Pulmonary Venous Vasculature in Neonatal Hyaline Membrane Disease

Abstract. *The pulmonary veins and venules in lungs of newborn infants with hyaline membrane disease can be completely filled with a barium sulfate solution. The same injection technique and combined roentgenological, histological, and histometrical investigations also reveal a defect in filling of many small muscular arteries and of most pulmonary arterioles. Hence, the latter pulmonary vessels are assumed to be important in pulmonary ischemia, as demonstrated in the respiratory distress syndrome of the newborn infant.*

We have reported our difficulties in injecting the pulmonary arteries, to arteriolar level, of infants who died from hyaline membrane disease (HMD), and have concluded that in this disease, often fatal to newborns, respiration and pulmonary hemodynamics are disturbed (*1*). This morphological observation was supported by physiological (*2*) and clinical (*3*) data from a number of investigators who suggested that the most notable lesion of HMD may be a pathological pulmonary vasoconstriction, which could take place along several pathways of physiological feedback. As Chu *et al.* (*3*) emphasized, the complex nature of the functional and structural relations and the self-perpetuating, progressive course of the syndrome could be summarized in the following pathogenetic sequence: pulmonary vasoconstriction → alveolar hypoperfusion → deficient anabolism in alveolar cells (*4*, *5*) [→ surfactant-deficient alveolar surfaces (*6*) → atelectasis] → increased alveolar wall permeability [→ effusion into airspaces (*5*, *7*) → fibrin deposition (*8*)]. They suggested that HMD be renamed the "pulmonary hypoperfusion syndrome" (*3*) or "neonatal pulmonary ischemia" (*9*).

In order to localize the level of the pulmonary vascular tree at which this presumed vascular observation exists, we undertook injection studies of the pulmonary arteries and reported that many small muscular pulmonary arteries and most pulmonary arterioles cannot be filled with a barium sulfate solution in lungs of newborns with HMD, but that they were easily completely injected in the lungs of normal 4-day-old lambs (controls) (*10*). We now report our attempts to discover the injection pattern of pulmonary veins in infants with HMD. It has been suggested (*11*) that reflex pulmonary venoconstriction is the "crucial functional abnormality" in HMD, which has been interpreted as an entity of the venous "pulmonary shutdown syndrome" (*12*). We no longer use 4-day-old lambs as controls. Such animals may have been a source of error in our earlier studies.

We now report findings of our comparative radiographical, microradiographical, histological, and histometrical studies of the pulmonary arterial (on one lung) and of the venous (on the other lung, of each infant) vasculature (after its injection with a solution of barium sulphate) in ten human newborns who had died from clinically and histologically proven HMD. Significant obstruction of the small muscular arteries and especially of the pulmonary arterioles is demonstrated in HMD, in which the pulmonary venous vasculature is easily and totally (that is, including all pulmonary postcapillary venules) injected.

After necropsy, the infants' lungs were kept in water (2° to 3°C) for 2 days and were then stored dry in a deepfreeze for 2 to 5 days. At the end of this period the lungs were immersed in tap water for 3 to 4 hours and then injected at room temperature. The injection was carried out in group 1 (injection of the pulmonary arteries) through a cannula inserted in the main pulmonary arterial trunk of the right lung at precisely 10, 20, 30, 40, 60, and 80 mm-Hg. In group 2, the pulmonary veins of the left lung of each infant were cannulated from the left atrium onward and injected at 5, 10, 15, 20, 30, and 40 mm-Hg. Each pressure was applied for 2 minutes and continuously controlled on a Dräger micromanometer. The bronchi and the pulmonary veins (of group 1, in contrast to pulmonary arteries of group 2) were left free to the air. The injection mass consisted of a barium sulfate solution [40 g of $BaSO_4$ (Alubar-Wander), 60 ml H_2O].

During the injection, an x-ray film was made after each change in pressure, by use of a fluoroscopy brilliancy amplified radioangiographic apparatus (Balteau) at 50 kv, 30 ma, 50 seconds. The radiographic films (Structurix D_2—Gevaert) were industrial, that is, high-contrast fine-grain emulsions that permit photographic enlargement of up to ten times.

