THE PATHOGENESIS OF EDEMA IN DIABETIC MACULOPATHY

Diabetic maculopathy is characterized by the accumulation of extra cellular fluid in Henle's layer and the inner nuclear layer of the retina. The localization of the edema is likely to be due, in part, to the relative barrier properties of the inner and outer plexiform layers. The origin of the extra cellular fluid is from the intravascular compartment. Although changes to retinal blood flow may partly explain the extravasations of fluid, the most important mechanism is breakdown of the blood retinal barriers. Both the inner blood retinal barrier formed by the retinal capillary endothelial cell tight junctions and the outer barrier formed by the retinal pigment epithelial cell tight junctions can be affected. The mechanism of breakdown of the blood retinal barriers is likely to be changes to the tight junction proteins including occludin and ZO-1. The biochemical messenger inducing these changes may be vascular endothelial growth factor. The origin of this or other cofactors may be the retinal glial cells. The underlying biochemical stimulus to the production of vascular endothelial growth factor is chronic hyperglycemia, but it is uncertain by what pathway this is effected **(Antcliff et al, 1999)**.

Physiologically, 2 circulations supply the retina ; the retinal capillaries and the choroid/choriocapillaris. The outer retina (photoreceptors) has an extremely high oxygen and metabolic requirement, which is mainly met by the choriocapillaris. The inner retina to the level of the outer plexiform layer (OPL) is supplied by the retinal capillaries through 2 major plexi, a superficial plexus in the axon and ganglion cell layers and a deep plexus in the inner nuclear layer **(Krebs, 1991)**. There are known to be 2 distinct types of diabetic maculopathy that can occur simultaneously and whose interrelation is unclear **(Whitelocke, 1979)**. The first is ischaemic maculopathy, which is relatively easily explained by capillary dropout. The second is edematous maculopathy: the pathogenesis of which still remains to be conclusively elucidated **(Whitelocke, 1979)**.

CYTOPATHOLOGICAL SITE OF FLUID

Controversy exists as to whether edema is intracellular or extra cellular. **Fine and Brucker (1981)** examined histologically 3 cases of cystoid macular edema (CME). Two patients had choroidal malignant melanoma, one of which was also diabetic and 1 further patient had diabetic retinopathy alone. They found widespread swelling and degeneration of the cytoplasm of the Müller cells and only found slight enlargement of intercellular spaces in 1 of the eyes. They also found degeneration of ganglion, bipolar, and photoreceptor cells **(Fine and**

Brucke, 1981). **Gass et al. (1985)** however, found through histological examination of CME in an eye with a choroidal melanoma that the fluid spaces were extra cellular with otherwise intact cytoarchitecture. They suggested that the findings of Fine and Brucker were caused by acute ischaemic changes or autolysis in the time from cessation of blood supply to adequate fixation **(Gass et al., 1985)**. In clinical studies, both angiographic leakage leading to edema and angiographic ischaemia are known to affect visual acuity, although when present simultaneously there effect on vision appears to be independent. **Arend et al.** examined several indices of ischaemia including capillary blood velocity and perifoveal intercapillary area, a measure of capillary density. They found that there was no difference between diabetics with or without CME. They further found that the size of the foveal avascular zone was larger in diabetics without CME than with CME. They concluded that inner retinal ischaemia does not contribute to CME **(Arend et al., 1995)**. **Smith et al. (1987)** measured retinal thickening with stereo fundus photography and showed that it was correlated with breakdown of the blood retinal barrier, as measured by either vitreous fluorophotometry or fluorescein angiography. However, ischaemia measured by the size of the foveal avascular zone was not correlated with retinal thickening, implying that the major cause of retinal thickening was extra cellular expansion **(Smith et al., 1987)**. **Ticho and Patz (1973)** showed that macular edema in the presence of capillary non-perfusion were associated with a worse prognosis after laser

treatment than macular edema with good perfusion **(Ticho and Patz, 1973)**. The conclusion from these studies must be that although ischaemia may well lead to intracellular edema, the amount of swelling is likely to be small and the major contribution to clinically significant macular edema is extra cellular expansion **(Antcliff, 1999)**.

