CAPILLARY BASAL LAMINA THICKENING

Its Relationship to Endothelial Cell Death and Replacement

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INTRODUCTION

Thickening of basal lamina[1] (BL) which occurs with advancing age (1) and various diseases (4, 7, 10) is most common and severe in patients with diabetes mellitus (2, 8, 6, 12, 14). The reasons for and the mechanism of this thickening have thus far escaped satisfactory explanation. In this communication, we wish to provide new evidence which elucidates the development of thick BL and which directs attention to the underlying defect.

In a detailed study of capillaries in skeletal muscle from 40 overt diabetics (15), 26 chemical diabetics, and 41 nondiabetics (14), several findings suggested the possibility that the BL thickening is the result of repeated episodes of cell death and cell regeneration. These are:

(*a*) The BL is not homogeneously thickened but is composed of concentrically arranged lamellae (6, 12, 14, 16), each measuring between 800 and 2000 A (Fig. 1). The thickness of individual lamellae increases with the distance from endothelium, while their electron opacity decreases. The innermost lamella is usually well defined, intimately applied to the endothelium, and encompasses the pericytes. The outermost lamella may be partly missing or it may be arranged in redundant folds.

(*b*) The interlamellar space is irregular in width or obliterated by fusion of lamellae, and it commonly contains cellular debris and droplets of lipid (6, 14, 12) (Fig. 1). Spaces also occur within individual lamellae, suggesting by their crescent shapes that they were occupied by pericytes. They, too, occasionally contain cellular debris.

(*c*) BL thickening between the pericytes and endothelium is generally absent (Fig. 1). As in normal capillaries, the pericytes are embraced by the innermost lamella of the BL, giving the impression that the endothelium, the pericytes, and the innermost lamella comprise a normal capillary which is located within a BL tube.

(*d*) Cylinders composed almost entirely of one or more concentric BL lamellae are found in the interstitium of diabetic skeletal muscle (14, 16). Only cellular debris may be present between the lamellae and within the central space. These cylinders when seen in the cross-section are generally the size of capillaries and very likely represent remnants of capillaries in which the cellular elements have degenerated.

(*e*) Area measurements of capillary cross-sections and lumina on electron micrographs (14, 15) have shown that, in general, the capillaries with thickened BL's have smaller luminal caliber than capillaries with normal investment of BL.

Two explanations for these findings seem plausible: (*a*) The machinery of endothelial cells which ordinarily makes only a single "normal" complement of BL may, under appropriate circumstances, be "turned on" and produce additional layers. Since the new layers are deposited centripetally with respect to the original layers of BL, the effect is a decreased inner capillary caliber and lamellated thickening of the BL. (*b*) Alternatively, it could be that each endothelial cell is capable of making its "normal" complement of BL only *once* in its life cycle. The latter explanation with the following added features seems to be a better fit with the data. After cell death, some cellular debris remains within the acellular BL tube. Regeneration of a new capillary composed of endothelium, pericytes, and BL then occurs within the old BL tube. The result is a capillary with two lamellae of BL between which cell debris may become trapped. Repetition of this cycle adds successive lamellae of BL and progressively narrows the caliber. As the BL widens, the older lamellae become looser in texture, increase in width, and decrease in electron opacity (14).

[1] Basal lamina is used synonymously with basal membrane.