After being injected, the lungs were stored overnight in the deepfreeze. The next day, they were cut with a large dissecting blade into several whole-lung sections 3 to 5 mm thick. The thawed slices were then microradiographed (50 kv, 30 ma, 45 seconds), and each microradiograph was later analyzed and enlarged with a Zeiss stereomicroscope with a variable power pod.

The lung sections were at the same time fixed in Bouin's fluid, and numerous samples of the injected and noninjected zones (as revealed by the microradiographs) were carefully selected and studied in serial histological sections; some samples were stained with hematoxylin-eosin-saffranin, and others with the elastica Verhoeff–Van Gieson stain. On two representative elastica-stained slides the main external diameter of all pulmonary arteries and arterioles present (of group 1, in contrast to pulmonary veins and venules of group 2) was recorded (*13*) with an ocular scale (ocular, × 10; micrometer, 0.01 mm; objectives, 10, 25, 40; Zeiss standard universal microscope). Only vessels cut completely or approximately at right angles to their axes were measured.

The histometrical measurements were made independently by two investigators. The pulmonary arteries (group 1) were classified into main pulmonary arteries (50 μ), small muscular pulmonary arteries (50 to 30 μ), and pulmonary arterioles (< 30 μ) (*14*). The pulmonary veins (group 2) were subdivided into venules and veins (*15*), and their precise dimensions were recorded. In each of the five groups so obtained, the individual vascular dimensions were noted, and the vessels were listed as "injected" or "noninjected." Both investigators recorded essentially the same results.

On injection of the pulmonary arterial trunk of HMD lungs, only the main pulmonary arteries (in the immediate vicinity of the lung hilus) were opacified fluoroscopically and radiographically at 10 mm-Hg pressure. At 20 to 30 mm-Hg, these main arteries were injected in the medial zones of the lungs; smaller side branches were usually not conspicuously filled. When the pressure was increased to 40 mm-Hg, we were able to inject some narrower and slightly tortuous side branches which corresponded histometrically to small pulmonary arteries. At 60 and 80 mm-Hg, we were able to reach more of the lung periphery with a more evenly distributed

