Retinal Hypoxia in Long-Term Diabetic Cats

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PURPOSE. To determine whether the retina is hypoxic in early stages of diabetic retinopathy in cats and to correlate intraretinal Po2 with fluorescein angiographic and histologic alterations.

METHODS. Intraretinal Po2 was measured with microelectrodes in three cats with long-standing diabetes (>6 years) that had been followed with fluorescein angiographs every 6 months. Average Po2 in the inner vascularized half of the retina was compared with similar measurements in 21 control animals. Photoreceptor oxygen consumption was also compared. The retinal vascular endothelium of the diabetic animals was stained for ADPase activity in flatmounts, and transverse sections were used to visualize microscopic alterations in vascular structure.

RESULTS. Po2 in the inner half of the retina was abnormally low in the diabetic cats, 7.7 ± 5.2 mm Hg (35 penetrations in 3 cats) versus 16.4 ± 9.3 mm Hg in normal cats (85 penetrations in 21 cats) (P < 0.001). Oxygenation was almost normal in some regions of the diabetic retinas, but little evidence of oxygen supply from the retinal circulation was observed in other regions. Inner retinal hypoxia was present in areas with no detectable capillary dropout in fluorescein angiograms or flatmounts. The worst changes histologically were microaneurysms, leukocyte and platelet plugging of aneurysms and venules, and degenerating endothelial cells in capillary walls. These histologic abnormalities were confined to small regions, some of which could be positively correlated with markedly abnormal Po2 profiles. Photoreceptor oxygen utilization was not affected in two diabetic cats, but was below normal in one animal in which choroidal Po2 was low.

CONCLUSIONS. This is the first direct demonstration of retinal hypoxia in early diabetic retinopathy, before capillary dropout was evident clinically. Hypoxia was correlated with endothelial cell death, leukocyte plugging of vessels, and microaneurysms. (Invest Ophthalmol Vis Sci. 1998;39:1647-1657)

Tissue hypoxia may play a significant role in the progression of diabetic retinopathy,1,2 but the time at which hypoxia occurs is unknown. By the time that capillary closure and nonperfusion become clinically apparent, inner retinal tissue is almost certainly hypoxic, because the choroidal circulation alone usually cannot provide adequate nutrition for the inner half of the retina in cats, primates, or humans.3-6 The hypoxia that exists as a result of pronounced capillary occlusion is thought to induce the synthesis of angiogenic factors, such as vascular endothelial growth factor (VEGF), which is present in increased amounts during proliferative diabetic retinopathy.7,8 It is possible that hypoxia occurs earlier in the disease, before large-scale capillary dropout, but the evidence is indirect. High resolution angiograms in humans have revealed increases in perifoveal intercapillary area and in the size of the foveal avascular zone before background retinopathy develops,9 and some recent measurements of retinal blood flow show early decreases in diabetes.10,11 These changes might be expected to produce hypoxia. Interestingly, several studies12-14 have reported that VEGF can be detected immunohistochemically in eyes showing no retinopathy or only early signs of retinopathy. In the absence of compelling evidence of hypoxia, Amin et al.13 suggested that other factors may stimulate VEGF synthesis.

Oxygen measurements have been made previously in the preretal vitreous humor of diabetic animals, but they have not supported the hypothesis that the retina becomes hypoxic after a relatively short duration of diabetes. No change in preretal vitreal Po2 was found in alloxan diabetic dogs compared with normal dogs after 8 months15 or 6 months16 of poor glucose control. Similarly, streptozotocin-treated rats (5- to 6-weeks duration) showed no difference in Po2 0.5 mm from the retinal surface when compared with normal animals, although Po2 was reported to be lower near retinal arterioles in diabetic animals.17 Finally, diabetic cats had preretal Po2s similar to those in normal cats up to 12 months after pancreatectomy.18

Diabetic cats maintained under poor glucose control develop alterations in the retina, which were detected with fluorescein angiography and microscopy, that are identical with those in diabetic humans, including thickening of the
fundus. This allows fluorescein angiography and other in vivo measurements to be made for years after the induction of experimental diabetes. The oxygen measurements reported here on diabetic cats differed in two important ways from those made previously in diabetic animals. First, they were made after a much longer duration of diabetes; second, intraretinal measurements were made for the first time. The main finding was that the inner half of the retina was hypoxic in these animals compared with historic control animals, even in regions showing few vascular abnormalities.

