PLASMA PROTEIN COMPOSITION OF HYALINE MEMBRANE IN THE NEWBORN AS STUDIED BY IMMUNOFLUORESCENCE

BY

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Hyaline membrane disease of the newborn has been a subject of ever-growing interest to paediatricians and pathologists as it is the major cause of death in newborn babies, particularly premature ones. Much more is known of the clinical picture and physiological changes in hyaline membrane disease, or respiratory distress syndrome of the newborn (Avery, 1962; Curtis, 1957; James, 1959; Miller, 1962; Rudolph and Smith, 1960), and there is a fair knowledge of the composition of the hyaline membrane (Buckingham and Sommers, 1960). There is, however, no agreement as to its aetiology and pathogenesis, though, according to the majority of investigators, the material constituting the membrane originates from the pulmonary vessels. These authors base their assumptions mainly on the work of Gitlin and Craig, who by means of immunofluorescence detected the presence of fibrin within the hyaline membranes (Gitlin and Craig, 1956). Electron microscope investigations suggest the presence of other plasma proteins as well (van Breemen, Neustein and Bruns, 1957; Campiche, Prod'hom and Gautier, 1961; Campiche, Jaccottet and Juillard, 1962; Groniowski and Biczyskowa, 1963), but this has not, however, been confirmed by Gitlin and Craig. Histochemical investigations have not established whether there are any other plasma proteins in the membrane (Aronson, 1961; Buckingham and Sommers, 1960; Duran-Jorda, Holzel and Patterson, 1956; Lieberman, 1961; Lynch and Mellor, 1956; Lynch, Mellor and Badgery, 1956).

Differences of opinion as to the exact plasma protein composition of hyaline membranes stimulated us to undertake further investigations on their structure.

Material and Methods

Investigations were undertaken on 29 newborn babies with respiratory distress. Of these, the lungs of 6 children with extensive and well-defined hyaline membrane syndrome constituted the material of the present study (Table 1).

TABLE 1

<table>
<thead>
<tr>
<th>Case No. and Sex</th>
<th>Gestation (wk.)</th>
<th>Weight (g.)</th>
<th>Length (cm.)</th>
<th>Age (hr.)</th>
<th>Delivery</th>
<th>Autopsy Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M</td>
<td>29 or 30</td>
<td>1,800</td>
<td>44</td>
<td>10</td>
<td>Normal</td>
<td>Hyaline membrane disease; minute meningeal haemorrhages</td>
</tr>
<tr>
<td>2 M</td>
<td>30</td>
<td>2,150</td>
<td>47</td>
<td>36</td>
<td>Normal</td>
<td>Hyaline membrane disease</td>
</tr>
<tr>
<td>3 F</td>
<td>31</td>
<td>1,370</td>
<td>41</td>
<td>15</td>
<td>Normal</td>
<td>Hyaline membrane disease; minute ependymal haemorrhage</td>
</tr>
<tr>
<td>4 M</td>
<td>33</td>
<td>2,470</td>
<td>47</td>
<td>36</td>
<td>Caesarean section; premature separation of placenta; born in asphyxia</td>
<td>Hyaline membrane disease; intraventricular bleeding</td>
</tr>
<tr>
<td>5 M</td>
<td>30</td>
<td>1,580</td>
<td>44</td>
<td>18</td>
<td>Caesarean section; placenta praevia; born in asphyxia</td>
<td>Hyaline membrane disease; small meningeal haemorrhage</td>
</tr>
<tr>
<td>6 M</td>
<td>30</td>
<td>1,200</td>
<td>38</td>
<td>14</td>
<td>Normal</td>
<td>Hyaline membrane disease</td>
</tr>
</tbody>
</table>

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The results obtained by means of immunofluorescence were absolutely uniform in all the six cases investigated. In the unstained sections the membranes showed greyish-blue autofluorescence. An intense yellowish-green (FIT-labelled reagents) or orange-red (LRB-labelled reagents) fluorescence was obtained in the reactions with antisera against fibrin and/or fibrinogen, γ-globulin and albumin (Figs. 4-9). Intense specific fluorescence of the membrane is proof of the presence of all these proteins within the membrane. In sections stained with anti-globulin or anti-albumin reagents, the fluorescence of membranes was uniform. Sections stained with antifibrin reagents showed inconsistent granular and laminar fluorescence of hyaline membranes, especially under higher magnification (Fig. 5). In these
FIG. 1.—Granular hyaline membrane, focally covering the preserved epithelium of terminal bronchiole (Case 2). (H. and E. × 400.)

