Vascular basement membrane changes in diabetic retinopathy

MONTGOMERY LECTURE, 1973

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Contributed by request and dedicated to Sir Stewart Duke-Elder

In recent years there have been many reports of changes in the capillary basement membranes in diabetes, particularly thickening of the membrane, and much speculation as to its role either in the aetiology of the diabetic state itself or in the pathogenesis of the all-important vascular complications. Basement membrane pathology should, therefore, be in the forefront of diabetic research to-day, and it is high time that we looked critically at the changes to be found in the vascular basement membrane in diabetic retinopathy, to attempt to correlate them with findings in extraocular vessels and to make some assessment of their possible significance. In the case of the eye, we are extremely limited by the lack of fresh material for ultrastructural studies; nevertheless, there is sufficient evidence in the literature and from our own work to justify consideration of a number of mechanisms which might be involved in the pathogenesis of diabetic angiopathy in general and diabetic retinopathy in particular.

(1) Basement membranes in general

It is interesting to recall that the term "basement membrane" was first introduced by the great ophthalmologist Sir William Bowman when describing, with Todd, subepithelial laminae in synovial and serous membranes (Todd and Bowman, 1857). In general, these membranes, although differing in some of their morphological, chemical and antigenic properties, have common features throughout the animal kingdom. They are all acellular hyaline sheets of gel-like plasticity, which are eosinophilic, argyrophilic, and intensely PAS-positive, and lie extracellularly in intimate relation to epithelial cells, smooth muscle cells, endothelial cells, pericytes, and nerve sheaths, etc., characteristically occurring at the interface between cells and connective tissue, but often, as in the case of vascular walls, between the cellular components.

It was originally believed that basement membranes were entirely formed by a condensation in the ground substance adjacent to the cell (Gersh and Catchpole, 1949), but electron microscopy has revealed that, in general, the basement membrane is not a single entity but has two main components. The first consists of a single sheet of interconnecting poorly resolved filaments (about 4 nm.) embedded in an amorphous homogeneous matrix, running parallel to the basal membrane of the cell and now called the *basal lamina*. It has been said to consist of two layers (Hall, 1955), an electron lucent layer near the cell (*lamina rara*) and an outer electron dense layer (*lamina densa*), but this has

since been held to be an artefact of fixation, only one *basal lamina* being present (Vernier, 1964).

The second component lies external to the basal lamina and consists of bundles of rather larger fibrils (10 nm.) lying in a protein-polysaccharide ground substance, which corresponds to reticulin and is thought to be largely responsible for the argyrophilia and positive PAS-reaction (Midgley and Pierce, 1963) that are probably attributable to the carbohydrate component (Tomlin, 1953; Irving and Tomlin, 1954). As was beautifully demonstrated in the voluminous work of Plenck (1927), this reticulin layer forms a fine meshwork of fibres around the vessels with coarser fibres extending into the surrounding tissue. The whole membrane, therefore, is to be regarded as a specialized form of connective tissue, arising from two sources; the amorphous material of the basal lamina is secreted by the associated cell (Pierce, Midgley, and Sri Ram, 1963), while the reticular-protein-carbohydrate layer is a product of the adjacent connective tissue with which it blends. Basement membranes are also formed in situations where no connective tissue is apparently present, as for instance between the epithelial cells of Reichert's membrane (Pierce, Beals, Sri Ram, and Midgley, 1964) or between endothelial cells and muscle cells of vascular walls, or between endothelial and glial cells in the nervous system, and in these situations only the homogeneous basal lamina has been identified (Midgley and Pierce, 1963).

Functionally, basement membranes provide support for their associated cells and act as a selective filtration barrier (Palade and Bruns, 1964; Kefalides, 1970; Spiro, 1970). Chemically, they consist of carbohydrate and peptide, containing substantial quantities of hydroxyproline, hydroxylysine, and glycine, indicating their collagenous nature, and may briefly be defined as tropocollagen in carbohydrate-rich mucoprotein. It may be concluded that basement membrane, reticulin, and collagen are all specialized forms of collagenous protein, in an ascending order of organization. The formation of basement membrane is highly complex, but the precursors, that is the peptide chain with the carbohydrate attached, are formed within the cell and then pass outside the cell where cross-links form the insoluble basement membrane (Spiro, 1969).

It seems probable that basement membranes are being continually removed by depolymerization and replaced by cellular secretion, and this was neatly demonstrated by labelling the glomerular basement membranes of rats by adding silver nitrate to the drinking water (Kurtz and Feldman, 1962). When the silver nitrate was discontinued, new increments of basement membrane could be identified and these were always on the epithelial side, indicating, in this instance, their origin from the epithelial cell.

(2) Basement membrane of retinal vessels

The structure of retinal vessels has been well described by many workers (Maeda, 1958; Hogan and Feeney, 1963; Ishikawa, 1963). The continuous lining of endothelial cells lies upon a homogeneous basal lamina, enclosing muscle cells or pericytes, which is particularly prominent where it separates the vessel from the nervous tissue. This is due to the fact, as can be seen at higher magnifications, that it here consists of two components, the basal lamina of the outermost vascular cell and the basal lamina of the investing glia. These may be separated by a dielectronic band, as noted by Hogan and Feeney (1963) in the human foetal retina, and this represents a potential space separating ectodermal and mesodermal basement membrane (Fig. 1). Indeed, as one traces the vessel backwards towards the disc, these two layers gradually separate and well-defined fibrils appear between them, so providing the typical basement membrane structure; that is, an inner

FIG. 1 Portion of wall of retinal capillary (monkey), showing endothelium (EN), intramural pericyte (P), basement membrane (BM), and perivascular Müller cells (M). Note that the outer basement membrane consists of two layers, the basal lamina of the outer vascular cells—mesodermal basement lamina (MBL), and that of the Müller cells—ectodermal basement lamina (EBL). They are separated by a dielectronic band (arrow) representing a potential space. Electron micrograph. $\times 42,000$

homogeneous basal lamina and an outer fibrillar coat corresponding to reticulin. As in the case of extraocular vessels, a perivascular reticulin network can be demonstrated in sections and retinal digests stained by silver impregnation methods.