**METHODS**

**Animals**

All work adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Three diabetic cats were used in this study. One (183) was pancreatectomized 7.71 years before the acute experiment described below, one (186) was pancreatectomized 6.67 years before the acute experiment, and the third (184) spontaneously became diabetic at least 8 years before the acute experiment. Methods for pancreatectomy and maintenance of the animals under poor glucose control (250 - 400 mg/dl) have been described previously. Blood glucose concentrations were checked at least every 7 days, and subcutaneous injections of appropriate amounts of long-acting insulin (range, 2-17 units) were given daily. Fluorescein angiography was performed every 6 months to track the progression of retinopathy. Angiography was performed with a fundus camera (TOPCON TRC-50VT; TOPCON, Paramus, NJ) while the animals were anesthetized with ketamine (30 mg/kg) and xylazine (2.5 mg/kg). The pupils were dilated with 2.5% phenylephrine and 1% cyclopentolate, and the corneas were kept moist. Photographs were taken with black and white film (TMAX-400; Eastman Kodak, Rochester, NY), after injection of 20 mg/kg of 10% fluorescein into the cephalic vein. To evaluate vascular abnormalities, original 35-mm negatives were read using an Agfa lupe with 8X magnification. These are similar methods in terms of settings and magnification to those used in humans.

**Preparation**

Intraretinal PO₂ was measured as in previous experiments. Briefly, animals were anesthetized intravenously with an initial dose of sodium pentothal (17 mg/kg). This was supplemented as necessary during surgery, and a transition was gradually made to urethane, the anesthetic used during recordings (200-400 mg/kg loading dose followed by 20-40 mg/kg per hour). Animals were paralyzed with gallamine triethiodide (20 mg/kg per hour) and artificially ventilated. Arterial blood pressure and the electrocardiogram were monitored continuously, and pH, PO₂, PaO₂, blood glucose concentration, and hematocrit were monitored intermittently through a femoral artery cannula. The mean values of these parameters are given in Table 1. Two of the diabetic cats had substantial metabolic acidosis compared with normal cats, in which the range is 7.35 to 7.45. PO₂, PaO₂, and blood pressures were normal, but blood pressures began to decrease earlier in the diabetic cats than is generally observed in normal animals. Recording was terminated when the mean arterial pressure decreased to less than 80 mm Hg because this value was regarded as unphysiological. No attempt was made to control blood glucose during the experiment, and hyperglycemia was observed. Mild hyperglycemia is also observed in normal cats. Blood glucose concentrations in normal animals average approximately 135 mg/dl over the first 4 hours of recording, but is sometimes as high as 190 mg/dl. Average blood glucose concentration then declines gradually to approximately 100 mg/dl approximately 14 hours after the onset of recording (Linsenmeier et al., unpublished observations). This mild hyperglycemia in normal cats is thought to be caused by urethane. The hematocrit in two diabetic cats was in the normal range (37 and 39); the low hematocrit in two observations). This mild hyperglycemia in normal cats is thought to be caused by urethane. The hematocrit in two diabetic cats was in the normal range (37 and 39); the low hematocrit in two observations). This mild hyperglycemia in normal cats is thought to be caused by urethane. The hematocrit in two diabetic cats was in the normal range (37 and 39); the low hematocrit in two observations). This mild hyperglycemia in normal cats is thought to be caused by urethane. The hematocrit in two diabetic cats was in the normal range (37 and 39); the low hematocrit in two observations). This mild hyperglycemia in normal cats is thought to be caused by urethane. The hematocrit in two diabetic cats was in the normal range (37 and 39); the low hematocrit in two observations). This mild hyperglycemia in normal cats is thought to be caused by urethane. The hematocrit in two diabetic cats was in the normal range (37 and 39); the low hematocrit in two observations). This mild hyperglycemia in normal cats is thought to be caused by urethane. The hematocrit in two diabetic cats was in the normal range (37 and 39); the low hematocrit in two observations). This mild hyperglycemia in normal cats is thought to be caused by urethane. The hematocrit in two diabetic cats was in the normal range (37 and 39); the low hematocrit in two observations). This mild hyperglycemia in normal cats is thought to be caused by urethane. The hematocrit in two diabetic cats was in the normal range (37 and 39); the low hematocrit in two observations). This mild hyperglycemia in normal cats is thought to be caused by urethane. The hematocrit in two diabetic cats was in the normal range (37 and 39); the low hematocrit in two observations). This mild hyperglycemia in normal cats is thought to be caused by urethane. The hematocrit in two diabetic cats was in the normal range (37 and 39); the low hematocrit in two observations). This mild hyperglycemia in normal cats is thought to be caused by urethane. The hematocrit in two diabetic cats was in the normal range (37 and 39); the low hematocrit in two observations). This mild hyperglycemia in normal cats is thought to be caused by urethane. The hematocrit in two diabetic cats was in the normal range (37 and 39); the low hematocrit in two observations). This mild hyperglycemia in normal cats is thought to be caused by urethane. The hematocrit in two diabetic cats was in the normal range (37 and 39); the low hematocrit in two observations). This mild hyperglycemia in normal cats is thought to be caused by urethane.

