Ultrastructural aspects of human and canine diabetic retinopathy

J. M. B. Bloodworth, Jr., and D. L. Molitor

Diabetic retinopathy has been examined in dogs made diabetic by bovine growth hormone or alloxan, and in humans. The thin-walled microaneurysm is a simple expansion of the circumference of the capillary with basement membrane thickening. The thick-walled microaneurysm represents the deposition of considerable debris between layers of thickened basement membrane, as well as compression phenomenon of the adjacent retinal tissues and a secondary reactive gliosis. These thick-walled microaneurysms are markedly permeable to all elements of the blood. No specific morphological change has been found to explain the focal nature of the initial dilation of capillaries to form microaneurysms, or the unusual permeability of these areas. Death of pericytes may play a role in development of diabetic retinopathy. The reason for the death of these cells is unknown but may be related to the metabolic derangements of diabetes itself.

Although diabetic retinopathy has been known for over a century, its exact nature and pathogenesis remain obscure. Ophthalmoscopy and pathologic examination using routine histologic techniques and flat preparations (whole mounts) of the retina examined by light microscopy revealed that diabetic retinopathy was a complex condition with patchy involvement—primarily of the posterior retina—by hemorrhages, exudates, microaneurysms, microinfarcts, capillary degeneration, and irregular degeneration of the neuroectodermal elements of the retina. Quite frequently the preceding changes may be associated with large hemorrhages which extend into the vitreous, and proliferation of new-formed capillaries into the vitreous or on the surface of the retina, so-called "retinitis proliferans." This condition almost inevitably leads to blindness if the patient lives long enough. Examination of the kidneys from diabetic patients has revealed that there is often a concurrent degeneration of the glomerular capillaries, as described by Kimmelstiel and Wilson, a condition now known as "diabetic glomerulosclerosis." More recently, electron microscopic observations of capillaries in other parts of the body have revealed that most capillary basement membranes are thickened in diabetic patients. The only exception reported to date is the basement membranes of capillaries supplying fat, which remain unaltered by diabetes. "Diabetic microangiopathy" has gained acceptance as the term...
to encompass all these capillary changes of the diabetic. During the last ten years, we have studied retinal vascular changes in human and experimental diabetes utilizing the electron microscope. This discussion is a summary of the results of these studies.

Materials and methods

Approximately 300 eyes from approximately 200 diabetic patients have been examined in this laboratory by “flat preparation” (whole mount). Routine paraffin-embedded histologic sections were made of the contralateral eye from many of these patients. Approximately ten of these paraffin-embedded eyes were serially sectioned and stained by numerous histochemical techniques. A few eyes were examined by the tryptic digestion technique of Kuwabara and Cogan. Ten human eyes have been examined by electron microscopy. For electron microscopy, the eye was opened and the retina removed in toto and placed in 1 per cent osmium tetroxide for approximately one hour. It was then dissected into small pieces approximately 1 mm. in diameter and embedded in Epon according to the method of Craig and associates. It is possible to identify major vessels and larger microaneurysms, as well as many hemorrhages and exudates in the osmium-fixed retina, if it is examined with the aid of a dissecting microscope. This method permits selection of pertinent areas of the retina for embedding. The Epon blocks were sectioned with a Porter-Blum microtome and examined with an RCA EMU-3G electron microscope. In most instances the sections were stained with uranyl acetate prior to examination. Blocks from each of two eyes were serially sectioned for electron microscopy. Eyes were obtained from autopsies and from surgical excisions. Most of the material to be presented in this study was obtained from a serially sectioned eye removed approximately four hours after death.

Dogs were made diabetic by administration of 3.5 mg. per kilogram body weight per day of bovine growth hormone for 30 days. Other dogs were made diabetic by the administration of 100 mg. per kilogram body weight of alloxan. These dogs were allowed to remain severely diabetic, requiring 10 to 20 units of insulin per day. Despite insulin therapy, they excreted as much as 200 Gm. of carbohydrate in the urine daily and were frequently in mild acidosis. Three dogs which survived over 4 years, two made diabetic by bovine growth hormone and one by alloxan, all showed the complete picture of diabetic retinopathy. Eyes from these animals were handled in the same manner as those from human patients.

Results and discussion

Our attention has been directed to the vascular network of the retina by the fact that almost all the pathologic changes of diabetic retinopathy occur in the inner layers of the retina, that portion supplied by the retinal capillaries. Although ophthalmoscopists have frequently described venous distention early in the course of diabetic retinopathy, pathologic changes of arteries and veins have not been delineated. As a result our studies are limited almost exclusively to the retinal capillaries and the degenerative changes found in them.

