Mechanisms Controlling the Peripheral Circulation of the Lung with Some Clinical Correlations*

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Introduction

Many of my earlier studies on pulmonary morphology employed conventional methods of tissue sectioning and staining. Because of their extreme thinness, tissue sections are often difficult to appreciate as parts of a three-dimensional structure; this is particularly true of the complex lung with its myriad branches and delicate, sponge-like parenchyma. If the lung is permitted to collapse prior to fixation, much distortion occurs; airways, their accompanying vessels, and air spaces are altered in shape and size. Depending upon the fixative solution used and the method by which it is applied, a variable amount of further distortion may occur. Many methods are now available to minimize fixation artifacts and to prepare lungs for various morphometric studies (Krahl, 1956; Blumenthal and Boren, 1959; Pratt and Klugh, 1961; Staub, 1961; Staub and Storey, 1962; Storey and Staub, 1962; Weibel and Vidone, 1961; Weibel, 1963, 1964). Students, especially, but investigators, too, have gained a far better appreciation of lung structure through the study of three-dimensional preparations. Dr. William Snow Miller (1947), among many others, helped us to make the transition from two- to three-dimensional studies through his meticulous and laborious plastic and graphic reconstructions of serially sectioned lungs. Now there are also excellent methods of displaying airways and their accompanying vessels in three dimensions by making plastic injections, followed by acid corrosion (Tompsett, 1956).

Today, as the borderland between structure and function becomes less and less distinct, still other methods are required. Dr. Melvin H. Knisely of The Medical College of South Carolina, the widely acknowledged "Dean of Microcirculation," impressed upon me quite forcefully the need to study the morphology and physiology of tissues simultaneously; i.e., in vivo, for, as he has often said and written, cells, tissues, and organs live not only in the three dimensions of space, but also in the important and too often neglected dimension of time. A thin, fixed section gives the viewer but a glimpse of a "frozen moment" in a tissue's long life history, tells little of its past, and nothing whatever of what it might have done at various times in the future.

We can now study virtually every tissue and organ of the body by in vivo techniques (Bourne, 1967). Observation of the living lung, however, has posed special technical problems because of its unique structure and because of the fact that its structure and function are altered profoundly when the chest is opened to expose the lung to view.

In Vivo Studies

In 1961 and 1962 I had the privilege of working for some months in the Cardiopulmonary Laboratory at The University of Colorado Medical Center under the direction of Dr. Giles F. Filley. There I devised a thoracic window which permitted me to make direct observations of the living lung in the closed chest of the rabbit (Krahl et al., 1961; Krahl, 1962, 1963a, b; 1964a, b; 1965a; Bourne, 1967). For the first time I was able to observe normal lung as it lived and breathed, long after any effects of surgical trauma, anesthesia, and other stressful influences had subsided (Krahl, 1962, 1963b, 1964a). Moreover, respiratory gases were transported in a natural manner by the animal's own thoraco-abdominal bellows mechanism. This prosthesis revealed to me an entirely different organ, a new, dynamic structure which I had never really seen before or even imagined. As a result, I had to begin to learn about the lung all over again.

In some of my publications I have described in detail the design of the thoracic window and the techniques of installing it and have pictured some subpleural alveolar sacs and alveoli with their accompanying vessels which feed the alveolar capillary networks (Krahl,

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1962, 1963b, 1964a; Bourne, 1967). The lung is illuminated by oblique, incident light from the focused beam of a Bausch and Lomb six-volt lamp fitted with a heat-filtering glass. Even with the unaided eye, a thoracic window enables one to make interesting and valuable observations on the lung, as I will describe presently. With relatively low magnification one can see and record photographically lung movements, changes in alveolar size and shape, and intravascular phenomena in peripheral pulmonary arterioles. These vessels taper rapidly; hence, they are readily plugged by thrombi or red cell aggregates ("sludge") which are known to form following burns, accidental and surgical trauma, and in a host of diseases (Knisely, 1965). W. H. and M. H. Knisely (1954) have described the catch-trap architecture of these arterioles. The vessels deserve much further experimental, in vivo study of their dynamic responses, in thromboembolism, not only to various gas mixtures at ambient and hyperbaric pressures, but to administration of a variety of vasoactive drugs. The thoracic window technique is recommended as a most useful experimental tool in these and other potentially fruitful investigations.

Observations

When I watched the lung through a thoracic window in unanesthetized, nonsedated, lightly restrained rabbits, I sometimes noted that certain polygonal areas (bases of secondary lobules) became a paler pink than those adjacent to them. Later, these took on the bright pink hue of well-perfused lung, or paler areas appeared elsewhere in the field. This suggested that some mechanism was altering the capillary perfusion of the lung on a lobular basis.

Upon sacrificing one of my rabbits, I injected a 1:1 mixture of india ink and physiological saline into the right heart and found that the lung became a mosaic of lobules which were either black, pink, or grayish pink. Following removal of the lungs and fixation by tracheal instillation of Zenker-formol solution (Krahl et al., 1959), I removed blocks of lung tissue containing adjacent ink-stained and ink-free lobules for sectioning. Microscopic study of the adjacent pink and black lobules revealed that the ink-blood mixture had perfused peripheral arterioles in all areas of the lung. However, while ink had filled alveolar capillaries in the black lobules, it had not passed through the last, right-angled, short feeders of the capillary networks in the pink lobules. A careful histologic examination of the origins of the right-angled arteriolar branches showed that they were encircled by smooth muscle cells. These sphincter-like structures were evidently able to regulate the perfusion of nearby capillary beds. Whether they were under sympathetic or parasympathetic (vagal) control remained to be seen.

Recalling that vagal motor fibers induce contraction of smooth musculature of the airways (sympathomimetic drugs are used for dilation), my associates and I surmised that vagal stimulation might also constrict the precapillary arteriolar sphincters in the lung. We, therefore, sectioned the rabbit's right cervical vagus nerve, stimulated it electrically, and then injected the ink-saline mixture. As the last drops of ink entered the right side of the heart, we increased the current in order to induce cardiac arrest. When the vagally stimulated, right lung was removed, it was predominantly pink, whereas the left lung showed a general mottling, characteristic of ink-injected, normal lungs. Histologic examination of the lungs showed well-perfused alveolar capillaries in the grossly black areas. The ink-blood mixture, however, had not passed through the last, right-angled arterioles leading to alveolar capillaries in the grossly pink areas.

