# EFFECTS OF VAGAL STIMULATION ON THE PULMONARY MICROCIRCULATION OF RABBIT, DOG, AND CAT

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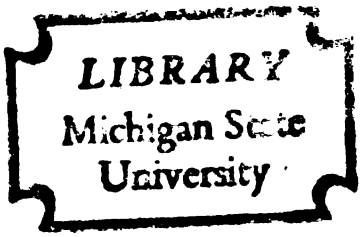
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# EFFECTS OF VAGAL STIMULATION ON THE PULMONARY MICROCIRCULATION OF RABBIT, DOG, AND CAT

By

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# INTRODUCTION

Traditionally, the pulmonary circulation has been considered a purely passive vascular bed. Any changes in pulmonary arterial or pulmonary venous pressures were attributed to alterations in cardiac output with complete disregard of neurally mediated vasomotor activity. Due to lack of a well-developed muscular layer, the pulmonary vessels were considered better constituted to accommodate large volumes of blood rather than to provide a great resistance to flow.

Recently, a number of investigators have provided information which indicates the pulmonary circulation is capable of offering resistance to blood flow by means of direct vasoconstrictor activity. Most of this recent data has been obtained by measurements of pressure differences and flow rates. Pulmonary arterial pressure increases or pulmonary blood flow decreases, in the absence of change in cardiac activity, have been defined as pulmonary vasoconstriction.

In contrast to the indirect evidence based upon pressure and flow rate changes, Krahl (1968) has presented information regarding the existence and actual site of direct pulmonary vasoconstriction. Employing techniques

of ink injection into the pulmonary vasculature, Krahl compared the extent of the ink's perfusion into the pulmonary circuit under approximately normal physiologic conditions with the ink's distribution in the pulmonary vessels following vagal stimulation or drug injection. Krahl demonstrated that stimulation of the vagus nerve in the mid-cervical region of the rabbit produced a constriction of smooth muscle precapillary sphincters. He also determined that intrapulmonary injections of acetylcholine in the rabbit had the same effect as vagal stimulation, while epinephrine injections resulted in relaxation of the smooth muscle sphincters. He concluded that the pulmonary circulation was regulated on a lobular basis by the autonomic nervous system. This indicated that pulmonary arterial vasomotion was an important factor affecting blood flow through the lungs.

With the studies of Krahl in mind, this investigation is an attempt to demonstrate similar responses to vagus nerve stimulation in the pulmonary vascular beds of the dog, rabbit, and cat. Intravenous ink injections given simultaneously with electrical stimulation of the vagus nerve were followed by photographic analysis of the gross lungs and microscopic study of the ink's perfusion into the pulmonary vasculature. These methods were employed to: (1) confirm the existence of an intrinsic control regulating pulmonary vasculature perfusion and (2)

determine the role of the vagus nerve in the controlling mechanism.

# REVIEW OF LITERATURE

## Physiologic Studies of Pulmonary Vasomotion

### Isolated Perfused Lungs

The work of Daly and Hebb (1966a) best exemplifies a most common physiologic method used in measuring pulmonary vasomotor activity. According to Daly and Hebb, isolated perfused lungs have been used extensively to study the actions of both the bronchial and pulmonary vascular smooth muscle. In this context, the term isolated does not necessarily mean that the lungs have been dissected free of other tissues, but it does mean that the circulation is confined to the lung vessels only. Even though no attempt is made to keep any other tissues or organs alive, it has been essential that the nerves supplying the lungs, in their extrapulmonary as well as their intrapulmonary course, be kept viable.

Simultaneous perfusion of the isolated pulmonary and bronchial circulations was achieved by using two separate pumps fed from one reservoir. One pump perfused the aortic arch and thoracic aorta down to $T_7$ or $T_8$. The second pump was inserted between the right heart and pulmonary artery supplying the perfused lung (perfusate was

animal's own blood). Blood reaching the left atrium, the pulmonary effluent, and blood from the bronchial circulation, which returned to the right atrium, was drained into a single external reservoir which served as a feed for the pumping apparatus. This technique affords the investigators a choice of delivering the perfusate at constant-volume or at constant-pressure inflow rates. When constant-volume inflow perfusion is practiced, the change in blood volume outflow from the lungs in response to a stimulus is assessed by recording the change in the volume of blood draining into the reservoir. When constant-pressure inflow perfusion is used, changes in pulmonary arterial pressure in response to a stimulus are determined by connecting the free end of a cannula (its opposite end inserted into the pulmonary artery) to a manometer. The recorded changes in outflow volume or pulmonary arterial pressure are believed to reflect changes in pulmonary vascular resistance resulting from either local pulmonary vasoconstriction or vasodilation.

Using this isolated perfused lung technique, Daly and Hebb observed that electrical stimulation of the cervical vagosympathetic trunk of atropinized dogs and cats (atropine blocks all vagal activity) produced a vasoconstriction. Aviado (1965a) using similar techniques, recorded a vasoconstriction with electrical stimulation of the postganglionic sympathetics ($T_1$ - $T_4$), while

similar stimulation of the vagus nerve produced a vasodilation. Dale and Narayana (1935), working with guinea-pigs, recorded decreased pulmonary outflow with both vagal and sympathetic stimulation; both effects were described as a vasoconstriction. Ingram (1968), studying the isolated lung of the dog perfused in situ, recorded an increased pulmonary arterial pressure (vasoconstriction) with sympathetic nerve stimulation.

Effects of intrapulmonary injections of sympathomimetic and parasympathomimetic drugs have also been recorded physiologically. Daly and Hebb (1966b) studied the effects of acetylcholine, epinephrine, and norepinephrine in the isolated perfused lung of the dog, cat and rabbit. The following results were recorded:

Rabbit: Vasoconstriction occurred with acetylcholine (fall in pulmonary outflow without change in perfusion pressure). Intravenous infusions of epinephrine and norepinephrine also produced a vasoconstriction.

Dog: After initial vasodilation with acetylcholine, increased doses of this drug resulted in vasoconstriction (with constant volume inflow, the initial dose caused a decrease in perfusion pressure but with increased dosage the perfusion pressure also increased). Epinephrine produced the same reaction as acetylcholine,

whereas the most common effect of norepinephrine was a vasoconstriction (decreased outflow with constant perfusion pressure).

Cat: Results of acetylcholine, epinephrine, and norepinephrine injections were similar to those in the dog.

Alcock and Daly (1935) observed decreased pulmonary venous outflow following acetylcholine injection and increased outflow with epinephrine injection into the pulmonary artery of an isolated perfused dog lung. Rose (1961) noted vasoconstriction following norepinephrine injection into the pulmonary artery of the isolated perfused lung of the dog. Increased pulmonary arterial pressure and decreased pulmonary venous outflow reflected the constriction. Previously, Rose (1957) had described a similar response to acetylcholine, but the concomitant rise in airway pressure appeared to be the primary response. Borst (1957) recorded a decreased blood volume entering the isolated dog lung with constant perfusion pressure (vasoconstriction) following injections of epinephrine and norepinephrine. The response to acetylcholine was reported as weak and variable. Carlill (1957), employing methods similar to Borst, observed a marked increase in pulmonary arterial pressure with epinephrine injections in the cat. Takasaki and Ahlquist (1963) reported an increased pulmonary

arterial pressure with epinephrine injection but no response to norepinephrine.

Foggie (1937) observed the actions of epinephrine and acetylcholine on isolated perfused rat lungs. With small doses of epinephrine injected into the pulmonary artery, dilation occurred. However, with increased dosage constriction replaced the initial dilation. These responses were observed initially as an increased pulmonary venous outflow followed by decreased outflow as the dosage increased. Acetylcholine caused decreased outflow (vasoconstriction) but, as with epinephrine, the site of this vasomotor activity was not determined.

Hauge (1966), sutdying isolated perfused rabbit lungs, reported acetylcholine to be a most powerful vasoconstrictor of the pulmonary vasculature. In a subsequent study, Hauge et al. (1967) reported that norepinephrine increased pulmonary vascular resistance (PVR) while epinephrine decreased PVR in the isolated rabbit lung.

## Measurements of PVR in the Intact Animal

A second method of studying pulmonary vasomotion physiologically utilizes the intact animal, in which the heart is not replaced by a mechanical pump. With this method, changes in pulmonary arterial and left atrial pressures are considered indicative of changes in PVR. Pressure changes in the pulmonary artery or left atrium

are measured by arterial or cardiac catheters connected to a manometer as in the isolated perfused lung technique. In some cases (specific references will be listed later), cardiac output is measured and used as a factor in calculating PVR. Dollery and Glazier (1966) listed the following equation:

$$PVR = \frac{\text{mean pulmonary arterial pressure - left atrial pressure}}{\text{cardiac output}}.$$

McGaff and Leight (1963), working with intact animals, recorded decreased pulmonary arterial pressure following sympathetic ganglionic blockade in the dog. This pressure decrease suggested a vasodilation. Colebatch (1963), also working with intact animals, found an absence of pulmonary hypertension (increased pulmonary arterial pressure) with vagal stimulation in sheep, as evidenced by a concomitant fall in pulmonary arterial pressure with stimulation. Both investigators considered the changes in the pulmonary vascular bed passive responses to changes in cardiac activity.

Bauman and Fletcher (1967), working with adult dogs and newborn puppies, catheterized the pulmonary artery and femoral artery for pressure recordings. Sympathetic blockade by high epidural anesthesia resulted in decreased pulmonary and femoral arterial pressures. The decreased pulmonary arterial pressure was attributed directly to

decreased vasoconstriction or indirectly to a decrease in bronchiolar constriction.

Studies of effects of drug injections upon the pulmonary vasculature have also been conducted with the intact animal. Rudolph and Scarpelli (1964) reported mild vasoconstriction following epinephrine injection into the cannulated pulmonary artery of the dog. This constrictor effect was difficult to observe, as the immediate increase in cardiac activity following even small doses of epinephrine, tended to mask any local vasomotor effect. Acetylcholine, infused into the cannulated left pulmonary artery, produced marked decrease in ipsilateral flow without producing change in total pulmonary blood flow. A local vasoconstriction was considered the cause of this response. Attinger (1960), using pressure changes and cardiac output measurements to calculate PVR in dogs, recorded decreased PVR (vasodilation) following injection of acetylcholine. The decrease in PVR was declared independent of changes in cardiac output, which appeared after the fall in pulmonary arterial pressure.

Braun and Stern (1967), studying pulmonary venomotor activity in the dog via venous catheterization, reported definite increase in venous pressure (venoconstriction) following injections of epinephrine, norepinephrine, and acetylcholine. The exact site of the venometer activity was not determined.

Bell (1961), using a thin walled catheter wedged into a distal pulmonary artery and a cardiac catheter for monitoring left atrial pressure in the dog, observed an immediate rise in arterial pressure within the wedged segment following injections of acetylcholine and norepinephrine. This rise appeared prior to changes in left atrial pressure and, therefore, indicated direct vasomotor action within the pulmonary vessels. Shimomura et al. (1962), employing a procedure similar to Bell, injected acetylcholine into the wedged segment and reported an immediate rise in perfusion pressure within the wedged segment. Because the response preceded systemic effects (decreased systemic pressure and bradycardia), it was concluded that direct vasoconstriction had occurred in the pulmonary vessels.