FIG. 2.—Remnants of nuclei incorporated in hyaline membrane (Case 4). (H. and E. × 800.)

FIG. 3.—Granular hyaline membrane, focally covered with epithelium (Case 4). (H. and E. × 400.)

FIG. 4.—Fibrin content of hyaline membranes (Case 4). (Stain. Gγ anti H f. FIT* × 320.)

FIG. 5.—Patchy granular and laminar fluorescence of hyaline membranes (Case 4). Note fluorescence of fibrin in septal capillaries of atelectatic portions. (Stain. Gγ anti H f. FIT × 320.)

FIG. 6.—γ-Globulin content of hyaline membranes (Case 4). (Stain. Rγ anti Hy2. FIT × 320.)

* Abbreviations of staining methods in Figs. 4 to 12 are given in Table 2.
PLASMA PROTEINS IN HYALINE MEMBRANES

Fig. 7.—Hyaline membrane continuous with alveolar septum impregnated with γ-globulin (Case 4). (Stain. Rγ anti Hyg. FIT × 320.)

Fig. 8.—Albumin content of hyaline membranes (Case 1). (Stain. Rγ anti H a. LRB × 320.)

Fig. 9.—Alveolar septum impregnated with albumin (Case 4). (Stain. Rγ anti H a. FIT × 320.)

Fig. 10.—Positive effects of blocking procedure—disappearance of specific fluorescence with only autofluorescence preserved (Case 4). (Stain. Rγ anti H a + Rγ anti H a. FIT × 320.)

Fig. 11.—Negative effects of blocking procedure—fluorescence preserved (Case 4). (Stain. Rγ anti H a × Rγ anti Hyg. FIT × 320.)

Fig. 12.—Almost complete abolition of fluorescence in hyaline membranes where only few fluorescent nuclei can be seen; intensive fluorescence of nuclei in alveolar septa (Case 4). (Stain. LE-serum + Rγ anti Hyg. FIT × 320.)
sections, a thin fibrin layer was clearly fluorescent in septal vessels. The membranes were sometimes fused with alveolar septa impregnated with fibrin, γ-globulin and albumin (Figs. 5, 7 and 9).

A prolonged (25 minutes) rinsing of sections in buffered saline before fixing them resulted in a decrease in the number of membranes, whereas in the persisting membranes the intensity of fluorescence was either not diminished or only minimally so. This was true not only of the fibrin but also of albumin and γ-globulin, proving that all these proteins help to form the membrane.

By treating the sections with homogeneous unlabelled reagents, before treating them with the same labelled reagents, the reaction was completely abolished (Fig. 10). Moreover similar treatment with unlabelled sera against other fractions of human serum was without any influence on the intensity of specific fluorescence (Fig. 11). On the one hand this provides proof of the staining specificity, on the other hand proof of the antigen monospecificity of the histochemical reagents used.

In sections covered with lupus erythematous-serum (LE-serum, containing the antinuclear factor) and then stained with the reagent against human γ2-globulin, a considerable decrease, and even focal disappearance, of fluorescence of hyaline membranes was observed, whereas the nuclei of alveolar septa and nuclei or their fragments incorporated in the membranes showed intense fluorescence (Fig. 12). Each of several stainings gave the same result (always with duplicate sections stained only with reagent against human γ2-globulin as control). The reaction made it possible to detect cell nuclei in hyaline membranes.

Unfortunately the attempts to give more exact data as to the quantitative relations and location of individual plasma proteins within the membrane, by means of mixtures of labelled reagents in contrasting colours, failed. A uniform brownish fluorescence of the membranes could be seen. In some individual instances, albumin seemed to prevail slightly.

