PULMONARY HYALINE MEMBRANE

FURTHER OBSERVATIONS ON EPITHELIAL ORIGIN

BY

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In sections of lungs from some prematurely born infants who have died within a few hours of birth it is possible to observe necrosis of epithelial cells in the respiratory bronchioles (Fig. 1). Study of sections from a large number of cases of hyaline membrane disease reveals all stages of epithelial degeneration from that shown in Fig. 1 to structureless linings having the typical appearances of eosinophilic hyaline membranes. In 69% of 133 cases so studied nuclear remnants were found in some of the membranes and were occasionally observed to show regular distribution corresponding to the normal disposition of cells around the lumina of respiratory bronchioles. For these reasons it has been stated (Barter, 1959; Barter and Maddison, 1960) that hyaline membranes result primarily from necrosis of epithelial cells and form *in situ* in respiratory bronchioles. It has also been maintained that deposition of fibrin in membranes occurs secondarily to epithelial degeneration.

Since this last publication the lungs from two fatal cases of kerosene poisoning have been studied. In both cases there are thick hyaline membranes situated predominantly in respiratory bronchioles. A similar case in which hyaline membranes formed in the lungs following aspiration of kerosene was described by Foley, Dreyer, Soule and Woll (1954).

Study of these two cases has revealed features of the early stages of epithelial degeneration which further help to clarify the concept of a cellular lesion as the primary change in neonatal hyaline membrane disease. It is the purpose of this paper to describe certain aspects of cellular degeneration in the lungs resulting from the aspiration of kerosene in so far as they are related to the pathogenesis of hyaline membranes.

Case Reports

Case 1. A female child aged 15 months put an allegedly spent bottle of kerosene to her mouth. Shortly following the accident she was treated by gastric lavage, after which procedure her condition markedly deterior-

ated. She was admitted two hours later to the Princess Margaret Hospital for Children, Perth, on February 5, 1961. On admission she was cyanosed and semi-conscious. Crepitations were heard on both sides of the chest and radiological examination revealed bronchopneumonic lesions in both lungs. Death occurred 10 hours after admission to hospital. Autopsy was performed 27 hours after death by Dr. A. T. Pearson for the City Coroner. The lungs were heavy, red, and poured pink watery fluid from the cut surfaces.

Microscopic examination reveals widespread polymorphonuclear exudation with frequent formation of small abscesses and the presence of thick hyaline membranes in respiratory bronchioles. The latter are not conspicuous in areas of the most cellular inflammatory changes where the pulmonary tissues are also necrotic.

Degenerating epithelial cells and cellular contribution to hyaline membranes are seen (Fig. 2), and epithelial necrosis is evident in occasional terminal bronchioles (Fig. 3). Various stains for fibrin (picro-Mallory, phosphotungstic acid haematoxylin and Gram-Weigert) show it to be present as fine threads, together with the inflammatory exudate (Fig. 4), but hyaline membranes are mostly devoid of fibrin except for some patchy red staining with the picro-Mallory technique and the presence of occasional fine threads with the phosphotungstic acid and Gram-Weigert methods.

Case 2. A female child aged 18 months was admitted to the Adelaide Children's Hospital, South Australia, half an hour after putting her face into the top of a drum of kerosene. Dr. M. C. Fowler has kindly given me slides from the blocks of this case and the relevant clinical details. On admission to hospital the child was collapsed and cyanosed. Her condition waxed and waned with high fever and cyanosis and she died 72 hours after admission. Autopsy was performed by Dr. Fowler 24 hours after death.

Microscopic examination of the lungs shows a moderate exudation of fluid and leucocytes and hyaline membrane formation in respiratory bronchioles and occasional alveoli. In addition, marked swelling of alveolar lining cells is a striking feature throughout. There are tremendous increases in both cytoplasmic and nuclear mass, and the nuclei are of bizarre shapes and show irregular chromatin clumping and distribution (Fig. 5).

FIG. 1.—Prematurely born infant, weight 880 g., lived two hours and 45 minutes. In the centre is a respiratory bronchiole lined by necrotic epithelial cells and showing early hyaline membrane formation. Respiratory bronchioles with intact cellular linings are seen at both sides. (H. and E. $\times 130$.)

FIG. 3.—Case 1, the section includes a terminal bronchiole showing necrosis of the lining cells on the left side. Also to the left is portion of a respiratory bronchiole showing hyaline membrane, and inflammatory exudate is seen in the alveoli. (H. and E. $\times 130$.)

FIG. 2.—Case 1, kerosene poisoning. Showing air spaces filled with greatly enlarged cells from the respiratory lining. The cytoplasm is eosinophilic and fusion of the most degenerate cells into hyaline membrane material can be seen. (H. and E. $\times 325$.)

FIG. 4.—Case 1: Fibrin threads in a site showing necrosis and inflammatory exudation. (Phosphotungstic acid haematoxylin $\times 517$.)

FIG. 5.—Case 2, kerosene poisoning. Showing alveolar spaces lined by greatly swollen epithelial cells with degenerate nuclei. (H. and E. $\times 325$.)

FIG. 6.—Case 2, swollen alveolar lining cells. The largest near the middle shows displacement of the degenerate nucleus to the periphery by a mass of granular eosinophilic material in the cytoplasm. (H. and E. $\times 1,300$.)

FIG. 7.—Case 2, another swollen alveolar lining cell with granular eosinophilic material forming most of the cytoplasmic component and showing probable extrusion of the degenerate nucleus. (H. and E. $\times 1,300$.)

FIG. 8.—Case 2, marked degenerative change is seen in the cell in the centre. The original nature can be determined by comparison with the still intact cell at the left, and similarity of composition to the hyaline membrane material at the right is apparent. (H. and E. $\times 1,300$.)