These results suggested that peripheral pulmonary arteriolar dilatation should occur during a period of sympathetic predominance. It seemed likely that if a rabbit were to become so excited that it breathed maximally, then adrenergic influences should overcome the vagus and permit optimal alveolar capillary perfusion. Therefore, rabbits were bounced 100 times in a large cardboard carton requiring vigorous contraction of all their antigravity muscles upon each descent in order to break their fall. When cardiac and respiratory rates seemed maximal, the india ink-saline mixture was injected into the right heart through the chest wall. The lungs, when exposed, were almost entirely blackened by the ink; virtually no pulmonary reserve remained, as evidenced by a few pink, poorly perfused lobules of lung tissue.

Having learned that the vagus, a cholinergic nerve, could markedly reduce flow through alveolar capillaries, we decided to inject a dosage of .01 mg/kg acetylcholine into the right heart and, after an interval of 15 seconds, inject a mixture of india ink, physiological saline, and a few crystals of KCl. When the KCl arrived in the left heart and the coronary arteries, there was a prompt cardiac arrest. This left the ink-blood mixture in the pulmonary vascular units which had been well-perfused at the moment of the last systole. The lungs of rabbits, treated in this way, were predominantly of a pink hue, although a few dusky areas suggested that ink was present somewhere in deeper vessels, having bypassed the peripheral alveolar capillaries.

When, on the other hand, .01 mg/kg of epinephrine was injected into the right heart and followed 15 seconds later by the ink-saline-KCl mixture, the exposed lungs became coal black—that is, maximally perfused.

Repetitions of these experiments

in a series of animals confirmed the results just described. It is evident, therefore, that the precapillary, arteriolar, smooth muscular sphincters of the lung *contract* upon cholinergic stimulation but *dilate* with conditions of stress (sympathetic predominance) or after the administration of epinephrine.

Clinical Correlations

Pulmonary Embolism

I have previously discussed the lung as a target organ in thromboembolism (Krahl, 1965b) and emphasized that the rapidly tapering peripheral pulmonary arterioles serve as catch-traps (Knisely and Knisely, 1954) in which even small emboli may easily become impacted.

Clinicians have observed both marked elevation of pulmonary arterial pressure and pulmonary resistance when a small shower of emboli have lodged in the pulmonary vasculature. Now, a few small emboli plugging a small proportion of the thousands of peripheral pulmonary arterioles could not directly account for this increase in pressure. However, if receptors in these vessels were stimulated by the impaction of a few emboli and, thereby, initiated a burst of vagal afferent impulses, then a resultant widespread vagal motor discharge might well close large numbers of precapillary arteriolar sphincters. The vagus nerve contributes both sensory and motor fibers to the pulmonary plexuses; hence, one may envision a vago-vagal neuronal mechanism through which a relatively small shower of emboli to the lung could, reflexively, bring about a widespread shutdown of precapillary arteriolar sphincters. This could account for the observed increase in pulmonary arterial pressure in such cases (see Addenda).

Primary Pulmonary Hypertension

The very use of the term "primary" or "idiopathic" pulmonary hypertension infers that its etiology is obscure. In any fluid-conducting system, be it the plumbing in a house or the vasculature of the human body, a narrowing of the lumens of the conducting tubes requires increased pressure if flow is to remain constant. With time, pulmonary arteries under elevated pressures undergo medial hypertrophy. Once this has occurred, vasodilator substances may no longer be effective. However, if the pulmonary hypertension is, in fact, a consequence of generalized contraction of pulmonary vascular smooth musculature under vagal control, and if the contraction can be attributed to a hyperactive dorsal motor nucleus of the vagus nerve, then one can anticipate a reduction in pulmonary arterial pressure following the administration of antivagal agents. Such puzzling disorders as primary pulmonary hypertension obviously require much further study. I should like to suggest to internists trying to solve such puzzles that the vagus nerve may well be involved. If it is, and the condition is detected in its early stages—that is, prior to medial hypertrophy—it is reasonable to expect antivagal therapy to be effective.

Primary Pulmonary Vascular Obstruction

In recent years, increasing attention has been given to a serious pediatric problem—namely, primary pulmonary vascular obstruction (PPVO). Thus far, the etiology of the disease has not been discovered, and no effective therapy has been found. The victims usually succumb before reaching the age of two years. The disease is characterized by an elevated pulmonary arterial pressure, the cause of which is said to lie at the pulmonary arteriolar level (Thilenius, Nadas, and Jockin, 1965). As mentioned previously the sphincters of peripheral pulmonary arterioles receive their motor innervation from pulmonary branches of the vagus nerve. Preliminary investigations in which we have given intracardiac injections of atropine, followed by an india ink-saline-KCl mixture, have shown that atropine relaxes smooth muscular sphincters of pulmonary arterioles; there is a prompt improvement of alveolar capillary perfusion, as evidenced by a uniform blackening of the lung. In the light of these findings, it is tempting to consider a possible autonomic imbalance in cases of PPVO. Certainly in other organ systems when there is vagal hyperactivity, e.g. gastric ulcers, vagotomy or anti-vagal medication can be of some value. If there is a hyperactivity of the vagus nerve in PPVO, perhaps the administration of atropine or some other anti-vagal drug should be considered as a means of improving peripheral pulmonary blood flow and reducing pulmonary arterial pressure.

Respiratory Distress Syndrome of the Newborn (Pulmonary Hypoperfusion Syndrome)

The preceding information which I have given on the regulation of pulmonary vascular perfusion seems pertinent to the successful management of our "Number-One Baby Killer," respiratory distress syndrome of the newborn (RDS). Recently, Chu and her associates (1965) have renamed RDS pulmonary hypoperfusion syndrome (PHS). I believe that this new term is an excellent one, for my own observations have convinced me that the basic deficit in RDS or PHS is an inadequate perfusion of pulmonary alveolar capillaries. Henceforth, I shall use the term PHS.

The problem of PHS is comparable to a large, difficult jigsaw puzzle containing many pieces which have been roughly shaped and provided by many basic scientists and clinical investigators, working independently. This, in itself, makes the puzzle difficult to

assemble correctly. The familiar commercial puzzle is simply stamped out by machine and, when reassembled, reconstitutes the entire picture story. However, the PHS puzzle picture which has emerged from the literature of the past 50 years is a confusing montage of many themes, reflecting widely varying opinions regarding the etiology and management of the disease. Its many and distorted pieces make it difficult, indeed, to assemble and view as a meaningful picture.