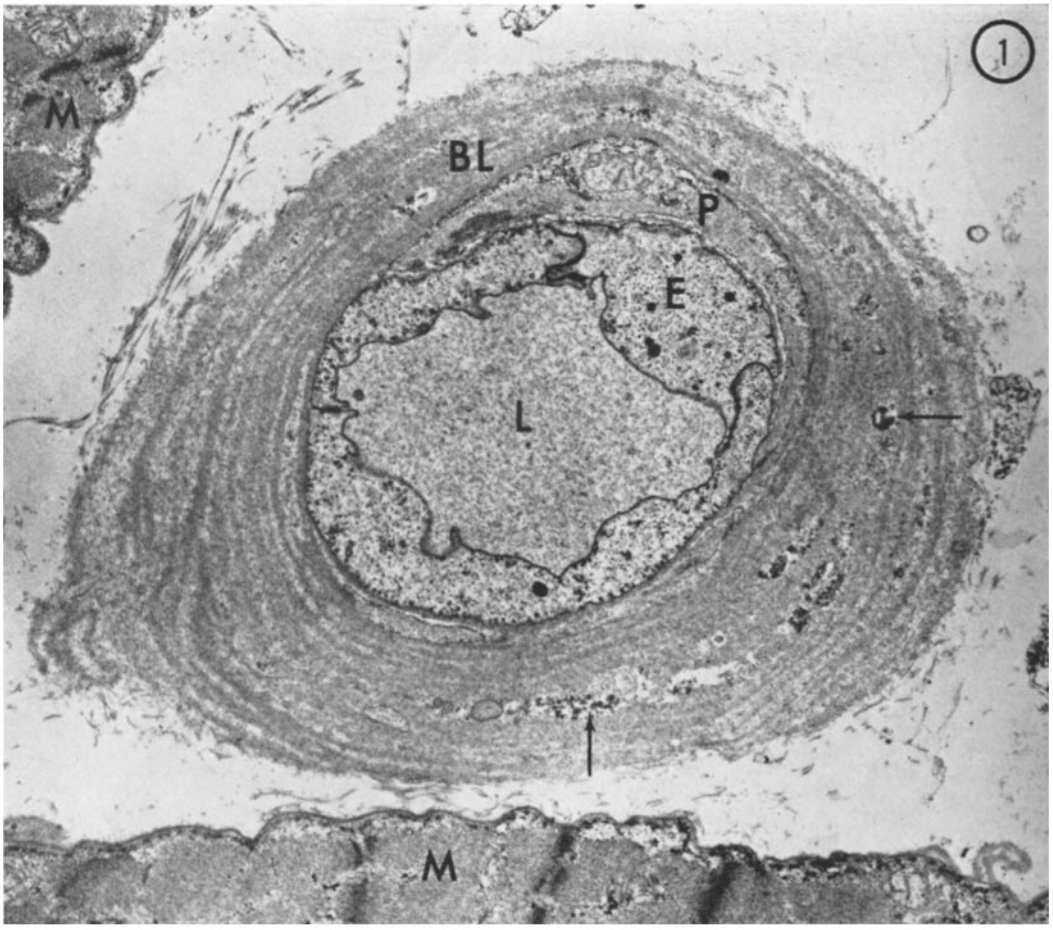

FIGURE 1 Cross-section of a capillary from plantar muscles of a 70-yr-old man who had overt diabetes mellitus for 20 years. The basal lamina (*BL*) is lamellated and contains cell debris (arrows) between lamellae. The basal lamina layer between pericyte (*P*) and endothelium is not thickened. The over-all appearance of this BL is suggestive of growth rings seen on cross-sections of trees. *L*, capillary lumen; *M*, muscle cells; *E*, endothelial cytoplasm. × 11,400.

According to this concept, the old, denuded BL serves as microskeleton or scaffolding for regenerating cells. That some preexisting scaffolding is needed for orderly regeneration has been demonstrated for skeletal muscle. Volkmann (13) observed that if the sarcolemma was not damaged, almost complete restoration of the structure and function of muscles occurred, whereas scar formation resulted if continuity of the sarcolemma was destroyed. Clark (5) in an ingenious experiment demonstrated that the regeneration of skeletal muscle fibers will occur in the direction of old fibers even if the piece of muscle was excised and then re-implanted at right angles to its original orientation.

EXPERIMENTAL OBSERVATIONS

In the present work, the experimental design of Clark (5) was used to test whether capillaries, as well as muscle cells, can regenerate along the old BL and produce a new layer of BL: Under phenobarbital anesthesia, a 1.5 cm^2 piece of rabbit gracilis muscle was removed, immediately returned to the defect, and sutured in place. Samples of tissue were removed thereafter in 5 1-wk intervals and examined by both light and electron microscopy. Under these circumstances, initially all cells died. Macrophages removed the bulk of the cellular debris but did not attack the BL. Regrowth of complete capillaries consisting of endothelium, pericytes, and BL occurred within the old capillary BL tubes within 5 wk. By repeating the

episodes of necrosis and regeneration, multiple layers or lamellae of BL were found to accumulate.

