HYALINE MEMBRANE DISEASE: A PATHOLOGICAL STUDY OF 55 INFANTS

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This study concerns the pathological changes found in the lungs of 55 newborn infants with hyaline membrane disease (HMD). The accepted appearances are a widespread resorption of air with collapse of many alveolar ducts and most alveoli, producing a severe atelectasis. The alveolar ducts and alveoli that remain open are distended and lined by a variable amount of homogeneous acidophilic material. There is intense vascular engorgement (Morison, 1952; Potter, 1953; Nesbitt, 1957).

Numerous hypotheses have been proposed to explain the formation of the hyaline membranes: these include the aspiration of amniotic fluid (Farber and Sweet, 1931; Farber and Wilson, 1933; Blystad, Landing, and Smith, 1951; MacGregor, 1953), defect in pulmonary circulation with plasma transudate (Rudolph, Auld, Drorbaugh, Rudolph, Nadas, and Smith, 1959; Neligan, 1959), and a disturbance of alveolar surface tension (Avery and Mead, 1959; Klaus, Reiss, Tooley, Piel, and Clements, 1962).

In this study an attempt has been made to determine the relation between the possible aetiological factors and the morphological findings. Particular attention has been paid to the degree of pulmonary maturation, the degree of pulmonary expansion or atelectasis, the extent of the hyaline membranes, the pulmonary lymphatics, the amount of aspirated amniotic fluid, the presence of alveolar oedema, the occurrence of pulmonary haemorrhages, and the relation of the hyaline membranes to the alveolar septa.

Material and Methods

Studies were carried out on 55 babies with HMD, born in the University Maternity Unit of Louvain (Belgium) during the past 6 years, during which period there were 15,000 deliveries. The distribution of the cases related to foetal age is shown in Table 1.

The frequency of HMD was maximal (22.9%) in the group of foetal age 29-30 weeks.

Necropsy included systematic histology of the lungs,

heart, liver, spleen, kidney, pancreas, adrenal, and thymus. Afterwards, each case was discussed at the weekly seminar on perinatal mortality, when the obstetric, paediatric, and pathological data were correlated.

The lungs were fixed in Bouin's fluid. To allow extensive study, blocks were taken covering the complete cut surface of a lobe. In 22 cases blocks were taken from each of the five lobes.

Lung sections were stained with haematoxylin and eosin, haematoxylin and eosin and safranin, Masson's trichrome, Mayer's mucicarmine, PAS, Mallory's phosphotungstic acid-haematoxylin, Verhoeff-van Gieson elastica stain, and Bielschowsky's method as modified by Foot for the staining of the reticular fibres. These methods are described in the *Manual of Histologic and Special Staining Techniques* (1957).

Results

Naked-eye Appearance. The lungs had a dark-red, purplish colour. Their consistency was usually firm. On gentle manual palpation no crepitation could be felt. A 'hydrostatic lung test' was carried out on each lung and lobe in 25 cases. All the lobes sank in water in 17 cases; all the lobes floated in 2 cases; and in 7 cases parts only of the lungs floated.

Histology. Pulmonary Maturation. One can easily distinguish lung parenchyma that is either

TABLE 1
DISTRIBUTION OF 55 CASES OF HYALINE MEMBRANE DISEASE (HMD) RELATED TO FOETAL AGE

Foetal Age (wk.)	Number of Live Births	Deaths from HMD*
26	33	5 (15)
26-28	42	5 (12)
29-30	61	14 (23)
31-32	91	13 (14)
33-34	156	7 (4)
35-36	250	5 (2)
37-38	1,200	1 (0.8)
39 +	13,167	5 (0.4)
	15,000	55

* Percentages are given in parentheses.

bronchiolar or immature, *canalicular* and *canaliculo-alveolar* or premature, and *alveolar* or mature. The pulmonary parenchyma of the 11 babies born before the 29th week of gestation showed a canalicular structure. Alveolar differentiation occurred in our series about the 29th to 30th week of gestation (Potter, 1953).

