UNUSUAL DEVELOPMENT OF BASEMENT MEMBRANE ABOUT SMALL BLOOD VESSELS

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Basement membrane is usually found where connective tissue comes into contact with epithelial structures (1). It occurs on the outer surface of the endothelium of small blood vessels, though it is flimsy and deficient about sinusoidal vessels of the spleen, liver, bone marrow, and frog heart (2–6). Consisting of a feltwork of fine filaments embedded in a homogeneous matrix (4, 5), it comprises three layers: *viz.*, the lamina rara interna, lamina densa, and lamina rara externa (5, 7–9). Under normal or physiological conditions, endothelial basement membranes are known to vary in thickness with age (9–13), species (9, 11, 13–17), and the site and nature of the vessel (2, 3, 9–20). Pathological thickening of the glomerular basement membranes occurs in both spontaneous and experimentally induced lesions (9, 11, 21–23). However, in the present report an exceedingly thick basement membrane, without any recognizable pathological cause, was found in small vessels of the frog. In addition, the ultrastructural organization of the basement membrane was quite unusual in that it was related not only to endothelium but also to pericytes and adventitial cells.

MATERIALS AND METHODS

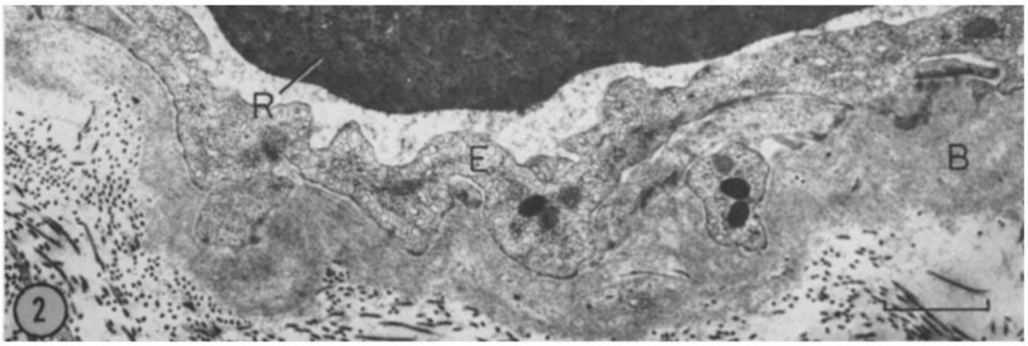
Queensland green tree frogs (*Hyla caerulea*) were pithed or decapitated prior to the removal of the tongue and the interdigital webs of the hind limbs. The tissues were cut into small blocks, fixed for 2 hours in 1 to 2 per cent osmium tetroxide buffered according to Zetterqvist's method (24), and then dehydrated in ethanol and embedded in Araldite. Thin sections were cut on an LKB (Type 4800A) ultramicrotome, mounted on copper grids, stained with lead (25) and 1 per cent uranyl acetate in ethanol, and examined in a Siemens Elmiskop I.

RESULTS

The thin-walled blood vessels (capillaries and small venules) studied in the web and tongue were in the main 5 to 15 μ in diameter. The basement membrane on the external surface of the endothelium of these vessels was frequently thin, being no more than 700 A thick. On one side, it was applied to the outer surface of the endothelium, and on the other it merged with the surrounding connective tissue space.

In some cases the basement membrane of vessels 5 to 9 μ in diameter varied from 700 to 1,500 A in width. In the tongue, one vessel 8 μ in diameter had a pericyte basement membrane 0.5 μ wide merging with the basement membrane of the endothelium where the latter was not covered by a pericyte.

