Retinopathy in Monkeys with Spontaneous Type 2 Diabetes

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PURPOSE. Type 2 diabetes occurs spontaneously in rhesus monkeys and shows an extraordinary similarity to human diabetes in clinical features and relative time course. The purpose of this study was to investigate clinically and histopathologically the ocular changes in these monkeys.

METHODS. Ophthalmoscopic examinations were performed on aged normal and diabetic monkeys. Retinas from 16 diabetic monkeys and 6 nondiabetic monkeys were incubated postmortem for adenosine diphosphatase (ADPase) activity (labels viable retinal blood vessels) and flat-embedded in JB-4. Tissue sections were cut through areas of interest.

RESULTS. Cotton-wool spots, intraretinal hemorrhages, and hard exudates in the macula were observed by ophthalmoscopy in some diabetic monkeys. Dot/blot hemorrhages, cotton-wool spots, and small nonperfused areas were the earliest histologically documented changes in the retinas. Large nonperfused areas extending from optic disc to midfovea were observed in four diabetic monkeys. Formation of small intraretinal microvascular abnormalities (IRMAs) and microaneurysms were associated with the areas of nonperfusion. There were apparent fluid-filled spaces in the outer plexiform layer in three of these maculas, suggesting macular edema. There was a significant correlation between the occurrence of retinopathy and hypertension (P = 0.037 for systolic pressure; P = 0.019 for diastolic pressure). In elastase-digested retinas, the ratio of pericytes to endothelial cells was 0.66:1 in diabetic and 0.64:1 in nondiabetic (P = 0.75) retinas.

CONCLUSIONS. This is the first detailed analysis of retinopathy in a colony of spontaneous type 2 diabetic monkeys. Monkeys with type 2 diabetes have many of the angiopathic changes associated with human diabetic retinopathy. Hypertension correlates with the severity of the diabetic retinopathy. (Invest Ophthal Vis Sci. 2004;45:4543–4553) DOI:10.1167/iovs.04-05119

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Obesity has recently been declared a national health risk in the United States.1 One consequence of obesity is type 2 diabetes. More than 12 million people in the United States have diabetes, and that number is increasing rapidly. Diabetes is the leading cause of blindness in working Americans. The most common cause of visual loss in diabetics is macular edema, followed by retinal neovascularization. The paucity of animal models that have all the pathologic characteristics of diabetic retinopathy has delayed therapies for early stages of diabetic retinopathy and subsequent proliferative retinopathy.

Most models of diabetes mimic type 1 diabetes, which is caused by destruction of the β-cells in pancreatic islets.2 Mice or rats given streptozotocin (STZ) or alloxan lose β-cells and become insulin dependent.3 The life of these rodents is greatly shortened. They lose retinal pericytes and capillaries, have thickened vascular basement membranes, and have increased vascular permeability in the retina. When pancreatectomy is performed on cats, thickened vascular basement membranes develop with loss of pericytes and retinal capillary segments, which eventually results in a large area of vascular nonperfusion temporal to the optic disk.4 Although microaneurysms and intraretinal microvascular abnormalities (IRMAs), which some interpret to be intraretinal neovascularization, occur in cats and some rats, preretinal neovascularization has never been observed in an animal model of diabetes. These models of type 1 diabetes have elevated plasma glucose. However, elevations in dietary galactose in dogs, rats, or mice produce retinal changes similar to those observed after destruction of β-cells.5,6 One benefit of galactose feeding is that the animals are more easily maintained and have a longer lifespan.

There are only a few models that truly represent type 2 diabetes, which is associated with obesity and insulin resistance. Zucker obese rats7 and the Otsuka Long-Evans Tokushima fatty rats8 are examples of rats that undergo spontaneous development of type 2 diabetes. Ob/ob mice9 and KKAY mice10,11 are good models for some metabolic changes that occur in type 2 diabetes. However, neither the rats nor mice appear to have substantial changes in the retina similar to diabetic retinopathy in type 2 diabetic humans.

The intent of this study was to characterize histologically the retinopathy that occurs in spontaneous type 2 diabetic, obese rhesus monkeys. Most prior studies of diabetes in monkeys was in streptozotocin-induced diabetic monkeys. Changes in the retinal vasculature were studied in flat-embedded retinas after incubation for adenosine diphosphatase (ADPase) enzyme histochemical activity. The retinal histologic findings were then compared to the severity of diabetes and presence of hypertension in the monkeys.

MATERIALS AND METHODS

The animals were maintained in an animal colony at the University of Maryland in accordance with the NAS/NRC guide for the care and use of laboratory animals and the tenets of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Sixteen obese diabetic monkeys and six nondiabetic monkeys that went to necropsy were studied (Tables 1 and 2). Age, diabetic history (duration and degree),
the presence of hypertension, and other abnormalities were carefully evaluated using the historic and medical records of each monkey. Most eyes were enucleated and dissected immediately after euthanasia with intravenous sodium pentobarbital, and tissues were fixed within 1 hour of enucleation.