The pathologist, for whom the hallmark of PHS is the presence of the so-called hyaline membranes in the air spaces of infant lungs, has focused attention upon this piece of the puzzle. Some clinicians have tried in various ways (lavage, enzymes, etc.) to remove the membranes, hoping thereby to improve gaseous diffusion across the air-blood pathway. It should be realized, however, that the hyaline membrane is a post-fixation representation of proteinaceous, fibrinous exudate from pulmonary vessels and is, in reality, a *fluid* prior to the death of the victim. Nevertheless, some authors have considered the presence of hyaline-like membranes to be the sole cause of death, assuming they were made of some impermeable substance preventing alveolo-capillary gas transfer. Membranes are present only as by-products of capillary hypoxia resulting from hypoperfusion. Even if it were possible, in some safe manner, to remove the membrane-producing material in the infant suffering from PHS, more exudate would promptly leak from the still hypoxic capillaries.

Other scientists, concerned with the infant's cyanosis, have attempted to assist his respiration by intermittent positive pressure breathing (IPPB) with air or with air-oxygen mixtures. Assuming that one could effectively and safely ventilate all of the unstable alveoli of the victim's lungs, it would seem to be of little avail if pulmonary capillaries are not transmitting CO_2-laden blood to the alveoli or carrying O_2 away to the body's hypoxic tissues. I am convinced by the appearance of specimens in my collection of PHS lungs that excessive use of IPPB has, in some cases, hastened the demise of infants in respiratory distress by producing an overwhelming interstitial pulmonary emphysema. There is good evidence that breathing high concentrations of O_2 may actually impair pulmonary alveolar capillary perfusion; the lungs are known to be among the prime target organs in O_2 toxicity. A number of writers (Bruns and Shields, 1954; Tran-Dinh-De and Anderson, 1954; Berfenstam, Edlund, and Zettergren, 1958) have reported pulmonary lesions quite like those of PHS in animals exposed to 100% O_2 for several days. Potter (1952) noted that all of a group of infants whose lungs showed hyaline membranes had been kept in incubators under atmospheres rich in O_2 for some time before death. Tran-Dinh-De and Anderson (1954) reported that O_2 poisoning actually produced hyaline membranes in adult guinea pigs and rats. In newborn animals, O_2 produced the membranes plus atelectasis. In my own in vivo studies of rabbit lungs, even 15-20 minutes exposure to 100% O_2 has been followed by several days of extreme hyperemia in the peripheral vasculature of the lung, with marked engorgement of alveolar capillary networks. Thus, the use of high concentrations of O_2, with or without IPPB, may actually compound the problem by diminishing an already scanty alveolar capillary perfusion.

In recent years, pediatricians have administered bicarbonate or various buffers in attempts to correct the metabolic acidosis which often accompanies PHS. The change of pH is another by-product of the underlying capillary hypoperfusion and, while correction of pH is considered helpful by some, it does not appear to modify the ultimate cause of the infant's hypoxia. There is still an inadequate oxygenation of mixed venous blood and an inadequate removal of CO_2, while precapillary arterioles remain closed and right-to-left shunting continues through thousands of open arteriovenous anastomoses.

From the relatively vast literature on PHS there has come little agreement on the etiology of the disease or a reliable, uniformly effective method of treating it. For further discussion of this controversial subject and an excellent survey of the recent literature, the works of Silverman (1961) and Avery (1964) are highly recommended.

Through the excellent cooperation of pathologists in Baltimore hospitals, I now have a sizable and well-documented collection of lungs of premature infants who succumbed to PHS. While the so-called hyaline membranes were present in all the lungs of this series and have some diagnostic significance, I have accorded to them only slight importance, as they are only a consequence of alveolar capillary hypoxia and could not be the "sole cause of death," as some believe (Latham, Nesbitt, and Anderson, 1955). Other features of these lungs, seldom mentioned by pediatricians and pathologists, appear to be of far greater importance as guides in solving the PHS puzzle.

1) Of primary significance is the presence in all PHS lungs of a generalized constriction of precapillary arterioles with lumens so markedly or completely occluded as to retard or even halt blood flow through alveolar capillaries. Some of the precapillary vessels, seen in serial, 10μ sections, are present in only one section of a series and have no discernible lumens; thus, in such a state of contraction, they could not possibly have been transmitting red blood cells of 7.5μ diameters to the capillary beds. (Fig. 1 and 2).