Larger vessels 10 to 15 μ (probably venules) had thick basement membranes (Fig. 1 to 4), frequently up to 0.6 μ. This was particularly so in the tongue, for in the web the membranes did not exceed a thickness of 0.5 μ. The basement membrane was not of uniform thickness in each vessel nor was the more translucent lamina rara interna between the basement membrane and the endothelium always discernible (Fig. 2), though this may have been caused by the plane of section. The thick basement membranes were continued onto the external surface of pericytes. However, in some vessels a thin basement membrane closely related to endothelium could be distinguished. It was separated from one or several bands of basement membrane material by a zone of lesser density (Fig. 3). These bands invested fragments of unidentified adventitial cells closely related to the vessel, but they were not restricted to the immediate vicinity of the cells.



Scale line = 1 μ in the electron micrographs.

FIGURE 1 Small blood vessel from frog tongue. A thin basement membrane (*B*) is related to the endothelium (*E*) in centre of illustration, but is much thicker at the sides. Note the thick basement membrane on lower surface of adventitial cell (*A*). Lumen (*L*) is at top. × 13,000.

FIGURE 2 Thick basement membrane on adventitial surface of endothelium of blood vessel from frog tongue. Note the variable density of the basement membrane and the incorporation of a few collagen fibres. The absence of lamina rara interna in some regions may be only apparent and due to the plane of section. Dark object at top is a red cell (*R*) in the lumen. × 14,000.

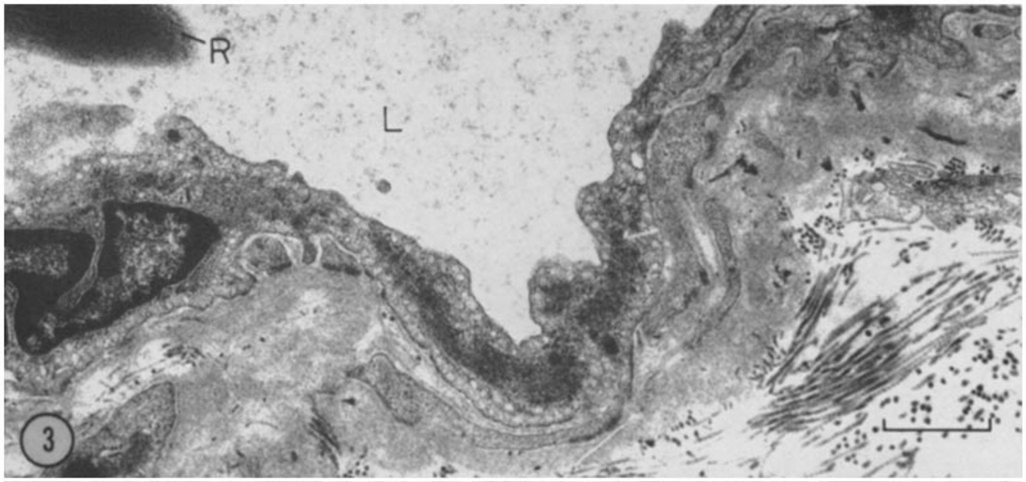
They were often convoluted and seemingly redundant, in places coalescing with each other or the endothelial basement membrane. In this manner, a laminated perivascular zone of basement membrane material, at times 2 μ wide, was formed about vessels in the tongue. In the web the limit was 0.5 μ. Scanty collagen fibres and occasional fragments of densely stained material resembling elastic tissue were often incorporated in the thick basement membrane.

A thick basement membrane (up to 0.13 μ thick) was also observed about a few vessels which were surrounded by a layer of muscle fibres and more plentiful elastic tissue. However, the majority of vessels showing a thick basement membrane were not arterioles but rather capillaries and small venules.

DISCUSSION

In the frog vessels, the remarkably thick basement membrane and its arrangement are of considerable interest. The thickness of the broad bands (morphologically basement membrane) could not be accounted for by the plane of section. Furthermore, as the animals were only small or medium-sized, it is improbable that the thickness is a factor of age. The possibility exists that it is pathological. Yet even if this were so, the findings emphasize the need for adequate control material in experimental investigations involving this species.