THE EFFECT OF BLOOD FLOW

The effect of diabetes on retinal blood flow has been extensively investigated. The retinal blood flow in diabetics without retinopathy has been variably reported as being decreased by some authors but increased by others **(Sinclair, 1991 and Clermont, 1997)**. Once retinopathy develops it has been generally shown that retinal blood flow increases **(Kohner, 1975)**. It is uncertain whether this is because of a loss of autoregualtion **(Antcliff, 1999)**. This is attendant with elongation and dilation of the retinal arterioles and venules **(Kristinsson, 1997)**. The effect of this would be to decrease the resistance of the arterioles, thus decreasing the pressure drop within this part of the circulation. As highlighted by Kristinsson et al., this would lead to increased vascular pressure in the capillary bed, which would of itself lead to increased passage of fluid into the extracellular space by Starling's law **(Kristinsson et al., 1997)**. This, however, can only be part of the story, as it would lead to fluid expansion of the extracellular space without the

attendant proteins that are known to be present in diabetic macular edema and particularly in exudates **(Antcliff, 1999)**.

Langham et al. (1991) examined the ocular haemodynamics and found the choroidal circulation to be reduced in diabetics with background retinopathy compared with normal controls and diabetics without retinopathy **(Langham et al., 1991)**. It is not known what effect this has on the outer blood retinal barrier or on the ability of the retinal pigment epithelium (RPE) and choroid to remove any fluid leaking from the retinal capillaries **(Antcliff, 1999)**.

BREAKDOWN OF BLOOD RETINAL BARRIER

Vitreous fluorophotometry has shown that in both the early stages of the rat and monkey models for diabetes and in humans with no retinopathy detectable by fluorescein angiography, increased concentrations of fluorescein can be detected in the vitreous near the retinal surface compared to normals. This implies that one of the earliest changes in diabetic retinopathy is breakdown of the blood retinal barrier (BRB) **(Jones et al., 1986)**.

This is conventionally believed to involve mainly the retinal capillaries, but clinical evidence has shown that some patients have a diffuse epitheliopathy that stains in the late phase of fluorescein

angiograms in the absence of retinovascular leakage **(Weinberger, 1995)**. This may be a common occurrence but once the inner BRB breakdown has occurred it would mask fluorescein leakage from the outer BRB **(Antcliff, 1999)**. Extensive experimental evidence also supports a role for the RPE **(Tso et al., 1980 and Vinores, 1990)**. In diabetic rats, by using horseradish peroxidase and vitreous fluorophotometry **Tso et al. (1980)** showed leakage from RPE lesions in the absence of horseradish peroxidase leakage from the retinal capillaries, and **Vinores et al. (1990)** showed structural changes to the RPE of diabetic rats **(Vinores et al., 1990)**. **Wong et al. (1988)** have also shown that retinal pigment epithelial cells in culture stimulated the proliferation of bovine retinal capillary endothelial cells, implying that the inner and outer BRB may be interrelated and that alterations of function in 1 cell type may affect the other **(Wong et al., 1988)**.

POSITON OF FLUID

Histological studies of cystoid macular edema have shown that the location of the extracellular fluid is usually in 1 or both of 2 layers, in the inner nuclear layer in the inner retina and the photoreceptor inner connecting fiber layer or Henle's layer in the outer retina, sometimes erroneously called the outer plexiform layer **(Tso, 1982)**. In the case of large amounts of fluid being present in this region it may also disrupt the outer nuclear layer. The recent introduction of optical coherence

tomography shows the cysts as areas of low or no signal with occasional high-signal elements bridging the retinal layers **(Hee et al., 1995)**. It must be remembered that the concept of multiple discrete cysts has developed as a result of examining histological sections and optical coherence tomography scans. Both these modalities give an erroneous picture in that they sample a single confined plane through the retina **(Otani et al., 1999)**. In 3 dimensional examination of cysts such as those derived from scanning electron microscopy, it is clear that the cysts are a single compartment spanned by the trunks of Müller fibers. As these are some 10 to 30 μm in diameter they are artificially identified as apparent compartmental barriers in sectioning techniques **(Mashall, 1991)**.

