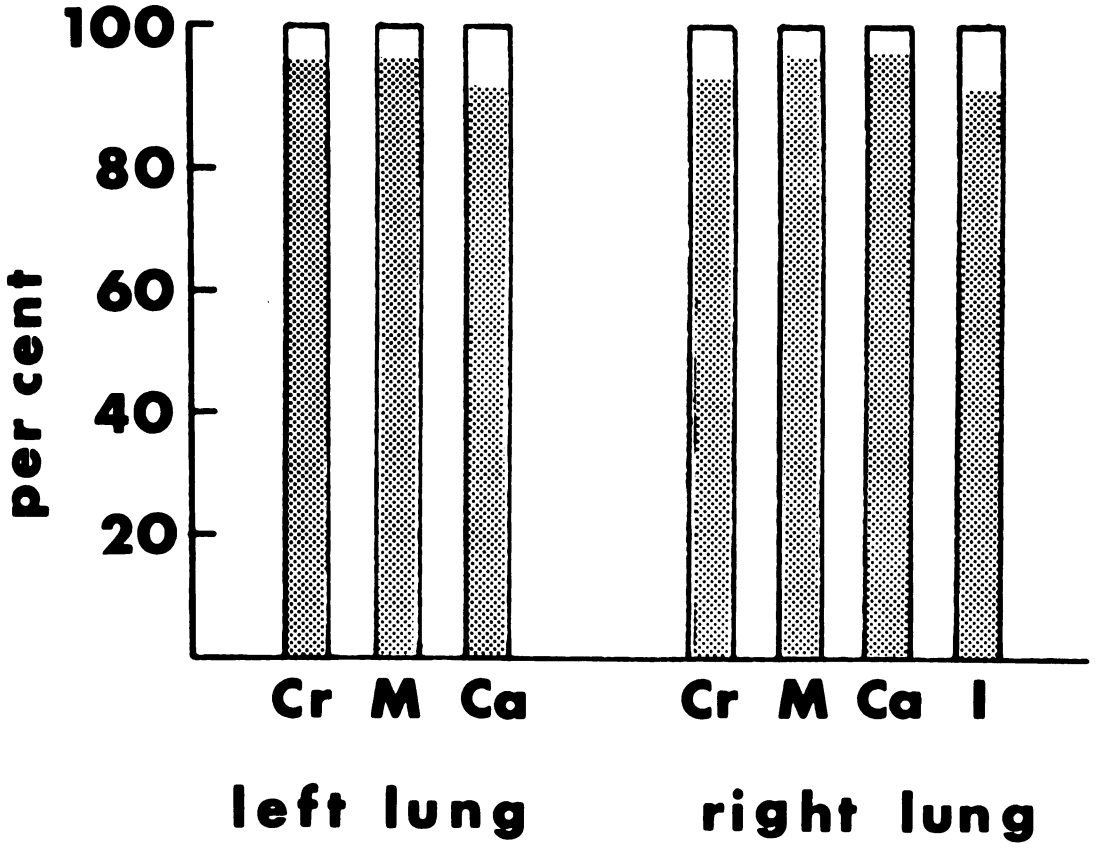


Figure 27

parasympathetic investigations. The virtually unaltered tracheal pressure, minute ventialtion, and airway compartmental volumes were demonstrated in conjunction with the substantially reduced tidal volumes (Tables 5 and 6). Thus, the degree of bronchoconstriction elicited after vagal activation was again inconsequential in passively creating ipsilateral changes in pulmonary vascular perfusion.

In both stimulated and control animals vessel lengths of the right side exceeded those on the left. In addition, the volumes occupied by ink-filled capillaries were substantially less on the left side in both the control and vagally stimulated animals. Thus, as discussed in the cat parasympathetic studies, a greater flow through the right lung appeared to expand its conductive vessels and alveolar capillary compartments. The vessel compartmental volumes, however, failed to display the right to left differences. Hence, the unequal flow patterns changed vessel lengths without substantially effecting vessel capacity. That is, the increased flow through the right side was accommodated by the small intra-alveolar vessels. The diminished volume of the left side's ink-filled capillary compartment also concurred with Engelberg and DuBois' conclusion that significant changes in pulmonary blood volume were primarily felt in the small intrapulmonary vessels.

The present statistical analysis eliminated existing size differences between the left and right lungs. That is,

the greater flow through the right lung was not accounted for by the right side's natural size advantage. The animals were secured in the supine position which also eliminated biased gravitation effects favoring an increased right lung perfusion. Vagal stimulation also failed to alter the right side's predominance. Therefore, physical and mechanical effects were not portrayed as precursors to the uneven flow distribution. Thus, the present studies suggested greater perfusion of the right lung in rabbits. Further experimental evidence would be required to establish this as an inherent tendency.

Sympathetic Studies

Left inferior cervical ganglion stimulation produced an increased mean left ventricular pressure while mean left pulmonary arterial pressure remained virtually unchanged (Table 5 and Fig. 31). That increased cardiac output failed to elevate pulmonary arterial pressure again reflected ample pulmonary reserve capacity. The even bilateral ink distribution (Figs. 27 and 28) after unilateral sympathetic activation established essentially equal perfusion increases in the left and right lungs.

As discussed in the parasympathetic studies, the control animals demonstrated greater vessel lengths and anatomic capillary volumes on the right side. Evaluations of the stereologic measurements (Table 6 and Appendix C)

Figure 28

Even bilateral ink distribution appears after left sympathetic stimulation in the rabbit. The figure's upper half displays the dorsal and ventral views while the lower half depicts the lateral surfaces. This surface perfusion pattern is comparable to the vagal preparations.