Aviado (1965b) reported a varied response to acetylcholine injection. In the dog, reduction in pulmonary arterial pressure occurred only if the dose was large enough to slow heart rate or reduce cardiac output. In the rabbit and guinea-pig, however, increased pulmonary arterial pressure occurred following the infusion of acetylcholine. The response in rabbit and guinea-pig was considered a passive response to the accompanying bronchoconstriction. With injections of epinephrine and norepinephrine, a constriction was the only response reported. However, the marked increase in pulmonary arterial pressure

was again considered a passive reaction to an increased cardiac output.

As an adjunct to the above studies involving the intact animal, fetal lambs delivered by cesarean section and maintained on placental flow (lungs were unexpanded) have been investigated. Dawes (1962) determined that PVR in fetal lambs could be reduced with acetylcholine injections. PVR in this case was calculated by monitoring pressures in the pulmonary artery and left atrium, and by determining the volume of segmental blood flow within the cannulated artery. It was determined that flow greatly increased while arterial pressure fell following acetylcholine injections. McMullen et al. (1968), using similar methods, reported that acetylcholine injection and vagal stimulation reduced PVR in fetal lambs.

## Isolated Pulmonary Blood Vessels

Additional information concerning pulmonary vasomotor activity has been reported from studies of isolated pulmonary blood vessels. Smith (1951) recorded the mean percentage change in internal volume of pulmonary blood vessels removed from the dog's lung and placed in a muscle chamber bath at 37°C. It was reported that epinephrine caused vasoconstriction (decreased internal volume) in the extrapulmonary arteries and veins, whereas acetylcholine caused vasoconstriction in the extrapulmonary veins only.

Smith concluded that the intrapulmonary vascular response was either extremely weak or absent entirely.

Somlyo and Somlyo (1964) measured isotonic contractions in helically cut strips of canine main pulmonary artery. Both epinephrine and norepinephrine demonstrated significant vasoconstrictor properties by intensifying smooth muscular contractions in the main pulmonary artery.

## Study of Pulmonary Vasomotion in Humans

The effects of sympathomimetic and parasympathomimetic drugs on the pulmonary vascular bed of man have been studied under normal as well as pathologic conditions. The method of study involves catheterization of the pulmonary artery, and in some cases the left atrium, to record pressure changes following infusion of the drug in question. In cases where PVR was calculated, cardiac output was also recorded. The brief resumé that follows is intended to provide information necessary to make a basic comparison to the previously described studies involving the lesser mammalian species.

Fowler (1951) noted increased pulmonary arterial pressure following infusion of norepinephrine. However, the increased pressure was attributed to increased left atrial pressure which produced back pressure in the pulmonary veins. Fowler (1960) later proposed a somewhat different theory claiming that norepinephrine was a

pulmonary vasoconstrictor while increased cardiac output and increased left atrial pressure (both produced by norepinephrine) tended to vitiate the constrictor effect. Cudkowicz and O'Neill (1964) also reported an increase in pulmonary arterial pressure with norepinephrine injections. The response was again attributed to increased left atrial pressure. Bousvaros (1962) reported that increased pulmonary arterial pressure following norepinephrine infusion preceded the increase in left atrial pressure by 20-40 seconds, which implied a direct pulmonary vasoconstrictor action.

Harris (1957) and Stanfield (1960) studied effects of acetylcholine upon pulmonary arterial pressure under normal conditions and in patients with mitral stenosis. It was concluded that acetylcholine decreased pulmonary arterial pressures in patients with pulmonary hypertension (result of mitral stenosis), but its action in normal individuals was too feeble to be detected. Charms (1962), on the other hand, believed the decreased PVR following acetylcholine injection in patients with chronic pulmonary disease was insufficient and concluded that mitral valve surgery was more beneficial. Fritts (1958) reported direct vasodilator action of acetylcholine under normal conditions as evidenced by decreased pulmonary arterial pressure without changes in cardiac output or left atrial

pressure. Dollery and Glazier (1966) calculated a decrease in PVR following acetylcholine injection into the pulmonary artery.

## Anatomical Studies of Pulmonary Vasomotion

*In vivo* observations of the pulmonary microcirculation and studies employing ink or radiopaque solution injections into the lesser circulation represent attempts to directly visualize pulmonary vasomotor activity. The previously mentioned work of Krahl warrants a careful analysis, both in methods used as well as conclusions drawn.

Krahl (1962) observed the surface of the lung *in vivo* by combining the fused quartz rod illuminator (Knisely, 1967) and a transparent thoracic window. The thoracic window, consisting of a piece of teflon stretched across a circular lucite frame, was placed at the level of the third rib in the rabbit. Krahl detected that certain polygonal areas (bases of secondary lobules) became distinctly more pale in color than their surroundings. They remained relatively ischemic for periods ranging from a few hours to a day or more, then returned to the normal bright pink color of the well-perfused lung. Krahl believed that some mechanism was altering capillary perfusion on a lobular basis and investigated this further by means of India ink injections. Krahl (1963) injected an

ink-saline-KCl mixture into the right ventricle of an effectively beating rabbit heart and observed that some lobules became entirely black with ink, some remained pink, and others appeared grayish-pink. The lung surface was a mosaic of discrete small areas of black, grayish-pink, and pink. The lungs were fixed to permit a histologic search for structures which had limited the flow of ink to discrete portions of the pulmonary capillary beds. Examination of histologic sections demonstrated the muscular arterioles, that accompany the respiratory bronchioles, contained similar quantities of ink in both the black and the normal-pink lobules. In blackened areas, ink particles were found in all subsequent branches and in the alveolar capillaries. In the pink areas, however, the smaller arterioles, which directly supply the alveolar capillaries through their short right-angled branches, appeared kinked and constricted while the ink had not gained access to the alveolar capillaries.

Working with the hypothesis that the vagus nerve was responsible for controlling alveolar capillary perfusion on a lobular basis, Krahl (1965) injected ink during unilateral electrical stimulation of the cervical vagus in the rabbit. On the stimulated side, great areas of the lung remained devoid of ink, while contralaterally, the lung showed the mottled pattern of lobular ink distribution that occurred normally (without vagal stimulation).

Histologic sections through adjacent black and pink areas of both the normal and vagal stimulated lungs revealed that ink had been prevented from entering the pulmonary alveolar capillaries of the pink areas by a marked contraction of a smooth muscle mechanism consisting of bundles of longitudinal and circular fibers located at the origins of the short, right-angled supply vessels of alveolar capillaries. In blackened areas this muscular apparatus was relaxed, permitting unobstructed entry of ink into the alveolar capillary networks.

Krahl (1966), using adrenergic and cholinergic agents, presented more conclusive evidence favoring a regulatory role of pulmonary capillary perfusion by the autonomic nervous system. When a small volume of acetylcholine was injected into the right ventricle of a rabbit and followed after 15 seconds by an India ink injection, the lung surface appeared as pink (poorly perfused) as following vagal stimulation.

These results suggested that sympathetic stimulation and/or increased secretion of epinephrine might permit widespread perfusion of pulmonary alveolar capillaries. Following vigorous exercising of rabbits (bounced approximately 100 times in a cardboard box), ink was injected through the chest wall into the right ventricle. On examination, the lungs appeared deeply blackened (well perfused) by the carbon suspension. Likewise, when a

small amount of epinephrine was injected into the right ventricle, followed after 15 seconds by an ink-saline-KCl solution, the lungs were entirely blackened by complete vascular perfusion with ink.

Krahl (1968), in summary, stated that the smooth muscle precapillary sphincter mechanism was under control of the autonomic nervous system, i.e. the vagus constricted the sphincters while the sympathetics dilated them. He concluded that neurally mediated vasomotion does occur in the pulmonary vascular bed.

Prior to the work of Krahl, Hall (1925) studied the pulmonary circulation by a transilluminator method based upon the principle of the fused quartz rod illuminator. He reported that, even though epinephrine caused cardiac acceleration, a definite decrease in size of the pulmonary arterioles was observed. Vagal stimulation, on the other hand, decreased pulmonary blood flow, only because it decreased heart rate. These results were obtained from observations of the pulmonary vasculature of rabbits, dogs, and cats. Wearn (1934) observed the behavior of cat pulmonary blood vessels by using the fused quartz rod illuminator. He reported an intermittent blood flow through the arterioles with spontaneous opening and closing of alveolar capillary beds. This intermittent flow was believed to be controlled by contractility of the arterioles, but regulation of this contractility was

unknown. Wearn also reported that following epinephrine injections into the pulmonary circuit, arterioles, that prior to the injection were allowing single-file RBC passage, dilated sufficiently to allow their passage at eight or more abrest.

More recently, Irwin and Burrage (1958) studied the microcirculation of rabbit with the quartz rod and observed the behavior of pulmonary arterioles, capillaries, and venules (arterioles and venules ranging from 10 - 160 micra in diameter). Intermittent, marked arteriolar constriction resulting in complete cessation of blood flow was observed. In other instances, less arteriolar constriction, leading to decreased rate of linear flow, and arteriolar dilation, causing an increased linear flow rate, were recorded. The capillaries demonstrated an intermittent blood flow (presumedly the result of arteriolar or venular constriction). The changes induced with intravascular epinephrine injections were also reported. An immediate arteriolar and venular constriction occurred but was quickly replaced with a dilation (linear flow rate decreased with the constriction and increased with the dilation). Kanematsu (1960) also made microscopic observations of the pulmonary capillary beds of the rabbit. He observed a marked increase in pulmonary blood flow and in the number of perfused capillaries following injections of epinephrine. Decreased blood flow and number of perfused

capillaries was reported following acetylcholine injections. Kanematsu concluded that changes in pulmonary capillary circulation produced by the administration of such drugs were secondary to changes in cardiac function.

Kilburn (1964), employing the quartz rod illuminator and thoracic window in the dog, recorded an intermittent blood flow through the pulmonary arterioles and capillaries. His in vivo observations revealed a dynamic pulmonary circulation in which velocity and distribution of blood flow appeared to be regulated individually by arterioles and precapillaries. Wagner and Filley (1965), using similar methods, studied the effects of epinephrine and vagal stimulation on the pulmonary circulation of the dog. Because these agents affected cardiac output, their effects upon pulmonary blood flow were thought to be caused by "upstream" events, i.e. epinephrine increased and vagal stimulation decreased cardiac output. There was no detection of change in vessel linear size.

Means other than the fused quartz rod illuminator have been employed to elucidate a direct confirmation of pulmonary vasomotor activity. Hirschman and Boucek (1963) injected at early systole 1-2ml of an opacifying material into the catheterized pulmonary artery of the dog. At the end of diastole a roentgenogram of the lung's vascular tree was recorded. After observing the pulmonary vascular bed under normal conditions, epinephrine was infused

into the pulmonary artery just prior to the radiopaque material. The resulting angiogram depicted a segmental narrowing and gnarling of the smaller pulmonary arteries and arterioles, while the larger proximal vessels passively dilated. Parker (1964) studied pulmonary venomotor activity in dogs through angiographic demonstration. A pulmonary vein was catheterized for serotonin injection, followed immediately by delivery of 15ml of a radiopaque material. The results indicated a kinking and local narrowing of small pulmonary veins and venules while the larger central veins showed a dilation.

Staub (1963) applied a technique of rapidly freezing lungs in vivo to study vasomotor action of the pulmonary vascular bed. Histologic sections of rapidly frozen lungs proved valuable in observing the effects of hypoxia (arteriolar constriction), but the effects of sympathomimetic or parasympathomimetic drugs were not observed.