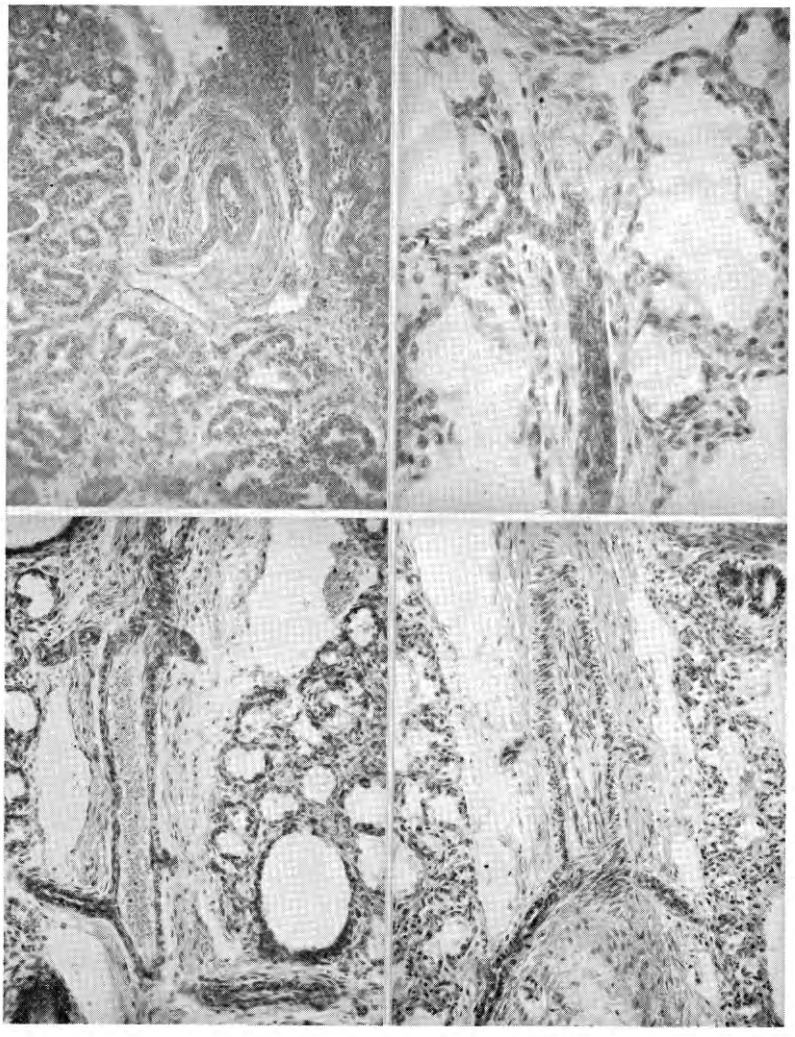

Fig. 1—Salient features in lungs of infants who died of pulmonary hypoperfusion syndrome (PHS). *Upper left:* Arteriole, cut in cross-section (near center), provides a precapillary arteriole which is too constricted to have carried blood to adjacent parenchyma. Alveoli, retaining original cuboidal epithelium, are only partially expanded. Extravasated blood cells lie above arteriolar sheath; at immediate right is an autonomic nerve twig. H & E, × 114. *Upper right:* At center, contracted arteriole's side-branch to parenchyma has no visible lumen and is not seen in adjacent sections of the series; therefore, it was unable to perfuse alveolar capillaries. Note proteinaceous coagulum in air spaces. H & E, × 248. *Lower left:* Blood-filled arteriole could not have perfused adjacent alveolar capillaries because of constriction of its various side-branches. Note widened lymphatic vessels and accumulation of blood cells in the upper right-hand corner. H & E, × 114. *Lower right:* Strikingly similar picture in another infant's lung in which distributing arteriole (off-center) shows four contracted branches. H & E, × 114.

2) There is evidence of hemoconcentration in the relatively wide pulmonary alveolar capillaries, as fluid leakage leaves blood cells tightly packed in these vessels. Potter (1952) is one of the few authors who remarks about the obvious capillary engorgement in lungs of infants who have died of PHS. She states that intense capillary engorgement is responsible for the color and increased weight of the lungs and is one of the most striking findings on histologic examination. On the other hand, Avery (1964) writes that such lungs are not significantly heavier than lungs of most infants at autopsy, although she adds that control data are lacking on lung weights of infants who succumbed to nonpulmonary diseases.

3) Many of the lungs show a spilling of blood cells into air spaces and connective tissue planes from fragile subpleural venules and from alveolar capillaries (Fig. 3). Extravasation of blood cells into the connective tissue sheaths of peripheral pulmonary arterioles may, thus, impair by compression an already diminished flow (Fig. 4). Pulmonary vessels may become exceptionally fragile when they are hypoxic, although there is ample evidence that cerebral vessels may also rupture in PHS (Blystad, 1951; Ambrus et al., 1963). In a series of autopsied infants with proven PHS, Ambrus et al. (1963) reported that 67% had cerebral hemorrhage and 53% had pulmonary or visceral hemorrhages. Avery (1964) suggests that such cerebral hemorrhages may be associated with profound tissue hypoxia, depression of clotting factors, or an elevation of cerebral venous pressure consequent to the vigorous respiratory struggles of the infant. Hutchison (1965), citing Inall et al. (1965), stated that an as yet unexplained finding in their cases was a statistically significant lowering of the hematocrit in babies with respiratory distress syndrome (PHS). I should like to suggest here a

plausible explanation for the observed lowering of the hematocrit. Not only is there a trapping of millions of blood cells in the rapidly tapering pulmonary arterioles (Knisely and Knisely, 1954) proximal to the muscular precapillary sphincters, but there is a statis of millions more in the engorged capillaries seen in PHS (Potter, 1952) and in filled but non-perfused postcapillary venules. This could, at once, account for the increased weight of the lungs in PHS observed by Potter and the lowered hematocrits observed by Hutchison (1965) and Inall et al. (1965).

4) Postcapillary venules were well filled in my PHS lung series, but the next-larger venules into which they emptied had few or no blood cells in them (Fig. 5). This, I feel, is not an artifactual loss of cells during processing; for cells should then have been lost from *all* vessels, but were not. Rather, this indicates stasis of blood in the alveolar capillaries and their postcapillary venules. The radiographic studies of Lauweryns (1966, 1968) confirm the present findings of precapillary constriction and venular emptiness. In lungs of PHS infants injected with a barium suspension via the pulmonary arteries, Lauweryns (1966) showed the peripheral pulmonary arterioles as having a pruned or "winter tree" appearance. On the other hand, the opposite lungs, injected with barium via the pulmonary veins, showed a complete filling, giving a "summer tree" appearance (Lauweryns, 1968). The constricted pulmonary precapillary arterioles would not transmit the barium mixture, but the empty, postcapillary venules accepted it readily (Lauweryns, 1968).

5) There is a marked engorgement of all pulmonary lymphatics in PHS. This is rarely noted by those interested in PHS but is a significant finding in lungs of PHS victims (Lauweryns, 1965a, b; Lauweryns, Claessens, and Boussauw, 1968). This filling of lymphatic