(3) Basement membrane changes in non-diabetic diseases

Thickening of the perivascular basement membrane has been shown to develop with advancing age (Gersh and Catchpole, 1949; Maynard, Schultz, and Pease, 1957; Ashworth, Erdmann, and Arnold, 1960; Kurtz, 1961; Fuchs and Scharnweber, 1968; Williamson, Vogler, and Kilo, 1971) and to be related to hydrostatic pressure, as has been strikingly demonstrated in man and giraffe (Williamson and others, 1971) and in the rat retina (Sosula, Beaumont, Jonson, and Hollows, 1972), and in various pathological conditions, such as oxygen-poisoning (Cedergren, Gyllenstein, and Wersall, 1959), hypertension (Ashworth and Grollman, 1959; McGee and Ashworth, 1963), myxoedema (DiScala, Salomon, Grishman, and Churg, 1967), polymyositis (González-Angulo, Fraga, and Mintz, 1968), and lupus erythematosus (Miescher and Paronetto, 1969).

(4) Basement membrane changes in diabetes mellitus

Basement membrane thickening, although inconstant in and non-specific for diabetes (Friederici, Tucker, and Schwartz, 1966; Vracko and Strandness, 1967; Yodaiken, Seftel, Kew, Lillenstein, and Ipp, 1969), seems undoubtedly to be most common and most pronounced in this condition. Such thickening, either homogeneous or stratified, has now been described in diabetes in the kidney (Bergstrand and Bucht, 1957, 1959; Hartmann, Goetz, and Lazarow, 1959; Bloom, Hartmann, and Vernier, 1959; Farquhar, Hopper, and Moon, 1959; Sakaguchi, Suzuki, and Ri, 1959; Kimmelstiel, Kim and Beres, 1962; Camerini-Davalos, Caulfield, Rees, Lozano-Castañeda, Naldjian, and Marble, 1963; Dachs, Churg, Mautner, and Grishman, 1964; Lannigan, Blainey, and Brewer, 1964; Siperstein, 1964; Fisher, Perez-Stable, Amidi, Sarver, and Danowski, 1967), in the skin of the forearm, fingers, toes, and ear lobe (Aagenaes and Moe, 1961; Handelsman, Morriane, and Ghitman, 1962; Banson and Lacy, 1964; Camerini-Davalos and others, 1963; Siperstein, 1964), in muscle (Goldenberg, Alex, Joshi, and Blumenthal, 1959; Zacks, Pegues, and Elliott, 1962; Fuchs, 1964; Bencosme, West, Kerr, and Wilson, 1966; Fuchs and Scharnweber, 1968; Siperstein, Unger, and Madison, 1968; Vracko, 1970; Vracko and Benditt, 1970; Pardo, Perez-Stable, Alzamora, and Cleveland, 1972), in nerve (Fagerberg, 1956), and in rectal capillaries (Farid, Wilkinson, Constable, and Anderson, 1973).

Similar changes have also been observed in pre-diabetics (Camerini-Davalos and others, 1963; Siperstein and others, 1968), and as early as 5 weeks after the onset of acute diabetes (Sabour, MacDonald, and Robson, 1962), and it has, therefore, been suggested that basement membrane thickening is the basic genetic defect rather than a complication of diabetes. The contrary, however, would seem to be indicated by the fact that microangiopathy has been noted in every type of diabetes, including that due to pancreatitis (Duncan, MacFarlane, and Robson, 1958), haemochromatosis (Becker and Miller, 1960), and in dogs rendered diabetic with anterior pituitary extract (Lukens and Dohan, 1946)—including basement membrane thickening (Bloodworth, Engerman, and Powers, 1969). Basement membrane thickening, however, according to Siperstein, Norton, Unger, and Madison (1969) and Siperstein (1970), does not occur in hyperglycaemia in the absence of genetic diabetes mellitus even after many years. Nevertheless, the present consensus view seems to be in favour of regarding the angiopathy as a consequence of the metabolic disturbance.

(5) What is basement membrane thickening?

In considering the problem of basement membrane thickening, it is important to recognize that there are pitfalls of artefact, as may result from the tonicity of the fixing fluid, variations in processing, the physical conditions at the time of biopsy (Shakib, Cunha-Vaz, and Keith, 1967), the angle of section, and so on, but, assuming that the increase in thickness is a genuine observation, then it might arise in the following ways:

- (i) Overproduction of basement membrane material from increased cellular synthetic activity,
- (ii) Increased availability of basement membrane precursors from the blood also resulting in accelerated glycoprotein synthesis,
- (iii) Diminished absorption of basement membrane which has a slow turnover rate,
- (iv) Augmentation of the membrane by entrapped plasma proteins from leakage or abnormal transport.

It may be that we are not dealing with a single entity, for sometimes the thickening is ultrastructurally indistinguishable from normal basement membrane and at other times it may be thickened by electron dense material consisting of large or small granules or by microfibrillar material or cellular debris. Thus the electron microscopical appearances are not uniform and perhaps one should speak of thickening *in the region of* the basement membrane rather than *of* the basement membrane.

With these considerations in mind, I should now like to examine the present theories regarding the pathogenesis of extraocular manifestations of diabetic microangiopathy; first hyaline arteriolosclerosis, and secondly diabetic glomerulosclerosis, for it would seem more than likely that all these complications are at some point pathogenetically related.

(6) Hyaline arteriolosclerosis

It is now known that hyaline arteriolosclerosis or hyaline degeneration, which is a prominent feature in diabetes, begins with a subendothelial deposition of lipohyaline material, and it is recognized that this change and that of fibrinoid necrosis, which is an acute form of hyaline degeneration, result from leakage of blood components into the vascular wall: these have been demonstrated tinctorially, histochemically, autoradiographically, immunologically, and electron microscopically (*cf.* Dustin, 1962). The predominant component of hyalin, according to Lendrum (1969), is fibrin; he, therefore, rejected the terms "hyaline degeneration" and "fibrinoid necrosis" and included them both as examples of "fibrinous vasculosis". According to Lendrum (1961), the fibrin gradually becomes converted into a substance tinctorially resembling collagen, which he called "pseudo-collagen". Similar views were also expressed by McKinney (1962), who stated that hyalin consisted initially of fibrin and plasma protein. It seems to me that a fundamentally important suggestion here, although not established, is that fibrin and presumably many other proteins deposited from the circulation into the vessel wall, can there convert or be converted into collagen-like material.