## Anatomy of the Pulmonary Vascular Bed

Although the anatomy of the pulmonary blood vessels as well as their innervation has been studied by various investigators, the conclusions reached have shown a degree of variance. It is imperative that the structural aspects of the pulmonary circulation be known before conclusive evidence of its functional behavior can be presented. Those favoring a passively reacting pulmonary vascular bed

have claimed the small pulmonary arteries and arterioles are devoid of smooth muscle. In contrast, those believing that the pulmonary circulation is capable of actively constricting or dilating, have reported the presence of sufficient amounts of smooth muscle in such vessels.

William Snow Miller (1947), a pioneer of research in pulmonary anatomy, reported the pulmonary artery, after reaching a point distal to the alveolar duct, branched to supply each alveolar sac associated with the alveolar duct. Each saccular artery gave rise to small radicles which traversed the sulci between alveoli and supplied the alveolar capillary networks.

Knisely (1960), using the quartz rod illuminator, observed the architecture of blood vessels supplying and draining the pulmonary alveoli of the rabbit. He described a system of rapidly tapering pulmonary arterioles from which two types of branches arise at right angles (this differs from the simply dichotomous branching of systemic arterioles supplying capillary beds). The first type of right-angled branch is called a precapillary (50 micra diameter) which supplies alveoli contiguous to its parent stem. From the precapillary ten to twenty alveolar capillaries (10 - 12 micra diameter) arise to form the alveolar capillary networks. The second type of right-angled branch, which is termed a precapillary arteriole, may have a diameter up to 100 micra and supplies alveoli

more distant to its parent stem. Knisely (1969) also reported that alveolar capillaries may branch at right angles directly from a pulmonary artery, thereby bypassing the above described precapillary and precapillary arteriolar pattern.

The arrangement of capillary drainage was described as being similar to the precapillary pattern, i.e. capillaries uniting to form postcapillaries or venules which in turn join forming the larger pulmonary veins. In addition, capillaries were seen entering directly into the veins at right angles from contiguous alveoli.

Von Hayek (1960a) reported a similar pre-and postcapillary architecture in humans and, in addition, described the quantity of smooth muscle found in each segment. The pulmonary arterioles, which run parallel with the respiratory bronchioles, appear as a "string of beads," due to their incomplete muscular layer consisting of circular and longitudinal muscle fiber bands separated by spaces void of muscular tissue. The precapillaries, considered a common source from which several capillary beds arise, lie between the alveolar ducts and are completely devoid of smooth muscle. Postcapillaries, arising from the union of alveolar capillaries, lack any muscular component in their walls. Venules, similar to the arterioles, have an incomplete muscular tunica media, which produces their "beaded" appearance.

Highlighting the work of von Hayek is his description of a complete muscular ring surrounding both the exit of the precapillaries from the arterioles and the entrance of the postcapillaries into the venules. The existence of such a muscular arrangement appears indicative of a sphincter-like mechanism.

Reid (1968) studied the smooth muscular structure of the pulmonary artery in man by tracing it from the hilus out to its peripheral extent. The first 43 percent of its length was void of smooth muscle and was termed an elastic artery. The next 10 percent of its length, called a transitional artery, consisted of elastic fibers and smooth muscle in nearly equal proportions. The last 47 percent was called a muscular artery as the quantity of smooth muscle was comparatively greater. This latter segment of the pulmonary artery included those branches associated with the terminal and respiratory bronchioles. The segment accompanying the respiratory bronchiole reduced in diameter at a faster rate than at more proximal levels, i.e. the greatest reduction rate of the total reduction process from hilus to alveoli occurred within this segment. It was from this segment that the precapillary arterioles and precapillaries arose at right angles to serve the surrounding alveoli.

Fishman (1961) and Hyman (1966), contrary to Reid and von Hayek, were unable to assign a sphincteric function

to the small precapillary vessels of the dog and cat. A lack of accumulation of smooth muscle in these vessels was the basis for their contradiction. However, it was agreed that the larger pulmonary vessels (150-1000 micra) did possess an organized layer of smooth muscle very capable of vasoconstriction.

Sobin (1966) studied the pulmonary vasculature of dog, cat, and rabbit by perfusing a silicone rubber material into the vessels, followed by fixation and macrosectioning. Sobin called that segment of the pulmonary artery which gave rise to right-angled branches a distribution artery (thereby discarding the term pulmonary arteriole). These distribution arteries showed relatively few smooth muscle cells, the precapillary vessels showed almost no smooth muscle, and their origin from the distribution arteries was not marked by either an increase in smooth muscle cells or specific orientation to indicate precapillary sphincters. According to Sobin, this absence of smooth muscle almost excludes a role in regulation of capillary blood flow by the precapillary vessels.

## Innervation of the Pulmonary Vessels

As mentioned previously, the innervation of the pulmonary vascular bed remains a disputable topic. Von Hayek (1960b) concluded that innervation of pulmonary arterial and venous smooth muscle in man appeared well

established, however, pathways had not been clarified anatomically, nor had the disposition of vasodilator or vasoconstrictor nerve fibers been defined.

According to Daly and Hebb (1966c), Morel and Francois-Franck in 1879 found that stimulation of filaments from the first thoracic ganglion raised pulmonary arterial pressure and lowered left atrial pressure, which they attributed to pulmonary vasoconstriction. In 1889 according to Daly and Hebb, Bradford and Dean recorded similar results and concluded that pulmonary vessels were supplied with vasoconstrictor fibers.

Such experiments were criticized on the grounds that apparent increased PVR in response to nerve stimulation could have resulted from passive effects of changes in cardiac output and back pressure on the lungs. It was also pointed out that fall in left atrial pressure with stimulation may have been caused by increased efficiency of the left ventricle as a result of excitation of cardioaugmentor fibers in the absence of cardiac acceleration. These criticisms led many investigators to adopt the technique of artificially perfusing an isolated lung. However, the feebleness of the vascular responses observed under these conditions added further support to the view that results obtained from the intact animal were due not to pulmonary vasomotor nerve activity, but rather to passive cardiovascular events. It should be mentioned that

improvements made upon this technique now enable the collection of consistent pulmonary vasomotor responses over prolonged experimental periods (such information was described previously).

The most complete anatomical account of the nerve supply to pulmonary vessels was presented by Hirsch et al. (1968). Preganglionic vagal fibers and postganglionic sympathetic fibers join together at the hilus forming a large posterior pulmonary plexus. From this plexus a few fibers extend over the rostral border of the hilus to form a much smaller anterior pulmonary plexus. From these two plexuses arise peribronchial, periarterial and perivenous plexuses, and sensory fibers which sparsely innervate the visceral pleura.

The periarterial plexuses run in the adventitia of the pulmonary and bronchial arteries, intermingling freely with the peribronchial plexuses. The fibers extend distally to the pulmonary arterioles as well as into the interalveolar septa. In the larger arteries, thick sensory fibers terminate in the tunica adventitia and are thought to be pressor receptors. Thin motor fibers terminate in the tunica media along the entire extent of the arterial tree, i.e. motor fibers are found in the most distal segments of vascular smooth muscle.

The perivenous plexuses do not intermingle with the peribronchial plexuses because the veins remain

independent of the airways. Sensory fibers terminate in the adventitia (proposed pressor receptors) and the tunica intima (probable chemoreceptors). Motor fibers terminate in the tunica media ramifying throughout the muscular coat in the form of a terminal reticulum.

## Vagal Innervation of the Heart

Mizeres (1955) outlined the course of the cardioinhibitory branches from the right vagus nerve in the dog. These nerve fibers arose from the cervical vagus (via the recurrent laryngeal nerve) and the thoracic vagal trunk. They terminated in the dorsal and ventral walls of the right atrium. Mizeres (1957) reported the distribution of cardioinhibitory fibers from the left vagus nerve in the dog. These fibers originated from the left recurrent laryngeal nerve and from the thoracic vagal trunk. The cardioinhibitory branches were distributed to the left atrium or nerve plexuses which supplied it. Miller (1964), using electrical stimulation studies, traced the course of the cardiovagal fibers originating from both the right and left vagi in the dog. The results were in accord with the work of Mizeres.

Truex (1955) also employed electrical stimulation studies and compared the right vagus nerve to the left vagus regarding their cardioinhibitory potential. In adult dogs as well as young puppies, the right vagus

exerted more inhibition on cardiac activity than the left vagus, i.e. the right vagus had more influence than the left vagus upon activity of the S-A node.

Rushmer (1961), reporting results from a variety of mammalian species, concluded that vagal cardioinhibitory fibers were distributed to the S-A node, A-V node, both atria, but not to the ventricular myocardium. When stimulated, these parasympathetic fibers exerted a powerful inhibitory effect on heart rate. However, the stimulation did not directly effect the contractile properties of the ventricular myocardium.

Guyton (1966) summarized cardiac control via the autonomic nervous system in humans. The cardiovagal fibers (as reported by Rushmer) were distributed to the S-A node, A-V node, and myocardium of both atria; but not to the ventricular muscle. The sympathetic nerves were distributed to these same areas plus the ventricular myocardium.

Vagal stimulation caused decreased heart rate by slowing transmission of cardiac impulses from the conduction system into the ventricular musculature. Sympathetic stimulation, on the other hand, had essentially the opposite effect on the heart, i.e. it increased the rate of S-A nodal discharge and greatly increased the force of contraction of both atrial and ventricular myocardia.

# MATERIALS AND METHODS

Three animal groups were established according to species. Groups I, II, and III consisted of six rabbits, dogs, and cats respectively. Each group was further divided into two subgroups each containing three animals. The effects of left cervical vagus nerve stimulation upon the pulmonary circulation were studied in the first subgroup; and the pulmonary vasomotor effects of right cervical vagal stimulation were observed in the second subgroup. In both subgroups, the contralateral lung served as a control which afforded immediate comparison to the experimental (ipsilateral) lung.

## Group I

Six, female, New Zealand, white rabbits, approximately five pounds, composed Group I. The following procedure pertains to the first subgroup in which the pulmonary circulation was observed after left vagus nerve stimulation. The same procedure was carried out on the second subgroup after stimulation on the right side.

### Preliminary Procedures

The rabbits were anesthetized with sodium pentobarbital (32mg/kg) via the right or left marginal ear vein.

The anterior thorax and anterior cervical region were shaved with an electric clippers. Cheesecloth coiled into yard-long "ropes" were tied with a slip-knot to the animals' forelimbs and hindlimbs. Each animal was then placed on its dorsal (back) side and its limbs secured to a surgical table by fastening the free ends of the ties.

Surgical Procedures

A midline incision in the anterior cervical region was made through the skin and superficial fascial layers. Using a hemostat or blunt scissors, the cut was extended laterally to the right side. The right external jugular vein was located and freed from its surrounding fascia for two inches of its length. A short piece of umbilical tape was carefully passed beneath the vessel and left undisturbed until a later cannulation.

Next, the sternohyoid and sternothyroid muscles were separated at the midline to expose the trachea. A piece of umbilical tape was passed beneath the trachea and used in a later tracheal cannulation procedure. Lateral retraction of the left sternohyoid and sternothyroid muscles exposed the left common carotid artery and left vagus nerve immediately lateral to the trachea. By careful dissection, the nerve (positioned a few millimeters lateral to the artery) was separated from the artery and clearly exposed for approximately two inches of its length.