So as to differentiate between the old and the newly formed BL, a modification of this experiment was carried out. A rat which had been given 0.15 M silver nitrate in drinking water for 20 months prior to excision and reimplantation of a piece of gracilis muscle was operated upon as described above. After operation, the animal received fresh water. Shown in Fig. 2 is a regenerated capillary from this animal. The original, now outer layer of BL, is labeled with several aggregates of silver, in contrast to the new silver-free inner layer of BL.

DISCUSSION AND CONCLUSIONS

The experiments show that concentric multilayered thickening of capillary BL can be produced by cell death and repopulation of the preexisting BL scaffolding. Moreover, they provide adequate explanation for the presence of residual cell debris between the layers of thickened BL

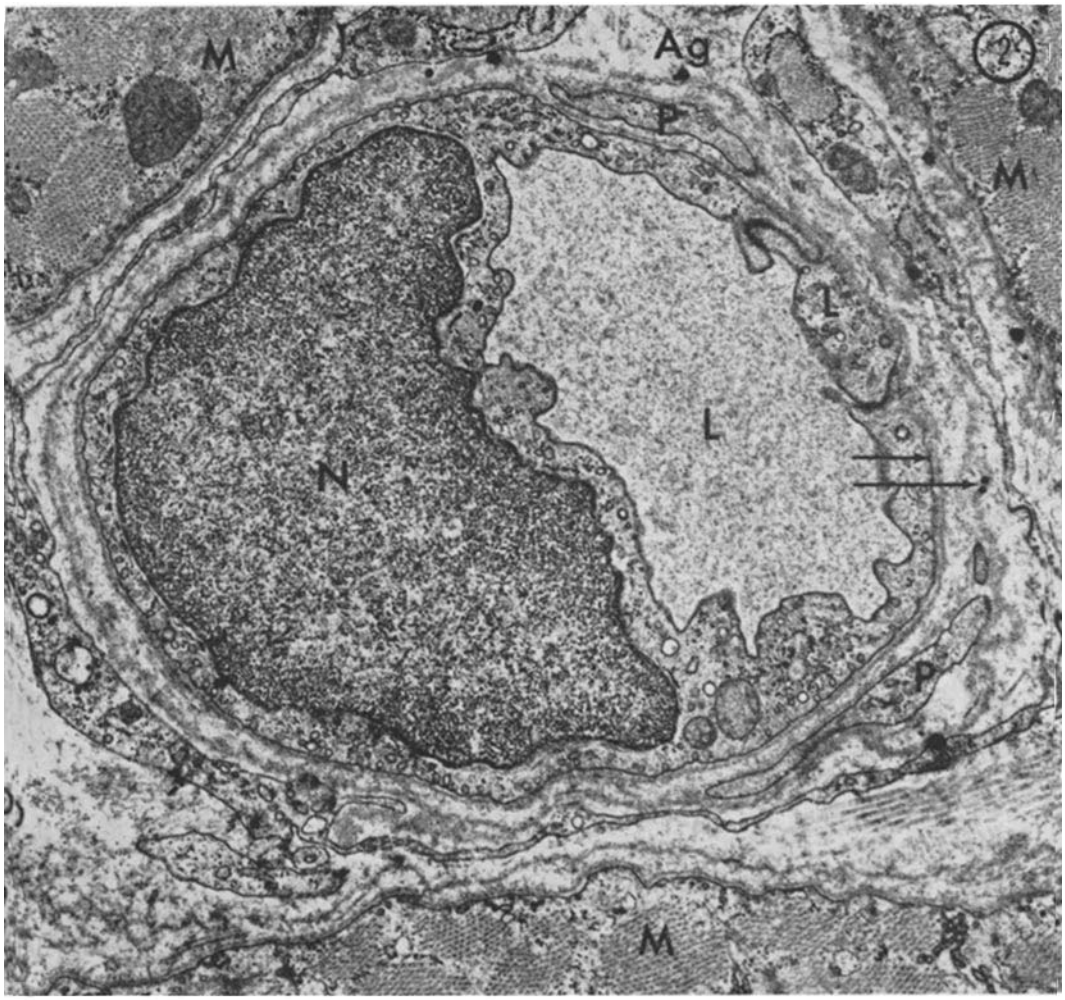

FIGURE 2 Cross-section of a capillary from gracilis muscle of rat 5 wk after a piece of muscle was excised and reimplanted. The 20-month-old rat was given 0.15 M silver nitrate solution as drinking water from weaning until the day of operation, and only fresh water after operation. The resulting accumulation of silver granules (*Ag*) is limited to the old, now outer layer of basal lamina (long arrow), indicating that the inner silver-free layer (short arrow) formed following the operation. Similar reduplication of BL is also apparent in two of the three muscle cells. *P*, pericyte; *M*, muscle cells; *L*, capillary lumen; *N*, endothelial cell nucleus. × 13,900.

as well as for lack of BL thickening between pericytes and endothelium and for narrowing of inner calibers in capillaries with thickened BL.

To our knowledge, the concept that BL formation is a "quantized" event and that thickening could be the result either of cell death and cell replacement or of a discrete "turn on" and "turn off" of BL production has not been considered previously. The ordinary concept has dwelt mainly on excessive accumulation due to either overproduction or reduced removal. The role and significance of BL removal in this scheme are unknown. While our short term experiments suggest that the acellular BL remains intact indefinitely after serving as a scaffolding for regenerating cells, it is possible that in the normal process of cell replacement the acellular BL eventually disappears. If this occurs, then an abnormal accumulation of BL could be caused by a defect in the resorption of the old, acellular BL rather then by an accelerated cell death and cell production, as we have suggested, or by a combination of the two.