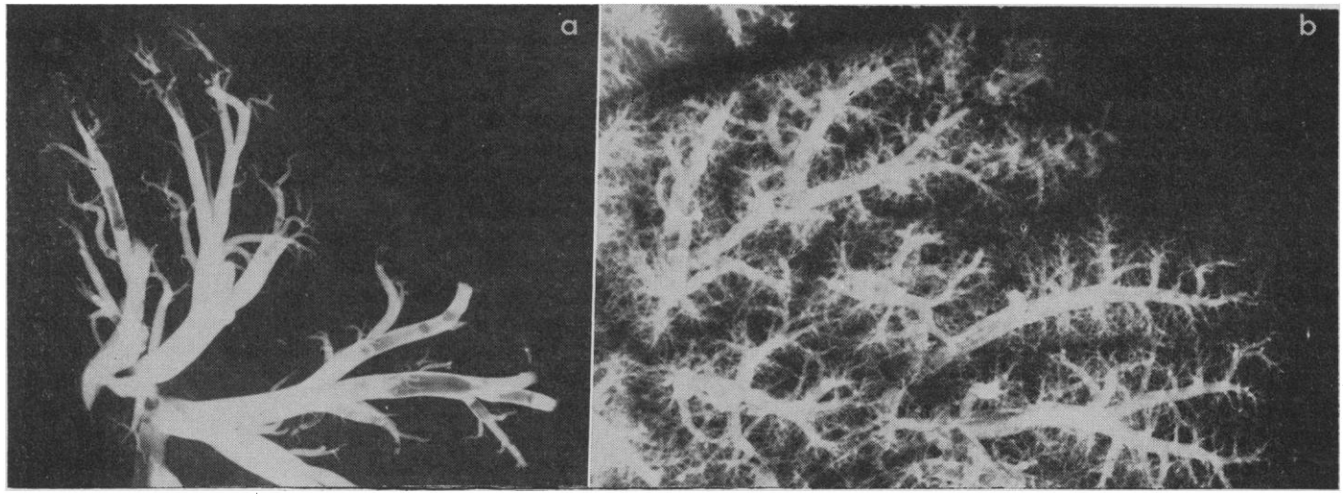

Fig. 1. Microradiographs of the right lung (injection of the pulmonary arteries) and of the left lung (injection of the pulmonary veins) in a case of neonatal hyaline membrane disease (HMD). (a) Filling of the main pulmonary arteries (large and well-contrasted vessels) and of some small muscular pulmonary arteries (smaller, slightly tortuous vessels); no filling of pulmonary arterioles; "Winter-tree pattern"; injection at final pressure gradient of 80 mm-Hg. (b) Entire filling of the pulmonary veins (large, rather straight-coursing trunks) and the ramifying network of pulmonary venules; ("Summer-tree pattern"; injection at final pressure of 40 mm-Hg (× 6).

injection throughout the different parts of the lung parenchyma. This filling was still very incomplete, and the most distally injected arterial branches still had a large diameter, and no alveolar capillaries were opacified.

Microradiography (Fig. 1a) permitted identification of the different injected vessels and an appropriate selection of tissue blocks for histology. There was a clear filling of large and well-contrasted, rather straight running vessels (identified histometrically as main pulmonary arteries). Among the smaller vessels, only some, usually slightly tortuous, were focally injected (histometrically: small muscular pulmonary arteries). These continued exceptionally in fine and narrow injected branches (histometrically: pulmonary arterioles). There was practically no alveolar capillary filling.

Histology of multiple sections and histometry of the diameters of all injected and noninjected vessels on elastica-stained slides revealed that the barium sulfate solution had filled the lumen of nearly all main pulmonary arteries, of approximatively one-third of the small muscular arteries, but of almost no pulmonary arterioles.

The final pressure gradient (80 mm-Hg) has been applied during 2 hours (instead of 2 minutes) in four cases of HMD, as a result of which no important change in filling was observed. Only the roentgenologic contrast of vessels already injected was somewhat improved, but the contrast substance did not penetrate any further, and it did not fill more vessels.

The same technique produced different results after injection of the pulmonary veins of the left lung of each infant. Already at 5 mm-Hg pressure, not only the large pulmonary veins but also their very fine side branches (the pulmonary venules) were opacified fluoroscopically and radiographically in the hilar zones of the lungs; at 10 to 15 mm-Hg, the medial zones of the lungs were filled in the same way, and the injection reached the large venous and the small venular branches in these areas. Increasing the pressure to 15, 20, 30, and 40 mm-Hg progressively caused injection to more of the peripheral region throughout the entire lung parenchyma, and resulted in a widespread, complete filling of the delicately ramifying venous and venular pulmonary plexus.

Microradiography confirmed that this procedure had entirely filled the large, rather straight-coursing venous trunks (histologically: pulmonary veins) that went into smaller branches and an extensively developed, completely filled network of very fine side branches (the pulmonary venules, as revealed by subsequent histological study) (Fig. 1b). When we compared the radiographs and microradiographs of pulmonary arterial injection to venous injection of HMD-infant lungs, we could see "a Winter tree" (Fig. 1a) or "a Summer tree" (Fig. 1b) pulmonary vascular pattern.

Histology of multiple serial sections and histometry on elastica-stained slides demonstrated that all pulmonary veins and venules throughout the HMD lung parenchyma had been filled. Many alveolar capillaries were also injected. At the same time, a backward filling was observed in many bronchopulmonary veins, in bronchial venous plexuses, and in bronchial capillaries. In some instances even the bronchial arteries were injected in some areas. Careful investigation revealed that these bronchial vessels were also on the microradiographs.

In four lungs of this group a final pressure of 80 mm-Hg was also applied for 2 hours. This prolonged injection again did not markedly affect the vascular filling.

In our experiments we have sought to compare, always using the same technique, the pulmonary arterial and the pulmonary venous vasculature on the lungs of each infant who died from HMD. Although we were able to obtain a complete filling of the pulmonary veins and venules in these lungs, it was impossible to inject some main pulmonary arteries, approximately two-thirds of the small muscular pulmonary arteries, and nearly all pulmonary arterioles in the HMD lungs.