Cannulation of the external jugular vein was then performed as follows. First, cranial to the umbilical tape, a hemostat was clamped across the main venous trunk; care was taken to include any major tributaries in the area of the clamp. By exerting slight upward pressure on both the hemostat and umbilical tape, the vein was held taut and in position for an incision which extended through three-quarters of the vessel's circumference. A blunt probe was briefly inserted into the incision to facilitate its exact location. After removing the probe, a six to eight inch piece of polyethylene tubing (PE 190) was inserted into the vessel and pushed to the right atrium. The umbilical tape was then tied around both vessel and cannula to maintain a secure connection. The PE tubing's free end was connected via a Tuohy adapter to a 50cc plastic syringe containing 20cc of warmed (37°C) P likan ink (NO. C11-1431A, J. Henschel and Co., Inc., New York). The 20cc represented one tenth of the total blood volume or that volume found within the pulmonary circulation at any given time. The total blood volume of the animal was calculated as eight per cent of the total body weight measured in grams (Schermer, 1967).

Before injection of the Pelikan ink, the silver-wire stimulating electrode was properly positioned and insertion of the tracheal cannula was completed. Following a tracheotomy, a four inch glass cannula was inserted into

the trachea. The predetermined diameter of the cannula provided a tight fit within the trachael lumen and, coupled with tying of the umbilical tape, produced an efficient cannulation. The animal was able to respire through the open end of the cannula, which remained free until its later connection with the formalin-fixation apparatus.

At the cranial extent of the exposed left vagus nerve, two hemostats, separated slightly, were clamped onto the nerve. The nerve was then severed between the hemostats; the distal stump for subsequent stimulation and the proximal stump for a later dissection to verify the nerve's identity. The distal stump of the severed left vagus nerve was carefully placed upon wire loops of the stimulating electrode. The wires, excluding their small terminal loops (spaced about 5mm apart) were supported in a non-conducting material which insulated them from other tissues and served as a convenient handle for the electrode. The nerve was stimulated with a monophasic square wave current produced by a Grass S-8 stimulator. The parameters of the stimulus were designed to include volts/pulse duration in msec/frequency per second. The stimulus used, 10 volts/10msec/50 cycles per second was initiated 15 seconds prior to the ink injection and delivered continuously until its termination just prior to lung removal from the thoracic cavity (an approximate 3-5 minute period). Two checks were made to verify the occurrence of the

electrical stimulus. A Tektronix Type 564 storage oscilloscope was connected to the small wire loops of the electrode for verification of the amplitude, duration, and frequency of the electrical impulses. In addition, increased peristalsis of the abdominal viscera and gastrointestinal tract, as evidenced by visible movements of the ventral abdominal wall, was considered indicative of an effective vagal stimulation.

Fifteen seconds after initiation of the vagal stimulation, the entire volume of Pelikan ink was rapidly injected. After approximately 10-15cc of ink had been delivered, a cardiac puncture of the left ventricle was made via the anterior thoracic wall. Entrance into the thoracic cavity was gained by inserting a 25 gauge, stainless-steel needle through the fifth intercostal space, located approximately two inches caudad to the caudal border of the major pectoralis muscle and in the same plane with the thoracic nipples on the left side of the anterior thorax. A 2.5cc plastic syringe attached to the needle contained 0.5cc of a 25 percent solution of carbachol, a parasympathomimetic agent. Injection of carbachol into the left heart caused an immediate cardiac arrest which allowed ink to remain, for the most part, within the pulmonary circulation.

Immediately following the last one cc of ink injectate, the tracheal cannula's free end was connected to

a 12 inch piece of rubber tubing which led to the sidearm of a 500ml pyrex aspirator bottle, containing 20 percent buffered formalin. The bottle was supported by a ring-stand at a level 25cm above the position of the trachea. At this height, the formalin was instilled into the trachea at 25 cm $H_2O$ pressure which enabled fixation of the lungs in a physiologically inflated state (Heard, 1962). Allowing approximately 15 seconds for the initial volume of formalin to enter the airways, the procedure of removing the fixed-lungs from the thorax was begun. An incision was made into the abdominal cavity caudal to the diaphragm and ventral to the liver. The xiphoid process of the sternum was clamped with a hemostat and raised to enable an initial incision through the thoracic wall. Autopsy scissors were used to incise the costochondral junction of each rib. The cut edges were retracted and the lungs exposed for initial <u>in situ</u> photographs. The visceral contents of the thoracic cavity were then removed via careful dissection. The tracheal cannula was replaced with a hemostat to prevent formalin escape by retrograde flow and thus the need for direct handling of lung tissue was obviated.

The severed vagus nerve's proximal stump was then traced cranially to the nodose ganglion. In addition, the cervical sympathetic nerves were exposed to confirm the

stimulation procedure, i.e. to further verify vagus nerve identity.

Lung Photography and Gross Analysis

After lung removal from the thorax, the ink's distribution within the pulmonary circulation was recorded by observing areas of black and pink on the lungs' surface. Each lobe was carefully analyzed as to the percentage of black (ink perfusion) versus the percentage of pink (absence of ink perfusion) appearing on its surface. A brief, written account describing perfusion patterns on the surface of each lobe accompanied the perfusion percentage data.

As a permanent record of perfusion patterns, both color and black and white photographs were taken of the lungs (the heart remained intact) as they appeared immediately following removal from the thoracic cavity. The photographs included a view of: the ventral and dorsal surfaces, both the left and right lateral surfaces, and the diaphragmatic surface.

A Nikon F camera with a 55mm automicro Nikkor lens was used for all of the photography. High Speed Ektachrome B film (Kodak) was used for the color photographs. Panatomic-X film was used for black and white photography.

Microscopic Analysis

Upon completion of the photography, the lungs underwent a 24 hour fixation period as described by Weiss and Tweeddale (1966). The lungs were placed in a plastic pan containing 20 percent formalin. The tracheal hemostat was removed and the trachea was attached to the heavy-walled plastic outflow tubing of a 1,000ml pyrex aspirator bottle. The bottle was supported by a ringstand at a level 30cm above the position of the lungs. The bottle was continuously filled by a small aquarium pump (Little Giant Pump Co., Oklahoma City, Okla.) placed in the pan containing the lungs. A double hose system was employed by threading a segment of heavy-walled plastic tubing through a Penrose tubing drain. The heavy tubing, attached to the outflow part of the pump, rose to, and entered the top of the bottle. The tube's end was allowed to lie loosely near the base of the bottle. The surrounding Penrose drain was used as an overflow system, automatically regulating pressure at the 30cm level. It was securely attached to the neck of the aspirator bottle and was allowed to fall in an unobstructed fashion back into the formalin-filled pan. For the inflation-fixation, the pan was filled above the level of the lungs with 20 percent formalin and the pump was started. For safety purposes, this procedure was always performed within a well-ventilated hood system.

Following the 24 hour fixation period, representative lobes, or portions of these lobes, from both the right and left lungs were dehydrated in three changes of acetone (20 minutes each at room temperature). Lobes were chosen which demonstrated both black and pink areas, enabling a microscopic comparison, i.e. the extent of the ink's perfusion in the black versus the pink areas. Smaller sections (1/4 inch thick) were then taken from the dehydrated lobes and placed in liquid paraffin at 60°C and 20 inches mercury vacuum for two hours. Paraffin-infiltrated sections were embedded in paraffin blocks and sectioned at 10 micra with a Spencer rotary microtome. Two 10 micra sections were taken from each block. One of the sections was passed through two, three minute changes of xylene (for paraffin removal) and cover-slipped. The other section was routinely stained with hematoxylin and eosin.

## GROUP II

Six, ten to twelve pound, mongrel dogs of both sexes composed Group II. Except for a few minor alterations, the surgical procedures were similar to those previously described for Group I. Thus, a description of these changes will sufficiently encompass the procedures employed.

Preliminary Procedures

Preparation of the dogs for experimental procedures was identical to that described for Group I with the exception of anesthetic administration, i.e. sodium pentobarbital (32mg/kg) was given intravenously via either the right or left cephalic vein.

Surgical Procedures

In the cervical region of the dog the vagus nerve and sympathetic chain are invested by a common connective tissue sheath as the vagosympathetic trunk. Therefore, electric stimulation of this trunk would not elicit a sole response from the vagus nerve. To produce such a response, the sympathetic fibers must be prevented from exciting their reception sites within the pulmonary vascular system.

After both the right external jugular vein and trachea had been cannulated, the left vagosympathetic trunk was carefully freed of its fascial connection to the common carotid artery, clamped with a hemostat, and severed. The electrode was then properly positioned for subsequent stimulation. Prior to stimulator activation, 2.3cc of phenoxybenzamine hydrochloride (alpha-adrenergic receptor blocking agent; Dibenzyline, Smith, Kline and French) followed immediately by 2.3cc of propranolol hydrochloride (beta-adrenergic receptor blocking agent; Inderol,

Ayerst) were injected into the cannulated external jugular vein. This injection was accomplished by piercing the polyethylene cannula with a 25 gauge needle which was inserted over the open end of a three-way plastic stopcock; its remaining outlets were occupied by two 2.5cc plastic syringes containing the adrenergic receptor blocking agents. Stock solutions of phenoxybenzamine (10mg/cc saline) and propranolol (2mg/cc saline) supplied the 2.3cc injectates which accommodated the recommended doses of 5mg/kg and 1mg/kg respectively (Gebber, 1969).

Immediately following injection of the blocking agents, the monophasic square wave stimulus (10volts/ 10msec/50 cycles per second) was initiated and continuously delivered throughout the Pelikan ink injection. The ink injection (40cc total volume), cardiac puncture (carbachol injection), and tracheal instillation with 20 percent buffered formalin were performed as described previously.

Gross and Microscopic Analysis

The methods of recording the descriptive accounts of the ink's distribution within the pulmonary vascular bed as well as the photography of this distribution were previously outlined and need no further explanation. The preparation of histologic sections and their subsequent analysis were also previously explained.

## Group III

Six, five pound, female, mongrel cats comprised Group III. Aside from two essential modifications, the preliminary procedures and surgical techniques were similar to those previously described.

### Preliminary Procedures

The first alteration concerned the administration of the anesthetic. Sodium pentobarbital (32mg/kg) was given intraperitoneally rather than intravenously. The reamining preparatory steps were conducted according to the previously defined pattern.

### Surgical Procedures

As previously described, cannulation of the trachea as well as the right external jugular vein was performed prior to positioning the electrode for left cervical vagus nerve stimulation. As with the dog, the cervical portion of the feline vagus nerve is encapsulated with the sympathetic chain within a common connective tissue sheath. Contrary to the dog, however, it is anatomically feasible to grossly separate these two nerve trunks allowing for the stimulation of one component independent of the other (Gebber, 1969).

The left carotid sheath, was exposed immediately lateral to the trachea. An initial incision was made into

the sheath and fascial elements were carefully stripped from the carotid artery and vagosympathetic trunk. Using blunt scissors, the fascial connection between the two structures was severed and the nerve trunk was isolated from its surrounding connective tissue attachments. A visual examination clearly revealed a fine line of demarcation between the sympathetic trunk and the vagus nerve. A finely pointed scissors was used to exaggerate this demarcation and the two components were carefully separated. Vagus nerve identification was readily made because its diameter always exceeded that of the sympathetic trunk. After isolating a two to three inch segment of the vagus nerve, the electrode was positioned and the stimulus applied. Subsequent Pelikan ink injection, cardiac puncture (carbachol injection), and formalin-fixation procedures were identical to that outlined for the rabbit.

Gross and Microscopic Analysis

The descriptive account of the ink's perfusion pattern, the photographic procedures, and the histologic examinations were enacted according to predesignated methods.