In very occasional alveoli one or two cells differ conspicuously from the rest due to conversion of parts of the cytoplasm from clear to eosinophilic staining. In Fig. 6 a cell is depicted showing accumulation of eosinophilic granular material in the cytoplasm. The cell membrane is intact, and the nucleus occupies a position just beneath the free edge. A similar eosinophilic change is seen in the cytoplasm in Fig. 7. Again the cell appears intact, and there is a rim of clear cytoplasm on the periphery. Also it might be concluded from the appearances that the nucleus is in the process of extrusion from the cell. In Fig. 8 the eosinophilic remains of a cell lie between hyaline membrane material on one side and a degenerate but intact cell on the other. Comparison with the intact cell leaves little doubt as to the original epithelial cellular nature of the structure under discussion, the contents of which are also similar to the nearby hyaline membrane. Other alveoli show small masses of granular eosinophilic material apparently confined by a cell membrane and of the size of the surrounding degenerating epithelial cells.

Discussion

It is concluded from the recent literature on hyaline membrane disease that the concept of a primary epithelial origin is not generally accepted. Most workers support the view that the membranes are composed largely of fibrin. The two cases reported here were of such an age that there seems to be no valid argument that the form of the fibrin in the membranes, if in fact fibrin were present in large amounts, is incompatible with positive staining when in other parts of the lungs there are characteristically stained fibrin threads.

Previously it was postulated (Barter and Maddison, 1960) that the change from an epithelial lining to hyaline membranes in respiratory bronchioles

involved great increase in cell size, conversion of the cytoplasmic staining and loss of nuclear material. These changes can well be appreciated if sections of the lungs from a large number of neonatal cases are examined together when all the features of the cellular necrosis can be summated. In the second case reported in this paper the above changes can be precisely observed in single cells.

A rational view of the effects of kerosene in causing necrosis of the lining cells in respiratory bronchioles and alveoli from these two cases seems to be as follows. Intense action of the noxious substance at some sites leads to rapid necrosis of tissues with leucocytic exudation. In other less injured sites an apparently necessarily slower process results in hyaline membrane formation. As has been observed in Case 1, there are no hyaline membranes in the sites of maximal necrosis of lung tissue. In Case 2 more prolonged and probably less intense action of kerosene led first to exudation and to hyaline membranes in respiratory bronchioles and later to degenerative changes in the lining epithelial cells of the alveoli. These altered alveolar cells then seem to have been liable to a similar degenerative process to that observed in the lining cells of bronchioles with eosinophilic conversion of the cytoplasm, loss of nuclear formation and finally fusion into hyaline membranes.

Swelling of alveolar epithelial cells as the result of noxious stimuli has previously been described by Young (1930), Ross (1939) and others. A similar process results from the action of inhaled kerosene, and it is of interest to note that the same changes in the rabbit, as observed by Young, took approximately 72 hours to occur. The further cytoplasmic

eosinophilic changes in these cells do not appear to have been described before and particularly not in relation to the pathogenesis of hyaline membranes.

Agreement may not yet be reached that hyaline membranes in different diseases are the same. However, the observations made on these two cases of kerosene poisoning and on some hundreds of neonatal cases indicate an essentially similar composition and a remarkable correspondence in the vulnerable parts of the respiratory tract. Thus the respiratory bronchioles are the most severely affected in cases of kerosene poisoning as in neonatal cases. The membranes observed in alveoli with prominent cellular linings in Case 2 in the present study further amplify the concept of a primary epithelial origin and suggest that if a noxious agent acts long enough to cause marked alteration of alveolar lining cells, then these may later degenerate further and fuse together to form hyaline membranes.

It is again suggested that the pathogenesis of hyaline membranes can be correctly related to epithelial cell necrosis and that a massive fibrinous exudation is not responsible for the appearances. The changes which occur in the newborn require a period of breathing and occur at the same rate in predisposed sites throughout the lungs. One observes a similar stage of degeneration in all membranes in a given case. Thus, if one membrane is of the structureless eosinophilic type nearly all will be of the structureless eosinophilic type. If one membrane shows nuclear remnants the same features will be commonly observed among the others. Finally, in babies dying very shortly after birth the epithelial degeneration does not reach the stage of hyaline membrane formation, and it is in such cases that the cellular contribution is most convincingly observed.

In addition to the evidence gained from the study of the whole lining of the respiratory bronchiole in newborn infants, there is now the further observation that granular eosinophilic material, akin to hyaline membranes, forms in the cytoplasm of epithelial cells in the respiratory tract. It seems

reasonable to apply the findings in this case to the neonatal disorder, since what has been observed in individual alveolar cells as due to kerosene was forecast as the only possible mode of development of hyaline membranes from the observations made previously in neonatal cases. The fact that in both these cases of kerosene poisoning, and in that reported by Foley *et al.* (1954), there were also widespread hyaline membranes in respiratory bronchioles adds further to the evidence afforded by the degenerate alveolar epithelial cells in favour of a primary epithelial origin of hyaline membranes.

Summary

Hyaline membranes are described in the lungs of the two young children who died following aspiration of kerosene.

The membranes occur predominantly in respiratory bronchioles, but some are also present in the alveoli in Case 2, in which there are also degenerative changes in the lining epithelial cells.

Granular eosinophilic material has been observed to form within the cytoplasm of greatly swollen alveolar epithelial cells. Gradations can be traced through several cells from the early formation of this eosinophilic substance in the cytoplasm to hyaline membranes.

The observations in individual cells seem conclusive for establishing that cellular necrosis is the basis of hyaline membrane formation.

The changes due to the action of kerosene on the epithelial lining of the respiratory tract are pertinent to further understanding of neonatal hyaline membranes.

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