Pulmonary Arterial Vasculature in Neonatal Hyaline Membrane Disease

Abstract. *Many small muscular pulmonary arteries and most pulmonary arterioles in lungs of newborn infants with hyaline membrane disease cannot be filled with a barium sulfate solution. In the control group, composed of normal, 4-day-old lambs, such vessels were easily and completely injected. Hence these vessels are supposed to play an important role in the pulmonary hypoperfusion, recently demonstrated in the respiratory distress syndrome of the newborn.*

In the so-called hyaline membrane disease (HMD) of the newborn, most observations and experiments have been directed to the air interface of the alveolar parenchyma. Recent physiological (*1*) and clinical (*2*) data from a number of investigators, however, have suggested that the basic and crucial abnormality in this disease may be a pathological pulmonary vasoconstriction, confirming my previous morphological studies (*3*). As Chu *et al.* (*2*) emphasized, pulmonary vasoconstriction is indeed common to several pathways of physiological feedback, which may explain the well-known functional and structural characteristics of HMD in the following summarized 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*)]. Hence Chu *et al.* suggested that the HMD syndrome "may be renamed the pulmonary hypoperfusion syndrome."

In order to localize precisely at which level of the pulmonary vascular tree this presumed obstruction does exist, I undertook a radiological, microradiological, histological, and histometrical study of the pulmonary arterial vasculature (after its injection with a solution of barium sulfate) of ten infants who died of clinically and histologically proven HMD. Data obtained were compared with a control group of lungs from twelve 4-day-old normal Texel lambs. As a result, an important obstruction at the level of the small muscular pulmonary arteries, and especially of the pulmonary arterioles, is convincingly demonstrated in HMD.

After necropsy of the humans (group 1) the lungs were kept in water in a freezer (2° to 3°C) for 2 days, then stored dry in a deepfreeze for 2 to 5 days, and finally injected at room temperature, after a brief (3 to 4 hours) previous immersion at the laboratory in tap water. The injection was carried out through a cannula inserted into the main pulmonary arterial trunk at precisely 10, 20, 30, 40, 60, and 80 mm-Hg, the pressure being continuously controlled on a Dräger-micromanometer. Each pressure was applied for 2 minutes. The bronchi and the pulmonary veins were left free to the air. The injection mass consisted of a barium sulfate solution [40 g $BaSO_4$ (Alubar-Wander), 60 ml H_2O].

During the injection procedure an x-ray film was made stepwise 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 used (Structurix D_2-Gevaert) were of so-called industrial type, that is, highly contrasting and fine-grain emulsions, which permit an easy photographic enlargement up to 10 times.

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

The lung sections being meanwhile fixed in Bouin's fluid, numerous biopsies of the injected and noninjected zones (as revealed by the microradiographs) were carefully selected and studied on serial histological sections, some of which 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 was recorded, by the method described by Rosen *et al.* (*9*), with an ocular scale (ocular, 10×; micrometer, 0.01 mm; objectives, 10, 25, 40; Zeiss Standard Universal microscope). Only vessels cut completely or approximatively at right angles to their axes were measured.

The histometrical measurements were done blind and independently by two different investigators (slides from groups 1 and 2 being put together in a sequence unknown to both microscopists). The pulmonary arteries were classified into three distinct categories, namely, main pulmonary arteries (> 50 μ), small muscular pulmonary arteries (50 to 30 μ), and pulmonary arterioles (< 30 μ), according to the classic data (*10*). In each of these three groups, the individual vascular dimensions were noted and the vessels listed as "injected" or "noninjected." Finally, the total number of injected and noninjected vessels in each category was counted (Fig. 1). No significant difference has been noted between the results of both investigators.

The normal and healthy lambs comprising the control group (group 2) were killed by a rapid intraperitoneal injection of a lethal dose of pentothal. The lungs were then stored, injected, and investigated by exactly the same procedure as used with group 1.