Hogan and Feeney (16) found that the basement membrane of small vessels in the human retina was often unusually thick. In capillaries a thin basement membrane (500 A) was applied to the external surface of the endothelium, and a thicker basement membrane (4,000 A) related to pericytes. These two basement membranes coalesced where pericytes were absent. Such formations and thick composite basement membranes are not unlike those found in the present study. However, in the frog vessels pericytes were not so



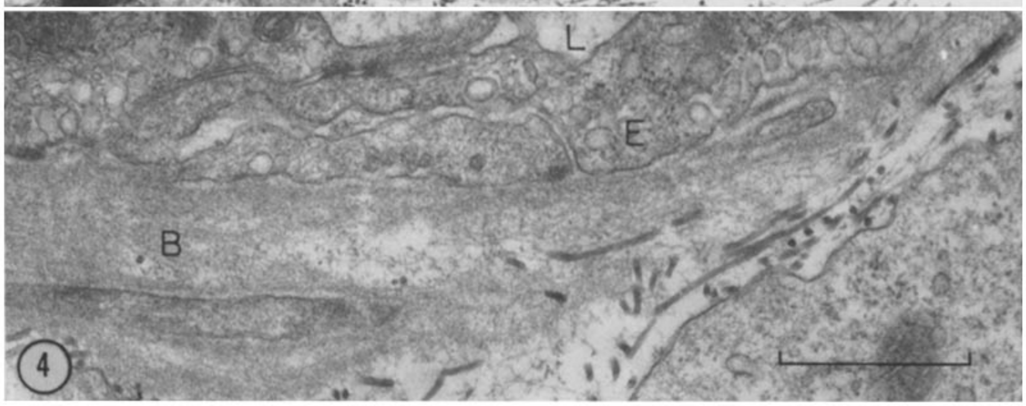


FIGURE 3 Broad band of basement membrane material about small blood vessel from frog tongue. Note multiple layers of basement membrane and the incorporation of some collagen fibres. Dark bodies in basement membrane at right of micrograph may be fragments of elastica. × 14,000.

FIGURE 4 Higher magnification to show fibrillary nature of thick basement membrane. × 25,000.

numerous as in the retinal vessels, nor was there cavitation or "Swiss cheese" appearance of basement membranes. In addition, the basement membranes were more loosely arranged and had a greater over-all thickness than in the retina.

Considerable widening of capillary basement membranes has also been found in muscle from human diabetics (22), and lamination similar to that observed in the frog was recorded, though the severity of the changes could not be correlated with the clinical duration or the severity of the diabetes. The functional significance of the present findings is not understood, but it would appear that frog tissue (especially the tongue) provides basement membranes sufficiently thick as to be highly suitable for comparative studies of their physiology.

SUMMARY

An electron microscope study of capillaries and small venules of the frog tongue and interdigital web often revealed basement membranes of extraordinary thickness. This thickness was due primarily to the coalescence of a thin endothelial basement membrane (700 A) and thicker accessory membranes associated with pericytes and adventitial cells. Multilayered structures at times surrounded small venules, producing broad perivascular zones of laminated membranes up to 2 μ thick in the tongue and 0.5 μ in the web.