These data, however, must be interpreted with utmost care. They constitute only an experimental approach to a much more complex situation in the living infant, and they must still be considered tentative and only valid in the given experimental setup, especially since they were obtained on postmortem material. Though carefully handled, the lungs and especially the vessels were to be left untouched before the injection, not even washed out to remove postmortem thrombi after necropsy. Hence, there was a rather long delay before in-

jection. Reduction of this delay—even investigation of the approach with another (injection) technique—should be attempted. The pulmonary arteries were injected according to the normally occurring blood flow, but the pulmonary veins could only be approached by an injection in a reverse flow manner (which did not seem to affect their complete filling, and hence can be considered unimportant).

Tissue perfusion may result from an equilibrated and extremely complex interplay of a wide spectrum of various morphological and functional factors, many of which are still unknown in health and disease. It may be an intricate "mosaic" which could possibly only be analyzed by a programmed computer tape (*16*).

Among the many mechanisms controlling tissue perfusion, an important morphological factor is the specific anatomical pattern of the organ's nutritive blood supply, which is peculiar to the lung and even changes with age. In this sense, the functional significance of the filling of the bronchial venous plexus, which we have again (*17*) observed in HMD lungs only after the injection of the pulmonary veins, should be further elucidated. Embryological (*18*), histological (*17, 19*), and pathological (*20*) studies have repeatedly revealed many widespread connections between the bronchial and the pulmonary circulation; they are even especially developed during embryogenesis and in the newborn infant may be important (even if technically difficult to assess) in collateral microcirculatory pathways during a hypoperfusion.

As important in the tissue perfusion of HMD lungs is the lymphatic microcirculation, which is an important factor in tissue-fluid balance and exchange, and to which, until now, only a casual interest has been paid. In HMD, the pulmonary lymphatics are frequently dilated and filled with an eosinophilic, presumably proteinaceous, possibly fibrinous fluid (*5*), as confirmed mathematically by histometrical measurements of the mean lymphatic diameter which is consistently greater in the idiopathic respiratory distress syndrome than in a control group of other newborns (*20*).

To summarize, although much information is still lacking, my observations support the argument for a disturbance of pulmonary perfusion localized largely at arterial level, the small muscular pulmonary arteries (50 to 30 μ) and especially the pulmonary arterioles ($< 30\ \mu$). The pulmonary venous vasculature does not seem to be the most important primary obstructive factor. These observations, as well as the filling of the bronchial venous plexus after injection of the pulmonary veins and the lymphatic angiectasis in such lungs, should be completed by urgently needed further data concerning the blood and lymphatic microcirculation in HMD.

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Auxin and Wall Extensibility: Reversibility of Auxin-Induced Wall-Loosening Process

Abstract. *The reversible nature of the auxin-mediated loosening of the cell wall is shown by the ability of respiratory inhibitors to cause the loss of the auxin-induced increase in wall extensibility without affecting the basal extensibility of the wall. This reversibility makes it unlikely that wall loosening is mediated by enzymes, such as cellulase and β-glucanase, which degrade polysaccharides, since their action is essentially irreversible.*

Auxin is believed to control the rate of cell elongation by regulating the extensibility of the cell wall (*1–3*). In stem and coleoptile tissues, auxin induces an increase in the wall extensibility. This potential for extension can, in turn, be converted by turgor pressure into a finite amount of wall extension (*2*). The process of cell elongation is the result of a continual series of these wall loosenings and extensions.

The biochemistry of the process of wall loosening is still unknown, but it may be mediated by enzymes, such as cellulase and β-glucanase, which degrade wall polysaccharides. Indeed, auxin can increase the endogenous activities of cellulase (*4*) and hemicellulases (*5*), and a crude β-glucanase preparation has been reported to replace auxin in inducing cell elongation (*6*). In addition, the extensibility of isolated cell walls can be increased by their treatment with cellulase (*7*).

Since the action of enzymes that degrade wall polysaccharides is essentially irreversible (*8*), any wall loosening that is induced in this way will persist until converted into extension or until the cleavage of the polysaccharide chains is repaired by separate, synthetic processes. One method of assessing the participation of these enzymes in wall loosening is to determine whether the wall-loosening process is irreversible.

There is some evidence (*9*) that wall