The following discussion is based on studies of material from diabetic humans and dogs. The retinal lesions in dogs were generally milder, the microaneurysms were mostly thin-walled, and the hemorrhages and exudates were small. Otherwise, there was no significant difference in the two species.

The retinal capillaries, which have been well described in various species, consist of a lumen varying from 4 to 7 μ in diameter, which is surrounded by a relatively thin layer of endothelial cytoplasm varying from 0.1 to 0.3 μ in thickness. Occasionally the endothelial cell impinges upon the lumen due to the presence of a nucleus which has an elongated, sausage-like shape, with its long axis parallel to the long axis of the capillary. The usual organelles, including endoplasmic reticulum, free ribosomes, mitochondria, and Golgi apparatus, are present in modest quantities within these cells. In addition, there are numerous small, membrane-lined sacs varying from 300 to 600 Å in diameter, the pinocytic vesicles, which are found throughout the cytoplasm of the endothelial cells. Around the outside of the endothelial cell there is a continuous basement membrane which varies from 600 to 800 Å in thickness, in the normal adult human. Occasionally, this basement membrane splits to surround completely a cell which is external to the endothelial cell and separated from it by a layer of basement mem-
brane. This cell has a nucleus which is oval or round, and projects externally from the capillary. It has many spiderlike cytoplasmic processes which surround and cover approximately 50 per cent of the surface of the capillary. These cells were first called "Rouget cells" but are now more often referred to as pericytes. They have also been referred to as "mural cells." They contain the same organelles as the endothelial cells, including pinocytic vesicles. In man, the basement membrane of retinal capillaries is somewhat more irregular in the retinal periphery, and sometimes appears to have a "Swiss-cheese" appearance due to many small vesicles in its external portion.

In diabetes mellitus there is a gradual thickening of the capillary basement membrane of all retinal capillaries. This occurs concurrently with capillary basement membrane thickening throughout other portions of the retina.

Fig. 1. Electron micrograph of retinal capillary from human diabetic. The capillary lumen (CAP) is surrounded by a thin layer of endothelial cytoplasm (EN) which contains the usual organelles and shows several cell junctions (arrow). There is a lipid droplet (L) in one cell. No nucleus is present at this level. Basement membrane separates the endothelial cells from cell processes (P) which probably represent portions of pericytes. Note the variation in thickness of basement membrane (BM) and the marked thickening of the peripheral portions of this membrane. There is a moderate amount of nonspecific debris between the peripheral layers of the basement membrane. Capillary is completely surrounded by glial cells. (x12,000.)
of the body and is the retinal component of diabetic microangiopathy. It is diffuse in the sense that every capillary appears to be involved throughout the retina. However, it is quite irregular in its involvement of the capillary in any given area. For instance, one half of the circumference of a capillary may show a markedly thickened basement membrane, while other portions may be almost normal (Fig. 1). We have seen basement membrane thickening many times without other involvement of the capillary. In the severe, far-advanced cases of diabetic retinopathy, the periphery of the capillaries is composed of a series of layers of thick basement membrane between which are found varying kinds of debris. Some appears to be fragmented membranous material simulating the plasma membrane of a cell wall. Other amorphous and granular debris is unidentifiable. Fat vacuoles are frequently present and sometimes there is collagen between the layers of basement membrane. Not infrequently, "basement membrane-like" material which is continuous with the basement membrane projects externally from the capillary into the space between the glial processes and nerve fibers which surround the capillary. This "basement membrane-like" material may completely surround several nerve fibers adjacent to the capillary. Sometimes there is an outpouching of the external layer of basement membrane to surround a globular mass of debris. These areas resemble degenerated pericytes and, no doubt, represent the "ghost cells" of Cogan and co-workers. Viable pericytes are present on many capillaries but seem to be reduced in number in those areas where degenerative changes, exudates, and microaneurysms are most frequent. Despite the many proliferative and degenerative changes of the basement membrane region of the capillary in the diabetic, each capillary is usually completely lined by intact endothelial cells which are connected by the normal cell junctions with desmosomes. The organelles of these endothelial cells are definitely normal in quantity and sometimes appear to be increased.

Not infrequently, one finds P.A.S.-positive strands of connective tissue connecting viable capillaries. These so-called "mesodermal bridges" appear to represent collapsed, nonfunctional capillaries in which the cells of the capillary have completely degenerated and the only remaining component is the basement membrane. We have observed many capillaries by electron microscopy which consist of residual basement membrane filled by structures which appear to represent processes of nerve cells or, more likely, glial cells (Fig. 2). In many instances, these processes completely fill the residual basement membrane sheath. Occasionally one sees breaks in the residual basement membrane through which such processes pass. We have postulated that these changes represent an attempt by the glial cells to phagocytize the degenerated capillary wall. Thus, it would be a secondary phenomenon, not important in the etiology of the capillary degeneration. Occasionally one can see an attempt at regeneration by ingrowth of a new capillary through the basement membrane sheath of a degenerated capillary, or of a small arteriole or venule. Most such attempts appear abortive.