## RESULTS AND DISCUSSION

### Perfusion-Percentage Results

Lobar surface perfusion patterns created by the distribution of black (ink-perfused areas) versus pink (underperfused areas) were examined visually and recorded on a perfusion-percentage basis. The percentage data for the rabbit, dog, and cat are listed in Tables 1, 2, and 3 respectively. Each table presents the perfusion-percentages recorded following either left or right vagal stimulation.

Average perfusion-percentage values were determined for each lobe and were graphically analyzed for the rabbit, dog, and cat as shown in Figures 1, 2, and 3 respectively.

Krahl (1963) reported a mosaic pattern of black, grayish-pink, and pink areas, on the surface of both lungs in the rabbit following India ink injection. When unilateral vagal stimulation occurred simultaneously with the ink injection, the ipsilateral lung remained void of ink, while contralaterally, the lung showed the mottled pattern of lobular ink distribution. Histologic sections of these lungs demonstrated that the small muscular arterioles appeared kinked and constricted in pink areas and normally

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

| Vagal Stimulation | Rabbit Number | | |
|---|---|---|---|
| Left vagal stimulation | | | |
| Left lung | Rabbit-1 | Rabbit-2 | Rabbit-3 |
| cranial lobe | 100%- 0 | 40%- 60% | 70%- 30% |
| middle lobe | 100 - 0 | 70 - 30 | 90 - 10 |
| caudal lobe | 100 - 0 | 60 - 40 | 90 - 10 |
| Right lung | | | |
| cranial lobe | 99 - 1 | 5 - 95 | 60 - 40 |
| middle lobe | 10 - 90 | 85 - 15 | 100 - 0 |
| caudal lobe | 40 - 60 | 75 - 25 | 60 - 40 |
| intermed. lobe | 50 - 50 | 98 - 2 | 90 - 10 |
| Right vagal stimulation | | | |
| Left lung | Rabbit-4 | Rabbit-5 | Rabbit-6 |
| cranial lobe | 95 - 5 | 95 - 5 | 90 - 10 |
| middle lobe | 99 - 1 | 95 - 5 | 95 - 5 |
| caudal lobe | 98 - 2 | 85 - 15 | 95 - 5 |
| Right lung | | | |
| cranial lobe | 100 - 0 | 98 - 2 | 99 - 1 |
| middle lobe | 90 - 10 | 80 - 20 | 95 - 5 |
| caudal lobe | 95 - 5 | 75 - 25 | 80 - 20 |
| intermed. lobe | 30 - 70 | 90 - 10 | 20 - 80 |

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

| Vagal Stimulation | Dog Number | | |
|---|---|---|---|
| **Left vagal stimulation** | | | |
| Left lung | Dog-1 | Dog-2 | Dog-3 |
| cranial lobe | 98%- 2% | 97%- 3% | 90%- 10% |
| middle lobe | 100 - 0 | 10 - 90 | 90 - 10 |
| caudal lobe | 100 - 0 | 80 - 20 | 95 - 5 |
| Right lung | | | |
| cranial lobe | 100 - 0 | 98 - 2 | 90 - 10 |
| middle lobe | 99 - 1 | 85 - 15 | 85 - 15 |
| caudal lobe | 100 - 0 | 98 - 2 | 95 - 5 |
| intermed. lobe | 99 - 1 | 50 - 50 | 90 - 10 |
| **Right vagal stimulation** | | | |
| Left lung | Dog-4 | Dog-5 | Dog-6 |
| cranial lobe | 98 - 2 | 100 - 0 | 96 - 4 |
| middle lobe | 99 - 1 | 99 - 1 | 95 - 5 |
| caudal lobe | 98 - 2 | 100 - 0 | 96 - 4 |
| Right lung | | | |
| cranial lobe | 100 - 0 | 100 - 0 | 95 - 5 |
| middle lobe | 98 - 2 | 100 - 0 | 95 - 5 |
| caudal lobe | 99 - 1 | 100 - 0 | 75 - 25 |
| intermed. lobe | 95 - 5 | 99 - 1 | 65 - 35 |

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

| Vagal Stimulation | Cat Number | | |
|---|---|---|---|
| **Left vagal stimulation** | | | |
| Left lung | Cat-1 | Cat-2 | Cat-3 |
| cranial lobe | 98%- 2% | 100%- 0 | 95%- 5% |
| middle lobe | 100 - 0 | 98 - 2 | 98 - 2 |
| caudal lobe | 99 - 1 | 98 - 2 | 95 - 5 |
| Right lung | | | |
| cranial lobe | 99 - 1 | 100 - 0 | 95 - 5 |
| middle lobe | 70 - 30 | 45 - 55 | 90 - 10 |
| caudal lobe | 80 - 20 | 90 - 10 | 99 - 1 |
| intermed. lobe | 95 - 5 | 85 - 15 | 90 - 10 |
| **Right vagal stimulation** | | | |
| Left lung | Cat-4 | Cat-5 | Cat-6 |
| cranial lobe | 20 - 80 | 99 - 1 | 100 - 0 |
| middle lobe | 60 - 40 | 98 - 2 | 99 - 1 |
| caudal lobe | 99 - 1 | 100 - 0 | 99 - 1 |
| Right lung | | | |
| cranial lobe | 100 - 0 | 99 - 1 | 100 - 0 |
| middle lobe | 55 - 45 | 98 - 2 | 85 - 15 |
| caudal lobe | 100 - 0 | 100 - 0 | 98 - 2 |
| intermed. lobe | 100 - 0 | 100 - 0 | 100 - 0 |

Figure 1.--Average lobar perfusion-percentages for the rabbit.

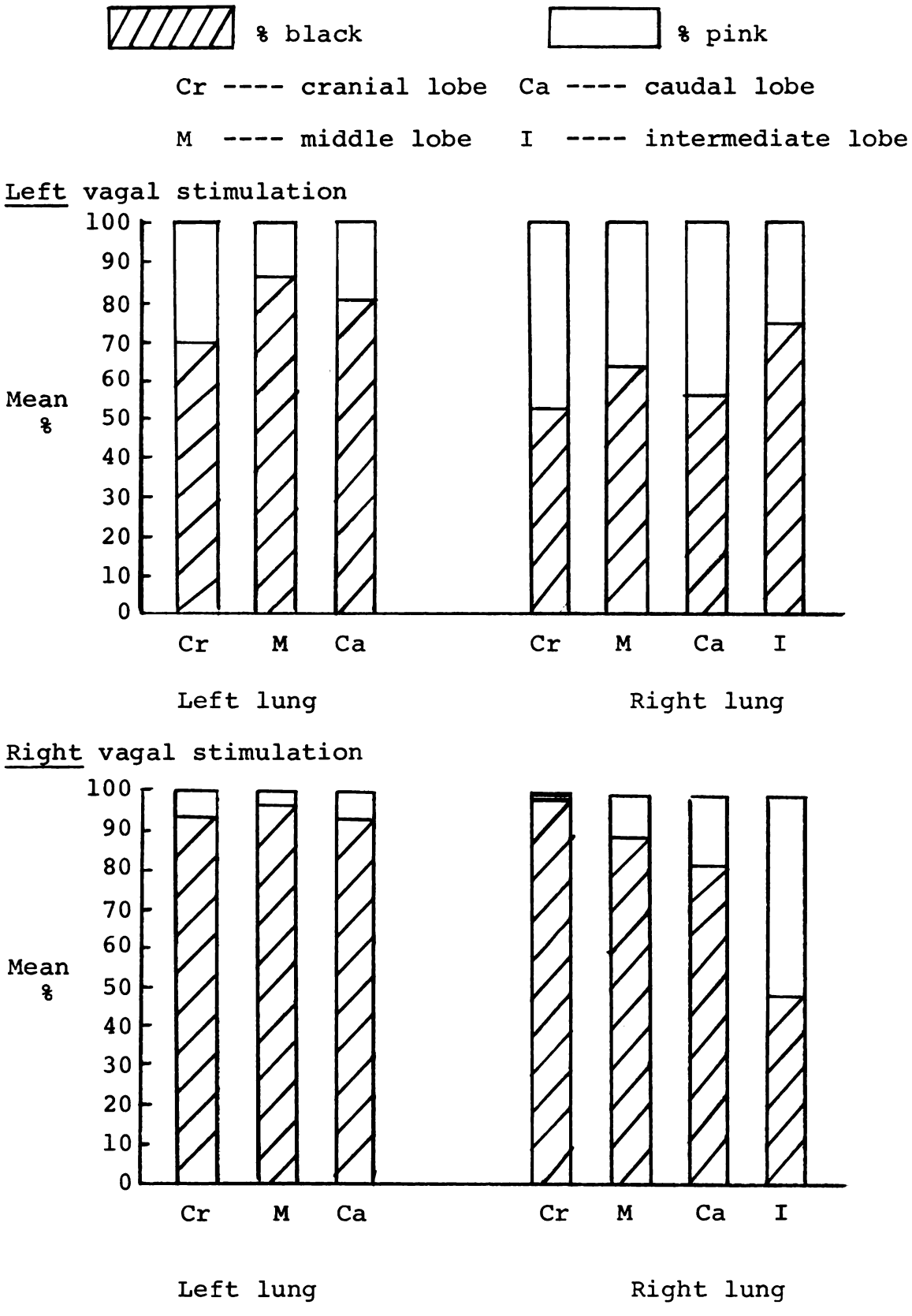


Figure 2.--Average lobar perfusion-percentages for the dog.