On injection of the pulmonary arterial trunk of HMD lungs, only the main pulmonary arteries (localized in

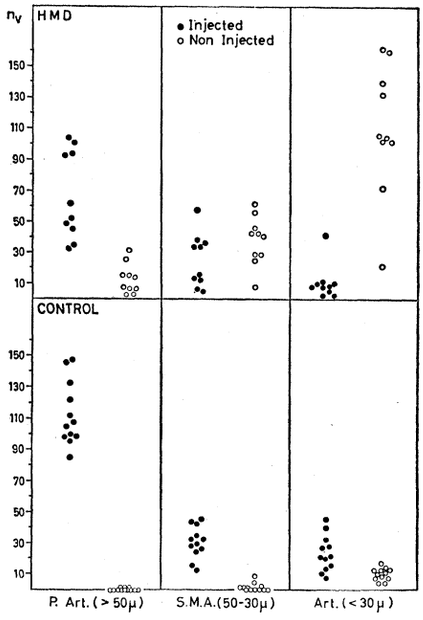

Fig. 1. Total number (n_v) of injected and noninjected pulmonary arteries in human lungs with hyaline membrane disease (HMD) and in a control group of normal neonatal lamb lungs. The pulmonary arteries are classified into three distinct categories, according to their main external diameter on elastica-stained sections: main pulmonary arteries (P.Art. > 50 μ); small muscular pulmonary arteries (S.M.A. 50-30 μ), and pulmonary arterioles (Art. < 30 μ).

the immediate vicinity of the lung hilus) were opacified on the radiographs at 10 mm-Hg pressure; at 20 to 30 mm-Hg the arterial filling progressed to the central and medial zones of the lungs; increasing the pressure stepwise up to 80 mm-Hg gave a still more peripheral and more evenly distributed injection throughout the different parts of the lung parenchyma. This filling of the arterial network was, however, very incomplete, the most distally injected branches having still a large diameter and no alveolar capillaries being opacified. Microradiography confirmed these findings (Fig. 2) and allowed an appropriate selection of tissue blocks for histology. Histometry of the diameters of all injected and noninjected vessels revealed that the barium sulfate solution had filled the lumen of nearly all main pulmonary arteries, of some small muscular pulmonary arteries (mean ratio: one-third injected), and of almost no pulmonary arterioles (Fig. 1). It is worthwhile to note that in four of these cases a final injection at 80 mm-Hg has been carried out during 2 hours continuously (instead of 2 minutes). The results obtained were practically the same as in the other experiments.