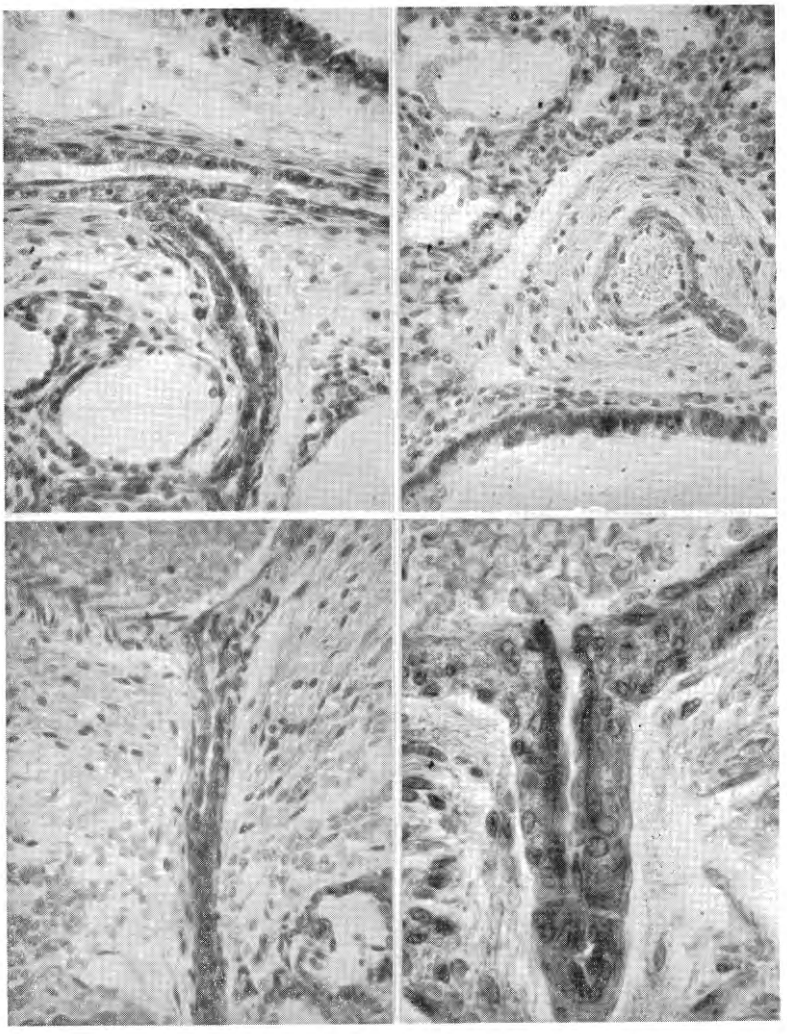

Fig. 2—*Upper left:* Constricted orifice of arteriole's side-branch prevented perfusion of adjacent alveolar capillaries. H & E, × 248. *Upper right:* Arteriole accompanying the bronchiole (below) gives off a constricted or occluded precapillary arteriole. Note hyaline material at upper left and lymphatic vessel arching over sheath of arteriole. H & E, × 248. *Lower left:* Well-filled distributing arteriole (top) shows a constriction of orifice of its side-branch (note thickened musculature), preventing perfusion of adjacent parenchyma. Note extravasated red blood cells in connective sheath of arteriole. H & E, × 248. *Lower right:* Arteriole, cut longitudinally through its lumen, gives off side-branch (bottom) which is seen in cross-section. Constriction of vessels and protrusion of endothelial cells narrow the lumen to less than the diameter of red cells (above). H & E, × 568.

vessels should not be surprising, for fluid leaks in large quantities not only into alveoli and airways from hypoxic capillaries, but also into the various connective tissue planes and sheaths of the lung. Furthermore, Drinker (1945) has shown in dog experiments that lymph does not flow from cannulated thoracic ducts of animals supported on positive pressure insufflation as it does during normal breathing. The rapid, vigorous, but ineffectual respiratory efforts of the infant suffering from PHS are evidently unable to propel the gathering excess of fluid in the lung through the many valved lymphatic vessels which lie in the subpleural connective tissue and in the plexuses which accompany pulmonary arteries, veins, and bronchi. The effects of higher than normal intrapulmonary pressures upon lymph transport, such as occur in IPPB, certainly deserve much further study. Many of the lungs of PHS victims, as received from pathologists, have engorged networks of subpleural lymphatic vessels which are readily seen with the aid of a hand lens or a dissecting microscope. Upon sectioning, such lungs show a majority of lymphatic vessels distended by a coagulum of proteinaceous fluid in which many blood cells are frequently suspended (Fig. 6).

Figure 7 is intended to re-emphasize some of the dominant features of PHS lungs, such as precapillary arteriolar constriction, loosening of bronchiolar epithelium by hyaline material, persistence of primary atelectasis, and extravasation of blood cells.

I have observed at autopsy and shortly after fixation a number of lungs of infants who died having shown all of the well-known clinical features of PHS. In each case, gross and low-power microscopic examinations of the whole lungs have enabled me to accurately predict which of the specimens would later show all of the characteristic features of PHS when sectioned, stained, and examined at higher magnifications. The lungs are heavy for their size; dark, purplish-red, because of capillary engorgement; airless (atelectatic); and readily sink in water or the fixing solution. Superficial lymphatic vessels are distended and, in places, show opacities representing coagulated proteinaceous contents, as do many of the subpleural alveoli under low magnification—especially with oblique, incident illumination. Reddish or pink specks later prove to be areas of minute hemorrhages into alveoli. Although lungs become exsanguinated to a variable degree during their removal at autopsy, some blood usually remains in the peripheral arterioles. Thus, under low magnification using incident illumination, the arterioles are rendered visible by their contents and are seen even more clearly with light transmitted through the thin edges of the lungs. In infants who die of PHS, the branching pattern is always that of the winter tree (Lauweryns, 1966). Hence, one can be reasonably certain of the final diagnosis