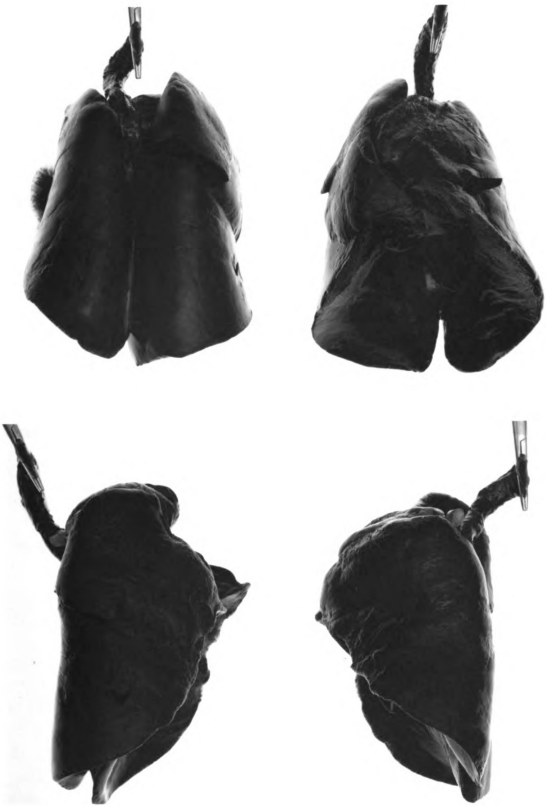


Figure 28

Figure 29

Histologic section from rabbit lung after vagal stimulation. Note the thorough ink-filling on the left and the absence of ink on the right. This microscopic picture is representative of relatively light areas on the lungs' surface. Active precapillary arteriolar constriction is not present as suggested by the staggered microvasculature filling. 225X.

Figure 30

A muscular pulmonary artery, located in the middle segment of the rabbit's middle lobe, is shown. The relatively thick tunica media is characteristic of the rabbit pulmonary vasculature. The patent, ink-filled lumen and the adjacent ink-perfused microvasculature suggest minimal constrictor activity after sympathetic stimulation. 225X.

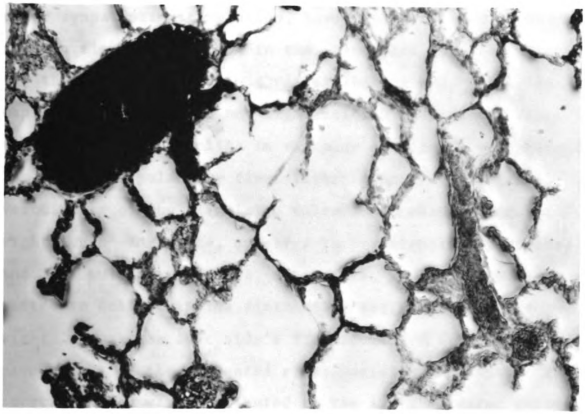


Figure 29

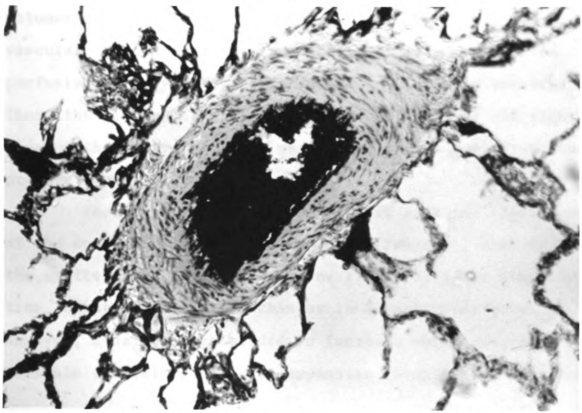


Figure 30

after sympathetic stimulation, however, further emphasized an even flow distribution in the stimulated animals. That is, the left and right lung vessel lengths and ink-filled capillary volumes were not significantly different. Thus, the most complete decline in vascular compliance was present. The increased pulmonary flow, rather than distending the vasculature of the stimulated animals and maintaining the right lungs' dominance, appeared to preferentially traverse the left pulmonary circuit. Therefore, less ink was permitted to collect in the distensible vasculature of the right lung as the left side's flow accounted for greater proportions of the increased right ventricular output. The increased flow was distributed to the intra-alveolar vasculature as evidenced by the virtually equal anatomic capillary volumes on the left and right sides. That is, unilateral vascular stiffening after sympathetic stimulation enabled perfusion of previously closed alveolar capillary networks. Thus, the pattern of greater capillary filling in the right side of the control animals was eliminated in the stimulated preparations.

The comparable airway volumes in left and right lungs of the control and stimulated animals (Table 6), coupled with the unaltered ventilation patterns (Table 5) after stimulation, negated substantial changes in bronchomotor tone. However, tidal volumes tended to increase which suggested a possible anatomic dead space expansion (bronchiolar dilation)

Figure 31

Physiologic pressure measurements during control and stimulation periods in the rabbit.

Legend:

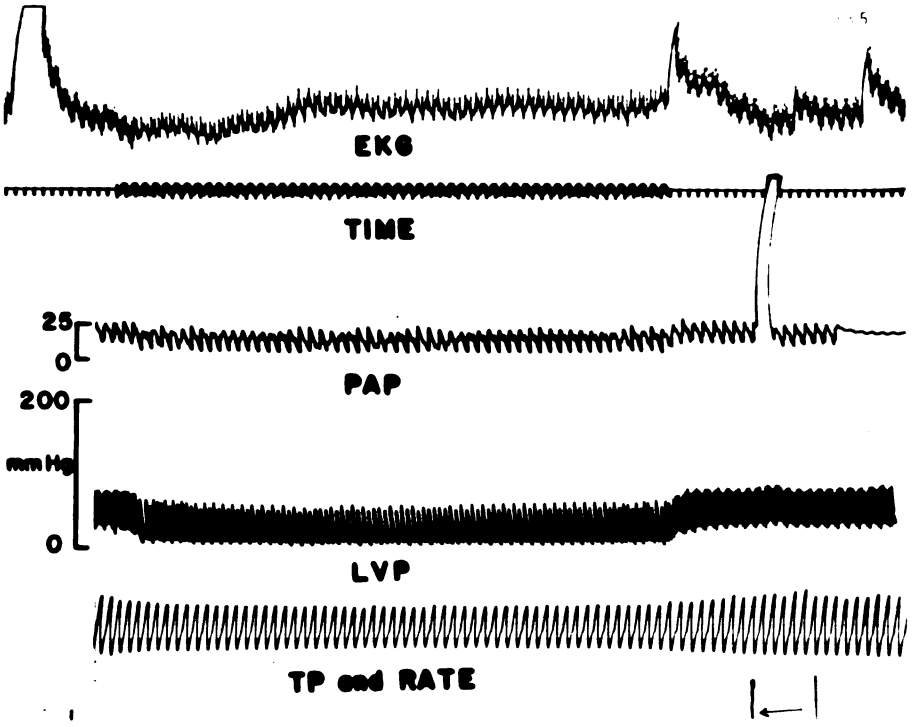
PAP: pulmonary arterial pressure

LVP: left ventricular pressure

TP and RATE: tracheal pressure and respiration frequency

Upper record depicts pressure changes after left vagal stimulation. Note the characteristic pulmonary and left ventricular pressure responses. Tracheal pressure and respiratory frequency were virtually unchanged.