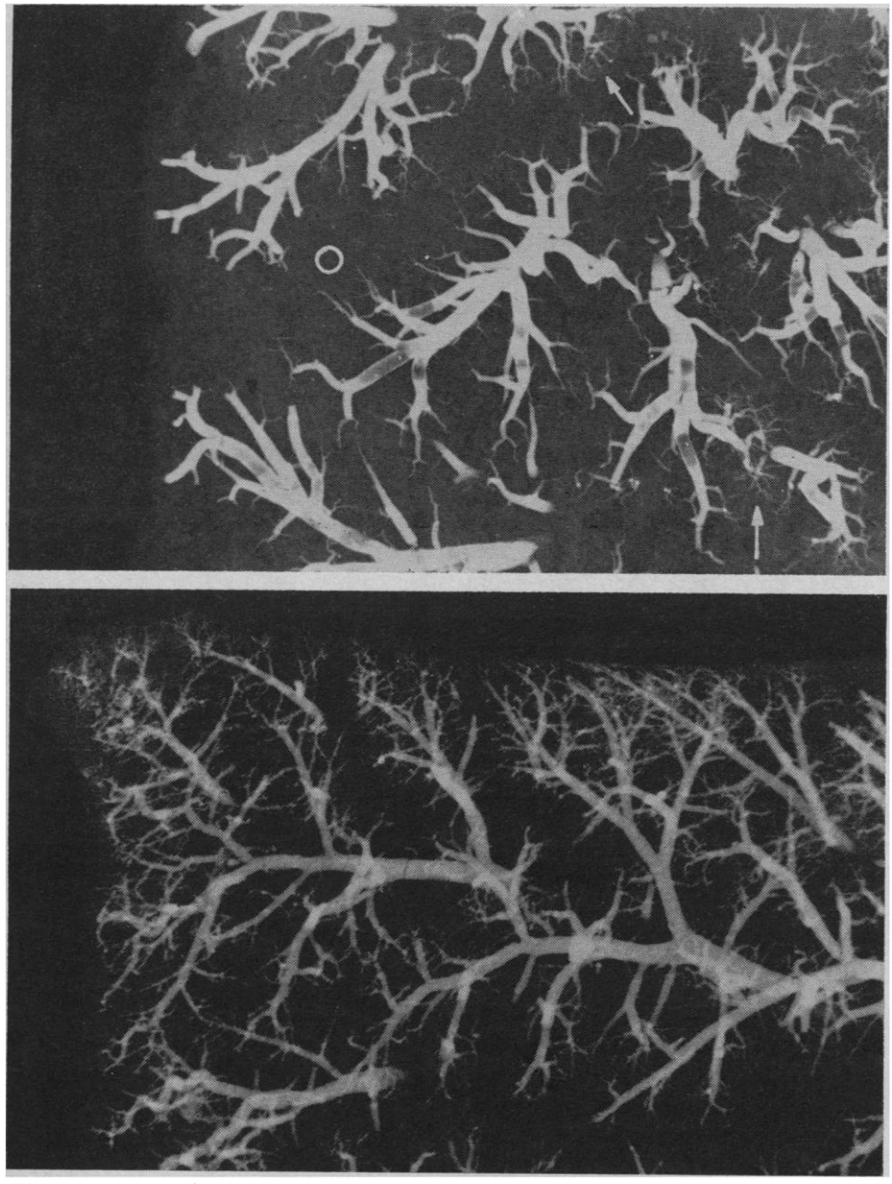

Fig. 2. Microradiographs (6×) of HMD lung (top) and of normal neonatal lamb lung (bottom) after injection of the pulmonary arteries, using the same technique, injection medium, and pressure (80 mm-Hg). (Top) Filling of the main pulmonary arteries, some small muscular arteries (circle), and practically no pulmonary arterioles (arrow) in HMD. (Bottom) Filling of all main pulmonary arteries, small muscular arteries, and pulmonary arterioles; distinct lobular pattern; normal neonatal lamb lung.

With the same technique, injection medium, and pressure, an entirely different result has been obtained in the lamb lungs used as a control group. At a pressure of 10 mm-Hg a filling of the main pulmonary arteries, the small muscular arteries, and some arterioles was obtained in the hilar zones of the lungs; at 20 mm-Hg the medial zones of the lungs filled in the same way, while showing a still more pronounced arteriolar filling; increasing the pressure to 80 mm-Hg provoked a progressive and at last entirely peripheral injection. On the radiographs and microradiographs the finest branches (Fig. 2) of the pulmonary arterial network, as well as a distinct lobular pattern, were observed. The measurements of the diameters of the vessel walls confirmed that practically all main pulmonary arteries and small muscular arteries were filled, as well as the majority of the pulmonary arterioles. Many alveolar capillaries were also injected. In four lungs of this group a final pressure of 80 mm-Hg was also applied for 2 hours; this prolonged injection did not affect markedly the vascular filling.

Finally, it is worthwhile to report that the injection of the pulmonary arteries of these normal neonatal lamb lungs filled, at the same time, many bronchopulmonary arterio-arterial anastomoses. These anastomoses are indeed known to be present in a rather large number in neonatal lungs (*11*). This filling, however, has not been observed in the HMD lungs.

The major interest of these injection experiments has been the possibility of comparing precisely the pulmonary arterial vasculature of infants dead of HMD with normal neonatal lamb lungs. Indeed, and in contrast with the normal 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.

Potential sources of error appearing in the processes of handling the lungs before injection and in the injection procedure itself can probably be excluded, as all technical manipulations were rigorously controlled and identical in both groups, the HMD lungs

and the neonatal lamb lungs. In any further comparative studies the possibility of reducing time and processes of handling before injection could be worth investigating. Indeed, and to avoid artifacts, the lungs and especially the vessels are to be left untouched before injection, not even washed out after necropsy to remove postmortem thrombi.