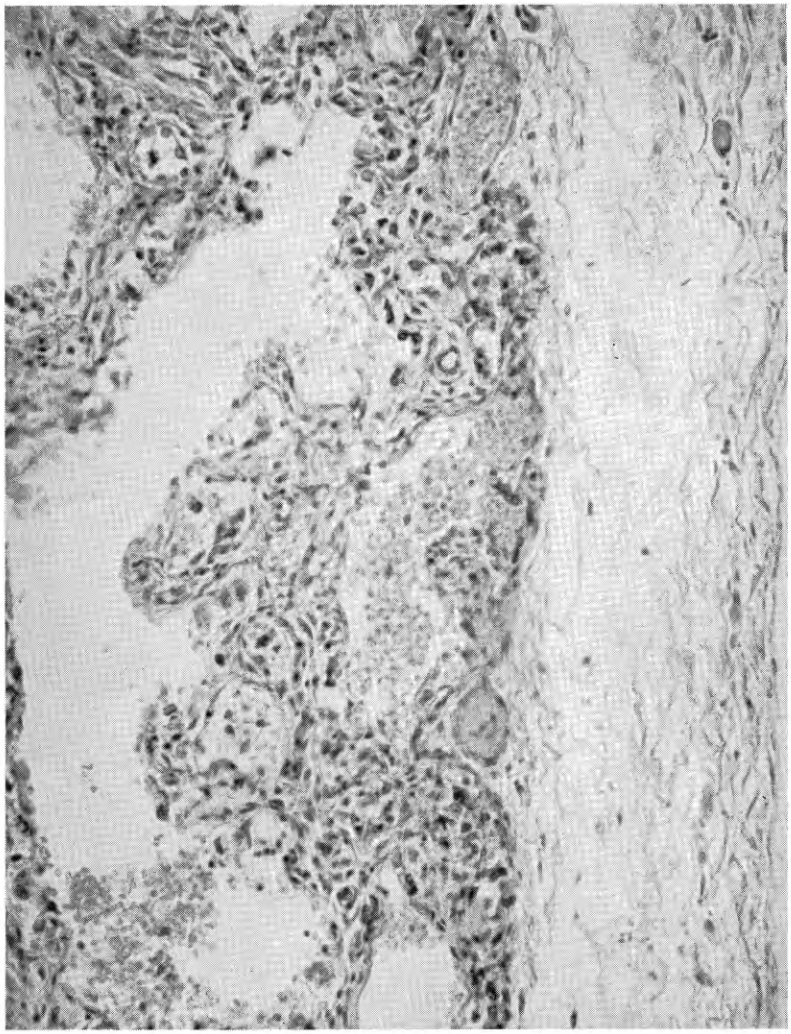

Fig. 3—Subpleural venules at right are engorged with blood cells. Near center, a venule has burst, spilling blood into peripheral air spaces. H & E, × 230.

even before the lungs are processed further for microscopic study by the pathologist.

Experimental Production of Pulmonary Hypoperfusion Syndrome

A number of workers have attempted to produce hyaline membrane disease in experimental animals and have claimed to have done so. Most of them have given as evidence of their success the presence of eosinophilic deposits in alveoli and peripheral airways. No attempt will be made here to review the literature on such experiments (see Tran-Dinh-De and Anderson, 1953, for review), but various substances, such as amniotic fluid plus HCl or other irritants, have been introduced into the lungs of experimental animals. Some investigators have done vagotomies, induced O_2 or CO_2 poisoning, or administered heavy doses of radiation; following such treatments, hyaline membranes have often been demonstrated. Actually, hyaline membranes may be produced in many different ways, but if a common factor exists in all of these studies, it is that the end result of the treatment has been the production of alveolar capillary hypoxia, which is all that is required to produce membranes following fixation. In their experimental production of hyaline-like membranes, Tran-Dinh-De and Anderson (1954) added the requirement that there should also be atelectasis. In my view, one should not be satisfied that the syndrome which occurs in certain premature infants has been reproduced unless one has not only caused eosinophilic membranes and atelectasis, but has also caused a widespread constriction of precapillary arterioles; stasis and engorgement of blood cells in alveolar capillaries and post-capillary venules; and intra-alveolar hemorrhages and lymphatic engorgement. All these characteristics are present

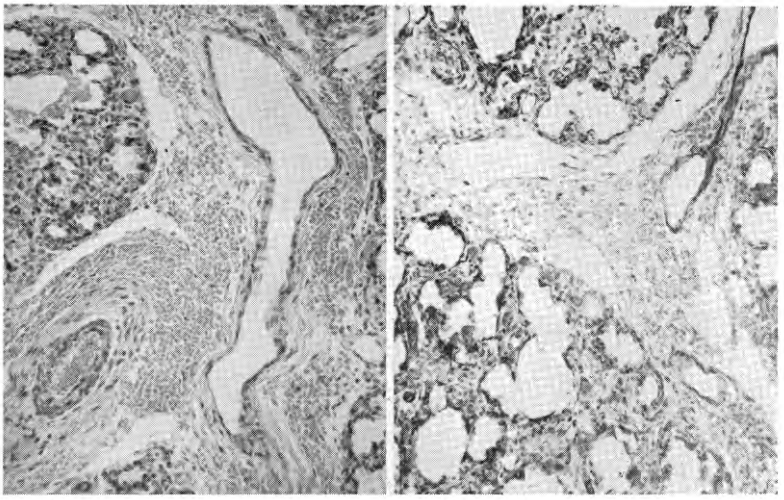

Fig. 4—Blood cells are often extravasated into connective tissue planes of PHS lungs, possibly impeding flow of lymph and venous blood by compression. *Left:* Note lymphatic channels and empty venule with many blood cells in the surrounding connective tissue. Some air spaces contain hyaline substance; many aveoli are atelectatic. H & E, × 118. *Right:* Similar conditions in subpleural lobules of another PHS victim's lung. Valve is seen in uppermost lymph vessel. H & E, × 118.

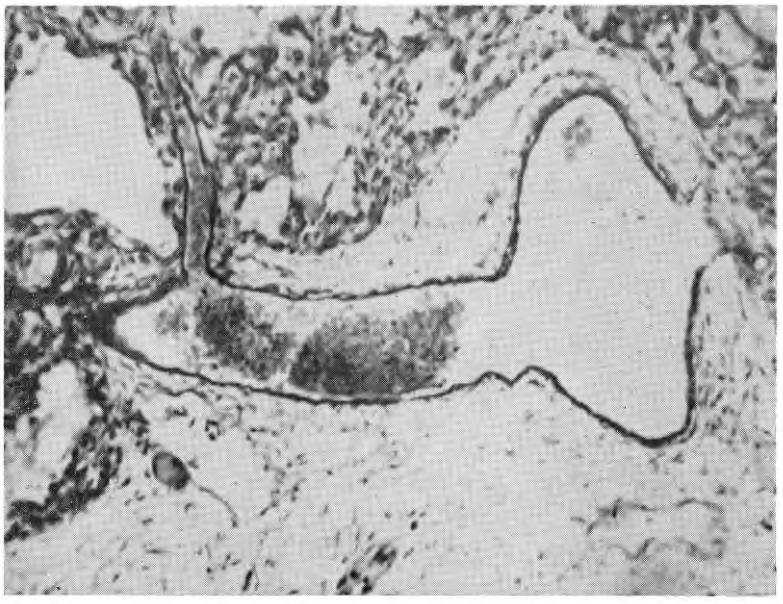

Fig. 5—Commonly seen in PHS lungs are well-filled postcapillary venules (upper left) but, because of stasis of blood in capillaries, the next larger venules are poorly filled or empty. H & E, × 183.

in lungs of infants who succumb to PHS and, therefore, must be considered in reproducing the syndrome. Prior to the death of the experimental animal, there should also have been obvious respiratory distress, an elevation of systemic arterial PCO_2, and a lowered PO_2 and pH. To my knowledge, such results have neither been obtained by others nor published thus far.