The microaneurysm is an abrupt, usually spherical dilatation of the retinal capillary wall. Both thick and thin-walled microaneurysms are usually present in the same retina. We have postulated for many years that the thin-walled microaneurysm is the initial stage. This hypothesis is supported by findings in our experimental dogs, in which most of the changes were postulated to be early and consisted primarily of thin-walled microaneurysms.

Exception for dilatation of the lumen, the wall of the thin-walled microaneurysm appears identical to the wall of the adjacent capillaries, as described above (Figs. 3 and 4). There is a thickened basement membrane lined by intact endothelial cells. There is no obvious change in the structure of the capillary as it leads into or out of
the microaneurysm. The thin-walled microaneurysm appears to compress the adjacent retinal elements to a mild degree but causes little or no glial reaction. There is no debris (or only a small amount) in the wall of most thin-walled microaneurysms.

The thick-walled microaneurysm is a highly variable structure (Figs. 5 to 8). However, all such microaneurysms which we have examined have been completely lined by a layer of intact endothelial cells with normal cell junctions and desmosomes. The endothelial cells of the microaneurysms frequently appear somewhat thicker than those of the normal capillary and they contain increased numbers of mitochondria, endoplasmic reticulum, and a prominent Golgi apparatus. Occasionally, lipid vacuoles are present in the endothelial cells, and sometimes large, clear vacuoles of unidentified type. Usually there is a thickened, amorphous basement membrane just outside of the endothelial cell. This basement membrane frequently has many ramifications or strata between which there are varying types of debris similar to those described in the wall of the capillary. In many areas of the outer portion

Fig. 2. Electron micrograph of a degenerated capillary in the retina of a human diabetic. The basement membrane (BM) is all that remains of the capillary. It is markedly thickened and contains some nonspecific debris between several layers. In addition, the lumen is completely filled by cell processes which resemble those of the glial cells (G) which surround the capillary. Note the basement membrane-like material which has infiltrated between the glial cells above the capillary. (×12,000.)
of the thick-walled microaneurysm there are large numbers of collagen fibrils between layers of basement membrane and interspersed collections of amorphous, granular, or fibrillar debris of great electron density. The appearance of this debris is consistent with that of plasma protein and/or fibrin fibrils. In addition, many oval or round, particulate-containing structures resembling platelets are present in the outer wall. These structures cannot be positively identified as platelets, but in conjunction with the fact that there are unusually large numbers of platelets present in the lumen of the microaneurysms, we suspect that they may have found their way into the wall. We have not observed platelets in retinal capillaries of nondiabetics, or in capillaries of diabetics except immediately adjacent to microaneurysms. The presence of plasma constituents in and around the microaneurysm wall is ex-
Fig. 4. Electron micrograph of small portion of the wall of microaneurysm in the retina of a metasomatotrophin-diabetic dog. Note the intact endothelial cells (EN) which completely line the microaneurysmal lumen. There is a normal cell junction with a desmosome present, at the arrow. Basement membrane (BM) is moderately thickened. Note that basement membrane-like material in continuity with the basement membrane extends laterally between some nerve fibers adjacent to the microaneurysm. There is no evidence of compression of the nerve fibers which surround the microaneurysm. (×31,000.)

Expected, in view of the marked leakage demonstrated by fluorescin injection. Intact red blood cells are not infrequently trapped in the wall of the microaneurysm or in the adjacent retinal stroma. There is marked lateral compression of the surrounding elements of the retina, and proliferation of cells in this area. These cells have moderately large nuclei, large amounts of cytoplasm, and sometimes contain debris. They are thought to represent microglial cells reactive in nature. Sometimes the debris in the wall of the microaneurysm resembles degenerating red blood cells and occasional deposits are composed of small, electron-dense particles compatible with ferritin. Cells identifiable as pericytes have been almost universally absent in the wall of microaneurysms. Occasional globular pockets of amorphous debris in the wall of the microaneurysm may well represent degenerating pericytes.
Fig. 5. Electron micrograph of an intermediate stage microaneurysm in the retina of a human diabetic. The lumen of the microaneurysm contains a platelet (P) and is surrounded by a thin layer of endothelial cytoplasm containing a portion of an endothelial nucleus (EN). A vesicle (V) of unknown type is present. The endothelial cells contain the usual organelles including many pinocytic vesicles, and appear completely normal. The basement membrane, which is the structure between the arrows, appears somewhat laminated, is markedly thickened, and shows an accumulation of various types of debris, some of which appears membranous. The processes of the adjacent glial cells (G) appear somewhat compressed but still viable. (×13,000.)