On the other hand, the selection of normal, 4-day-old lambs as a control group was based on the fact that in neonatal physiological studies the lamb is commonly used as a substitute for the human newborn, and on the assumption that both species have a morphologically similar pulmonary vasculature. As a matter of fact, no differences have been observed either on histological examination of the lung sections or on histometrical study of the arterial wall diameters in both groups. The use, as controls, of lungs of human newborns who died of nonrespiratory disease was my original plan, but it was discarded because such lungs nearly always show some pulmonary disturbance, either as a complication, an agonal manifestation, or an association (as in cardiac malformations) with the primary disease in our autopsy cases dealing with premature births. In larger hospitals handling numerous emergencies, it should perhaps be possible and extremely interesting to study a series of lungs from infants who died suddenly from accidents.

To summarize, even if much information is still lacking about the precise nature of this filling defect in HMD, my reported observation nevertheless argues strongly in favor of a disturbance of the pulmonary perfusion, localized largely at the level of the small muscular pulmonary arteries (50 to 30 μ) and even more at the level of the pulmonary arterioles ($< 30\ \mu$) in this disease. This phenomenon is in accordance with our previous findings (*3*) and with the recent findings of Chu *et al.* (*2*), which have shown that pulmonary hypoperfusion is the prominent characteristic of the respiratory distress syndrome of the newborn. Its description and localization seem to be of more than academic interest: the therapy of HMD could be greatly influenced by additional data on the lung vasculature of the human fetus and newborn.

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Polymorphism of Shock Loaded Fe-Mn and Fe-Ni Alloys

Abstract. *Addition of nickel or manganese to iron lowers the pressure of the "130-kb" dynamic polymorphic transition to about 55 kilobars at the limits of the body-centered cubic alloy phase.*

Since the initial discovery of the "130-kb" polymorphic transformation of iron (*1*) from body-centered cubic to a hexagonal structure (*2*), we have studied the polymorphic behavior of a number of binary iron alloys under explosive pressure loading (*3*). In general, the transformation pressures of these alloys increase as the amount of solute is increased. A mild violation of this rule in Fe-Ni alloys has been reported (*3*). We now report the more-dramatic transition-pressure lowering found in the Fe-Mn alloy system, and extend the range of the previous Fe-Ni investigation.

As in our earlier studies, our alloy samples were made by arc melting the constitutents together; then followed the heat treatments necessary to obtain uniform solid solutions. The requirement that all samples be entirely body-centered cubic limited the maximum solute concentrations.

Behavior of the alloys under explosive loading was studied by use of the pin technique (*3*, *4*), the object of which is to determine the successive velocities of the unconfined or "free" surface of the sample. Each free-surface velocity is induced by one of the three shock waves traveling through the sample; from these velocities the properties of the shock waves themselves may be calculated. The three shock waves are: the elastic wave, having the pressure of the dynamic elastic yield point of the material (typically less than 20 kb); the plastic-I wave, which has the characteristics of the onset of the polymorphic transition; and the plastic-II wave, which carries the balance of the initial shock pressure. Thus we are interested in determining the properties of the plastic-I wave, these being the conditions under which the polymorphic transformation begins.

We slightly modified the traditional calculation (*1*) of the shock characteristics, in which it is assumed that the interaction of the elastic wave with the free surface produces a rarefaction shock traveling back into the sample with the speed of the elastic wave (relative to the free surface). This assumption is used to find the point of intersection between the plastic-I wave and the rarefaction, from which the plastic-I shock velocity is determined (*5*). However, it is known that this treatment of the rarefaction is to some degree incorrect (*6*). The other extreme is to ignore the interaction between the rarefaction and the plastic-I wave, and to calculate the plastic-I velocity from the coordinates of the point at which the plastic-I wave arrives at the free surface (*7*). We have used this simplifying assumption in the calculations for this report. The two extreme methods of calculation result in transition-pressure values differing typically by 1 percent and bracketing the unknown true value. As the experimental errors are somewhat larger than this difference, improvement of the calculation is of little value.