Lower record represents the pressure responses to left inferior cervical ganglion stimulation. Again, the characteristic vascular responses are shown as were previously demonstrated in the dog and cat. The respiratory parameters were also unchanged.


EKG
TIME
25
0
PAP
200
mm Hg
0
LVP
TP and RATE

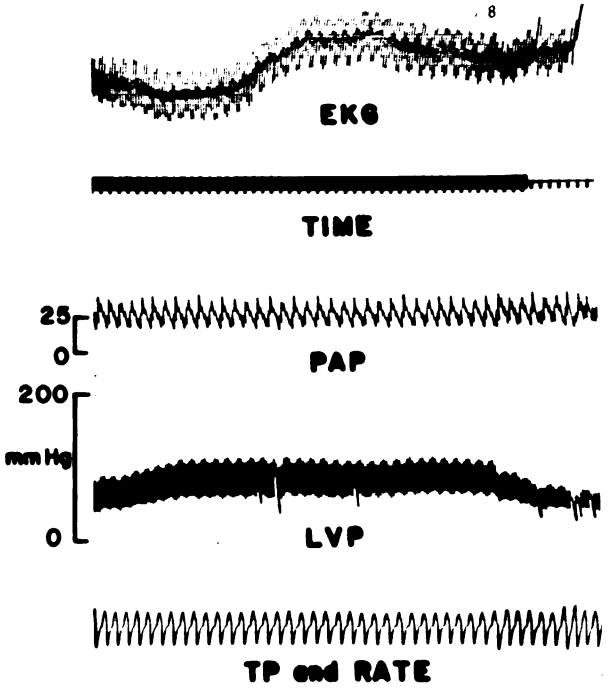

EKG
TIME
25
0
PAP
200
mm Hg
0
LVP
TP and RATE

Figure 31

after sympathetic activation. The volume increases were not significant and furthermore, substantial alterations in vascular perfusion were not demonstrated in the ipsilateral lung. Thus, a consequent pulmonary vascular response to slight bronchomotor tone alteration was also eliminated.

GENERAL DISCUSSION OF TECHNIQUE

The majority of previous investigations suggesting active nervous control of the pulmonary circulation were performed with isolated, artifically ventilated and perfused lungs. The elimination of normal physiologic conditions with these procedures was obvious.

The present studies, employing intact animal preparations, more accurately approximated the natural physiologic condition. However, aberrations from the normal state were included. First, Sharpless (1970) reported that sodium pentobarbital anesthesia depressed the central and peripheral nervous systems. In addition, respiration rate and systemic blood pressure were slightly diminished. Goldberg, Linde, Gaal, Momma, Takahashi, and Sarna (1968) reported minimal pulmonary circulatory response to pentobarbital in intact dogs. This state of general depression was equally felt by the control and stimulated animals. Thus, owing to the absence of direct barbiturate action on the pulmonary vascular bed, the electric nerve stimulations represented the essential variable in the experimental preparations.

The vagal stimulations in the dog also required prior intravenous injections of adrenergic blocking agents. According to Nickerson (1970), phenoxybenzamine, the

alpha-receptor blocker, caused little change in systemic blood pressure with moderate increases in cardiac output and slight decreases in total peripheral resistance in normal laboratory animals. Minor shifts of blood volume from the pulmonary to systemic vascular bed, due to a greater reduction of tone in the latter, were also reported. However, direct actions on the pulmonary vasculature were not demonstrated. In addition, Nickerson reported reduction in heart rate and mild systemic blood pressure decreases after injections of propranolol, the beta-receptor blocker. The cardiac depression was considered minimal in the resting animal, but more profound under stressful conditions. Again, a direct effect on the pulmonary circulation was not reported. Thus, the combined adrengic receptor blockade failed to establish critical pulmonary vascular reactivity while antagonizing its sympathetic input during the vagal stimulations.

Sympathetic activation in the dog, cat, and rabbit were also performed in conjunction with administration of atropine, an acetylcholine antagonist. According to Innes and Nickerson (1970), atropine inhibited the actions of acetylcholine on structures innervated by postganglionic cholinergic nerves while transmission at the autonomic ganglia or skeletal neuromuscular junctions was unaffected. In addition, atropine produced mild heart rate increases without accompanying changes in blood pressure or cardiac output in laboratory animals. Its direct effect on systemic and

pulmonary vessels was also minimal. The authors attributed the slight cardiovascular effects to sparse parasympathetic innervation of most vascular beds. Thus, the effects of sympathetic stimulation on the pulmonary vasculature were observed without initial vascular reactivity to atropine.

Finally, the intraventricular carbachol injections, which produced an immediate cardiac arrest and thereby enabled ink retention within the pulmonary circulation, required further discussion. Access of this parasympathomimetic agent to the pulmonary circulation was not controlled. That is, a single left ventricular contraction, after carbachol injection, suggested its presence in the pulmonary vascular bed, via the bronchial-pulmonary capillary anastomoses. The potential amount and concentration within the pulmonary system appeared extremely small and its possible effects would have arisen bilaterally. Thus, interference with interpretation of the nervous stimulation effects remained minimal.

SUMMARY AND CONCLUSIONS

The effects of vagal and sympathetic stimulation on the pulmonary circulation were observed in closed-chest dogs, cats, and rabbits. Pulmonary arterial and left ventricular catheterization provided records of the respective stimulation-induced pressure responses. Concomitant measurements of minute ventilation and tracheal pressure were also recorded. Thus, the sources of passive pulmonary vascular activity, namely cardiomotor and bronchomotor tone, were monitored. Secondary biologic Pelikan ink injections served to isolate the site(s) within the pulmonary vasculature responsible for any changes in pulmonary vascular pressures. Observations of ink perfusion patterns on the gross lung's surface and stereologic evaluations of the serial-sectioned middle lobes enabled final correlation between pulmonary flow distribution and the nerve stimulations.

Unilateral vagal stimulation produced bilateral decreases in pulmonary vascular perfusion. Pulmonary arterial pressure and tidal volumes were also diminished. Thus, the pulmonary vascular bed passively responded to the vagally-induced decline in cardiac output. Because minute ventilation and tracheal pressure were essentially unchanged,

the moderate bronchoconstriction (tidal volume decreased) contributed little to the passive response. This indirect vagal control of the pulmonary circulation was most evident in the cat. The regulatory mechanism was less profound in the dog and rabbit.