Our findings indicate that the wall of the thick-walled microaneurysm is composed primarily of exudative material from the plasma, cellular debris, and reactive gliosis. We also suspect that on occasion basement membrane-like material may be deposited between the adjacent nerve fibers and glial processes, surrounding them, and eventually interfering with their nutrition to the point that they degenerate and are incorporated into the wall of the microaneurysm to explain some of the membranous debris (Fig. 4). This hypothesis is based on observation of many sections of multiple vessels and aneurysms, rather than observation of the evolution of this process in a single location. It is, therefore, subject to considerable speculation.
Fig. 6. Electron micrograph of portion of wall of thick-walled microaneurysm in the retina of a human diabetic. The lumen of the microaneurysm contains several red blood cells (RBC) and is surrounded by intact endothelial cells which contain endothelial nuclei (EN). The usual organelles are present in the endothelial cells which appear completely normal. The plasma membrane of the endothelial cell, at the arrow, is quite evident, but there is no distinct basement membrane beneath it. This area is occupied by granular and fibrillar (F) debris. This debris resembles fibrin fibrils and other proteins compatible with plasma proteins. Occasional small black droplets of lipid are also present. The wall of the microaneurysm is surrounded by a layer of cells (G) thought to represent reactive glial cells. These cells contain various types of debris, including lipid vacuoles or lipid droplets. (×6,500.)

The deep waxy exudates which occur primarily in the plexiform layers and are strongly P.A.S.-positive can now be attributed with reasonable certainty to plasma leaks as demonstrated by fluorescein injection. Electron microscopic observations of exudates reveal irregular deposits of electron-dense material, some of which is finely granular while other portions are fibrillar and resemble fibrin. The finely granular material often resembles ferritin. Quite frequently one observes elongated mononuclear cells at the periphery of this material, apparently glial cells attempting phagocytosis. Hemorrhages observed by electron microscopy were small. We made no attempt to study large hemorrhages. Sometimes
there was evidence of diapedesis with 2 to 10 red blood cells in and around the wall of a microaneurysm. Several small hemorrhages of 50 to 100 cells were observed. These small hemorrhages were circumscribed by the retinal tissues which were therefore slightly compressed. No point of leakage was identified. Pockets of debris thought to represent ferritin mixed with other proteins and lipids were observed in the neighborhood of microaneurysms, and occasionally groups of phagocytes containing hemosiderin were present.

Electron microscopy has demonstrated that microaneurysms develop in the presence of apparently normal endothelial cells which completely line the feeder capillaries and microaneurysms. Most micro-
an aneurysm is completely free of viable pericytes and contain debris suggestive of degenerated pericytes or "ghost cells." There is also evidence of compression and probable degeneration of adjacent nerve and glial processes. Basement membrane material is quite prominent and increased both in thickness and number of layers in the diabetic patient. However, present information does not let us determine finally whether the basement membrane changes are primary or secondary. In some areas of thick-walled microaneurysms the endothelial basement membrane is absent.

Fig. 8. Electron micrograph of periphery of thick-walled microaneurysm in the retina of a human diabetic. The arrow points toward the lumen. There is a large cell containing debris thought to represent a glial cell with nucleus (GN). This cell appears healthy and apparently active in phagocytosis. Adjacent to this cell are several compressed nerve or glial fibers containing fine fibrils. On the right of the photograph there is a collection of debris including many lipid droplets (L). (×12,000.)
Electron microscopy has demonstrated quite well the increased permeability of the wall of the microaneurysm to all elements of the blood. Again we are unable to say whether this permeability change is primary or secondary. One must speculate that metabolic derangement due to the diabetic process in some way damaged the capillary cells or adjacent neuroectodermal cells of the retina, thereby causing a focal expansion of the capillary wall. Local changes responsible for the focal nature of this lesion are as yet undetermined.

Bovine pituitary growth hormone was furnished by the Endocrinology Study Section, National Institutes of Health. Much of the insulin used on the dogs was supplied by Dr. William R. Kirtley of Lilly Research Laboratories.

We are indebted to Mr. Kenneth Powers for technical assistance with the electron microscope, to Mr. James Young for the photography, and to Mrs. Marilyn Graville for editorial assistance.

REFERENCES