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REFERENCES

1. Pease, D. C., The basement membrane: substratum of histological order and complexity, *in* Fourth International Conference on Electronmicroscopy, Berlin, Springer-Verlag, 1960, **2,** 139.
2. Bennett, H. S., Luft, J. H., and Hampton, J. C., Morphological classification of vertebrate blood capillaries, *Am. J. Physiol.*, 1959, **196,** 381.
3. Farquhar, M. G., Fine structure and function in capillaries of the anterior pituitary gland, *Angiology*, 1961, **12,** 270.
4. Palade, G. E., Blood capillaries of the heart and other organs, *Circulation*, 1961, **24,** 368.
5. Fawcett, D. W., Comparative observations on the fine structure of blood capillaries, *in* The Peripheral Blood Vessels, (J. L. Orbison and D. E. Smith, editors), Baltimore, Williams and Wilkins Co., 1963, 17.
6. Stehbens, W. E., and Meyer, E., Ultrastructure of endothelium of the frog heart, *J. Anat.*, 1965, **99,** 127.
7. Yamada, E., The fine structure of the renal glomerulus of the mouse, *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 551.
8. Fawcett, D. W., The fine structure of capillaries, arterioles and small arteries, *in* The Microcirculation, (S. R. M. Reynolds, and B. W. Zweifach, editors), Urbana, University of Illinois Press, 1959, 1.
9. Bencosme, S. A., and Bergman, B. J., The ultrastructure of human and experimental glomerular lesions, *Internat. Rev. Exp. Path.*, (G. W. Richter and M. A. Epstein, editors), 1962, **1,** 139.
10. Maynard, E. A., Schultz, R. L., and Pease, D. C., Electron microscopy of the vascular bed of rat cerebral cortex, *Am. J. Anat.*, 1957, **100,** 409.
11. Farquhar, M. G., Vernier, R. L., and Good, R. A., An electron microscope study of the glomerulus in nephrosis, glomerulonephritis, and lupus erythematosus, *J. Exp. Med.*, 1957, **106,** 649.
12. Donahue, S., and Pappas, G. D., The fine structure of capillaries in the cerebral cortex of the rat at various stages of development, *Am. J. Anat.*, 1961, **108,** 331.
13. Bloom, P. M., Hartman, J. F., and Vernier, R. L., An electron microscopic evaluation of the width of normal glomerular basement membrane in man at various ages, *Anat. Rec.*, 1959, **133,** 251.
14. Bergstrand, A., Electron microscopic investigations of the renal glomeruli, *Lab. Invest.*, 1957, **6,** 191.
15. Farquhar, M. G., Glomerular permeability investigated by electron microscopy, *in* Small Blood Vessel Involvement in Diabetes Mellitus, published by The American Institute of Biological Sciences, 1964, 31.
16. Hogan, M. J., and Feeney, L., The ultrastructure of the retinal vessels. II. The small vessels, *J. Ultrastruct. Research*, 1963, **9,** 29.
17. Bergstrand, A., and Bucht, H., Anatomy of the glomerulus as observed in biopsy material from young and healthy human subjects, *Z. Zellforsch.*, 1958, **48,** 51.
18. Rhodin, J., Electron microscopy of the glomerular capillary wall, *Exp. Cell Research*, 1955, **8,** 572.
19. Policard, A., Collet, A., and Pregermain, S., Étude au microscope electronique des capillaires pulmonaires, *Acta Anat.*, 1957, **30,** 624.
20. Latta, H., Maunsbach, A. B., and Madden, S. C., The centrilobular region of the renal glomerulus studied by electron microscopy, *J. Ultrastruct. Research*, 1960, **4,** 455.
21. Kurtz, S. M., and Feldman, J. D., Experimental studies on the formation of the glomerular basement membrane, *J. Ultrastruct. Research*, 1962, **6,** 19.
22. Zacks, S. I., Pegues, J. J., and Elliott, F. A., Interstitial muscle capillaries in patients with diabetes mellitus: A light and electron microscope study, *Metabolism*, 1962, **11,** 381.
23. Lannigan, R., Blainey, J. D., and Brewer, D. B., Electron microscopy of the diffuse glomerular lesion in diabetes mellitus with special reference to early changes, *J. Path. Bact.*, 1964, **88,** 255.
24. Zetterqvist, H., The ultrastructural organization of the columnar absorbing cells of the mouse jejunum, Thesis, Department of Anatomy, Karolinska Institutet, Stockholm, 1956.
25. Millonig, G., A modified procedure for lead staining of thin sections, *J. Biophysic. and Biochem. Cytol.*, 1961, **11,** 736.