The unilateral sympathetic ganglion stimulations produced ipsilateral decreases in pulmonary vascular compliance. The sympathetically-induced elevation in cardiac output failed to distend the pulmonary vascular tree while alveolar capillary perfusion tended to increase. Pulmonary arterial pressure and ventilation patterns were virtually unchanged. Thus, the increased pulmonary perfusion appeared to utilize the vascular reserve capacity rather than to passively dilate the normally perfused channels. This vessel stiffening was more prominent in the rabbit than in either the dog or cat.

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APPENDICES

APPENDIX A

(DOG)

Individual Volumes and Pressures (Dog)

	Control	LVS*	Control	LMCGS*
PAP (mmHg)				
1.	20/8 (15)	15/5 (12)	25/15 (12)	25/15 (15)
2.	20/8 (15)	18/10 (12)	18/10 (15)	22/10 (15)
3.	26/12 (20)	22/12 (15)	40/25 (24)	35/20 (24)
4.	35/18 (25)	30/10 (18)	24/10 (15)	20/10 (15)
5.	25/12 (20)	25/12 (18)	20/10 (15)	20/10 (15)
LVP (mmHg)				
1.	100/5 (55)	75/5 (40)	75/10 (45)	120/15 (68)
2.	125/105	100/70	150/20 (85)	168/10 (89)
3.	175/10 (92)	148/5 (78)	115/10 (62)	125/10 (68)
4.	100/10 (55)	75/10 (42)	135/10 (72)	150/10 (80)
5.	100/10 (55)	80/10 (45)	135/15 (75)	145/10 (80)
HR (beats/min)				
1.	84	48	148	210
2.	86	57	150	172
3.	110	60	165	180
4.	94	62	132	148
5.	52	36	120	148
TP (cmH_2O)				
1.	14	8	8	10
2.	9	11	22	28
3.	15	13	9	13
4.	15	10	10	14
5.	10	10	15	17

Individual Volumes and Pressures (Dog)--Continued

	Control	LVS*	Control	LMCGS*
$\dot{V}_E$ (l/min)				
1.	2.00	1.75	3.20	3.00
2.	2.05	3.00	2.60	3.98
3.	2.50	2.45	4.80	4.30
4.	2.80	2.65	2.80	3.75
5.	0.60	0.55	6.95	7.40
f (breaths/min)				
1.	14	13	30	33
2.	12	18	18	23
3.	10	12	66	45
4.	16	16	25	37
5.	4	4	40	46
V_T (ml/breath)				
1.	143	135	107	91
2.	171	166	144	178
3.	250	175	73	96
4.	175	166	112	103
5.	150	138	174	162

*LVS = left vagal stimulation.
LMCGS = left middle cervical ganglion stimulation.

Lobar Perfusion Percentages (Dogs)
(Percent black)

	Cranial		Middle		Caudal		Intermed.
	L.	R.	L.	R.	L.	R.	R.
Control							
1. (20)*	100%	95%	100%	100%	95%	95%	90%
2. (20)	95	95	95	95	100	100	100
LVS							
1. (20)	100	100	100	95	100	100	100
2. (20)	90	50	90	30	85	95	50
3. (20)	100	95	100	95	100	100	100
4. (25)	100	100	100	100	100	100	100
5. (15)	100	100	95	95	100	100	100
LMCGS							
1. (15)	100	95	100	95	95	100	75
2. (15)	100	95	100	95	100	100	85
3. (20)	100	100	100	100	100	100	100
4. (15)	90	85	90	95	80	90	80
5. (15)	100	95	100	100	100	100	100

*Dog body weights (lbs.).

Stereologic Count Totals (Dogs)
(Middle Lobes)

Total Lobar Volumes (Water Displacement)		
	Left Lobe	Right Lobe
Control	25cc	35cc
Parasympathetic		
1.	25	35
2.	25	35
3.	20	30
Mean	23cc	33cc
Sympathetic		
1.	15	25
2.	20	30
3.	20	30
Mean	18cc	28cc

Stereologic Counts	Control		Vagal		Sympathetic	
	L.	R.	L.	R.	L.	R.
Sections Observed	236	217	259	270	221	184
Total Counts	652	602	706	739	634	529
Total Possible "Hits"	27,384	25,284	29,652	31,038	26,628	22,218

Airway Volume Fractions in Dog Lungs

	Control		Parasympathetic		Sympathetic	
	L.	R.	L.	R.	L.	R.
Hilar Portion						
Counts	211	212	195	243	217	169
"Hits"	395	547	237	557	622	439
Possible "Hits"	8,862	8,526	8,190	10,206	9,114	7,098
Fraction	.0443	.0669	.0289	.0238	.0682	.0618
Middle Portion						
Counts	222	204	297	270	210	180
"Hits"	694	516	443	324	299	211
Possible "Hits"	9,324	8,568	12,474	11,340	8,820	7,560
Fraction	.0745	.0602	.0355	.0285	.0338	.0279
Peripheral Portion						
Counts	219	186	214	226	207	180
"Hits"	279	268	200	164	186	205
Possible "Hits"	9,198	8,442	8,988	9,492	8,694	7,560
Fraction	.0303	.0317	.0222	.0172	.0214	.0271
Mean Lobar Values						
Counts	652	602	706	739	634	529
"Hits"	1,368	1,331	880	1,045	1,107	855
Possible "Hits"	27,384	25,284	29,652	31,038	26,628	22,218
Fraction	.0476	.0529	.0289	.0232	.0412	.0389

Large Vessel Volume Fractions in Dog Lungs

	Control		Parasympathetic		Sympathetic	
	L.	R.	L.	R.	L.	R.
Hilar Portion						
Counts	211	212	195	243	217	169
"Hits"	405	1,480	907	1,639	845	516
Possible "Hits"	8,862	8,526	8,190	10,206	9,114	7,098
Fraction	.0457	.1811	.1107	.1605	.0927	.0726
Middle Portion						
Counts	222	204	297	270	210	180
"Hits"	733	715	1,164	872	748	588
Possible "Hits"	9,324	8,568	12,474	11,340	8,820	7,560
Fraction	.0786	.0833	.0933	.0768	.0848	.0778
Peripheral Portion						
Counts	219	186	214	226	207	180
"Hits"	665	606	474	471	456	272
Possible "Hits"	9,198	8,442	8,988	9,492	8,694	7,560
Fraction	.0723	.0719	.0527	.0496	.0524	.0359
Mean Lobar Values						
Counts	652	602	706	739	634	529
"Hits"	1,803	2,801	2,545	2,982	2,049	1,376
Possible "Hits"	27,384	25,284	29,652	31,038	26,628	22,218
Fraction	.0657	.1107	.0856	.0956	.0769	.0619