This deposition or leakage from the vessels is made possible either by increased endothelial permeability, due to a raised intraluminal blood pressure, or by endothelial injury, or by a combination of the two. Hence fibrinous vasculosis may occur if the blood pressure rises or if endothelial permeability increases from any other cause, as in hyperergic, hypoxic,

or metabolic conditions. On this basis, even if the endothelial injury were uniformly distributed, one would expect the leakage or "insudation" to be most marked where the hydrostatic pressure was at its greatest, namely on the arterial side of the circulation; and this supposition may have relevance to the problem of diabetic retinopathy.

(7) Diabetic glomerulosclerosis

Most investigators now seem to agree that the diffuse and nodular forms of glomerulosclerosis in diabetes each represent a different degree of excessive production of basement membrane, the first being confined to the normal basement membrane situation and the second extending into the mesangium. The afferent arterioles to such affected glomeruli usually show pronounced hyaline arteriosclerosis; there is, in fact, a parallelism between the two (Thomsen, 1965), and it has been suggested that both abnormalities result from a common defect (Farquhar and others, 1959). Indeed, as reported by Anderson (1954), hyalinization of the afferent arteriole is often continuous with the hyaline nodules; Anderson thought that the nodular lesion was the result of "a deposition of fibrin or some other protein substance" and was identical with the hyalin of glomerular arterioles. Lendrum (1969) and Lendrum, Slidders, and Fraser (1972) also believed that the nodules were derived from fibrin which, as in the case of hyaline arteriosclerosis, undergoes an ageing change to "pseudo-collagen". Similarly, it has been shown immunohistochemically that the nodules contain fibrinogen and lipoprotein, once again suggesting that they are derived, at least in part, from circulatory plasma proteins by a process of filtration and arrest within the glomerular capsule wall (Davies, Woolf, and Carstairs, 1966; Thomsen, 1972). In addition to fibrin, globulins and albumin have been demonstrated in the glomerular basement membrane in diabetes (Larsson, 1968; Westberg and Michael, 1972). Further evidence comes from the most recent study in which fibrinogen and fibrin were clearly demonstrated in the affected glomeruli by light and electron microscopy and immunofluorescence, and it was suggested that fibrinogen and/or other macromolecules might initiate the basement membrane irregularities and nodular formation (Farquhar, MacDonald, and Ireland, 1972).

Another school of thought, in apparent contradiction, suggested that both arteriolar hyalinosis (Wiener, Spiro, and Lattes, 1965) and diabetic glomerulosclerosis (Farquhar, 1964) result from a local and excessive production of basement membrane, a concept which is in line with all the other findings of basement thickening throughout the capillaries in diabetes. Because there is a similarity between the chemical constitution of basement membrane fractions isolated from diabetic and non-diabetic kidneys, it has been assumed that thickening of this membrane must necessarily relate either to an increased formation or to a decreased rate of removal of basement membrane material (Lazarow and Speidel, 1964; Pierce and Nakane, 1969). One might have thought that electron microscopy would have resolved this conflict, but it is, in fact, often impossible to distinguish ultrastructurally fibrin and other plasma proteins from either basement membrane hyalin or basement membrane proper (Bencosme and Bergman, 1962), and it is the exception rather than the rule to find the specific periodicity of the banding of fibrin (Prose, Lee, and Balk, 1965; Haust, Wyllie, and More, 1965).

This difficulty is reflected in the literature. For instance, in electron microscopical studies of hyaline arteriosclerosis, the hyaline material has been reported both as basement membrane thickening (McGee and Ashworth, 1963) and as deposited plasma protein (Biava, Dyrda, Genest, and Bencosme, 1964). In a very recent paper on glomerular base-

ment membrane thickening, this difficulty in distinguishing by electron microscopy the exact nature of protein deposits is again emphasized, and the authors refer to the deposition of granular or fibrillar material apparently continuous with the basement membrane, which they have identified by immunofluorescence as fibrin (Davison, Thomson, MacDonald, Rae, Uttley, and Clarkson, 1973). In our own experiments in hypertensive retinopathy, it was often quite impossible to differentiate plasma insudation from basement membrane.

To sum up, Lendrum has for long insisted that the hyaline material of both hyaline arteriosclerosis and nodular glomerulosclerosis consists initially of fibrin which gradually undergoes a change to a substance staining for collagen; this he calls "pseudo-collagen". No collagen fibrils, however, were found in the electron microscopical study of diabetic glomerular nodules (Lacy, 1964; MacDonald, 1964), but chemical analysis of glomeruli in diabetic nephropathy has since shown that the lesions consist of a complex glycoprotein containing as its major protein a form of collagen, and so closely corresponding to the chemical composition of normal basement membrane (Odin and Törnblom, 1959; Lazarow and Speidel, 1964). Although there are distinct differences, in that the diabetic basement membrane material has an increased amount of hydroxylysine (Spiro, 1969; Beisswenger and Spiro, 1973), there is now sufficient electron microscopical evidence that the changes in diabetic glomerulopathy are indistinguishable from basement membrane material. It would, therefore, appear that the "pseudo-collagen" of Lendrum, although lacking the typical fibrillar banding of mature collagen, is in fact a true collagenous glycoprotein related to basement membrane material. Assuming then that all these observations are correct, it logically follows that basement membrane thickening may develop not only by a local increased synthesis (or a decreased absorption), but also by the transformation of deposited fibrin and other plasma proteins. Indeed, in the past it was believed that fibrin was converted into collagen (Baitsell, 1915) or that fibrinogen was an essential precursor or a source of protein for collagen production (Nageotte and Guyon, 1934). In more recent times, however, it has been found that fibrinogen and fibrin do not belong to the collagen group of proteins and have distinct chemical differences, so that a direct conversion would seem unlikely; they might, however, be broken down and their fractional products used in the synthesis of collagen (Jackson, 1953). At the present time, we can pursue this possible relationship between fibrin and collagen no further, but it is clear that there are no facts to contradict the thesis that all these lesions—hyaline arteriosclerosis, diabetic glomerulosclerosis, and basement membrane thickening—may in whole or in part be haematogenous in origin.

Thus basement membrane thickening and diabetic glomerulosclerosis may be the capillary equivalents of arteriolar hyalinosis, just as arteriolar hyalinosis may be the arteriolar equivalent of atheroma in the larger arteries (Dustin, 1962). All these manifestations may, therefore, be linked by some modification of the permeability of vascular walls or of their protein transportation which may also involve increased basement membrane synthesis.