Tso (1982) showed that vascular diseases are more likely to lead to accumulation of fluid in the inner retina, whereas diseases of the RPE are more likely to lead to pooling in Henle's layer, although the cases of end stage diabetes that he presented mainly had cysts in Henle's layer **(Tso, 1982)**. A possible explanation for the distribution of fluid pooling within the neuroretina seen in histological studies is as follows. Fluid accumulation can only occur within the neuroretina in the presence of resistance barriers to outflow. The morphological appearance will be determined both by the location of the high resistance barriers and by the physical constraints imposed by the organization of the retina. These physical constraints may be identified as positions where cells are

joined by junctional complexes or where their processes invaginate into the surface of themselves **(Antcliff, 1999)**. In the outer retina the Müller's fibers display junctional complexes between themselves and the photoreceptors, which collectively form the outer limiting membrane (OLM), and in the inner layers they exhibit junctional complexes between themselves and the inner limiting membrane **(Hogan et al, 1971)**. Thus, these cells constrain x, y-plane displacement as a result of their junctional complexes and z-plane displacement as a result of their cell body processes **(Antcliff, 1999)**. In the OPL, the invaginated synapses of the rods, and cones would theoretically limit displacement as would the tortuosity and interwinging of the dendritic processes in the inner plexiform layer **(Hogan et al., 1971)**. This layer does not include the inner connecting fibers of the photoreceptor cells, which in the macula give rise to the fiber layer of Henle. Cell movement would then occur in the inner and-outer nuclear layers, in the fiber layer of Henle, and in the nerve fiber layers. If this concept was correct, then in the early stages of capillary leakage, fluid would be constrained between the inner plexiform layer and the OPL and pool in the inner nuclear layer. Similarly with an outer BRB leak, fluid would pool between the OPL and the OLM, thus causing displacement in the outer-nuclear layer and the fiber layer of Henle **(Antcliff, 1999)**.

Under normal physiological conditions fluid must move across the entire retina and through the high resistance barriers. However, there

is evidence that diffusion limits for metabolic supply in the system are approximately 150 µm. This would explain the thickness of the retina within the capillary free zone where it rarely exceeds this value, and it would also explain cell loss in relation to vascular closure in either the retinal or choroidal supply **(Antcliff, 1999)**. In large areas of capillary closure all the layers internal to the OPL are lost and replaced by gliosis **(Hamilton et al., 1975)**, whereas choroidal infarction may lead to loss of RPE and photoreceptor cells but preservation of all internal layers **(Parrish et al., 1982)**. Therefore, it could be argued that fluid leaking from a given vascular system may easily diffuse up to 150 µm. If such fluid was leaking at an abnormal rate and pooling within this diffusion limit, it could rapidly change the microanatomical configuration of the tissue components and its boundaries. Increasing pressure within the pool would lead to increasing compactness in the surrounding tissues, thus in conditions leading to macular edema, fluid leaking from retinal capillaries would result in displacement of nuclei in the INL and ultimately compression of fibers within both plexiform layers. If pressure related barrier changes in the OPL were slower or of less magnitude than those in the inner plexiform layer then fluid could pass through this layer into the region o photoreceptor inner segments and nuclei before compressing junctions in the OLM and enhancing its barrier properties. In conditions in which the outer BRB leaked, fluid would rapidly reach the photoreceptor inner segments and outer aspect of the OLM. It could pass through this barrier before becoming further

impeded by the OPL. Again as fluid progressively accumulated, resistance to fluid movement through the OLM and OPL would ultimately govern the size of any given cyst **(Antcliff, 1999)**.