Large Vessel Relative Lengths* in Dog Lungs

	Control		Parasympathetic		Sympathetic	
	L.	R.	L.	R.	L.	R.
Hilar Portion						
Counts	211	212	195	243	217	169
Profile Counts	376	551	608	568	630	383
Mean Profiles	1.782	2.714	3.117	2.337	2.903	2.266
Middle Portion						
Counts	222	204	297	270	210	180
Profile Counts	574	584	786	641	679	435
Mean Profiles	2.585	2.714	2.646	2.374	3.233	2.416
Peripheral Portion						
Counts	219	186	214	226	207	180
Profile Counts	793	552	549	485	709	396
Mean Profiles	3.621	2.746	2.565	2.146	3.425	2.200
Mean Lobar Values						
Counts	652	602	706	739	634	529
Profile Counts	1,743	1,684	1,943	1,694	2,018	1,214
Mean Profiles	2.673	2.784	2.776	2.286	3.189	2.284

*Length/$_{volume}$ $(L_V) = 2. \frac{\text{Mean Profiles (P)}}{\text{Test Square Area (A)}}$

Ink-Filled Capillary Volume Fractions in Dog Lungs

	Control		Parasympathetic		Sympathetic	
	L.	R.	L.	R.	L.	R.
Hilar Portion						
Counts	211	212	195	243	217	169
"Hits"	2,270	1,610	1,959	1,951	2,719	2,085
Possible "Hits"	8,862	8,526	8,190	10,206	9,114	7,098
Fraction	.2562	.1967	.2391	.1911	.2983	.2937
Middle Portion						
Counts	222	204	297	270	210	180
"Hits"	2,582	2,453	3,040	2,941	3,167	2,576
Possible "Hits"	9,324	8,568	12,474	11,340	8,820	7,560
Fraction	.2769	.2863	.2437	.2593	.3591	.3407
Peripheral Portion						
Counts	219	186	214	226	207	180
"Hits"	3,450	2,554	2,396	2,281	3,102	2,779
Possible "Hits"	9,198	8,442	8,988	9,492	8,694	7,560
Fraction	.3750	.3025	.2665	.2403	.3453	.3675
Mean Lobar Values						
Counts	652	602	706	739	634	529
"Hits"	8,302	6,617	7,395	7,173	8,988	7,440
Possible "Hits"	27,384	25,284	29,652	31,038	26,628	22,218
Fraction	.3027	.2618	.2498	.2302	.3375	.3359

Individual Volumes and Pressures (Cat)

	Control	LVS*	Control	LSGS*
PAP (mmHg)				
1.	22/15 (20)	20/8 (16)	18/12 (20)	26/16 (22)
2.	25/12 (18)	25/10 (15)	22/15 (20)	25/16 (22)
3.	20/12 (15)	20/10 (12)	15/10 (15)	18/12 (17)
4.	20/10 (16)	18/8 (13)	25/12 (17)	26/16 (20)
5.	20/12 (15)	20/12 (15)		
LVP (mmHg)				
1.	95/30 (62)	80/10 (45)	110/15 (62)	150/15 (82)
2.	165/25 (95)	132/10 (72)	140/25 (82)	180/40(111)
3.	140/15 (78)	100/10 (55)	85/10 (48)	100/20 (60)
4.	120/10 (65)	110/5 (58)	150/35 (92)	160/32 (95)
5.	150/25 (88)	125/15 (70)		
HR (beats/min)				
1.	180	120	150	175
2.	120	85	155	180
3.	120	65	160	185
4.	72	60	150	165
5.	120	105		
TP (cmH_2O)				
1.	6	5	8	15
2.	10	8	6	6
3.	10	12	10	12
4.	10	14	6	7
5.	13	15		

Individual Volumes and Pressures (Cat)--Continued

	Control	LVS*	Control	LSGS*
$\dot{V}_E$ (l/min)				
1.	.55	.50	1.00	1.85
2.	.85	.80	1.50	1.15
3.	1.05	1.25	1.90	2.00
4.	.60	.80	1.75	1.25
5.	1.20	1.55		
f (breaths/min)				
1.	17	10	16	14
2.	18	15	22	24
3.	20	21	22	24
4.	19	21	42	40
5.	17	19		
V_T (ml/breath)				
1.	40	32	62	72
2.	47	47	68	46
3.	60	52	86	83
4.	35	32	42	30
5.	82	71		

*LVS = left vagal stimulation.
LSGS = left stellate ganglion stimulation.

Lobar Perfusion Percentages (Cats)
(Percent black)

	Cranial		Middle		Caudal		Intermed.
	L.	R.	L.	R.	L.	R.	R.
Control							
1. (8)*	95%	100%	95%	95%	90%	100%	90%
2. (8)	80	100	95	95	95	95	90
LVS							
1. (9)	100	100	75	75	100	100	100
2. (8)	100	100	100	100	100	100	100
3. (8)	100	100	100	100	100	100	100
4. (9)	100	100	100	100	100	100	100
5. (9)	95	95	100	95	90	75	70
LSGS							
1. (8)	100	100	95	95	100	95	85
2. (8)	90	95	90	85	95	95	95
3. (9)	100	95	95	95	95	100	95
4. (8)	95	95	85	95	90	95	95

*Cat body weights (lbs.).

Stereologic Count Totals (Cats)
(Middle Lobes)

Total Lobar Volumes (Water Displacement)		
	Left Lobe	Right Lobe
Control	9cc	11cc
Parasympathetic		
1.	8	11
2.	7	10
3.	8	10
Mean	8cc	10cc
Sympathetic		
1.	7	10
2.	7	10
3.	8	10
Mean	7cc	10cc

Stereologic Counts	Control		Vagal		Sympathetic	
	L.	R.	L.	R.	L.	R.
Sections Observed	159	195	163	181	150	159
Total Counts	439	555	388	502	435	456
Total Possible "Hits"	18,438	23,310	16,296	21,084	18,270	19,152