Pulmonary Expansion. The pulmonary parenchyma always showed the classical picture of secondary or resorption atelectasis, distended emphysematous alveolar ducts and alveoli alternating with extensive atelectatic areas. In large sections covering a whole lobe, areas with other varieties of disturbed pulmonary expansion were present. For example, localized areas of interstitial emphysema were seen in 7 cases: in each instance the baby's foetal age was more than 30 weeks.

Hyaline Membranes. The membranes stained red with haematoxylin and eosin, purple-brown with phosphotungstic acid haematoxylin, and varying shades of purple with a predominance of either red or blue, with Masson's aniline blue trichromic stain. The PAS stain yielded either a positive reaction (21 cases), a slightly positive reaction (22 cases), or a negative reaction (12 cases). The membranes are usually not stained positively with Mayer's mucicarmine, but a positive reaction was noted in 7 cases.

The hyaline membranes varied in extent, as described by Latham, Nesbitt, and Anderson (1955), who placed them in four grades according to their individual thickness and extent and their distribution in large lung sections. The membranes of the 55 cases have been classified according to the same criteria. It was found that the hyaline membranes were nearly always less extensive and less developed (grades 1 and 2) in the very premature group of less than 28 weeks, while the more developed and more extensive membranes (grades 3 and 4) were observed in more mature infants.

Pulmonary Oedema. A conspicuous, homogeneous or slightly granular fibrinous exudate was present in the alveoli and alveolar ducts. It was seen in nearly all cases (45), but with varying intensity: slight in 24 cases, moderate in 12 cases, severe and diffuse in 9 cases.

Pulmonary Haemorrhage. Pulmonary haemorrhages were common. They were usually localized, either in the alveoli (10 cases), in the alveoli and septa (7 cases), or in the alveoli, septa, and bronchi (10 cases).

Aspirated Amniotic Material. Numerous cells and

debris of amniotic fluid origin were seen in the alveoli or alveolar ducts in only 7 cases. No correlation between the extent of this aspiration on the one hand and the degree of the hyaline membranes on the other could be established. Nor was there any relation between the degree of aspiration and the foetal age.

Relationship of Hyaline Membranes to the Inter-alveolar Septum. We have investigated the relation of the hyaline membranes to the walls of the alveoli and alveolar ducts, and in particular their relation to the reticular fibres. After silver impregnation, it was clearly seen that the hyaline membranes were directly apposed to the reticular fibres of the alveolar walls and alveolar ducts (Figs. 1, 2, 3, and 4). The membranes frequently contained necrotic cellular material (Figs. 1 and 5) which likewise appeared to rest directly upon the reticular fibres. To our knowledge this phenomenon has not been described previously; its possible significance is discussed below.

Pulmonary Lymphatics. Impregnation of the reticular fibres is also an established method for outlining the lymphatic vessels (Lauweryns, 1962, 1964). In most of the cases the lymphatic vessels were strikingly distended with fluid, sometimes appearing angiectatic and congested (Figs. 6, 7, 8, and 9). Their lumina contained eosinophilic material, which was sometimes granular and sometimes homogeneous fibrinous. These ectatic lymphatic vessels were observed at all sites, including the peribronchial, periarterial (Fig. 6), and perivenous (Fig. 7) and pleural (Figs. 8 and 9) lymphatics. Lymphatic angiectasis was most conspicuous in cases where the hyaline membranes were extensive (Table 2). This observation has, to our knowledge, not been recorded in the literature.

TABLE 2
LYMPHANGIECTASIS RELATED TO HYALINE MEMBRANES

No. of Cases	Degree of Lymphangiectasis	Case Distribution According to Grade of HMD	
		Grades 1-2	Grades 3-4
28	Severe Moderate Slight	12	16
15		6	9
9		8	1

Discussion

Importance of Histological Examination. This has to be emphasized once again, since the so-called hydrostatic lung test is still used in forensic medicine to determine whether a baby found dead has breathed or not, and as evidence in possible cases of

FIG. 1.—Hyaline membranes directly apposed to the reticular fibres; cellular material containing prominent nuclei enmeshed within the hyaline membranes themselves (marked with arrow). (Reticular fibres impregnated according to the method of Bielschowsky as modified by Foot; eosin counterstain. $\times 250$.)