Recent work on the hydraulic conductivity of human retina after progressive excimer ablation through the retina from both the vitreous and photoreceptors has indicated the presence of 2 high-resistance barriers to fluid flow vertically through the retina residing in the inner and outer plexiform layers. These observations would confirm that fluid leaking from the deep retinal capillary plexus in the inner nuclear layer would be relatively restrained between the inner and outer plexiform layers. Likewise fluid leaking from the RPE would be constrained by the OPL **(Antcliff, 1999)**

MECHANISM OF BRB BREAKDOWN

The mechanism of BRB breakdown has not yet been definitively established, and the biochemical mechanisms that damage the BRB in diabetics have also not been comprehensively elucidated. Barrier dysfunction could arise through either changes in the membrane state or pumping capacity of the barrier cells or in the junctional complexes between them. The most likely cause is changes to the tight junctions **(Gardner et al., 1997)**. However, other mechanisms that have been postulated include fenestration of the endothelial cell cytoplasm,

increased infoldings of the RPE leading to increased surface area and increased transport by vesicles **(Vinores, 1993**). **Gillies et al. (1997)** showed that high glucose concentrations reduced the electrical resistance of cultured bovine retinal capillary endothelial cells and induced breakdown of the capillary BRB by the paracellular route by using inulin, which doesn't cross cell membranes **(Gillies et al., 1997)**. This strongly suggested damage to the interendothelial tight junctions **(Antcliff, 1999)**. **Vinores et al. (1993)** however, with morphological studies of galactosaemic rats by using immunocytochemical labeling of albumin, suggested a transendothelial route for breakdown of the BRB as they showed diffuse staining of vesicles in the cytoplasm. They did not show labeling in the extracellular space distal to the tight junctions. As this was a morphological study, this does not rule out leakage through the tight junctions, which was not evident either at the time of sectioning or in the plane of sectioning **(Wallow, 1977)**.

The molecular constituents of tight junctions that have been identified include the proteins ZO-1, ZO-2, cingulin, 7H6, and occluding **(Anderson et al., 1995)**. ZO-1 is found in retinal capillary endothelial cell tight junctions and is believed to reflect tight junction function **(Gardner et al., 1997)**. Gardner et al., showed that in culture astrocytes both induce tight junction expression, as measured by enzyme-linked immunosorbent assay of ZO-1, and increase vascular endothelial cell barrier function, probably through a soluble factor

(Gardner et al., 1997). They also observed glial reactivity in short-duration diabetes, suggesting a possible link between diabetes induced glial cell changes and increased BRB permeability **(lieth et al., 1998)**. Occludin is another tight junction protein that is specific for vascular endothelial cells with strong barrier properties **(Antonetti et al., 1998)**. **Antonetti et al. (1998)** showed that occludin content decreased in diabetic rats coincident with increased BRB permeability. This permeability was selective with albumin with a molecular weight of 66 kD allowed to pass through, whereas rhodamine-dextran with a molecular weight of 10 kD was not. This may be a function of hydrophobicity **(Antonetti et al., 1998)**.

BIOCHEMICAL BASIS OF BRB BREAKDOWN

The underlying biochemical cause of diabetic maculopathy would seem to be chronic hyperglycaemia. The Diabetes Control and Complications Trial showed 23% less macular edema in those on tight control than those on looser control of blood sugars **(DCCT, 1993)**. However, how this might lead to macular edema remains unclear. Biochemical pathways that have been implicated in the pathogenesis of diabetic retinopathy for some time include the sorbitol pathway consisting of the enzymes aldose reductase (AR) and sorbitol dehydrogenase, non-enzymatic glycation of proteins, and the nitric oxide pathway **(Schmetter et al., 1997)**. AR inhibitors have prevented