Airway Volume Fractions in Cat Lungs

	Control		Parasympathetic		Sympathetic	
	L.	R.	L.	R.	L.	R.
Hilar Portion						
Counts	148	186	129	165	147	153
"Hits"	361	510	88	121	380	541
Possible "Hits"	6,216	7,812	5.418	6,930	6,174	6,426
Fraction	.0581	.0653	.0162	.0174	.0615	.0842
Middle Portion						
Counts	147	183	144	183	144	153
"Hits"	292	296	238	166	335	273
Possible "Hits"	6,174	7,686	6,048	7,686	6,048	6,426
Fraction	.0473	.0385	.0393	.0215	.0553	.0425
Peripheral Portion						
Counts	144	186	115	154	144	150
"Hits"	91	223	97	65	110	234
Possible "Hits"	6,048	7,812	4,830	6,468	6,048	6,300
Fraction	.0148	.0285	.0200	.0100	.0182	.0371
Mean Lobar Values						
Counts	439	555	388	502	435	456
"Hits"	744	1,029	423	352	825	1,048
Possible "Hits"	18,438	23,310	16,296	21,084	18,270	19,152
Fraction	.0403	.0441	.0252	.0163	.0451	.0547

Large Vessel Volume Fractions in Cat Lungs

	Control		Parasympathetic		Sympathetic	
	L.	R.	L.	R.	L.	R.
Hilar Portion						
Counts	148	186	129	165	147	153
"Hits"	532	563	368	372	389	302
Possible "Hits"	6,216	7,812	5,418	6,930	6,174	6,426
Fraction	.0855	.0721	.0679	.0536	.0630	.0469
Middle Portion						
Counts	147	183	144	183	144	153
"Hits"	649	1,010	525	629	338	512
Possible "Hits"	6,174	7,686	6,048	7,686	6,048	6,426
Fraction	.1051	.1314	.0868	.0818	.0558	.0796
Peripheral Portion						
Counts	144	186	115	154	144	150
"Hits"	355	525	202	195	286	295
Possible "Hits"	6,048	7,812	4,830	6,468	6,048	6,300
Fraction	.0587	.0672	.0418	.0301	.0472	.0468
Mean Lobar Values						
Counts	439	555	388	502	435	456
"Hits"	1,536	2,098	1,095	1,196	1,013	1,109
Possible "Hits"	18,438	23,310	16,296	21,084	18,270	19,152
Fraction	.0831	.0902	.0655	.0552	.0554	.0578

Large Vessel Relative Lengths* in Cat Lungs

	Control		Parasympathetic		Sympathetic	
	L.	R.	L.	R.	L.	R.
Hilar Portion						
Counts	148	186	129	165	147	153
Profile Counts	454	640	321	489	550	513
Mean Profiles	3.067	3.660	2.488	2.964	3.741	3.353
Middle Portion						
Counts	147	183	144	183	144	153
Profile Counts	521	681	339	437	560	552
Mean Profiles	3.612	3.716	2.354	2.278	3.889	3.607
Peripheral Portion						
Counts	144	186	115	154	144	150
Profile Counts	654	865	294	309	621	628
Mean Profiles	4.541	4.650	2.556	2.006	4.312	4.186
Mean Lobar Values						
Counts	439	555	388	502	435	456
Profile Count	1,629	2,186	954	1,235	1,731	1,693
Mean Profile	3.740	4.008	2.466	2.416	3.981	3.715

*Length/$_{volume}$ $(L_v) = 2. \frac{\text{Mean Profiles (P)}}{\text{Test Square Area (A)}}$

Ink-Filled Capillary Volume Fractions in Cat Lungs

	Control		Parasympathetic		Sympathetic	
	L.	R.	L.	R.	L.	R.
Hilar Portion						
Counts	148	186	129	165	147	153
"Hits"	1,480	1,699	986	1,350	1,527	1,469
Possible "Hits"	6,216	7,812	5,418	6,930	6,174	6,426
Fraction	.2218	.2174	.1819	.1948	.2473	.2286
Middle Portion						
Counts	147	183	144	183	144	153
"Hits"	1,588	1,916	941	1,262	1,538	1,309
Possible "Hits"	6,174	7,686	6,048	7,686	6,048	6,426
Fraction	.2572	.2492	.1555	.1641	.2543	.2037
Peripheral Portion						
Counts	144	186	115	154	144	150
"Hits"	1,645	2,046	727	1,090	1,588	1,426
Possible "Hits"	6,048	7,812	4,830	6,468	6,048	6,300
Fraction	.2719	.2619	.1505	.1685	.2625	.2263
Mean Lobar Values						
Counts	439	555	388	502	435	456
"Hits"	4,713	5,661	2,654	3,702	4,653	4,204
Possible "Hits"	18,438	23,310	16,296	21,084	18,270	19,152
Fraction	.2556	.2428	.1626	.1758	.2557	.2191

APPENDIX C

(RABBIT)

Individual Volumes and Pressures (Rabbit)

	Control	LVS*	Control	LICGS*
PAP (mmHg)				
1.	28/10 (20)	22/8 (15)	28/12 (20)	30/14 (20)
2.	30/15 (18)	20/10 (12)	25/10 (20)	25/12 (22)
3.	25/8 (18)	25/10 (18)	25/10 (27)	32/20 (30)
4.	25/5 (17)	25/8 (12)		
5.	20/5 (15)	17/3 (10)		
LVP (mmHg)				
1.	80/50 (65)	70/42 (56)	80/25 (52)	86/42 (64)
2.	75/25 (50)	54/10 (32)	75/25 (50)	80/28 (54)
3.	120/25 (72)	120/30 (75)	80/45 (62)	92/50 (71)
4.	135/30 (82)	125/10 (68)		
5.	95/15 (58)	75/5 (40)		
HR (beats/min)				
1.	195	145	220	245
2.	135	110	200	285
3.	160	144	210	245
4.	240	185		
5.	210	95		
TP (cmH_2O)				
1.	11	13	10	6
2.	12	12	6	7
3.	8	10	8	8
4.	22	14		
5.	24	15		

Individual Volumes and Pressures (Rabbit)--Continued

	Control	LVS*	Control	LICGS*
$\dot{V}_E$ (l/min)				
1.	1.80	1.90	1.22	1.24
2.	2.80	2.04	1.94	2.46
3.	1.70	1.85	2.38	1.85
4.	3.30	2.89		
5.	3.60	3.30		
f (breaths/min)				
1.	46	43	42	56
2.	70	56	64	94
3.	48	50	52	48
4.	62	70		
5.	72	78		
V_T (ml/breath)				
1.	39.1	44.4	29.0	22.1
2.	40	36.8	30.6	27.4
3.	35.4	37.0	45.8	25.6
4.	53.2	42.3		
5.	50	41.2		

*LVS = left vagal stimulation.
LICGS = left inferior cervical ganglion stimulation.