Double-barreled oxygen electrodes were used to record intraretinal PO₂ and the local electroretinogram from the intact right eye. The main objective was to obtain profiles of PO₂ across the retina under dark-adapted conditions while the animal was breathing air. Penetrations of the retina were made in areas of interest chosen from a recent fluorescein angiogram of the right eye in each cat. The areas studied were always in the central retina or directly superior to the optic nerve head. Ophthalmoscopically visible vessels were avoided. Profiles in control animals were measured in exactly these same areas. The local electroretinogram was recorded to give an index of retinal depth and adequacy of each retinal penetration. Once the electrode reached the choriocapillaris, as signaled by a negative DC shift of the voltage signal, it was slowly withdrawn from the retina in 1-μm steps at 2 μm/sec, generating a profile of PO₂ across the retina. Only a few profiles were obtained during the light-adapted state. Each profile was stored on

**TABLE 1. Arterial Parameters during Oxygen Experiments in Diabetic Cats**

<table>
<thead>
<tr>
<th>Cat</th>
<th>pH</th>
<th>PaCO₂ (mm Hg)</th>
<th>PaO₂ (mm Hg)</th>
<th>Mean Pressure (mm Hg)</th>
<th>Glucose (mg/dl)</th>
<th>Hct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>183</td>
<td>7.17 ± 0.01</td>
<td>28.6 ± 3.1</td>
<td>100.1 ± 14.7</td>
<td>101 ± 25</td>
<td>369 ± 86</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>184</td>
<td>7.22 ± 0.06</td>
<td>27.8 ± 3.5</td>
<td>99.5 ± 6.3</td>
<td>115 ± 18</td>
<td>220 ± 69</td>
<td>37 ± 3</td>
</tr>
<tr>
<td>186</td>
<td>7.35 ± 0.02</td>
<td>26.9 ± 2.4</td>
<td>99.7 ± 14.2</td>
<td>116 ± 14</td>
<td>212 ± 51</td>
<td>39 ± 1</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Hct, hematocrit.
magnetic tape and a chart recorder, and was digitized on-line to create a computer file of Po2 as a function of position. Two types of analysis were performed on the oxygen profiles. First, the profile in the outer avascular retina was fitted to the model of oxygen diffusion and consumption described previously,25,26 to allow a determination of photoreceptor oxygen consumption (Qave), called Qave. As in previous work, the consumption values were corrected for the fact that the penetration was not perpendicular to the retinal surface and that there was distortion as a result of electrode drag during the withdrawal. This latter component of the correction was made by multiplying the value of Qave obtained from the fit by (L/100)^2,27 where L is the thickness (in micrometers) of the outer half of the retina during electrode withdrawal and 100 μm is the approximate average actual thickness of this part of the retina. The second analysis was to average the Po2 values recorded over the inner half of the retina (0% to 50% retinal depth) to determine the mean Po2 in the vascularized retina in each profile.28

Control Animals

The oxygen data from the diabetic animals were compared with measurements made under identical conditions in two previous studies on normal animals, and replotted appropriately here (Figs. 2, 3, 4, 5, 6). One was a study of the effects of hypoxemia on the retina,29 and the other was a study of the effects of retinal arterial occlusion on the oxygen distribution.4

Histologic Analysis

At the conclusion of recording, animals were killed with an overdose of pentobarbital, and their eyes were removed for ADPase staining of the retinal vasculature.29 Retinas were removed from the eye cup, fixed in 2% paraformaldehyde in cacodylate buffer, and shipped in 10% sucrose in cacodylate on ice to Baltimore, Maryland. Retinas were then incubated for demonstration of ADPase activity and flat-embedded in glycol methacrylate as previously described.29 Transverse sections of retina containing sites of electrode penetration were serial sectioned for histologic analysis.

