The problem of the splitting of the glomerular basement membrane originated with the observations of Jones (1953, 1957) on the histogenesis of membranous thickening, made possible by means of a new method of staining (silver-methenamine). In his opinion this thickening was caused by the splitting of the basement membrane due to the deposition of a hyaline substance between the two layers. Jones’ hypothesis was based on the fact that the basement membrane is constructed normally of two layers, one epithelial and the other endothelial in origin, being separated by a ‘virtual space’. This space could become apparent in some pathological conditions.

This concept of splitting of the basement membrane is not generally accepted. Electron microscopists have not been able to demonstrate similar lesions. On the contrary, electron microscopy has shown that the basement membrane in normal and in pathological conditions appears to be a single entity. We believe that only one of the two layers, as shown by the Jones’ stain, represents the true basement membrane while the other layer corresponds to a newly laid-down argyrophilic substance with staining properties similar to those of the basement membrane, but with different electron density.

Churg and Grishman (1959), in their studies on the membranous glomerular lesion, were able to demonstrate three different morphological entities. The first was due to the ‘splitting’ of the true basement membrane while the second, also called splitting, was caused by the deposition of a substance between the endothelial cells (‘lamina fenestrata’) and the basement membrane. The third type consisted of the deposition of material between the epithelial cells and the basement membrane. This material, of which the irregular appearance is caused by the spike-like protrusion of the cytoplasm of the epithelial cells, was recognized by Movat and McGregor (1959) as being the basic lesion of the chronic membranous glomerulitis of Allen (1951).

Associated with the splitting is the presence of cellular elements scattered between the two layers.

**Material and Methods**

- One hundred and ninety kidney biopsies from patients with diffuse renal disease were examined. Specimens were fixed in Bouin solution, embedded with paraffin and bees-wax mixture and all sections of 0.5 to 1.0 micron were stained with PAS, silver-methenamine and hematoxylin according to the Bignami and De Matteis technique (1959), as modified by us. Studies on such preparations were done with the light microscope and in phase-contrast to visualize better the cells and cellular components which otherwise would be poorly stained because of the very thin sections.*

This report is based upon 23 cases demonstrating splitting of the wall of the capillary loops.

**Results**

The splitting observed was divided into five types according to the following histological criteria:

**Type 1:** Splitting was observed with no cellular elements included in the space which varied from 2 to 4 micra in width. In addition, the two layers resulting from the splitting were located around the free edge of the wall of the glomerular loops. The external layer seemed more irregular, tortuous and not of uniform thickness.

Scattered between the two layers were connecting filaments consisting of a substance with the same staining characteristics as the two layers (Fig. 1 (a)).

**Type 2:** With this type of splitting there was observed inclusion of cellular elements in the resulting space which was about 8 to 10 micra in width. The external layer was thin and the internal

* The light-field microphotographs were taken with a Leitz-Ortholux microscope furnished with wide range lens (objective: immersion C Pl, 100 : 1, 170/0.17 ocular: Periplan 8 X). For the phase-contrast a Leitz lens was used (objective: Pv El Oel, 70 : 1 h, 170/0.17; ocular: Periplan 8 X; condenser for phase-contrast in black and white and in colour).
Types of 'false splitting' of the basement membrane with Jones’ technique, using silver methenamine.

(a) Type 1: Two concentric layers separated by a restricted space.
(b) Type 2: Two layers separated by a wider space with inclusion of cellular elements.
(c) and (d) Type 3: The external layer appears scalloped in configuration.
(e) Type 4: Two concentric layers separated by a wide space inside which are sometimes found cellular elements. (× 1,600)

Type 3: In these instances, the space resulting from the splitting was subdivided by numerous, more or less well-defined, cavities (Fig. 1 (c), 1 (d)). The external layer appeared scalloped in configuration. As a rule, the individual cavities appeared empty but occasionally small cellular elements (3 to 4 micra in diameter) were seen. These elements had an oval or round nucleus with dense chromatin material.

Type 4: In this last type, the two layers of the splitting were of equal thickness and showed the same staining properties and would at times appear to be arranged in a perfectly concentric manner. The lumen in such a case was clearly delineated by the inner layer. Between the two layers were often found cells of elongated form with oval or round nuclei containing lightly stained chromatin material (Fig. 1 (e)).

The relationship between the histological and clinical pictures is summarized in Table 1.
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<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Cases</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
<th>Type 4</th>
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<tbody>
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<td>8</td>
<td>8</td>
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<td>-</td>
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<tr>
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<tr>
<td>Subacute glomerulonephritis with nephrotic syndrome</td>
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<td>6</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Subacute glomerulonephritis</td>
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<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Focal nephritis</td>
<td>1</td>
<td>1</td>
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<td>-</td>
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</tr>
<tr>
<td>Renal rickets</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Type 1* lesions were observed in the following instances:

Eight cases of chronic glomerulonephritis with the nephrotic syndrome; three cases of chronic glomerulonephritis without the nephrotic syndrome; six cases of subacute glomerulonephritis with the nephrotic syndrome; two cases of subacute glomerulonephritis without the nephrotic syndrome; one case of focal nephritis; one case of disseminated lupus erythematous; one case of chronic pyelonephritis; and one case of renal rickets.