With these possibilities in mind, it will now be appropriate to return to the problem of the eye.

(8) Basement membrane changes in the eye in diabetes

With regard to our own observations, I had shown many years ago, with PAS-staining and Indian ink injections, the lipohyaline material which commonly forms around and

FIG. 2 Diabetic retina injected with Indian ink and stained with periodic acid-Schiff (PAS) showing hyaline material within the arteriolar and precapillary arteriolar wall and forming a cap on the aneurysm (arrows). $\times 190$

FIG. 3 Digested diabetic retina stained with PAS. Note that the mural thickening is on the arterial side of the circulation and particularly affects the precapillary arterioles (arrows). $\times 90$

within the walls of microaneurysms and is of a similar composition to the exudates found in the outer molecular layer, and had drawn an analogy between these changes and those of nodular diabetic glomerulosclerosis (*cf.* Ashton, 1958) (Fig. 2). In such preparations, in sections and digests, it is not difficult to see that an exactly comparable material is present within the walls of the posterior precapillary arterioles and is even seeping through them (Fig. 3). I pointed out 20 years ago that capillary closure was seen selectively on the arterial side of the circulation and is in fact related to attenuation and occlusion of these vessels (Ashton, 1953).

FIG. 4 Diabetic retinopathy. Capillary containing a red cell (RBC) and showing an intact endothelium (EN) and marked thickening of the basement membrane (BM) which shows many vacuoles and electron dense particles, probably lipid. At one point the membrane appears to extend outwards between the Müller cells (arrows). EM. $\times 15,000$

Electron microscopy has largely been concerned with basement membrane thickening of the vessels, which has been repeatedly demonstrated in diabetic retinopathy in man and animals (Kissen and Bloodworth, 1961; Bloodworth, 1962, 1963, 1964, 1965a,b; 1967; Bloodworth and Molitor, 1965; Toussaint and Dustin, 1963) and by light microscopy in the ciliary basement membrane in diabetes (Yamashita and Becker, 1961).

FIG. 5 *Diabetic retinopathy. High-power view of capillary wall, showing marked thickening in the region of the basement membrane (BM) which is due to lipid inclusions (arrows) lying in a finely granular substance indistinguishable from basement membrane. The structure of the endothelium (EN) appears comparatively normal. RBC—red blood cell. M—Müller cells. EM. $\times 24,000$*

All investigators have demonstrated a great thickening of the capillary basement membrane, which sometimes extended outwards into the retina between the nervous elements, just as it does between the endothelial cells of the glomerulus. The altered membrane may be vacuolated, and may contain osmiophilic and cellular debris (probably from degenerate pericytes) and occasionally reticulin fibres. The changes in the walls of microaneurysms

FIG. 6 *Diabetic retinopathy. Blood vessel, showing disruption of endothelium (EN) and marked laminated thickening in the region of the basement membrane (BM). This is due to fine granular and fibrillar material, indistinguishable from basement membrane, containing cholesterol crystals (arrows), lipid (L), and red cell fragments (RBC)—clear evidence of vascular leakage. EM. $\times 4,000$*

are of a similar nature but more marked in degree, and the greatly thickened laminated basement membrane contains in addition blood elements such as lipid, fibrin, platelets, and red cells. The exudates in the outer molecular layer also contains these components.

Our own observations on the ultrastructural changes of diabetic retinopathy entirely confirm these findings (Figs 4 and 5) and it is interesting to note, as pointed out by Bloodworth (1967), that the basement membrane of the pericyte within the wall does not usually share in the thickening seen in the membrane around the vessel which might argue against a hyperactivity of the cells themselves. I would particularly emphasize the association of

FIG. 7 Diabetic retina injected with Indian ink, showing several occluded precapillary arterioles (arrows). One of them (A) was removed for electron microscopy and is shown in Fig 8. $\times 44$

basement membrane thickening with haematogenous elements—lipid, plasma, fibrin, haemosiderin, red cells, platelets, cholesterol crystals—most of which can have arrived at this situation only by way of abnormal leakage through the retinal endothelial barrier (Fig. 6). The appearances often suggest that the potential spaces between the vessel wall and the investing glia have opened to contain this heterogeneous leaking material which gradually flows between the glial cells to form the exudates which are therefore similarly constituted.

Even the demonstration of insulin-anti-insulin complexes within the basement membrane

FIG. 8 *Transverse section through injected vessel shown in Fig. 7. Ink particles may be seen in the lumen (L) and the narrowing of the vessel is seen to be due to a gross thickening in the region of the basement membrane which consists of granules and fibrils of varying density. The endothelium (EN) is attenuated. EM. $\times 12,000$*

of eye and kidney, thought by some to be of aetiological significance (Freedman, Peters, and Kark, 1960; Berns, Owens, Hirata, and Blumenthal, 1962; Coleman, Becker, Canaan, and Rosenbaum, 1962; Becker, Coleman, and Keates, 1962; Blumenthal, Goldenberg, and Berns, 1965; Larsen and Werner, 1969; Werner and Larsen, 1969; Bloodworth and Engerman, 1971), is in my view more likely to be due to non-specific haematogenous depositions, and further evidence of abnormal endothelial permeability (Deckert, 1967; Kniker, 1967, a,b; Larsson, 1968; Thomsen, 1972; Westberg and Michael, 1972). Certainly, in the case of the retinal vessels, the mere presence of these protein complexes beneath the endothelium indicates a grossly abnormal permeability. Thus, in the case of the retinal vessels, in incriminating the deposition of immune subendothelial complexes (Bloodworth and Engerman, 1971), one at the same time necessarily postulates a preceding breakdown of the blood-retinal barrier. I would, therefore, stress that in the diabetic retina there are many reasons to suspect that the basement membrane thickening and the associated changes we attribute to leakage are not independent manifestations, but may have a common pathogenesis.

We have recently started to study the topographical distribution of basement membrane thickening in diabetic retinopathy which had been described as diffusely distributed throughout the retinal vessels (Bloodworth and Molitor, 1965). Apparently this is not so, for, as may be seen in digests of diabetic retinopathy stained with PAS for basement membrane, the intensity of staining is more evident on the arterial than on the venous side of the circulation (Fig. 3), and this is being borne out in our electron microscopical studies.