Recently a group of freshman medical students and I designed an experiment intended to produce in the adult rabbit all of the antemortem and postmortem phenomena which are seen in PHS in the human infant. The experiment was based upon the assumption that the basic, etiologic factor in PHS is a hyperactive vagus nerve. Rather than attempt the technical problems of maintaining a chronic electrical stimulation of the cervical vagus or the infusion of higher than physiologic levels of acetylcholine (small amounts would be rapidly inactivated by the cholinesterase present in the lung), we elected to infuse small amounts of eserine sulfate (physostigmine), as required, to bind the animal's pulmonary acetylcholinesterase. This permitted vagally-produced acetylcholine to accumulate and, at vagal terminals, act upon precapillary arteriolar sphincters in order to mimic vagal hyperactivity.

Through a polyethylene catheter advanced from the rabbit's femoral vein to a position in the inferior vena cava near the heart, we infused small amounts of eserine sulfate. Respiratory distress appeared promptly, as evidenced by labored respiration, flaring of the nostrils, and gasping movements of the mouth accompanied by the transient bradycardia also observed in severe cases of PHS in human infants. Blood samples taken at frequent intervals showed a progressive rise in PCO_2 and a drop in PO_2. A number of the animals died during the severe respiratory distress produced in this manner. Their lungs had been poorly perfused near death, as evidenced by their paleness, despite an injection of india ink-saline solution given just as death seemed imminent. Furthermore, the peripheral pulmonary arterioles, viewed by transillumination of the thin lung margins, had the pruned or winter tree appearance noted in the arterial injection studies of Lauweryns (1966) in human infants who had died of PHS.

We were gratified, of course, when our experimental procedure reproduced in a laboratory mammal the antemortem distress, the blood gas changes, and the alveolar capillary hypoperfusion which have been demonstrated in infants suffering from PHS; but, having done this, we hoped also to be able to show that the use of an anti-vagal drug could restore perfusion of alveolar capillary networks and permit the experimental animal to survive.

In still other animals in which respiratory distress had been produced by infusions of eserine sulfate, as described above, we were successful in relieving the distress and returning the blood gas and pH levels to normal values by intravenous infusions of atropine, as required.

The Assembled Puzzle

In my own method of assembling the PHS puzzle, I make the central etiologic piece a hyperactive vagus nerve. I believe that it is hyperactive not only because of the immaturity of vago-vagal reflex mechanisms, but because of dorsal motor nuclei of vagus nerves which still lack the restraints normally imposed upon them in more mature fetuses or term infants. Hyperactive vagi could constrict large numbers of precapillary arterioles and their specialized smooth muscular sphincters, which I have described and have shown to be under vagal control (Krahl, 1965b). They are closed or markedly contracted in the lungs of infants who died of PHS (Lauweryns, 1966; Krahl, 1968). This view is supported by Buckingham, Sommers and Sherwin (1967), who demonstrated histologic peculiarities in neurone cell bodies in the dorsal vagal motor nuclei of PHS victims, particularly

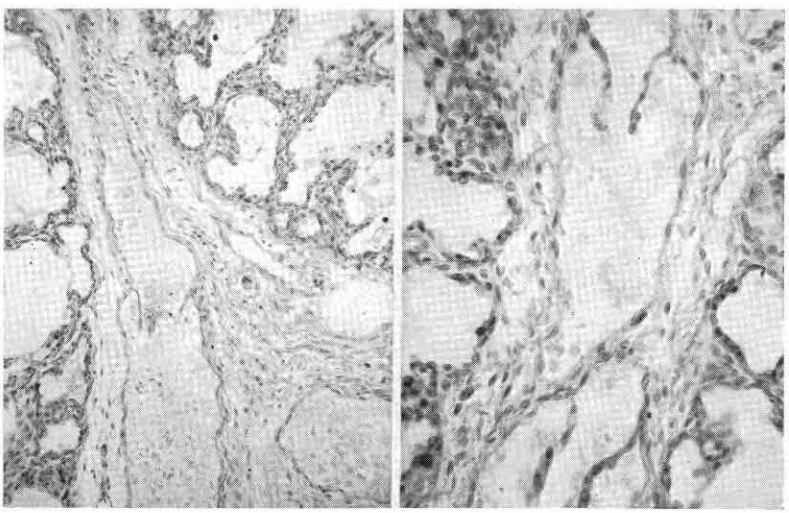

Fig. 6—Capillary hypoxia in PHS lungs results in leakage of proteinaceous fluid into: a) air spaces where, upon fixation, it appears as eosinophilic hyaline material, and b) widened lymphatic channels where it forms a coagulum which often contains a few or many blood cells. Note valves. Some alveoli have retained their original cuboidal epithelium. From two tissue samples of one lung. *Left:* H & E, × 114; *Right:* H. & E, × 248.

those weighing less than 2,500 gm. This was suggestive of cell exhaustion arising from excessive activity. Thus, arteriolar constriction, in turn, would reduce or halt the flow of blood through alveolar capillaries.

Capillary hypoxia can be held accountable for the leakage of proteinaceous, fibrinous exudate into alveoli and connective tissue areas of the lung. Following fixation some of the fluid forms the eosinophilic, so-called hyaline membranes in peripheral alveoli and airways. Fluid containing protein and blood cells also distends vast numbers of pulmonary lymphatic vessels (Lauweryns, 1965a, b; Lauweryns et al., 1968), which are poorly drained because of the infant's abnormal respiratory mechanics.

Stasis of blood in pulmonary alveolar and other peripheral capillary beds deprives the epithelial cell "factories" of the raw materials required for production of the critically important surfactant film necessary for normal alveolar stability. Its deficiency in the lungs of PHS infants accounts for the stiff, noncompliant lungs which are so difficult for the infant and the inhalation therapist to inflate. Widespread alveolar collapse eventuates in the airless, liver-like lungs seen at autopsy. Stasis of blood in pulmonary alveolar capillary networks also explains why there is insufficient *vis a tergo* to propel blood through postcapillary venules onward into the larger venules and pulmonary veins.