RESULTS

Angiography

Figure 1 shows a fluorescein angiogram of the cat that was spontaneously diabetic (184), taken 5 weeks before oxygen measurements. Four of the sites of oxygen recording are indicated with numbers. Of the three animals studied, this cat had the most abnormal angiogram, with a few dot hemorrhages and microaneurysms in both eyes. An early phase angiogram of this eye was not available, and an early phase angiogram from the other eye of this cat revealed no areas of nonperfusion. Angiograms of cat 185 (2 months before the acute experiment) revealed one microaneurysm in each eye, but no hemorrhages or regions of nonperfusion. Angiograms from cat 186, taken 1 week before the acute experiment, were the most normal, showing no microaneurysms, hemorrhages, or areas of nonperfusion.

Inner Retinal Oxygenation

Figure 2 presents examples of retinal oxygen profiles. The retinal pigment epithelium/choriocapillaris boundary is on the left, at 100% retinal depth, and the retinal/vitreous interface is at zero. The top left profile (60W2) is from a normal, dark-adapted animal and shows features reported previously.22,27 In general there are two peaks in Po2 in the inner half of the retina, corresponding to the superficial and deep capillary layers. In the outer retina there is a minimum in Po2 that is at or near 0 mm Hg. The distal part of this minimum corresponds to the position of the inner segments.25 Sometimes, as in this profile, the minimum extends proximally from the inner segments through part of the outer nuclear layer. There is a steep gradient of Po2 from the choriocapillaries and typically a shallower gradient from the retinal circulation toward the minimum Po2.

All other profiles in Figure 2 are from diabetic cats. The second through fourth profiles on the left are from cat 184 and correspond to the recording locations indicated in Figure 1. The profiles in the right column are from cats 183 and 186. In some profiles from diabetic animals (184W18 and 186W9) the inner retina had Po2 distributions similar to those found in normal animals. Other profiles from the diabetic animals (184W7 and 183W1) were more like profiles recorded previously in retinas with an occluded retinal circulation.4 Many of the profiles that showed peaks in Po2 associated with retinal capillaries had mean values of Po2 that were unusually low (184W19, 183W2), and the more distal portion of the inner retina tended to have lower Po2s than the proximal portion.

The mean Po2 was computed from the inner half of the retina in each profile, and a histogram of these values from the 35 profiles of diabetic animals is shown in Figure 3A. Figure 3B shows similar data in 82 profiles from 21 normal animals. One
FIGURE 2. Profiles of oxygen tension from a normal animal (60W2) and animals with diabetes (all other traces). The second through fourth traces on the left are from cat 184. On the right are two profiles each from cats 183 and 186. Each trace represents a continuous electrode withdrawal from the choroid into the vitreous humor. Dashed vertical lines indicate the position of the retinal pigment epithelium/choriocapillaris boundary at 100% retinal depth and the retina/vitreous boundary at 0% retinal depth. In profile 60W2 the electrode was stopped just after entering the vitreous, and the part of the trace marked with an asterisk was obtained while the electrode was stationary.

expects a broad distribution in both histograms, because even if retinal blood flow and oxygen utilization are identical across profiles, the mean Po2 in a profile depends on the proximity of the electrode to vessels and on whether those vessels are arterioles, or venules, or capillaries. The two histograms overlap, but the mean of the diabetic distribution was 7.7 ± 5.2 mm Hg (mean ± SD), whereas the distribution from normal animals was significantly different, with a mean of 16.4 ± 9.3 mm Hg (P < 0.001; two tailed Student's t-test). Because each cat contributed a different number of profiles to these histograms, the average inner retinal Po2 was obtained for each cat by calculating the mean of the profile means. This is shown in Figure 4. All values for diabetic cats were at or below the bottom of the normal distribution. The difference between the normal and diabetic cats in Figure 4 was significant (P = 0.012; two tailed t-test).