FIG. 2.—Reticular fibre impregnation as Fig. 1, low power view. ($\times 26$.)

FIG. 3.—Magnified area I of Fig. 2; direct apposition (A) of hyaline membranes to the reticular fibres. Compare on the same field with the reticular fibre structure of normal alveoli (B) where the alveolar epithelial cells rest upon the reticular fibres. (Staining as Fig. 1. $\times 200$.)

FIG. 4.—Magnified area II of Fig. 2. Compare the reticular fibre structure of an alveolar duct (A) lined by a hyaline membrane, with that of the normal (but collapsed) alveoli (B). (Staining as Fig. 1. $\times 200$.)

FIG. 5.—Nuclear material (marked with pointers) enmeshed within a hyaline membrane, apposed to the reticular fibres. (Staining as Fig. 1. $\times 314$.)

infanticide. The complete fallibility of the test has been pointed out by others (Gonzales, Vance, Helpert, and Umberger, 1954; Fournier, 1957; Parmentier, 1962) and has been confirmed here.

Pulmonary Expansion. In addition to the so-called secondary or resorption atelectasis, localized areas of interstitial emphysema were observed in 7 cases. As this phenomenon was observed only in the more mature group (with a foetal age of more than 30 weeks), interstitial emphysema is perhaps partially due to the greater strength of respiratory movements in these babies as compared to the younger age-group (E. Eggermont, personal communication, 1964). (In 3 of the 7 cases showing interstitial emphysema it was known that positive-pressure resuscitation had been used, and in 3 it was known that it had not.)

Pulmonary Oedema. Though the intensity and distribution of pulmonary oedema varied in individual cases, its constant presence strongly favours some failure of the 'alveolocapillary barrier' in hyaline membrane disease. (This same phenomenon is also discussed in relation to the pulmonary lymphatics—see below.)

FIG. 6.—Marked distension and ectasia of many peri-arterial lymphatic vessels; some lymphatic vessels contain a granular precipitate. A, B, C, D are pulmonary arteries. (Staining as Fig. 1. $\times 26$.)

FIG. 7.—Unusual case of extreme peri-venous and septal lymphangiectasia and lymphatic congestion, mimicking congenital pulmonary lymphangiectasis. (Staining as Fig. 1. $\times 26$.)

FIG. 8.—Collapsed area of lung with neonatal hyaline membrane disease; pleural lymphatic congestion; lymphatic vessels contain a fibrinous fluid, and valves pointed to the pleura (marked with pointers). (Staining as Fig. 1. $\times 31$.)

FIG. 9.—Magnified area from Fig. 8. Pleural lymphatic vessel, congested and containing a fibrinous exudate. Valves pointed to the pleura. (Staining as Fig. 1. $\times 190$.)

Pulmonary Haemorrhages. These were observed in about half the cases. In a recent review of 30 cases of primary neonatal pulmonary haemorrhage, pulmonary haemorrhage was found in a broad variety of fatal neonatal conditions (Lauweryns, Eggermont, and Romelart, 1963). Here also, these haemorrhages probably represent merely a terminal complication of severe neonatal distress.

Aspiration of Amniotic Fluid. Intra-alveolar aspiration of amniotic fluid was in the past considered an important factor in the formation of hyaline membranes (Farber and Wilson, 1933; Farber and Sweet, 1931; Blystad *et al.*, 1951; MacGregor, 1953). The hypothesis is not supported by these observations. Aspirated amniotic material in considerable amounts was observed in only 7 cases, and there was no close correlation between the extent of this aspiration and the degree of hyaline membrane formation.

Relation of Hyaline Membranes to Alveolar Wall. The observed relation of the hyaline membranes to the reticular fibres of the walls of the alveoli and alveolar ducts (Figs. 1, 3, and 4) seemed important in connexion with the pathogenesis of the disease. The relation is clear and well defined. A comparison in the same microscopic field of alveoli lined by hyaline membranes with normal alveoli shows how in a normal alveolus it is the alveolar epithelial cells that are resting upon the reticular fibres, while the hyaline membranes are directly apposed to these fibres (Figs. 3 and 4).