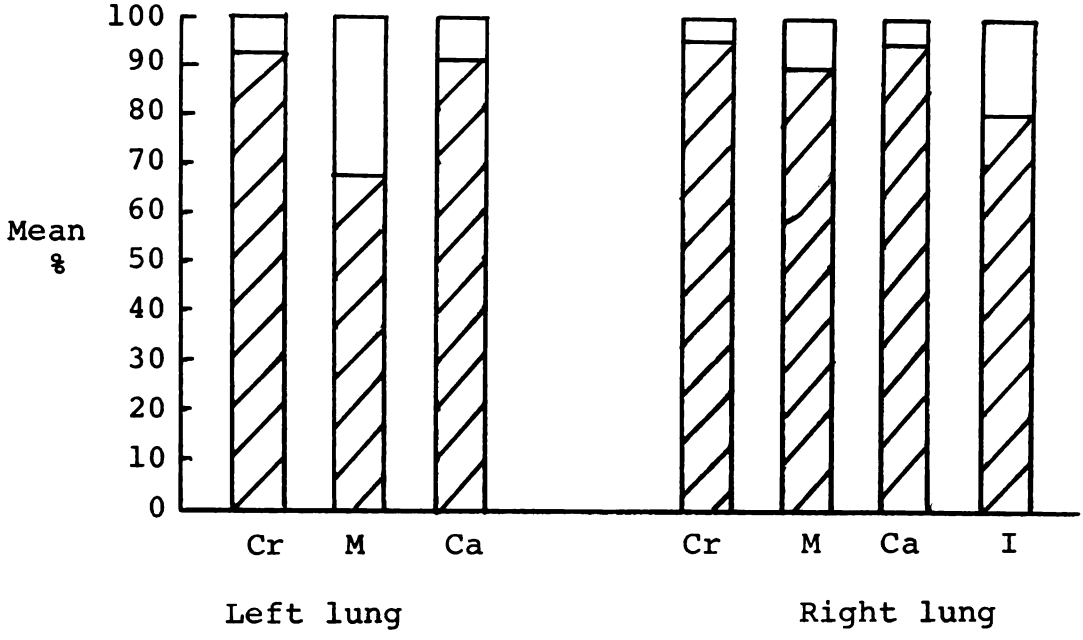
% black

% pink

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

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

Left vagal stimulation




Right vagal stimulation

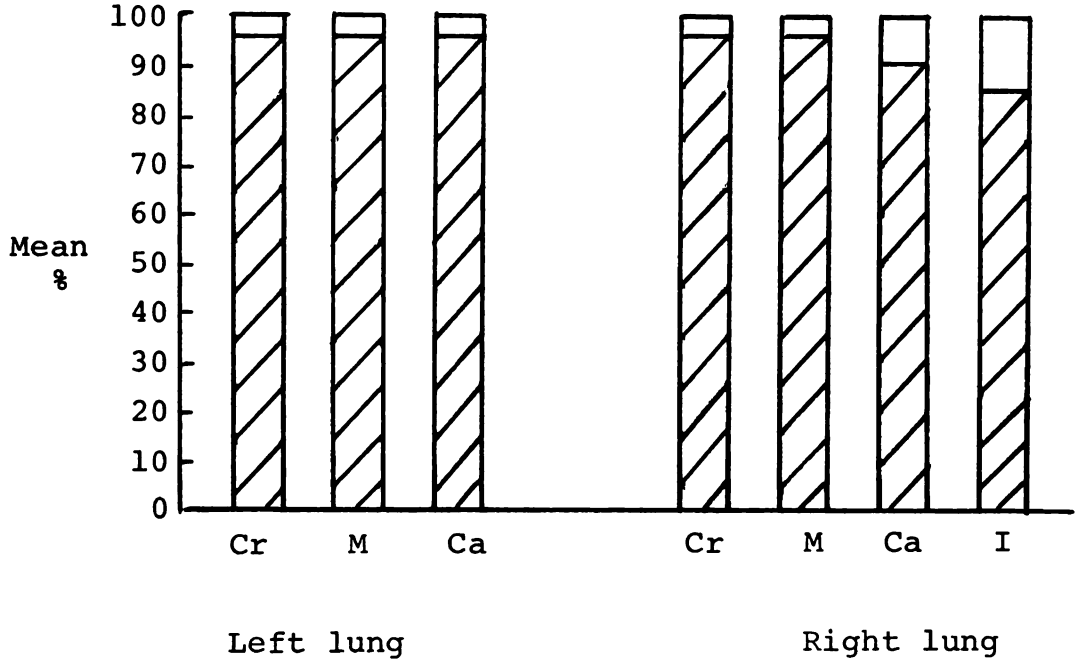
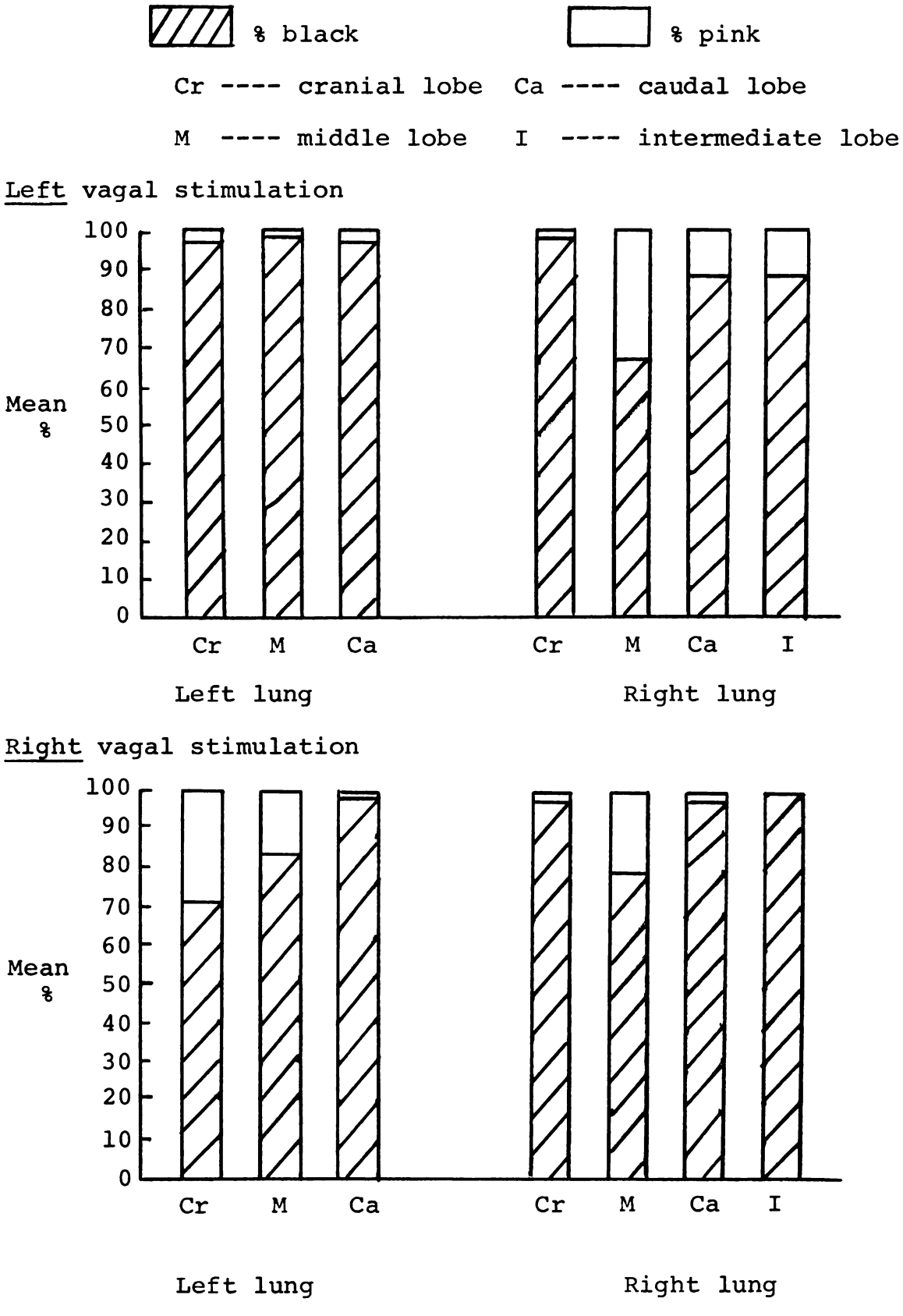


Figure 3.--Average lobar perfusion-percentages for the cat.



patent in black areas. Krahl concluded that vagal stimulation produced direct vasoconstriction of the muscular pulmonary arterioles, i.e. pink areas represented lobular regions underperfused as a result of active vasoconstriction.

Assessment of the present results is based upon the following: black lobar colorization indicates alveolar capillary perfusion while pink lobar surfaces represent hypoperfusion of pulmonary capillary networks. According to Krahl (1969), hypoperfused capillary beds (pink surface colorization) result from pulmonary arteriolar constriction. This constriction diverts blood flow through arterio-venous anastomses which readily allows return of pulmonary arterial blood to the left heart. Prinzmetal (1948), von Hayek (1960c), and Weibel (1963) reported numerous arteriovenous shunts in the mammalian lung.

Contrary to Krahl, the present histologic sections do not confirm arteriolar smooth muscle contraction. Differentiation between pulmonary arterioles and venules in histologic sections is often difficult. However, the present sections lacked visual evidence of any vascular smooth muscle constriction. This lack of vasoconstriction coupled with high perfusion-percentages in experimental lungs implies, according to Krahl's conclusions, that black areas represent alveolar capillary perfusion, while pink areas reflect hypoperfusion of pulmonary capillary beds.

In the absence of demonstrated morphologic vasoconstriction, however, is pink colorization indicative of capillary hypoperfusion? The ink's rapid transit through the pulmonary capillary networks (pink colorization would result) may reflect alveolar capillary hyperperfusion, i.e. the Pelikan ink injectate is pumped past the pulmonary capillary beds. Three conditions vitiate this assumption: (1) immediate cardiac arrest via carbachol injection decreases effectiveness of the ink's pumping mechanism. (2) Hyperperfusion indicates that pink areas had been thoroughly washed out with blood containing no experimental ink. The large volume of ink injected, coupled with immediate cardiac arrest obviate this condition. (3) A preliminary in vivo investigation demonstrated the permanent nature of pink color patterns. Circulation of ink through the pulmonary vascular bed was observed via open thorax (lungs were inflated under constant positive oxygen pressure) in the rabbit. The mottled pattern consisted of pink and black areas which maintained their colorization throughout the entire ink infusion. However, effects of positive pressure and high oxygen concentrations upon the pulmonary circulation must be considered in interpretation of this study.

Species Variation

The ipsilateral lung of the rabbit following unilateral vagus nerve stimulation appears well-perfused as

depicted by its black colorization. Examination of its average lobar perfusion-percentage values and histologic sections soundly supports this statement.

With left vagal stimulation (Figure 4), the left lung indicates greater vascular perfusion than its counterpart on the right side. Areas of pink appear upon the surfaces of both lungs. However, the contralateral lung accounts for a much greater percentage of these underperfused areas. A lobar comparison following right vagal stimulation (Figure 5) indicates an even ink distribution between right and left side, i.e. both lungs are black with scattered areas of pink.

Unilateral cervical vagus nerve stimulation in the dog resulted in vascular perfusion patterns indicating an overall decrease in percent pink relative to the rabbit. Following left vagal stimulation (Figure 8) a comparison of the ink's perfusion into left lung versus right lung reveals little difference. In each case, lobar color patterns reflect a well-perfused pulmonary vasculature. The left middle lobe's decreased perfusion represents the only exception to this pattern. The lobar differences between right and left lung following right vagal stimulation are minor (Figure 9).

The effects of unilateral vagal stimulation in the cat parallel those of the dog regarding the overall increased perfusion relative to the rabbit. Left vagus

nerve stimulation (Figure 12) indicates complete perfusion of the ipsilateral lung. The contralateral lung, excluding the middle lobe, follows the typical perfusion pattern of a black field edged with small areas of pink. The middle lobe shows a considerably greater average hypoperfusion percentage than the remainder of the right lung. Vast pulmonary vasculature perfusion appears in the ipsilateral lung following right vagal stimulation (Figure 13). Contralaterally, the extent of vascular perfusion is limited by decreased perfusion values for the cranial and middle lobes.

Observation of histologic sections taken from adjacent black and pink areas failed to reveal evidence of arteriolar smooth muscle contraction. In each of the three species studied, the lack of evidence was consistent. Black areas (Figures 6, 7, 14) were totally perfused, i.e. capillaries and the larger pulmonary vessels were evenly filled with ink. Pink areas, however, lacked complete vascular perfusion as the ink's distribution in the pulmonary vessels (arteries, arterioles, venules, and veins) was variable, i.e. such vessels were either completely filled, partially filled, or entirely void of ink (Figures 10, 11, 14). The capillary networks within the pink areas were void of ink; however, cause for the absence of ink was not ascertained.

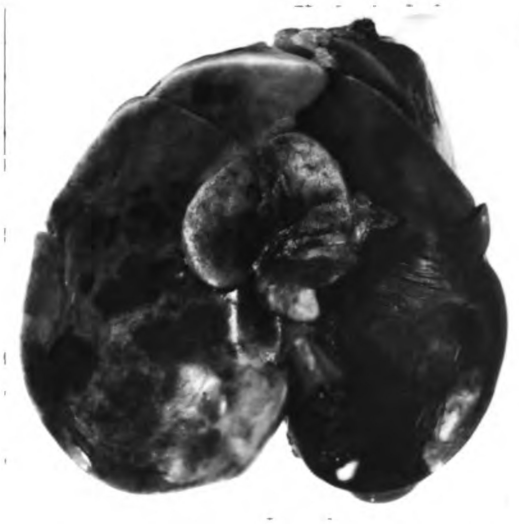


Figure 4.--Rabbit lung (left vagal stimulation). Diaphragmatic surface (lungs' ventral surface faces top of photograph) showing uneven ink distribution between right and left lungs. Note greatest perfusion in the left (vagally-stimulated) lung.