Oxygen profiles (Fig. 2) usually showed a gradient in which the vitreal Po2 was higher than the mean inner retinal Po2. The Po2 in the vitreous, obtained from the end of each withdrawal, averaged 14.1 ± 6.0 mm Hg (mean ± SD) in the diabetic cats, almost twice the inner retinal mean value of 7.7 ± 5.2 mm Hg (P < 0.001, paired Student's t-test). As shown in Figure 5, the vitreal value was higher than the inner retinal mean value in almost all profiles. Data for a subset of the normal profiles, for which comparable information was available, are also shown in Figure 5. Like inner retinal Po2, the vitreal Po2 was significantly lower in diabetic cats than in normal cats (14.1 ± 6.0 mm Hg versus 21.0 ± 8.6 mm Hg; P < 0.01). In normal animals, vitreal values were often higher than average intraretinal values, 21.0 ± 8.6 mm Hg in the vitreous versus 15.4 ± 9.3 mm Hg in the inner retina (P = 0.004), but the percentage difference was not as great in normal cats (vitreal/retinal = 1.36) as in diabetic cats (1.83). Thus, while the values of both vitreal and intraretinal Po2 were lower in the diabetic animals than in normal animals, retinal hypoxia was more apparent in intraretinal measurements.

**Outer Retinal Oxygenation**

Two parameters obtained from fitting the profiles to a diffusion model characterize the main aspects of oxygenation in the
outer half of the retina. These are the choriocapillaris PO2 (called PC in earlier studies) and the average oxygen consumption of the photoreceptors, Qav. Qav varies directly with PC in normal cats,27,28 because the flux of oxygen to the photoreceptor inner segment is supplied almost entirely by the choroid and is therefore reduced at low levels of PC. A range of values of PC was observed in the diabetic animals, as in normal animals, although one diabetic animal (186) had lower values of PC than we have ever observed in a normal animal. The reason for these low values in one diabetic is not known. (Cat 183 had the lowest inner retinal PO2, whereas cat 186 had the lowest choriocapillaris PO2, as is reflected in Fig. 2.) Because of the dependence of Qav on PC, a simple comparison of average values of Qav between normal cats and diabetic cats is not meaningful. Instead, Figure 6 illustrates Qav as a function of PC for both groups. These data reveal no abnormality in photoreceptor oxygen utilization in the diabetic animals, that is, the values of Qav are as expected from the values of PC.

Although insufficient data were collected in the light-adapted state to fully characterize dark-light differences in Qav in the diabetic cats, it appeared that Qav was reduced by light, as expected from previous work.22 This conclusion is based on the shape of profiles recorded in light adaptation (not shown) and on the observation that PO2 in the outer retina increased transiently during the flashes of light used to elicit the local electroretinogram.

**Histologic Analysis**

An area of the ADPase flat-embedded retina from the spontaneously diabetic cat (184) is shown in Figure 7A. The center of
FIGURE 4. Mean inner retinal Po$_2$ in each cat, averaged over the profiles available from each cat. The mean for the diabetic cats was 6.37 ± 4.13 mm Hg, and for normal cats was 17.69 ± 9.55 mm Hg (means ± SD).

this area is approximately 1 mm from the edge of the optic nerve head, and the vessels are running in the superior-temporal direction. The loss of capillaries near the optic nerve head at the lower right is probably an artifact of tissue processing. The capillary density in this micrograph is representative of that in the entire central retina. Exact locations of most of the oxygen profiles were difficult to determine on micrographs, but vascular landmarks allowed a positive identification in a few cases. Areas 18 and 19 in Figure 7A were the site of profiles 184W18 and 184W19 in Figure 2. Region 18, with a relatively normal profile, had no detectable vascular abnormalities. Region 19, in which inner retinal Po$_2$ was low, had several microaneurysms that corresponded to hyperfluorescence on the angiogram (Fig. 1).

These two areas were sectioned transversely for histopathologic analysis. Area 18 had patent vessels with normal wall structure and no adherent blood elements (Fig. 7B). Area 19 had platelets in microaneurysms and leukocytes adhering to the wall of post-capillary venules (Fig. 7C). Figure 7D shows another view of a microaneurysm from area 19 extending into the outer nuclear layer and containing platelets and an endothelial cell that appears to be degenerating based on its pale nuclear staining. There were few endothelial cell nuclei in serial sections of this aneurysm. Some of the capillaries in the deeper (secondary) capillary layer were acellular and had thickened basement membranes (Fig. 7E). A similar group of abnormalities could be seen in another area that was positively identified with an abnormal oxygen profile. However, in that area (area 23), aneurysms were observed in the nerve fiber layer, and the lowest Po$_2$ values were also in that layer (Fig. 8). This can be compared with the profile for area 19 (Fig. 2) where the lowest Po$_2$ values were at approximately 40% depth and the aneurysms originated from the secondary, or deeper, vascular layer (Fig. 7D). The comparison of these two areas suggests that aneurysms and hypoxia may be causally linked.