In one diabetic retina injected with Indian ink, an attenuated precapillary arteriole was removed for electron microscopy at a point where it connected with a closed capillary circulation (Fig. 7). The severe narrowing of this vessel was found to be due to the deposition of electron dense granular material immediately beneath the endothelium (Fig. 8). In another case, small retinal discs—one containing a precapillary arteriole and the other its corresponding postcapillary venule—were removed *post mortem* from a retina with advanced diabetic retinopathy and examined in cross-section by electron microscopy. While the small artery showed an extensive subendothelial deposition of granular material partly resembling basement membrane, with narrowing of the lumen, the venule showed only collagenous degeneration of the wall, with no intramural depositions (Fig. 9). Another precapillary arteriole from this retina again showed a gross deposit of coarse and fine granular material merging with the basement membrane (Figs 10 and 11). Obviously a great deal more evidence than this is required before one can be certain of this distribution of basement membrane changes in the retina; nor at the present time can this finding be supported from studies on extraocular vessels as, curiously, no one appears to have carried out a comparable investigation.

(9) Basement membrane changes in the retinal vessels in non-diabetic subjects

It has already been pointed out that all these changes seen in the basement membrane region of diabetic vessels are not confined to diabetes, and the same applies in the case of the retinal vessels. Changes similar to those I have shown occur in varying degrees in the ageing eye, especially at the retinal periphery, in hypertension, and particularly around the telangiectatic leaking vessels in Coats's disease, which show grossly thickened laminated basement membranes containing plasma, fibrin, and formed blood elements, and there is here no doubt that the general changes are due to leakage (Tripathi and Ashton, 1971). They may also be seen in cases of corneal vascularization around growing vessels (Fig. 12), which again are known to leak freely.

FIG. 10 *Diabetic retinopathy. Another precapillary arteriole and branch from the same case as shown in Fig. 9. Note subendothelial homogeneous and laminated thickening of granular and fibrillar material which encroaches upon the lumen and apparently occludes the branch vessel. EM. $\times 5,600$*

FIG. 9 *Diabetic retinopathy. Representative cross-sections through the walls of a precapillary arteriole (A) and of its corresponding postcapillary venule (B). Note that the arteriole shows gross granular and fibrillar thickening in the region of the basement membrane, whereas the venule, although degenerate and collagenized, shows no similar subendothelial material. EM. $\times 13,200$*

It is noteworthy, especially in studies of diabetes, that the descriptions of basement membrane thickening almost always relate to *vascular* basement membranes and not to basement membranes more remote from vessels, such as those of the epithelium of skin or cornea, or of lens capsule or Descemet's membrane; this would seem to be against the likelihood that the changes described could be due to a generalized metabolic disturbance of basement membrane-forming cells. Moreover, if one believes that the membrane thicken-

FIG. 11 High-power view of vessel shown in Fig. 10. Note the varying electron density of the subendothelial material and its fibrillar (F) and organelle-like inclusions (arrows). In some areas the material is indistinguishable from basement membrane (BM). EM. $\times 32,000$

ing is due to the abnormal diabetic metabolism, other explanations must be found for the very similar changes in the basement membrane in non-diabetic conditions. Thus, while the various suggested mechanisms of basement membrane thickening are not mutually exclusive, and all may occur separately or together, our observations on the retinal vessels are more readily interpreted in terms of an abnormal endothelial permeability, possibly with heightened synthetic activity.

FIG. 12 Cross-section of a growing vessel in a non-diabetic case of corneal vascularization. Note around the vessel a laminated thickening of basement membrane-like material. This change corresponds very closely with that described as basement membrane thickening in diabetic capillaries. Lumen—L. Endothelium—EN. Pericyte—P. (Courtesy of Dr R. Tripathi). EM, $\times 8,400$

(10) Significance of basement membrane changes in diabetic retinopathy

In conclusion, I should like to consider the role that basement membrane thickening, whatever its cause, may play in the evolution of diabetic retinopathy. It has always been a puzzling question whether the retinopathy is or is not a part of the general angiopathy. One would expect it to be so; and yet two decades of research have shown that its characteristic components—microaneurysms, capillary closure, pericyte degeneration, shunts, exudation, neovascularization, etc.—are non-specific pathological reactions peculiar to the retina and that all may occur in the absence of generalized vascular disease. In diabetic retinopathy, however, they must at some point be initiated by a pathological change attributable to the diabetes. There may be many points, but in this study I have focused on thickening in the region of the basement membrane.

As I have demonstrated in these preliminary considerations, this would seem to occur predominantly on the arterial side of the circulation, narrowing the affected vessels, and particularly encroaching upon the lumina of the precapillary arterioles and their immediately related capillaries. This must be an insidious process, gradually interfering with metabolic exchange and with nutrition of the inner retina and of the vessel walls themselves.

If this is due, as seems possible, to glycoprotein insudation, it presupposes an abnormality of vascular permeability as the underlying cause of diabetic vascular disease as a whole, which in the retina necessarily points to endothelial dysfunction as the first stage—as was emphasized by Cunha-Vaz (1972). It is, however, difficult to know to what extent the individual lesions of diabetic retinopathy are attributable to this initial abnormality or to the secondary ischaemia, with its attendant effects on cellular metabolism, which gradually develops as the precapillary arterioles become involved. We know only that many of them can result from arteriolar insufficiency, such as capillary closure, shunts, cotton-wool spots, and retinitis proliferans, which would account for much of diabetic retinopathy, but to which stage or stages microaneurysms, pericyte degeneration, and exudation belong is not at present clear.

When this involvement of the precapillary arterioles was first described, on evidence obtained by light microscopy, it was found only in advanced cases (Ashton, 1953), which was against the concept originally advanced by Cristini and Tolomelli (1946) that diabetic retinopathy was initiated by “precapillary sclerosis”, and the possibility was considered that the affected vessels might previously have been functionally constricted. Electron microscopy, however, has since shown basement membrane thickening to develop quite early in the diabetic state, and it may be, therefore, that the retinal precapillary arterioles are also structurally involved early in the retinopathy, but we must await the electron microscopical findings in the incipient stages of retinopathy before this can be established.