Lobar Perfusion Percantages (Rabbits)
(Percent black)

	Cranial		Middle		Caudal		Intermed.
	L.	R.	L.	R.	L.	R.	R.
Control							
1. (7)*	95%	90%	90%	90%	90%	90%	80%
2. (7)	100	100	100	100	100	100	100
LVS							
1. (8)	60	50	55	10	95	50	30
2. (8)	95	80	70	75	75	90	90
3. (8)	100	100	70	100	100	100	95
4. (8)	80	95	70	70	90	60	85
5. (8)	95	90	75	75	95	80	80
LICGS							
1. (7)	95	90	90	90	80	80	90
2. (7)	95	90	90	90	95	95	80
3. (7)	85	90	90	95	90	95	90

*Rabbit body weights (lbs.).

Stereologic Count Totals (Rabbits)
(Middle Lobes)

Total Lobar Volumes (Water Displacement)	Left Lobe	Right Lobe
Control	9cc	11cc
Parasympathetic		
1.	9	12
2.	9	11
3.	9	12
Mean	9cc	12cc
Sympathetic		
1.	6	9
2.	7	10
3.	7	10
Mean	7cc	10cc

Stereologic Counts	Control		Vagal		Sympathetic	
	L.	R.	L.	R.	L.	R.
Sections Observed	120	133	160	196	141	120
Total Counts	338	368	354	522	387	330
Total Possible "Hits"	14,196	15,456	14,868	21,924	16,254	13,860

Airway Volume Fractions in Rabbit Lungs

	Control		Parasympathetic		Sympathetic	
	L.	R.	L.	R.	L.	R.
Hilar Portion						
Counts	107	122	120	177	129	108
"Hits"	590	471	260	529	392	335
Possible "Hits"	4,494	5,124	5,040	7,434	5,418	4,536
Fraction	.0518	.0772	.0515	.0711	.0723	.0738
Middle Portion						
Counts	117	123	141	198	129	111
"Hits"	480	687	233	377	428	292
Possible "Hits"	4,914	5,166	5,922	8,316	5,418	4,662
Fraction	.0403	.0230	.0393	.0453	.0789	.0738
Peripheral Portion						
Counts	114	123	93	147	129	111
"Hits"	392	474	92	126	195	129
Possible "Hits"	4,788	5,166	3,906	6,147	5,418	4,662
Fraction	.0273	.0284	.0235	.0204	.0359	.0276
Mean Lobar Valves						
Counts	338	368	354	522	387	330
"Hits"	572	662	585	1,032	1,015	756
Possible "Hits"	14,196	15,456	14,868	21,924	16,254	13,860
Fraction	.0403	.0429	.0381	.0463	.0624	.0544

Large Vessel Volume Fractions in Rabbit Lungs

	Control		Parasympathetic		Sympathetic	
	L.	R.	L.	R.	L.	R.
Hilar Portion						
Counts	107	122	120	177	129	108
"Hits"	590	471	411	663	453	388
Possible "Hits"	4,494	5,124	5,040	7,434	5,418	4,536
Fraction	.1312	.0919	.0815	.0892	.0836	.0851
Middle Portion						
Counts	117	123	141	198	129	111
"Hits"	480	687	474	868	333	276
Possible "Hits"	4,914	5,166	5,922	8,316	5,418	4,662
Fraction	.0956	.1329	.0800	.1044	.0614	.0592
Peripheral Portion						
Counts	114	123	93	147	129	111
"Hits"	392	474	182	275	325	148
Possible "Hits"	4,788	5,166	3,906	6,174	5,418	4,662
Fraction	.0818	.0917	.0466	.0445	.0599	.0317
Mean Lobar Values						
Counts	338	368	354	522	387	330
"Hits"	1,462	1,632	1,067	1,806	1,111	812
Possible "Hits"	14,196	15,456	14,868	21,924	16,254	13,860
Fraction	.1029	.1054	.0693	.0793	.0683	.0586

Large Vessel Relative Lengths* in Rabbit Lungs

	Control		Parasympathetic		Sympathetic	
	L.	R.	L.	R.	L.	R.
Hilar Portion						
Counts	107	122	120	177	129	108
Profile Counts	438	585	408	624	416	409
Mean Profiles	4.093	4.795	3.400	3.525	3.224	3.786
Middle Portion						
Counts	117	123	141	198	129	111
Profile Counts	467	583	464	632	483	446
Mean Profiles	3.991	4.739	3.291	3.192	3.744	4.018
Peripheral Portion						
Counts	114	123	93	147	129	111
Profile Counts	557	586	307	453	541	326
Mean Profiles	4.886	4.763	3.236	3.081	4.193	2.937
Mean Lobar Values						
Counts	338	368	354	522	387	330
Profile Counts	1,462	1,754	1,179	1,709	1,440	1,181
Mean Profiles	4.323	4.765	3.309	3.266	3.720	3.583

*$\text{Length}/_{\text{volume}}$ $(L_v) = 2. \frac{\text{Mean Profiles (P)}}{\text{Test Square Area (A)}}$

Ink-Filled Capillary Volume Fractions in Rabbit Lungs

	Control		Parasympathetic		Sympathetic	
	L.	R.	L.	R.	L.	R.
Hilar Portion						
Counts	107	122	120	177	129	108
"Hits"	1,451	1,711	1,446	2,109	1,825	1,571
Possible "Hits"	4,494	5,124	5,040	7,434	5,418	4,536
Fraction	.3228	.3339	.2869	.2836	.3368	.3463
Middle Portion						
Counts	117	123	141	198	129	111
"Hits"	1,506	1,864	1,688	2,581	2,141	1,617
Possible "Hits"	4,914	5,166	5,922	8,316	5,418	4,662
Fraction	.3064	.3569	.2850	.3103	.3951	.3468
Peripheral Portion						
Counts	114	123	93	147	129	111
"Hits"	1,708	1,953	946	1,837	1,958	1,363
Possible "Hits"	4,788	5,166	3,906	6,174	5,418	4,662
Fraction	.3567	.3780	.2422	.2975	.3614	.2923
Mean Lobar Values						
Counts	338	368	354	522	387	330
"Hits"	4,685	5,528	4,080	6,527	5,924	4,551
Possible "Hits"	14,196	15,456	14,868	21,924	16,254	13,860
Fraction	.3286	.3563	.2713	.2971	.3644	.3271

APPENDIX D

ANALYSIS OF COVARIANCE TABLES FOR DOG, CAT, AND RABBIT

Dog Parasympathetic Trials (Analysis of Covariance Tables)