Angiopathic changes were not observed in cat 183 or 186 using flat perspective analysis of ADPase flat-embedded retinas. However, there were elevated numbers of leukocytes in retinal blood vessels of both of these animals.

DISCUSSION

Inner Retina

The main finding of this work is that Po$_2$ in the inner half of the retina in cats with long-standing diabetes was, on average, only approximately half of the value in normal animals. Some profiles in the diabetic cats were similar to those recorded during experimental retinal arterial occlusion$^4$ in showing almost no oxygen in the inner retina. There was considerable heteroge-
neity, however, and other regions in the same retinas appeared normal or nearly normal. Retinal hypoxia was observed in the absence of angiopathy or the presence of mild angiographic changes, including microaneurysms and a few dot hemorrhages, which would be considered to be early background retinopathy in a human. No areas of capillary nonperfusion could be detected with angiographic techniques that provided resolution of capillaries comparable to human angiograms. This is the first direct demonstration of reduced retinal tissue PO2 at any stage of retinopathy. It suggests that local ischemia and tissue hypoxia may occur earlier in diabetic retinopathy than has often been thought, although some other investigators have already argued on the basis of other types of evidence that hypoxia occurs early in the disease.9-11,30

Experiments performed on the diabetic animals were identical to those performed on the control population, but it is worth considering whether there were any important differences in the two groups other than diabetes. Diabetic animals may have been older than the control animals, on the whole, and it is not possible to completely rule out an effect of age. Control animals tended to be relatively large animals (4-5 kg) from random sources, rather than purpose bred, so it is likely that some of them were similar in age to the diabetic animals. Arguing against a major effect of age is the observation that normal and abnormal profiles could be correlated with specific changes in the vasculature, and were not generalized. Another difference between control and diabetic animals is that one diabetic cat had a low hematocrit, but a reduction of hematocrit per se can lead to improvement in retinal oxygenation51 rather than causing hypoxia. Intraocular pressure was not measured, but the eyes were sealed in all cases, allowing normal regulation of intraocular pressure. At least in normal animals, inner retinal PO2 is not strongly dependent on intraocular pressure.37

An important conclusion is that tissue responses to ischemia, such as production of VEGF, do not require large areas of visible capillary dropout and may occur simultaneously with early signs of disease, as recently shown by Lutty and associates12,14 and Amin et al.15 The latter group concluded that factors other than hypoxia may be involved in the induction of VEGF, but, on the basis of the results presented here, it seems unnecessary to postulate the involvement of other factors, at least in diabetes. It is still difficult to explain the finding of increased VEGF in the inner retina in cases of age-related macular degeneration,15 in which one would not expect inner retinal hypoxia.

Possible Mechanisms of Retinal Hypoxia
Although the angiograms did not appear grossly abnormal, altered vascular histopathologic features in certain areas could
be identified with abnormal oxygen profiles in cat 184. Blood flow patterns in microaneurysms could certainly be abnormal, which might cause tissue hypoxia. In addition, local flow in these areas was probably reduced by adherent leukocytes and platelets. Several leukocyte abnormalities occur in diabetes. First, diabetic cats have increased numbers of white blood cells, mostly because of increased numbers of polymorphonuclear leukocytes (PMN) compared with normal cats, and these PMNs are less easily deformable than those in normal cats.\(^{32}\) Two years before the experiments reported here, the three cats studied had 10,720 to 17,510 PMNs/mm\(^3\) compared with an average of 7,150 PMNs/mm\(^3\) in 13 normal cats.\(^{32}\) PMNs are also less deformable in diabetic humans.\(^{33}\) Second, increased levels of endothelial cell/leukocyte adhesion molecules (ICAM-1 and P-selectin) have been observed in human subjects with diabetes.\(^{34}\) This is probably the reason that leukocyte activation and adhesion are increased in diabetic humans\(^{35}\) and rats.\(^{36}\) The presence of activated leukocytes is, in turn, known to increase vascular resistance,\(^{37,38}\) which would reduce local blood flow. The observation of adherent white blood cells suggests that hypoxia was prolonged rather than transient in at least some of the regions affected. Unfortunately, it was not possible to perform a correlation of histology and Po\(_2\) at every location studied, so the extent of vascular impairment necessary to affect tissue Po\(_2\) is still uncertain. However, in two cats the only detectable histologic change was an increase in the number of leukocytes, and Po\(_2\) was still low.