Regardless of the role that resorption may have in the process of BL thickening, the present proposal provides a new frame of reference for consideration of the kinetics of BL production and its relationship to the genesis of this important pathological process. The increased frequency of cell death and regeneration which we deduce from our observations and experiments as occurring with diabetes mellitus could be related to the defects in cell proliferation which have been noted in cell cultures obtained from patients with diabetes mellitus. In one study (11), a reduced number of cell generations were elicited in fibroblasts in a culture from a juvenile diabetic and in cultures from three patients with Werner's syndrome and diabetes. In another study (9), fibroblasts from prediabetics exhibited decreased plating efficiency. These concepts are also consistent with the clinical observations of accelerated aging in patients with diabetes mellitus (3). Shortened life span of fibroblasts from diabetics in cell cultures could be due to accelerated rate of cell death and cell replacement. Whatever the cause, we believe that the evidence presented here on the BL, like the rings in the cross-section of a tree trunk, gives some index of the number of cell generations which have occurred in a particular capillary.

SUMMARY

Capillaries with thickened basal lamina (BL) from patients with diabetes mellitus exhibit structural characteristics which indicate that the accumulation of BL is caused by repeated episodes of cell death and regeneration. The characteristics are (*a*) lamellation of BL, (*b*) presence of cell debris between the lamellae, (*c*) absence of BL thickening between pericytes and endothelial cells, (*d*) narrowing of the inner capillary caliber, and (*e*) presence of acellular BL cylinders in skeletal muscle interstitium.

Experimentally, lamellation of BL was produced by excising and reimplanting a piece of rat skeletal muscle. The regenerating capillaries grew within the old, acellular BL tubes and formed a new second layer of BL.

This evidence indicates (*a*) that, following cell death, BL provides a scaffolding for regenerating cells; (*b*) that each new cell generation produces only a "normal" complement of BL; and (*c*) that excessive accumulation of BL is produced by accelerated rate of cell death and replacement.

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