Figure 5.--Rabbit lung (right vagal stimulation). Ventral and diaphragmatic surfaces depicting thorough bilateral filling of the pulmonary vascular beds.

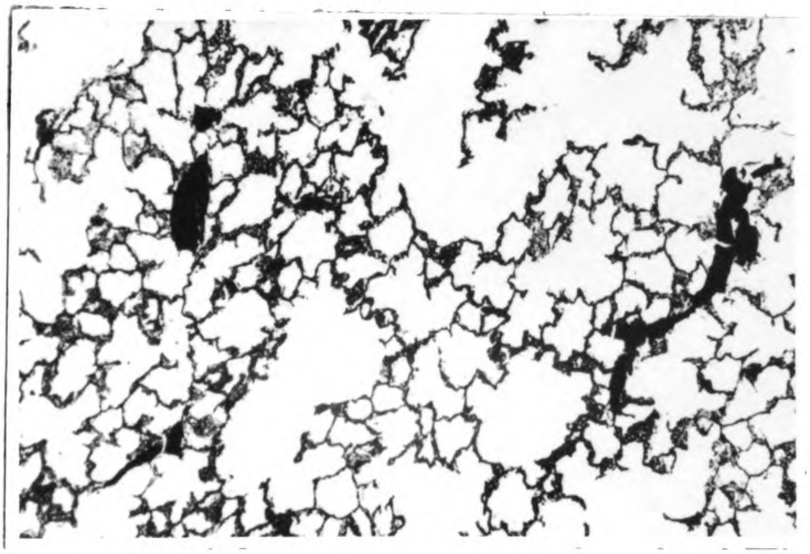


Figure 6.--Histologic section from the ipsilateral lung following left vagal stimulation showing thorough ink perfusion of the rabbit's pulmonary microcirculation. Grossly, this same area appeared black. About 96X.

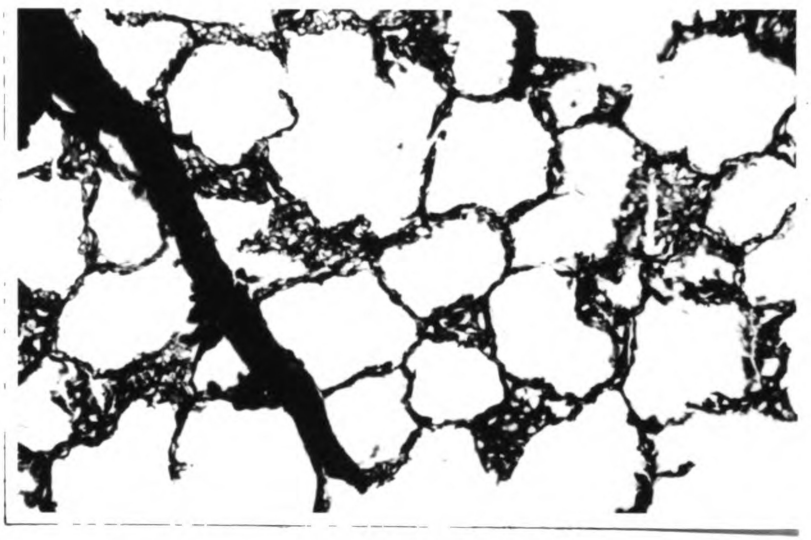


Figure 7.--Rabbit lung (section from left lung after left vagal stimulation). Complete vascular perfusion of large pulmonary vessels and capillary beds is shown. Note right-angled branching pattern, a consistent feature of the pulmonary microcirculation. About 380X.

Figure 8.--Dog lung (left vagal stimulation). Diaphragmatic surface (lungs' ventral surface faces top of photograph) showing small, evenly scattered areas of hypoperfusion. The vascular perfusion patterns appear uniform in both lungs.

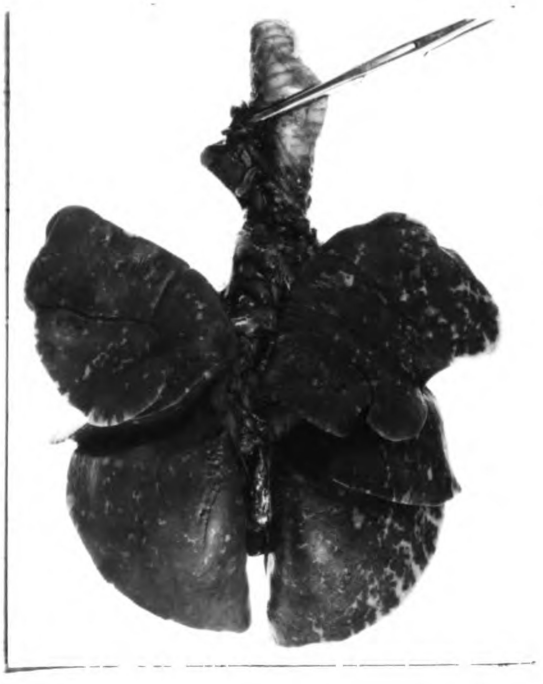


Figure 9.--Dog lung (right vagal stimulation). Dorsal and lateral surfaces showing a similar pattern to Figure 3. Note even bilateral ink distribution and the overall increased perfusion relative to the rabbit lung.

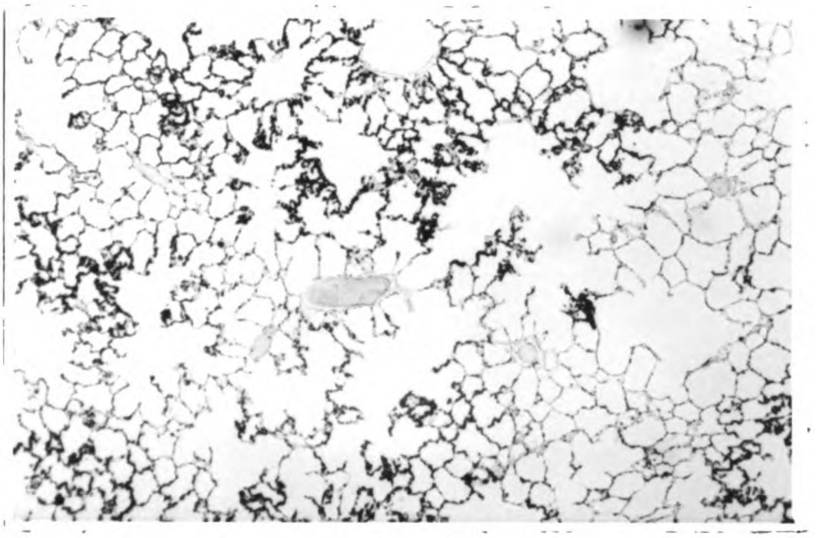


Figure 10.--Low power magnification of an unevenly perfused segment of dog lung (right lung following left vagal stimulation). Note larger vessels seem to lack ink while the capillary beds present a "patchy" appearance. About 80X.

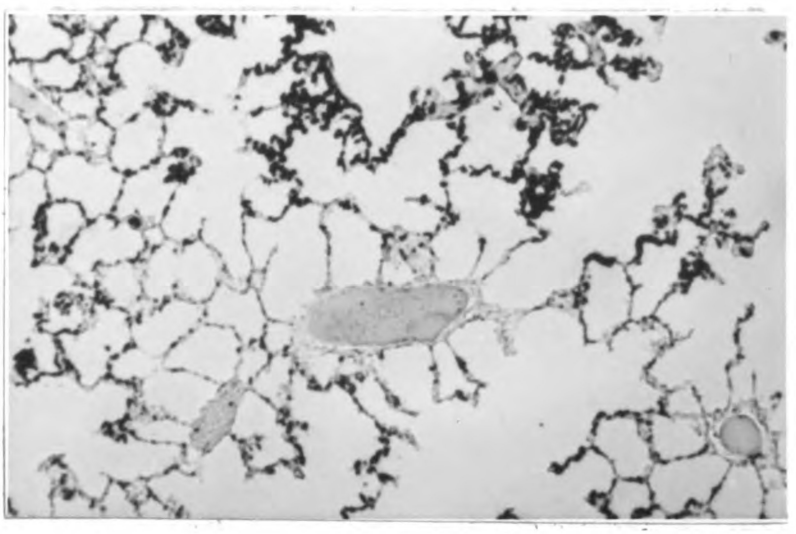


Figure 11.--Higher magnification of central area of Figure 10. Larger vessels show traces of ink while the capillary beds appear ink perfused. This same area appeared grayish-black grossly. About 160X.

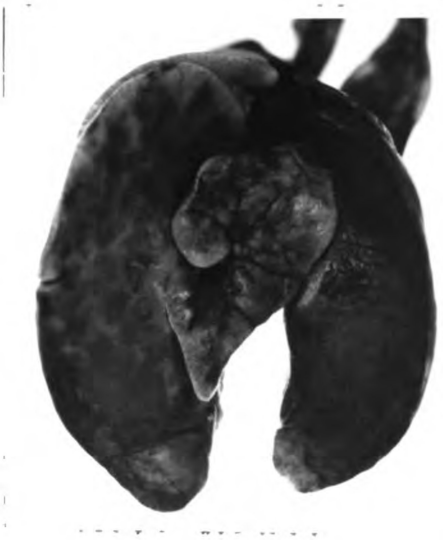


Figure 12.--Cat lung (left vagal stimulation). Diaphragmatic surface (lungs' ventral surface faces top of photograph) demonstrating nearly complete ipsilateral perfusion. The right lung, including the intermediate lobe (positioned in center) appears relatively hypoperfused.

Figure 13.--Cat lung (right vagal stimulation). Dorsal surface showing the "pooling effect" of down-lung segments. Note ink's thorough perfusion into both right and left lungs.

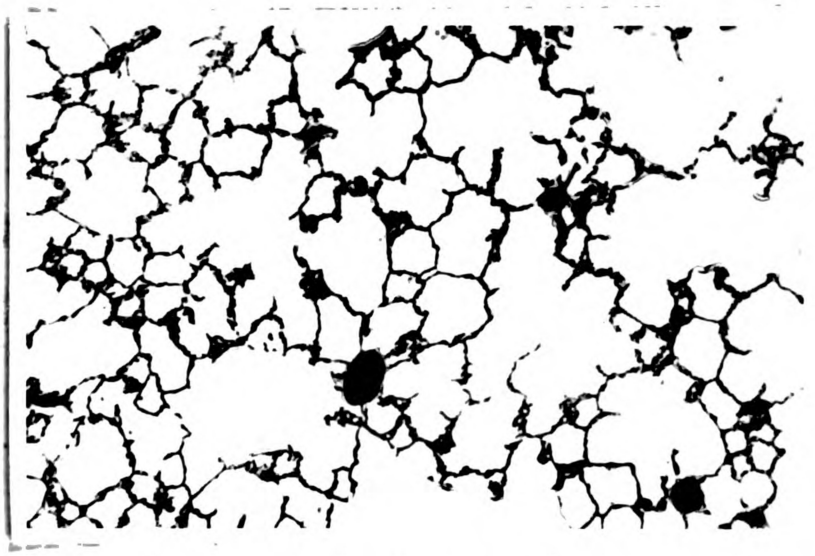


Figure 14.--Histologic section of cat lung (left lung after right vagal stimulation) demonstrating thorough perfusion of the larger vessels and alveolar capillary beds. About 80X.