Other vascular changes, particularly degeneration of cells in the vessel wall, which was also observed here, could have further impaired oxygen transport. Mizutani et al.\(^{39}\) demonstrated accelerated death by apoptosis of retinal microvascular cells in humans with background diabetic retinopathy and also in short-term (24 and 31 weeks) galactosemic- and alloxan-induced diabetic rats. Excess cellular death was observed at early stages of retinopathy or even in its absence. Loss of endothelial cells and pericytes leads to capillary nonperfusion.\(^{40}\)

**Outer Retina**

The inner retina is the main site of pathology in diabetes, but there are many reports of changes in the retinal pigment epithelium, outer retina, and choroid.\(^{41-48}\) In vitro studies have shown that retinal oxygen consumption (Q\(_{O_2}\)) is decreased in an alloxan-induced model of diabetes\(^{49,50}\), and since the total Q\(_{O_2}\) in rabbit retina is primarily attributable to photoreceptors,\(^{51}\) the changes shown in diabetic rabbits are likely

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**Figure 6.** Photoreceptor oxygen consumption (Q\(_{AV}\)) obtained from fitting the oxygen profile through the outer half of the retina to a diffusion model, as a function of the fitted value of choriocapillaris Po\(_2\) (Po\(_c\)). The cluster of points at the lower left is from one diabetic cat that had low Po\(_c\) and, consequently, low Q\(_{AV}\). All values were obtained in dark adaptation.
Figure 7. (A) Region of the ADPase flat-embedded retina from cat 184 indicating sites from which two oxygen profiles were recorded (arrows 18 and 19). (B) Transverse section taken through the region indicated by arrow 18 in (A). This area of retina had a relatively normal profile (profile 184W18 in Fig. 2), normal retinal architecture, and no apparent angiopathy. (C) Transverse section taken through the region indicated by arrow 19 in (A). This area had the lowest PO₂ at a retinal depth of 40%. Angiopathic changes consisting of a secondary capillary network microaneurysm (short arrow) and adherent leukocytes in a postcapillary venule draining the secondary capillary network (long arrow) are evident in this region. (D) Higher magnification of a different section of area 19 with low PO₂ shows accumulation of platelets and a degenerating endothelial cell (arrow) within a different microaneurysm. (E) Acellular capillaries with thickened basement membranes (arrows) in area 19. Magnification, (A) × 120; (B) × 300; (C) × 300; (D) × 450; (E) × 1100.
When the choroids from the three diabetic cats were examined using the alkali ne-phosphatase flat-embedding technique, no influence the choroid only during the acute experiment. Without regard to Pc, the diabetic average would be lower. Measurements available, and showed no distinct effect of diabetes under the conditions examined. Two of the three diabetic animals had Pc in the range typically observed in normal cats. However, one had extremely low Pc, and this led to reduced Qo2 in that animal, just as hypoxemia leads to reduced Qo2 in normal cats. If one were to compare average values of oxygen consumption between control and diabetic animal groups without regard to Pc, the diabetic average would be lower. Differences in choroidal Po2 cannot account for the lower Qo2 in diabetic versus normal diabetic rabbits, because both groups were studied in vitro under controlled oxygenation. It is not known whether the low choroidal Po2 in one cat was actually the result of diabetes, or whether it was a result of unusually active sympathetic activation or some other mechanism that influenced the choroid only during the acute experiment. When the choroids from the three diabetic cats were examined using the alkaline-phosphatase flat-embedding technique, no areas of capillary dropout were observed (GA Lutty and DS McLeod, unpublished data), suggesting that choroidal nonperfusion was not an issue. The basically normal status of the outer retina is supported by the observation that in all three diabetic cats the amplitude of the c-wave of the electroretinogram was normal, in vitreal and intraretinal recordings (RA Linsenmeier, MA McRipley, LB Padnick, DL Hatchell, unpublished results). This is quite different from the c-wave in diabetic rats, which is lost or inverted in polarity early in diabetes. Typically, diabetic rats are not given insulin at all, and may therefore have worse glucose control than the cats studied here. Whether differences in glucose control or species differences explain discrepancies in the results obtained on the outer retina remains to be determined.

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**References**


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