The precise histological structure of the alveolar wall, as revealed by the electron microscope (Low, 1953; Karrer, 1956; Bargmann and Knoop, 1956; Schulz, 1957; Policard, Collet, and Pregermain, 1957; Divertie and Brown, 1964) shows that the 'alveolo-capillary membrane' is made up of three continuous and distinct layers: (1) external, the alveolar cells (both small and large), (2) the interstitium, (3) the capillary endothelium (Weibel, 1963). The interstitium, limited on one side by the endothelial and on the other by the epithelial basement membrane, contains the reticular fibres.

The hyaline membranes as seen by conventional microscopy lie directly upon the reticular fibres of the alveoli and alveolar ducts, so it follows that there must be necrosis of the epithelial part of the wall. This hypothesis is strengthened by the finding in the same sections of areas with hyaline membranes and absent epithelium, along with normal alveoli with an epithelial lining but no membranes.

This view is also supported by the well-known fact, confirmed by this study, that the hyaline

membranes often include necrotic nuclear and cellular material, DNA-positive (Buckingham and Sommers, 1960). Moreover, some of these nuclei and cell remnants have the cytological characteristics of alveolar cells and macrophages (Figs. 1 and 5). This interpretation is supported by the electron microscope studies of Van Breemen, Neustein, and Bruns (1957) and Campiche, Jaccottet, and Julliard (1962), which demonstrated necrotic changes in the alveolar epithelial cells in cases of HMD.

It is of course impossible by light microscopy to specify if this superficial necrosis of the alveolar wall also involves the alveolar basement membrane. Indeed the hyaline membrane cannot be certainly differentiated from the alveolar basement membrane, which covers superficially the alveolar interstitium as such. The intimate relationship of the hyaline membranes to the reticular fibres does suggest also that there is necrosis (at least partial) of the basement membrane, and this receives some support from the work of Van Breemen *et al.* (1957), who observed a partial disintegration and necrosis of the basement membranes in electron micrographs.

Pulmonary Lymphatics. The ectasia and congestion of the pulmonary lymphatic vessels (Figs. 6, 7, 8, and 9) observed in most of our cases, particularly in those with extensive hyaline membranes, are perhaps a consequence of transudation through the alveolo-capillary wall. The demonstration of fibrin as a constituent part of the hyaline membranes (Gitlin and Craig, 1956) provided the first evidence of such alveolo-capillary transudate. The pulmonary congestion and oedema, demonstrated by Shanklin (1963) and also observed in the present cases, are in keeping with such a process.

Summary

A detailed analysis has been made of the pathological lesions in the lungs of 55 human newborn infants with hyaline membrane disease.

The highest incidence in this series was in the groups of foetal age, 29-30 weeks.

At necropsy, a hydrostatic lung test was often negative. The inconsistency of this test as evidence of live-birth is re-emphasized.

Histology included total sections of the different lung lobes. In addition to the accepted lesions of hyaline membrane disease, this study stresses the following features.

- (1) Localized areas of interstitial emphysema in 7 babies who were relatively mature. This may be the result of the greater force of their inspiratory movements.

- (2) Hyaline membranes were less extensive and less developed in the very young premature age-group (less than 28 weeks).
- (3) Pulmonary oedema was almost constant.
- (4) Localized or extensive pulmonary haemorrhages were present in approximately half the cases, and were thought to be a terminal complication.
- (5) Aspiration of amniotic fluid was usually not impressive; when present, it did not correlate with the extent of the hyaline membranes.

In addition, two previously undescribed features have been observed. The hyaline membranes are directly apposed to the reticular fibres of the alveolar walls and alveolar ducts which they line, suggesting a superficial necrosis of the walls of alveoli and alveolar ducts in hyaline membrane disease. The lymphatic vessels of the lungs were in most cases distended with fluid and often appeared angiectatic and congested. This, and the pulmonary oedema, are in keeping with the concept of an abnormal alveolo-capillary transudation in hyaline membrane disease.

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