Fats possessing autofluorescence could also be observed within some of the membranes. This method of fat detection does not, however, seem to be more sensitive than routine histological stainings.

**Discussion**

Investigations were carried out by means of a very specific and sensitive method of immunofluorescence. The results were consistent in all the six cases investigated, and we conclude that all the three elementary serum proteins (fibrin, γ-globulin and albumin) are integral parts of hyaline membrane.

Washing tissue sections before fixing them, according to Gitlin and Craig, removes all the proteins not closely bound with the tissue (Gitlin and Craig, 1956). Prolonged washing of completely unfixed sections resulted in the disappearance of albumin and γ-globulin in the vessels of alveolar septa, and of the oedema fluid in the alveoli; it also completely removed a part of the hyaline membranes. However, in the remaining membranes, a large amount of albumin and γ-globulin was still to be found. Taking the intensity of fluorescence as criterion of the quantity of protein in washed sections stained with the same immuno-histochemical reagent, the amount of albumin and γ-globulin in hyaline membranes remained unchanged or changed only minimally.

The detection of fibrin, albumin and γ-globulin as integral components of the hyaline membrane makes it seem likely that the other plasma proteins (α-globulin and β-globulin) are equally present. In other words, the hyaline membrane consists of a plasma clot, in which some cell remnants and fats can also be found.

The existence of cell nuclei within the hyaline membrane has been confirmed by means of immuno-fluorescence with the use of antinuclear serum (LE-serum). A considerable decrease or complete disappearance of fluorescence of the whole membrane may be interpreted as follows. The antibodies contained in a drop of the reagent covering the section are absorbed by the cell nuclei, previously subjected at LE-serum. In view of the character of the fluorescence, it may be presumed that γ-globulin and albumin impregnate the whole membrane homogeneously, whereas the fibrin preserves a granular, slightly laminar structure. The method applied is a qualitative one and allows conclusions to be drawn as to the relevant amount of antigen only when the same immuno-histochemical reagent is used.

The fusion of hyaline membranes and of alveolar walls focally impregnated with albumin, γ-globulin and fibrin (Fig. 7) seems to imply capillary damage.

The results of the present study confirm the observations made with the electron microscope. They support the pathogenetic theories that assume the endogenous origin of hyaline membranes. Gitlin and Craig who represent the same view do not mention explicitly why fibrin should be the only component of the hyaline membrane.

Lungs of newborn babies only were studied. The detection of several plasma components in the hyaline membranes of the newborn establishes a strong relation between hyaline membrane disease and other conditions of children and adults in which the same membranes can be observed in a light microscope. A temporary increase of capillary
permeability is the feature common to these conditions, and for this reason fibrin and other serum proteins escape from the vessels, as has also been observed in experimental studies on hyaline membrane (Buckingham and Sommers, 1960; De and Anderson, 1954; Farber, 1937).

The lack of a consistent occurrence of general oedema in hyaline membrane disease prompts us to search for the causes of this seemingly selective injury of the pulmonary vessels. An increased surface tension in the alveoli may account for the increased permeability (Buckingham and Sommers, 1960; Pattle, Claireaux, Davies and Cameron, 1962).

An inflammatory origin of the exudate, on the other hand, cannot be entirely excluded; Groniowski and co-workers support the inflammatory origin of hyaline membrane disease (Groniowski, Gabryel and Grembowicz, 1957; Groniowski and Biczyskowa, 1963), while Laughton (1963) claims to have isolated a specific organism from a case of hyaline membrane disease.

Summary

The lungs of six newborns with well-defined hyaline membrane disease were studied by means of immunofluorescence. It was found that the hyaline membranes were composed of fibrin, γ-globulin and albumin, which also focally impregnated the alveolar septa. Moreover, cell nuclei were found within the membranes by means of fluorescent antibody procedure.

It was concluded that the hyaline membrane may be formed in the plasma-clotting process resulting from capillary wall damage and increased capillary permeability.

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References


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