Many years ago it was customary to contrast diabetic retinopathy with hypertensive retinopathy; the first, it was thought, primarily affected the venous side of the circulation, and the second the arterial side, but on the present analysis their pathogenesis is rather to be compared. Both predominantly affect the posterior fundus, both are associated with narrowing or occlusion of the posterior precapillary arterioles, and both may develop capillary closure, exudation, microaneurysms, and cotton-wool spots. In the case of hypertensive retinopathy, we have shown that the ischaemic foci are due to acute fibrinous vasculosis of the precapillary arterioles (Ashton and Harry, 1963; Garner and Ashton, 1971; Ashton, 1972), and here it is postulated that diabetic retinopathy is in the main an ischaemic condition due to chronic fibrinous vasculosis affecting the same vessels, and

possibly aggravated by involvement of the large arteries supplying the eye (Garner and Ashton, 1972). In both cases the question arises—how is the endothelium damaged so that intramural leakage can occur? In hypertensive retinopathy we believe it is due to actual rupture of the endothelial cells (Garner and Ashton, 1971; Ashton, 1972; Garner, Ashton, Kohner, Bulpitt, and Dollery, 1974), but in diabetic retinopathy this is the challenging problem that still remains.

This hypothesis that diabetic vascular disease stems from an abnormal vascular permeability raises more questions than it answers but, if correct, it at least points in the direction where the answers are most likely to be found.

In the past I have drawn attention to a number of mechanisms which might be involved in the pathogenesis of the individual components of diabetic retinopathy and this particular concept has for me the merit of embracing them all. Whether it is true or not time will tell, but as Claude Bernard wrote "If an hypothesis is not verified and disappears, the facts which it has enabled us to find are nonetheless acquired as indestructible materials for science".

It is a pleasure to acknowledge the technical assistance of Mr. G. E. Knight throughout these studies and of Mr. D. Wood with the electron microscopy. I am indebted to Miss E. FitzGerald for secretarial help. This work was generously supported by the Wellcome Trust.

References

- AAGENAES, Ö., AND MOE, H. (1961) *Diabetes*, **10**, 253
 ANDERSON, G. S. (1954) *J. Path. Bact.*, **67**, 241
 ASHTON, N. (1953) *Brit. J. Ophthalm.*, **37**, 282
 ——— (1958) *Advanc. Ophthalm.*, **8**, 1
 ——— (1972) *Trans. Amer. Acad. Ophthalm. Otolaryng.*, **76**, 17
 ——— and HARRY, J. (1963) *Trans. ophthalm. Soc. U.K.*, **83**, 91
 ASHWORTH, C. T., ERDMANN, R. R., and ARNOLD, N. J. (1960) *Amer. J. Path.*, **36**, 165
 ——— and GROLLMAN, A. (1959) *A.M.A. Arch. Path.*, **68**, 148
 BAITSELL, G. A. (1915) *J. exp. Med.*, **21**, 455
 BANSON, B. B., and LACY, P. E. (1964) *Amer. J. Path.*, **45**, 41
 BECKER, B., COLEMAN, S. L., and KEATES, E. U. (1962) *Trans. Amer. ophthalm. Soc.*, **60**, 260
 BECKER, D., and MILLER, M. (1960) *New Engl. J. Med.*, **263**, 367
 BEISSWENGER, P. J., and SPIRO, R. G. (1973) *Diabetes*, **22**, 180
 BENCOSME, S. A., and BERGMAN, B. J. (1962) *Int. Rev. exp. Path.*, **1**, 139
 ———, WEST, R. O., KERR, J. W., and WILSON, D. L. (1966) *Amer. J. Med.*, **40**, 67
 BERGSTRAND, A., and BUCHT, H. (1957) *Lab. Invest.*, **6**, 293
 ——— (1959) *J. Path. Bact.*, **77**, 231
 BERNIS, A. W., OWENS, C. T., HIRATA, Y., and BLUMENTHAL, H. T. (1962) *Diabetes*, **11**, 308
 BIAVA, G. B., DYRDA, I., GENEST, J., and BENCOSME, S. A. (1964) *Amer. J. Path.*, **44**, 349
 BLOODWORTH, J. M. B., JR. (1962) *Diabetes*, **11**, 1
 ——— (1963) *Ibid.*, **12**, 99
 ——— (1964) In "Small Blood Vessel Involvement in Diabetes Mellitus", ed. M. D. Siperstein, A. R. Colwell, and K. Meyer, p. 81. American Institute of Biological Sciences, Washington
 ——— (1965a) *Diabetes*, **14**, 441
 ——— (1965b) *Arch. Path.*, **79**, 113
 ——— (1967) In "Vascular Complications of Diabetes Mellitus", ed. S. J. Kimura and W. M. Caygill, p. 73. Mosby, St. Louis
 ——— and ENGERMAN, R. L. (1971) *Acta diabetol. lat.*, **8**, Suppl. 1, p. 263
 ———, ———, and POWERS, K. L. (1969) *Diabetes*, **18**, 455
 ——— and MOLITOR, D. L. (1965) *Invest. Ophthalm.*, **4**, 1037