*Type 2* was observed in five biopsies associated with *type 1*. Two of the cases were also associated with *type 4*. The latter were cases of chronic glomerulonephritis with and without the nephrotic syndrome. The other three were cases of subacute glomerulonephritis, all associated with the nephrotic syndrome.

*Type 3* was observed in three cases, one of which was clinically diagnosed as chronic glomerulonephritis, one as subacute glomerulonephritis with the nephrotic syndrome and the third as disseminated lupus erythematous. These changes were always associated with one of the other types of splitting. Intracapillary proliferation was evident in all these cases.

*Type 4* was observed in three cases: two of chronic glomerulonephritis, one with the nephrotic syndrome.

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**Fig. 2.**—Possible histogenesis of the newly-formed layer due to deposition of argyrophilic material on the inner surface of the endothelial cytoplasm during the intracapillary proliferative process.  
(a) The endothelial cells proliferating.  
(b) The cells acquiring argyrophilic substance.  
(c) Some elements, increasing in size, break the newly-formed layer and protrude into the capillary lumen; this corresponds to what is seen by micro-photography.  

\(× 1,600\)
syndrome and the other one without; the third one was of subacute glomerulonephritis with the nephrotic syndrome. Type 4 was seen associated with type 1 in all three cases. There was also observed in two of these cases, type 2 in one and type 2 and type 3 in the other.

Discussion

Systematic studies done on very thin sections of biopsy material makes it possible for us to clarify some of the morphological aspects which up to now have been classified by others as 'membranous glomerulitis'.

These were based on observations made on specimens of such a thickness as to make the two layers hardly distinguishable. As a result of these new studies we now define 'false splitting of the basement membrane' as the histological appearance characterized by the presence of two or more layers concentrically arranged, sometimes discontinuous and of variable thickness, separated at times by a narrow space and at other times by a wider cavity and occasionally containing cellular elements. This false splitting appears to us to be caused by the presence of newly-formed layers with the same staining properties as the basement membrane. The hypothesis, that the doubling of the basement membrane is caused by the splitting of the epithelial from the endothelial layer with the deposition of protein material and that scattered cells between the two layers are mesangial is not accepted by us for the following reasons:

1. Under the electron microscope the basement membrane is seen as a unit.
2. The existence of the mesangium is not generally accepted.
3. Continuity between the space enclosed by the two layers ('pericapillary space' according to Churg and Grishman) and the intercapillary space is not demonstrable even though it is a theoretical possibility.

In our opinion the newly-formed layer represents argyrophilic material deposited on the epithelial or endothelial cytoplasm. The histogenesis of the various types of splitting could then be explained on the basis that the argyrophilic substance is deposited on the inner surface of the endothelial cytoplasm and the endothelial cell is included between the basement membrane and the newly formed layer. Whenever there is a proliferative process from the endothelial cells we can find two or more cellular elements between the layers. This could be the histogenesis of some of the appearances described by Churg and Grishman (1959). Therefore in our opinion they are endothelial and not mesangial cells. A similar process seems to be the basis of the 'splitting' of

![Fig. 3.—Peritubular 'false splitting' with inclusion of cells. (x 1,250)](image)

![Fig. 4.—Formation of the newly-formed layer due to the deposition of argyrophilic material on the capsular surface of the epithelial cell.](image)
the basement membrane of the tubules, where fibroblastic-like cells are found. In other instances the argyrophilic material is deposited in a concentric manner around the basement membrane on the outside of the endothelial cytoplasm. The space between the basement membrane and the newly-formed layer is very narrow and devoid of cellular elements. At times this splitting seems to be characterized by the apposition of the argyrophilic material on the outer side of the basement membrane. In this case the epithelial cells would remain outside or be included in the space between the two layers. Material accumulated on the membranous side of the epithelial cells has an irregular edge due to the presence of primary processes (trabeculae of Hall) (Fig. 1 (b)). On
other occasions the argyrophilic material is deposited on the capsular side of the epithelial cells (Fig. 4).

These observations and the reports by various other authors have convinced us that similar morphological appearances are not infrequent. For this reason, the hypothesis of the epithelial origin of the cells included between the two argyrophilic layers is substantiated in practice. However we must not forget that similar changes are also caused by more complicated structural alterations especially associated with cellular proliferation.

Finally, we would like to mention the relationship between false splitting and the clinical picture and to point out the probable significance of the various aspects of these particular structural alterations. The functional consequences of these lesions must be correlated with the altered permeability of the basement membrane. The false splitting, therefore, seems to be a particular appearance of membranous glomerulitis. At other times it is related to the reduction of the lumen of the capillary loops identified with those cases of intracapillary glomerulitis.

The first condition could be realized by a particular splitting which we designated 'type i', while the second condition could correspond to the other types of splitting which are associated with the most serious morphological aspects of the type i. This can be verified by examining the figure.

However the validity of this anatomic-functional correlation is limited because of the relatively small number of observed associations between the type i and the other three types.

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Splitting of the Glomerular Basement Membrane

A. Fabbrini, G. A. Cinotti and F. Giacomelli

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