the histological change of basement membrane thickening **(Frank et al., 1983)**, and Tilton et al., showed that AR inhibition could prevent the leakage of albumin in diabetic rats **(Tilton et al., 1989)**. The same group showed that inhibition of nitric oxide formation could prevent vascular leakage of albumin **(Tilton et al., 1989)**. **Vinores et al. (1980)** showed that in galactosaemic rats' inhibition of AR decreased the cytoplasmic albumin staining of retinal capillary endothelial cells but not the vesicle formation. They showed AR expression increased in perivascular astrocytes **(Vinores et al., 1993)**. They also showed that AR could be immunohistochemically shown in diabetic human retina and RPE and that its expression correlates with the severity and duration of diabetic retinopathy **(Vinores et al., 1988)**. Gillies et al., however, showed that the increase in paracellular permeability of cultured bovine retinal capillary cells was likely to be independent of the aldose reductase/ sorbitol pathway or because of advanced glycosylated end products **(Gillies et al., 1997)**.

More recent attention has focused on the peptide vascular endothelial growth factor (VEGF). This is known to be produced by a number of cells in the retina including the RPE, pericytes, retinal capillary endothelial cells, Müller cells, and astrocytes **(Aiello et al., 1995)**. It is well established as a causative agent in proliferative retinopathy but it is also a powerful vasopermeability factor, having about 50,000 times the potency of histamine **(Tolentino et al, 1996)**. It

is increased in rats with induced diabetes at 6 moths and in patients with non-proliferative diabetic retinopathy **(Murata et al., 1996)**. **Murata et al., 1996** showed that it was present in diabetic rats coincident with hyperpermeability confirmed by immunohistochemical staining of albumin **(Murata et al., 1995)**. **Aiello et al. 1997** showed that intravitreal injection of VEGF in rats activated protein kinase C leading to increased vasopermeability **(Aiello et al., 1997)**. This was confirmed in primates by **Tolentinto et al. (1996)** they also showed that VEGF injection led to retinal ischaemia **(Tolentinto et al., 1996)**. It is known to cause conformational changes in the tight junctions of retinal vascular endothelial cells, and **Antonetti et al. (1998)** showed that VEGF reduced the occludin content of cultured bovine retinal endothelial cells **(Antonetti et al., 1998)**. **Clermont et al. (1997)** showed that VEGF might be implicated in the retinal vasodilation in non-proliferative retinopathy **(Clermont et al., 1997)**. The underlying mechanism stimulating VEGF production is certain. It is known to be up regulated by ischaemia, but also by reactive oxygen intermediates, advanced glycation end products, and insulin-like growth factor **(Punglia et al., 1997)**. Other newer factors have also been implicated. Histamine itself may also play a role and has been shown to reduce expression of ZO-1 **(Gardner, 1995)**, and Endothelin-1 has been implicated in changes to retinal blood flow **(Takagi et al., 1996)**.

Which cells might be responsible for the release of VEGF or other factors remains largely a matter for speculation. Possible candidates include the retinal glial cells. Both Müller cells and astrocytes are known to have a regulatory effect on the tight junctions of bovine retinal capillary endothelial cells so that either ischaemic injury or edema itself could lead to a worsening of the breakdown of the BRB **(Gardner, 1995)**.

Müller cells have been suggested as a possible target for hyperglycaemia partly as a result of their elevated rate of glycolysis **(Poitry-Yamate et al., 1995)**. They have been shown to have selective biosynthetic changes in human diabetic eyes, and abnormalities to the b-wave of the electroretinogram in early diabetic retinopathy also suggest Müller cell changes **(Mizutani et al, 1998)**. Experimentally, Müller cells have been shown in animal models to be able to induce properties of the BRB on other vascular endothelial cells, notably impermeability in albumin and horseradish peroxidase **(Tout et al., 1993)**. Thus, if Müller cells are possibly important in maintenance of the BRB, and they are known to be affected in early diabetic retinopathy, they may have an important role in the pathogenesis of macular edema **(Antcliff, 1999)**.

In summary, whatever the origin of the fluid, its distribution within the retina is defined by physical parameters that only allow pooling in areas of low resistance to tissue displacement.