Source of Variation	sxx	sxy	syy	$syy - \frac{(sxy)^2}{sxx}$	df	MS	F
Airway Volumes:							
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:							
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:							
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				
Capillary Volume:							
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				

Cat Parasympathetic Trials (Analysis of Covariance Tables)

Source of Variation	sxx	sxy	syy	$syy - \frac{(sxy)^2}{sxx}$	df	MS	F
Airway Volumes:							
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:							
among treatments	15	5.33	3.29	1.40	3	.467	4.67*
within treatments	0	0	.70	.70	7	.100	
totals	15	5.33	3.99				
Vessel Volume:							
among treatments	15	1.64	.36	.18	3	.060	0.82
within treatments	0	0	.51	.51	7	.073	
totals	15	1.64	.87				
Capillary Volume:							
among treatments	15	5.41	3.13	1.18	3	.393	8.35*
within treatments	0	0	.33	.33	7	.047	
totals	15	5.41	3.46				

*Analysis continued.

Cat Parasympathetic Trials--Continued

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

μ_1 = left lung of control animal

μ_2 = right lung of control animal

μ_3 = left lung of stimulated animal

μ_4 = right lung of stimulated animal

I am 95% confident that the following hold simultaneously:

Vessel Length	Capillary Volume
$-1.47 \leq \mu_1-\mu_2 \leq .37$	$-1.02 \leq \mu_1-\mu_2 \leq .28$
$-.11 \leq \mu_1\ \mu_3 \leq 1.73$	$.34 \leq \mu_1\ \mu_3 \leq 1.64$
$-.38 \leq \mu_1\ \mu_4 \leq 1.46$	$-.12 \leq \mu_1\ \mu_4 \leq 1.18$
$.44 \leq \mu_2\ \mu_3 \leq 2.28$	$.71 \leq \mu_2\ \mu_3 \leq 2.01$
$.17 \leq \mu_2\ \mu_4 \leq 2.01$	$.25 \leq \mu_2\ \mu_4 \leq 1.55$
$-1.19 \leq \mu_3\ \mu_4 \leq .65$	$-1.11 \leq \mu_3\ \mu_4 \leq .19$

Rabbit Parasympathetic Trials (Analysis of Covariance Tables)

Source of Variation	sxx	sxy	syy	$syy-\frac{(sxy)^2}{sxx}$	df	MS	F
Airway Volumes:							
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:							
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:							
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				
Capillary Volumes:							
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				

*Analysis continued.

Rabbit Parasympathetic Trials--Continued

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

μ_1 = left lung of control animal

μ_2 = right lung of control animal

μ_3 = left lung of stimulated animal

μ_4 = right lung of stimulated animal

I am 95% confident that the following hold simultaneously:

Vessel Length	Capillary Volume
$-1.24 \leq \mu_1-\mu_2 \leq -.30$	$-1.67 \leq \mu_1-\mu_2 \leq -.25$
$.03 \leq \mu_1\ \mu_3 \leq .97$	$-.19 \leq \mu_1\ \mu_3 \leq 1.23$
$-.50 \leq \mu_1\ \mu_4 \leq .44$	$-1.24 \leq \mu_1\ \mu_4 \leq .18$
$.80 \leq \mu_2\ \mu_3 \leq 1.74$	$.77 \leq \mu_2\ \mu_3 \leq 2.19$
$.27 \leq \mu_2\ \mu_4 \leq 1.21$	$-.28 \leq \mu_2\ \mu_4 \leq 1.14$
$-1.00 \leq \mu_3\ \mu_4 \leq -.06$	$-1.76 \leq \mu_3\ \mu_4 \leq -.34$

Dog Sympathetic Trials (Analysis of Covariance Tables)

Source of Variation	sxx	sxy	syy	$syy-\frac{(sxy)^2}{sxx}$	df	MS	F
Airway Volumes:							
among treatments	447	28.93	2.08	.21	3	.070	0.25
within treatments	0	0	1.95	1.95	7	.264	
total	447	28.93	4.03				
Vessel Lengths:							
among treatments	447	55.92	8.85	1.86	3	.620	0.45
within treatments	0	0	9.71	9.71	7	1.39	
total	447	55.92	18.56				
Vessel Volumes:							
among treatments	447	65.30	12.47	2.93	3	.980	0.66
within treatments	0	0	10.33	10.33	7	1.48	
total	447	65.30	22.80				
Capillary Volumes:							
among treatments	447	89.40	22.42	4.54	3	1.51	0.67
within treatments	0	0	15.83	15.83	7	2.26	
total	447	89.40	38.25				

Cat Sympathetic Trials (Analysis of Covariance Tables)

Source of Variation	sxx	sxy	syy	$syy - \frac{(sxy)^2}{sxx}$	df	MS	F
Airway Volumes:							
among treatments	26.25	1.39	.11	.04	3	.013	0.27
within treatments	0	0	.34	.34	7	.048	
total	26.25	1.39	.45				
Vessel Lengths:							
among treatments	26.25	5.72	1.30	.05	3	.017	0.17
within treatments	0	0	.69	.69	7	.098	
total	26.25	5.72	1.99				
Vessel Volumes:							
among treatments	26.25	3.15	.53	.15	3	.050	0.89
within treatments	0	0	.39	.39	7	.056	
total	26.25	3.15	.92				
Capillary Volumes:							
among treatments	26.25	5.26	1.19	.14	3	.047	1.21
within treatments	0	0	.27	.27	7	.038	
total	26.25	5.26	1.46				

Rabbit Sympathetic Trials (Analysis of Covariance Tables)

Source of Variation	sxx	sxy	syy	$syy - \frac{(sxy)^2}{sxx}$	df	MS	F
Airway Volumes:							
among treatments	26.25	0.51	.06	.05	3	.017	0.28
within treatments	0	0	.41	.41	7	.058	
total	26.25	0.51	.47				
Vessel Lengths:							
among treatments	26.25	8.47	3.36	.63	3	.210	3.68
within treatments	0	0	.40	.40	7	.057	
total	26.25	8.47	3.76				
Vessel Volumes:							
among treatments	26.25	3.48	.89	.43	3	.143	2.64
within treatments	0	0	.38	.38	7	.054	
total	26.25	3.48	1.27				
Capillary Volumes:							
among treatments	26.25	8.51	3.00	.24	3	.080	1.08
within treatments	0	0	.52	.52	7	.074	
total	26.25	8.51	3.52				