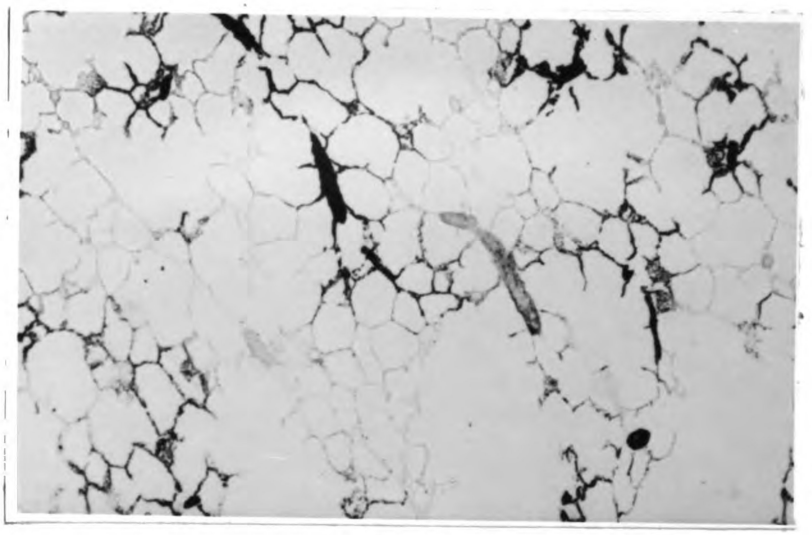


Figure 15.--A section taken immediately adjacent to that shown in Figure 14. Note uneven ink distribution (some large vessels appear ink perfused while others are void of ink). Perfusion of capillary beds appears equally mixed, i.e. some beds are filled, others are lacking ink. About 80X.

## Analysis of Perfusion-Percentages

Stimulation of the vagus nerve generates a response in the pulmonary vascular bed either actively or passively. Krahl reported a vagally-induced vasoconstrictor effect; however, the present results are not in agreement. Interpretation of these results is made by designating the contralateral lung the control. Vagal effects are determined by comparing the ipsilateral vascular perfusion patterns to those of the control. Verification of the contralateral lung serving as the control requires an examination of vagal distribution to the lungs. According to Daly and Hebb (1966d), Krahl (1964), and von Hayek (1960b), the lung innervation remains the least understood aspect of pulmonary anatomy and physiology. The anatomical disposition of the pulmonary nerves remains unclear. An ipsilateral innervation is certain, while the existence of contralaterally directed nerve fibers remains questionable. Daly and Hebb reported that in the dog vagal and sympathetic fibers derived from one side of the body may cross to innervate the contralateral lung. Removal of one lung in the cat, however, caused chromatolysis in the ipsilateral stellate, middle cervical, and nodose ganglia and had no effect on these ganglia of the opposite side.

## Active and Passive Circulatory Responses

The existence of a vagally-induced, ipsilateral pulmonary vasoconstriction with unilateral vagal stimulation could not be demonstrated. The plausibility of vagally-mediated vasodilator activity is suggested by the present investigation. However, caution must be emphasized in interpretation of these results since conclusive evidence of direct vasodilation necessitates additional investigation. In the rabbit, ipsilateral vasodilation may result from left vagal stimulation. Experimental results from the cat may reflect an ipsilateral pulmonary vasodilation following right and left vagal stimulation. Accordingly, Daly and Hebb (1966e) and Aviado (1965c) presented evidence of ipsilateral vasodilation following vagal stimulation in dogs and cats.

In contrast to a direct response, reaction in the pulmonary vascular bed to vagal stimulation may be passive, i.e. distribution of the pulmonary blood flow depends on mechanisms extrinsic to the blood vessels. Rodbard (1966) believed this extravascular regulation operated via bronchiolar tone. On inspiration, bronchial dilation reduces intra-alveolar pressure permitting engorgement of the pulmonary capillaries. During expiration, bronchiolar constriction increases air pressure within the alveolus acting to compress the capillaries thus increasing their

resistance to blood flow. Therefore, the vagus nerve, a proven bronchoconstrictor, should, upon stimulation elicit decreased perfusion of the pulmonary capillary beds. In the present investigation, however, unilateral vagal stimulation failed to consistently demonstrate decreased perfusion in the ipsilateral lung.

Physiologic evidence indicates that pulmonary vascular response to vagal stimulation is mediated passively through vagally-induced changes in cardiac output. According to Guyton (1966), stimulation of the vagus nerve decreased heart rate by slowing the rate of impulse transmission from nodal tissue to the myocardium. Decreased heart rate reduces cardiac output which initiates a passive response in the pulmonary circulation.

Haddy (1969) defined the passive response generated by the vagally-induced bradycardia. Decreased heart rate elevates left atrial pressure which, in turn, increases pulmonary venous pressure. Pulmonary arterial pressure initially decreases with decreased cardiac output, then, in response to increased pulmonary venous pressure, it shows a marked increase. Therefore, marked evidence of pulmonary underperfusion with vagal stimulation appears indicative of decreased cardiac output, i.e. the pulmonary vascular bed reacts passively to vagal stimulation. Under these circumstances, response to unilateral

vagal stimulation would appear bilaterally, as the cardiac output traverses both lungs.

The present results do not conclusively reveal a passively-induced pulmonary hypoperfusion with either right or left vagal stimulation. Thoracic palpation indicated a decreased heart rate in each species, however, the decrease failed to effect the extent of pulmonary perfusion. This fact is accentuated by results obtained from the dog. Even though the administration of propranolol enhanced the vagally-induced cardiac inhibition by blocking cardiac beta-adrenergic receptor sites, decreased pulmonary perfusion was absent. This absence may be attributed to maintainence of an approximately physiologic cardiac output. Cardiac output is determined by the product of heart rate and stroke volume (Burton, 1965). Vagal stimulation decreases heart rate, which in the absence of an altered stroke volume, reduces cardiac output; however, in the present investigation stroke volume does not remain unchanged. Stroke volume varies directly with venous return, which directly effects the end-diastolic ventricular volume. By injecting a volume of ink equal to the total pulmonary blood volume, venous return rapidly increases, which in turn, initiates an increased end-diastolic volume. Increased end-diastolic volume stretches the ventricular myocardial fibers thus increasing their force of contraction. Combination of an increased

end-diastolic volume with an increased force of contraction elevates stroke volume thus compensating for the vagally-induced bradycardia and eliminating a marked decrease in cardiac output.

Evaluation of Experimental Techniques

First, responses in pulmonary perfusion patterns to the injected anesthetic require clarification. Pentobarbital sodium depresses respiratory rate by decreasing $CO_2$ sensitivity within the medullary-located respiratory center. However, chemoreceptors, such as the carotid and aortic bodies, remain sensitive to decreased $O_2$ levels thus preventing hypoxic conditions by stimulating an increased respiration (Goodman and Gilman, 1966). In addition, pentobarbital sodium is a depressant of the central nervous system. However, electrical stimulation of the mid-cervically severed vagus nerve should counter the depressant and sufficiently excite the vagal efferent fibers.

Secondly, evaluation of the ink-perfusion technique is required. Pelikan ink (No. C11/1431A), a biologic solution, closely parallels pH, viscosity, and osmolarity of the blood. Coupled with 37°C temperature and a volume comparable to total pulmonary blood volume, the ink injection attains approximate physiologic conditions.

According to Keith and Campbell (1961), gravitational effects produced pooling of blood within the downlung of laterally recumbent subjects. Ink distribution depicted by lobar surface color patterns demonstrates a similar effect in the present investigation. Each animal, positioned on its dorsal side, demonstrated the pooling effect within the lungs' dorsal segments. According to Rodbard (1966) and Dollery and Glazier (1966), gravitational effects in man determine a much larger basal blood flow than that passing through the lung apex. Application of this statement to the present investigation indicates that vagal stimulation offers slight opposition to regional hydrostatic pressure gradients, i.e. gravitational effects are not overcome with vagal stimulation.

Retention of ink within the pulmonary circulation, accomplished by left ventricular carbachol injection, also requires an explanation. Carbachol hydrochloride, a parasympatheomimetic drug, causes immediate cardiac arrest; however, its access to the pulmonary vascular bed is not controlled. A single left ventricular contraction, after carbachol injection, suggests its presence within the pulmonary vasculature, mediated via the bronchial-pulmonary capillary anastomoses. (Daly and Hebb (1966f) clearly defined the morphology of these capillary anastomes.) The potential amount and concentration within the pulmonary system appears extremely small and its

possible effects would arise bilaterally. Thus, interference with a vagally-induced response remains minimal (possibly nonexistent).

A comparison of carbachol and potassium chloride as cardiac arrest mediators clearly emphasizes the advantages of carbachol. As mentioned, Krahl injected a mixture of India ink-saline-KCl into the rabbit's effectively beating right heart. Therefore, the KCl traversed the pulmonary circulation before its effectiveness was fully realized. According to Haddy (1969), an extracellular potassium concentration which exceeds ten milliequivalents per liter unquestionably elicits smooth muscle contraction. Krahl (1969) used a potassium concentration of 100mg in the 2.5 cc India ink-saline-KCl injectate. This concentration when converted to mEq/liter presents a value 50 times greater than 10 mEq/liter.

Finally, it should be emphasized that the present method employing visual examination of pulmonary vascular perfusion patterns is mainly qualitative. Perfusion-percentage values were assigned each lobe based upon subjective evaluations. Statistical analyses of these results were not carried out due to the inherent variables existing in any qualitative examination.

# SUMMARY AND CONCLUSIONS

Effects of unilateral vagus nerve stimulation upon the pulmonary microcirculation were studied by means of Pelikan ink injections in the rabbit, dog, and cat. Visual estimations of lobar surface color patterns produced a qualitative evaluation of pulmonary vascular perfusion patterns. Color patterns of the contralateral lung served as a control allowing direct comparison to the ipsilateral (experimental) lung.

## Active Responses

Electric stimulation of the mid-cervically severed vagus nerve failed to produce constriction of the ipsilateral pulmonary arteriolar smooth muscle as was previously reported by Krahl (1963). Active pulmonary vasodilation was suggested by the experimental results from the rabbit and cat; however, such a response was negative in the dog.

## Passive Responses

Rodbard (1966) reported that vagally-induced reductions in bronchiolar diameter indirectly elicited pulmonary vasoconstriction. The present results failed to

elucidate a link between increased bronchiolar tone and pulmonary vasoconstriction.

Haddy (1969) attributed passively-mediated pulmonary vasoconstriction to vagally-induced bradycardia. This bilateral response (cardiac output traverses both lungs) was not demonstrated by the present investigation, however, the previously defined explanation warrants a cautious interpretation of this negation.

Obviously, the results of the present investigation are best defined as an introduction to the role of the vagus nerve in the controlling mechanism(s) of pulmonary vasculature perfusion. Original investigations by Krahl emphasized the need to investigate this function of the vagus nerve. Krahl's conclusions and those from the present investigation further emphasize the necessity of additional research. It is the author's intention to employ the ink injection technique with pulmonary arterial and venous pressure recordings in a subsequent study of neurogenic pulmonary vascular control.

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