- BLOOM, P. M., HARTMANN, J. F., and VERNIER, R. L. (1959) *Anat. Rec.*, **133**, 251
- BLUMENTHAL, H. T., GOLDENBERG, S., and BERNIS, A. W. (1965) In "On the Nature and Treatment of Diabetes", ed. B. S. Leibel and G. A. Wrenshall (Excerpta Medica International Congress Series No. 84), Ch. 27, p. 397
- CAMERINI-DAVALOS, R. A., CAULFIELD, J. B., REES, S. B., LOZANO-CASTAÑEDA, O., NALDJIAN, S., and MARBLE, A. (1963) *Diabetes*, **12**, 508
- CEDERGREN, B., GYLLENSTEN, L., and WERSALL, J. (1959) *Acta paediat.*, **48**, 477
- COLEMAN, S. L., BECKER, B., CANAAN, S., and ROSENBAUM, L. (1962) *Diabetes*, **11**, 375
- CRISTINI, G., and TOLOMELLI, E. (1946) *Arch. Pat. Clin. med.*, **25**, 271
- CUNHA-VAZ, J. G. (1972) *Trans. ophthalm. Soc. U.K.*, **92**, 111
- DACHS, S., CHURG, J., MAUTNER, W., and GRISHMAN, E. (1964) *Amer. J. Path.*, **44**, 155
- DAVIES, M. J., WOLF, N., and CARSTAIRS, K. C. (1966) *J. Path. Bact.*, **92**, 441
- DAVISON, A. M., THOMSON, D., MACDONALD, M. K., RAE, J. K., UTTLEY, W. S., and CLARKSON, A. R. (1973) *J. clin. Path.*, **26**, 102
- DECKERT, T. (1967) *Acta med. scand.*, **182**, Suppl. 476, p. 29
- DISCALA, V. A., SALOMON, M., GRISHMAN, E., and CHURG, J. (1967) *Arch. Path.*, **84**, 474
- DUNCAN, L. J. P., MACFARLANE, A., and ROBSON, J. S. (1958) *Lancet*, **1**, 822
- DUSTIN, P., JR. (1962) *Int. Rev. exp. Path.*, **1**, 73
- FAGERBERG, S-E. (1956) *Acta med. scand.*, **154**, 145
- FARID, N. R., WILKINSON, E., CONSTABLE, F. L., and ANDERSON, J. (1973) *Lancet*, **1**, 837
- FARQUHAR, A., MACDONALD, M. K., and IRELAND, J. T. (1972) *J. clin. Path.*, **25**, 657
- FARQUHAR, M. G. (1964) In "Small Blood Vessel Involvement in Diabetes Mellitus", ed. M. D. Siperstein, A. R. Colwell, and K. Mayer, p. 74. American Institute of Biological Sciences, Washington
- , HOPPER, J., and MOON, H. D. (1959) *Amer. J. Path.*, **35**, 721
- FISHER, E. R., PEREZ-STABLE, E., AMIDI, M., SARVER, M. E. and DANOWSKI, T. S. (1967) *J. Amer. med. Ass.*, **202**, 291
- FREEDMAN, P., PETERS, J. H., and KARK, R. M. (1960) *Arch. intern. Med.*, **105**, 524
- FRIEDERICI, H. H. R., TUCKER, W. R., and SCHWARTZ, T. B. (1966) *Diabetes*, **15**, 233
- FUCHS, U. (1964) *Frankfurt Z. Path.*, **73**, 318
- and SCHARNWEBER, W. (1968) *Virchows Arch. path. Anat.*, **343**, 276
- GARNER, A., and ASHTON, N. (1971) "Ophthalmology: Proc. XXI int. Congr. Ophthalm. Mexico, D. F., 1970", vol. 1, p. 583. Excerpta Medica, Amsterdam
- (1972) *Trans. ophthalm. Soc. U.K.*, **92**, 101
- , KOHNER, E., BULPITT, J., and DOLLERY, C. T. (1974) *Brit. J. Ophthalm.*, **58** (in press)
- GERSH, I., and CATCHPOLE, H. R. (1949) *Amer. J. Anat.*, **85**, 457
- GOLDENBERG, S., ALEX, M., JOSHI, R. A., and BLUMENTHAL, H. T. (1959) *Diabetes*, **8**, 261
- GONZÁLEZ-ANGULO, A., FRAGA, A., and MINTZ, G. (1968) *Amer. J. Med.*, **45**, 873
- HALL, V. (1955) In "Proceedings of the Sixth Annual Conference on the Nephrotic Syndrome, 1954", ed. J. Metcalf, p. 1. National Nephrosis Foundation, New York
- HANDELSMAN, M. B., MORRIONE, T. G., and GHITMAN, B. (1962) *Arch. intern. Med.*, **110**, 70
- HARTMANN, J. F., GOETZ, F. C., and LAZAROW, A. (1959) "Electron microscopy of the human kidney in diabetes", in "Diabetes Mellitus: III Kongr. int. Diabetes Federation, Düsseldorf, 1958", p. 189. Thieme, Stuttgart.
- HAUST, M. D., WYLLIE, J. C., and MORE, R. H. (1965) *Exp. molec. Path.*, **4**, 205
- HOGAN, M. J., and FEENEY, L. (1963) *J. Ultrastruct. Res.*, **9**, pp. 10, 29
- IRVING, E. A., and TOMLIN, S. G. (1954) *Proc. roy. Soc. B.*, **142**, 113
- ISHIKAWA, T. (1963) *Invest. Ophthalm.*, **2**, 1
- JACKSON, S. F. (1953) In "Nature and Structure of Collagen", ed. J. T. Randall and S. F. Jackson. London
- KEFALIDES, N. A. (1970) In "Chemistry and Molecular Biology of the Intercellular Matrix", ed. E. A. Balazs, vol. 1, p. 535. Academic Press, London and New York