If the picture of PHS as I have portrayed it here is the correct one, and the experimental evidence which I have submitted appears to support this, then I feel that there is now a rational basis for treating PHS by anti-vagal therapy when the very first symptoms appear rather than as a last resort. Atropine or other effective anti-vagal agents should be infused via the umbilical vein, as required, to alleviate the clinical symptoms and adjust pH and blood levels of O_2

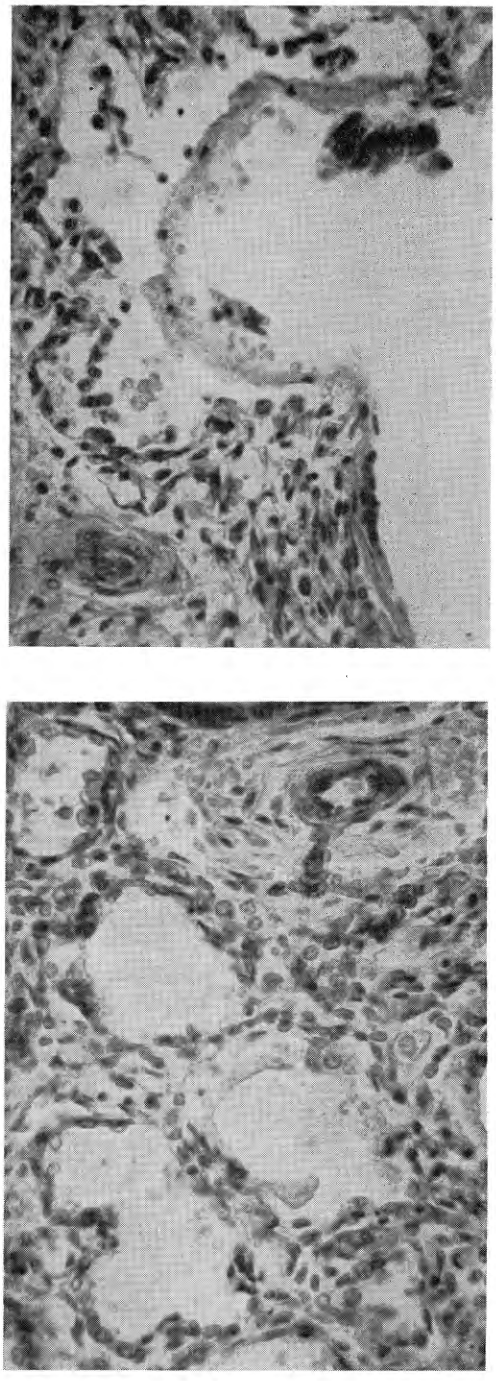

Fig. 7—Fluid exudate in PHS lungs loosens and disrupts epithelial linings of peripheral air spaces. *Upper:* Note clumps of bronchiolar epithelial cells lifted by band of hyaline material. *Lower:* Cuboidal alveolar epithelium is loosened at lower left. Precapillary arterioles in both photomicrographs have obliterated lumens as a result of vigorous constriction. H & E, × 340.

and CO_2 toward normal values. Once perfused, alveolar capillaries should stop leaking, respiratory gases should cross alveolocapillary membranes, and alveolar (bronchiolar?) epithelial cells should begin to produce sufficient quantities of the stabilizing surfactant film. With increased pulmonary compliance, respiratory struggles, sternal retraction, and expiratory grunting should subside. Oxygen therapy and IPPB, with their attendant hazards, would no longer be required. Metabolic acidosis should soon be corrected with adequate O_2 uptake and CO_2 removal from tissues and mixed venous blood.

In recent months, two of my former students in Baltimore and a clinical friend in North Carolina have pioneered in the use of umbilical infusions of atropine sulfate solution in infants who appeared to be dying of PHS. The dramatic initial successes in this small group of babies encourages me to suggest that atropine or other anti-vagal agents might now be tried in PHS cases by other pediatricians conducting prospective and double-blind studies.

Summary

I have described my in vivo studies of mammalian lungs, focusing on the peripheral pulmonary arterioles. These observations led to my current concept that peripheral pulmonary perfusion is regulated on a lobular basis by motor fibers of the vagus nerve.

Besides having purely academic interest, vagal regulation of pulmonary alveolar capillary perfusion has practical implications for clinical problems such as pulmonary embolism, primary pulmonary hypertension, primary pulmonary vascular obstruction and, particularly, PHS.

Supported by experimental evidence, I have presented the thesis that there is a stasis of blood in alveolar capillaries caused by a generalized constriction of precapillary arteriolar sphincters produced by vagal hyperactivity. This, alone, can be held accountable for every clinical and histopathological feature of the syndrome. Evidence adduced from my own and other studies supports the idea of vagal predominance in PHS. A rational basis for a simple and, hopefully, a generally successful treatment of PHS by anti-vagal agents such as atropine has been outlined.

Addenda

After the completion of this manuscript, the following chapter came to my attention: "Pulmonary Circulation in Pathological States" by A. M. Rudolph in *Paediatric Cardiology*, H. Watson (ed.). London: Lloyd-Luke, 1968, pp. 57–62. This excellent work brings together recent information regarding the pathologic physiology in PHS and the influences of hypoxia and acidosis on pulmonary circulation. It also emphasizes the clinical features, radiologic characteristics, and biochemical changes in PHS. No attempt is made here to summarize Rudolph's paper; it is simply highly recommended for those who wish an introduction to the complexity of the PHS problem, a statement of current methods of therapy, and an up-to-date bibliography on PHS and other types of pulmonary pathology.

While this paper was in press, I was reminded by Dr. W. H. Knisely that the question of whether pulmonary hypertension actually does follow a relatively small shower of emboli is quite controversial. Thorough discussion of this question and citation of all the pertinent literature cannot be included here. The reader is referred to an excellent book by R. Marshall: *Pulmonary Embolism. Mechanism and Management.* Springfield, Ill.: C. C. Thomas, 1965 (includes 484 bibliographic references).

Acknowledgement

In their freshman research project at The University of Maryland School of Medicine, R. Bardow, T. Detrich, A. Steele, and N. J. Wilson undertook the experimental production of PHS in the rabbit. Their findings, mentioned in this paper, are significant. It is a pleasure to acknowledge these contributions resulting from their enthusiastic efforts. A paper describing and illustrating their results in detail is in preparation.

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