- KISSEN, A. T., and BLOODWORTH, J. M. B., JR. (1961) *Exp. Eye Res.*, **1**, 1
- KIMMELSTIEL, P., KIM, O. J., and BERES, J. (1962) *Amer. J. clin. Path.*, **38**, 280
- KNIKER, W. T. (1967) In "Vascular Complications of Diabetes Mellitus", ed. S. J. Kimura and S. F. Caygill. (a) 184; (b) 201. Mosby, St. Louis
- KURTZ, S. M. (1961) *Lab. Invest.*, **10**, 1189
- and FELDMAN, J. D. (1962) *J. Ultrastruct. Res.*, **6**, 19
- LACY, P. E. (1964) In "Aetiology of Diabetes Mellitus and Its Complications" (Ciba Foundation Colloquia on Endocrinology, vol. 15), ed. M. P. Cameron and M. O'Connor, p. 297. Churchill, London
- LANNIGAN, R., BLAINEY, J. D., and BREWER, D. B. (1964) *J. Path. Bact.*, **88**, 255
- LARSEN, H-W., and WERNER, A. U. (1969) *Acta ophthalm. (Kbh.)*, **47**, 956
- LARSSON, O. (1968) *Acta med. scand.*, **183**, Suppl. 480
- LAZAROW, A., and SPEIDEL, E. (1964) In "Small Blood Vessel Involvement in Diabetes Mellitus", ed. M. D. Siperstein, A. R. Colwell, and K. Meyer, p. 127. American Institute of Biological Sciences, Washington
- LENDRUM, A. C. (1961) *Ned. T. Geneesk.*, **105**, 1359
- (1969) In "Trends in Clinical Pathology", p. 160. Brit. Med. Ass., London
- , SLIDDERS, W., and FRASER, D. S. (1972) *J. clin. Path.*, **25**, 373
- LUKENS, F. D. W., and DOHAN, F. C. (1946) *Arch. Path.*, **41**, 19
- MACDONALD, M. K. (1964) In "Aetiology of Diabetes Mellitus and Its Complications" (Ciba Foundation Colloquia on Endocrinology vol. 15), ed. M. P. Cameron and M. O'Connor, p. 297. Churchill, London
- MAEDA, J. (1958) *Acta Soc. ophthalm. jap.*, **62**, 90
- MAYNARD, E. A., SCHULTZ, R. L., and PEASE, D. C. (1957) *Amer. J. Anat.*, **100**, 409
- MC GEE, W. G., and ASHWORTH, C. T. (1963) *Amer. J. Path.*, **43**, 273
- MCKINNEY, B. (1962) *J. Path. Bact.*, **83**, 449
- MIDGLEY, A. R., and PIERCE, G. B. (1963) *Amer. J. Path.*, **43**, 929
- MIESCHER, P. A., and PARONETTO, F. (1969) In "Textbook of Immunopathology", ed. P. A. Miescher and H. J. Müller-Eberhard, vol. II, p. 675. Grune and Stratton, N.Y. and London
- NAGEOTTE, J., and GUYON, L. (1934) *C.R. Ass. Anat.*, **29**, 408 (quoted by Jackson, 1953)
- ODIN, L., and TÖRNBLOM, N. (1959) *Acta Soc. Med. upsalien.*, **64**, 313
- PALADE, G. E., and BRUNS, R. R. (1964) In "Small Blood Vessel Involvement in Diabetes Mellitus," ed. M. D. Siperstein, A. R. Colwell, and K. Meyer, p. 39. American Institute of Biological Sciences, Washington
- PARDO, V., PEREZ-STABLE, E., ALZAMORA, D. B., and CLEVELAND, W. W. (1972) *Amer. J. Path.* **68**, 67
- PIERCE, G. B., BEALS, T. F., SRI RAM, J., and MIDGLEY, A. R. (1964) *Ibid.*, **45**, 929
- , MIDGLEY, A. R., JR., and SRI RAM, J. (1963) *J. exp. Med.*, **117**, 339
- and NAKANE, P. K. (1969) *Lab. Invest.*, **21**, 27
- PLENK, H. (1927) *Ergebn. Anat. Entwickl.-Gesch.*, **27**, 302
- PROSE, P. H., LEE, L., and BALK, S. D. (1965) *Amer. J. Path.*, **47**, 403
- SABOUR, M. S., MACDONALD, M. K., and ROBSON, J. S. (1962) *Diabetes*, **11**, 291
- SAKAGUCHI, H., SUZUKI, Y., and RI, T. (1959) *Medicine (Tokyo)*, **16**, 541
- SHAKIB, M., CUNHA-VAZ, J. G., and KEITH, C. G. (1967) *Invest. Ophthalm.*, **6**, 198
- SIPERSTEIN, M. D. (1964) In "Small Blood Vessel Involvement in Diabetes Mellitus", ed. M. D. Siperstein, A. R. Colwell, and K. Meyer, p. 125. American Institute of Biological Sciences, Washington
- (1970) In "Pathogenesis of Diabetes Mellitus" (Nobel Symposium 13), ed. E. Cerasi and R. Luft, p. 81. Almqvist and Wiksell, Stockholm
- , NORTON, W., UNGER, R. H., and MADISON, L. L. (1969) In "Diabetes: Proc. VI Congr. int. Diabetes Federation, Stockholm, 1967", ed. J. Östman and R. D. G. Milner, p. 572. Excerpta Medica Foundation, Amsterdam

- , UNGER, R. H., and MADISON, L. L. (1968) *J. clin. Invest.*, **47**, 1973
- SOSULA, L., BEAUMONT, P., JONSON, K. M., and HOLLOWS, F. C. (1972) *Invest. Ophthalm.*, **11**, 916
- SPIRO, R. G. (1969) *New Engl. J. Med.*, **281**, 1043
- (1970) In "Chemistry and Molecular Biology of the Intercellular Matrix", ed. E. A. Balazs, vol. 1, p. 511. Academic Press, London and New York
- THOMSEN, A. C. (1965) "The Kidney in Diabetes Mellitus", p. 95. Munksgaard, Copenhagen
- THOMSEN, O. F. (1972) *Acta path. microbiol. scand.*, Sect. A., **80**, 193
- TODD, R. B., and BOWMAN, W. (1857) "The Physiological Anatomy and Physiology of Man", pp. 129, 523, 660. Blanchard and Lea, Philadelphia
- TOMLIN, S. G. (1953) *Nature (Lond.)*, **171**, 302
- TOUSSAINT, D., and DUSTIN, P. (1963) *Arch. Ophthalm. (Chicago)*, **70**, 96
- TRIPATHI, R., and ASHTON, N. (1971) *Brit. J. Ophthalm.*, **55**, 289
- VERNIER, R. L. (1964) In "Small Blood Vessel Involvement in Diabetes Mellitus", ed. M. D. Siperstein, A. R. Colwell, and K. Meyer, p. 57. American Institute of Biological Sciences, Washington
- VRACKO, R. (1970) *Circulation*, **41**, 271
- and BENDITT, E. P. (1970) *J. Cell Biol.*, **47**, 281
- and STRANDNESS, D. E. (1967) *Circulation*, **35**, 690
- WERNER, A. U., and LARSEN, H.-W. (1969) *Acta ophthalm. (Kbh.)*, **47**, 937
- WESTBERG, N. G., and MICHAEL, A. F. (1972) *Diabetes*, **21**, 163
- WIENER, J., SPIRO, D., and LATTES, R. G. (1965) *Amer. J. Path.*, **47**, 457
- WILLIAMSON, J. R., VOGLER, N. J., and KILO, C. (1971) *Ibid.*, **63**, 359
- YAMASHITA, T., and BECKER, B. (1961) *Diabetes*, **10**, 167
- YODAIKEN, R. R., SEFTEL, H. C., KEW, M. C., LILLENSTEIN, M., and IPP, E. (1969) *Ibid.*, **18**, 164
- ZACKS, S. I., PEGUES, J. J., and ELLIOTT, F. A. (1962) *Metabolism*, **11**, 381