**Clinical Observation**

The fundus of each monkey was examined periodically with an indirect ophthalmoscope while the monkey was under anesthesia for diagnostic tests. If media opacity due to advanced cataract prevented examination of the fundus, ophthalmoscopy was not possible, and we could perform only a physiological evaluation until surgical correction of the media opacity. The fundus was also examined during dissection of the enucleated eyes after death. Hypertension was graded on the following scale: systolic pressure from 120 to 129 mm Hg was considered mild hypertension; 130 to 149 mm Hg, moderate hypertension; and 150 mm Hg or greater, severe hypertension. The severity of diabetes was based on fasting plasma glucose (FPG) levels: FPG 126 to 200, severe; 201 to 250, severe; 150 to 250, moderate; and greater than 150 mg/dL, mild diabetes; 150 to 250, moderate; and greater than 250, severe.

**Elastase-Digestion Method**

Retinas from six monkeys (three diabetic, three nondiabetic) were digested with elastase, as described by Laver et al. The digested retinas were stained with PAS and hematoxylin and mounted on a slide with a photomicroscope (Carl Zeiss Meditec, Dublin, CA) at ×100 magnification (field area, 0.64 mm²). Endothelial cells and pericytes were counted in random fields throughout the retina until the total number was more than 1000. The ratio of pericytes to endothelial cells was then calculated. All slides were masked to observers and were not classified or identified before counting. Nuclei of pericytes stain more darkly, are smaller than endothelial cell nuclei, and lie in the outer aspect of the capillary wall. Endothelial cell nuclei were also distinguished from pericyte nuclei by their large pale nuclei being parallel to the long axis of the blood vessels. Pericyte ghosts (lacking hematoxylin staining of the nucleus) were also counted per 1000 endothelial cells.

**ADPase Enzyme Histochemistry**

After enucleation of the eye, a deep incision was made 1.0 cm posterior to the limbus with a #11 surgical blade, and the anterior segment was removed. After removal of the vitreous, the retina was separated carefully from the RPE and choroid and fixed in 2% paraformaldehyde in 0.1 M cacodylate buffer overnight at 4°C. The retina was washed in 0.1 M cacodylate buffer with 5% sucrose and then incubated for enzyme histochemical demonstration of adenosine diphosphatase (ADPase) activity, as previously published. The ADPase-incubated retina was washed in 0.1 M cacodylate buffer and kept in 2% paraformaldehyde in 0.1 M cacodylate buffer until further examination.

**Flat-Embedding of Retinas**

The regions from each retina were excised and placed between two pieces of screen (Nytex; Tetko, Elmsford, NY) in a holder, as previously published. The tissue was fixed again in 25% Karnovsky fixative (pH 7.4) at 4°C overnight or longer. The tissue was then washed, dehydrated, and infiltrated with freshly catalyzed glycol methacrylate (JB-4; Polysciences, Warrington, PA), as published previously.

**Table 1. Medical and Histologic Data of Nondiabetic Monkeys**

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† Throughout the posterior pole.

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† Throughout the posterior pole.
infiltration, the retina was embedded in the methacrylate and the blocks were stored in a desiccated environment.

The cured block was placed on a drop of immersion oil on a glass slide with the vasculature nearest the objective, and then immersion oil and a coverslip were applied to the upper surface. Under dark-field illumination, the blood vessels in the whole block were photographed and mapped. Sections 2.5/μm thick were cut on a dry glass knife with a microtome (NT2; Sorvall, Newtown, CT), and the sections were stained with PAS and hematoxylin or thionin to stain the cell nuclei blue and ammonium sulfide to develop the ADPase activity in viable blood vessels.12

Counting Retinal Ganglion Cells

Retinal ganglion cells (RGCs) were counted in 12 diabetic and 4 control monkeys. With a microscope (Carl Zeiss Meditec) at ×400 magnification (field area, 0.64 mm²), nuclei in the ganglion cell layer (GCL) were counted in three contiguous nasal and three contiguous temporal fields, one disc diameter inferior to the macular center and in four fields from the nasal margin of the optic disc.

All nuclei without segmentation or degenerative changes in the GCL were counted as RGC nuclei. No attempt was made to distinguish between RGCs and displaced amacrine cells. Ganglion cells were assumed to have a larger size, more irregular outlines, and clumped Nissl substance and could be distinguished from non-neuronal cells, which were either elongated endothelial cells in blood vessels or smaller, more darkly stained glial cells with sparse cytoplasm.13

Statistical Analysis

Comparisons between groups were performed using the two-tailed Student’s t-test. P ≤ 0.05 was considered significant. Exact logistic regression analysis was used to analyze the relationship of hypertension, severity of diabetes, and retinopathy.

RESULTS

One of the earliest changes observed ophthalmoscopically in the diabetic monkey retina was cotton-wool spots. These appeared very similar to human cotton-wool spots ophthalmoscopically and histologically in the flat perspective and had an area around them that had nonviable capillary segments (lacked ADPase activity; Fig. 1). When cut in cross section, characteristic cytoid bodies were observed within the lesions (Fig. 1D). Most cotton-wool spots were close to the optic disk. Another pathologic change observed early in retinopathy was small areas of capillary dropout (lack of ADPase activity). The nonviable capillary segments were in both the superficial and deep capillary networks (Figs. 2A, 2B). Larger areas of capillary...
loss always involved arteriolar pruning (Fig. 2D). Small hemorrhages were also observed ophthalmoscopically early in retinopathy.

At later stages in retinopathy, small microaneurysms were observed histologically. These were usually associated with areas that had apparent nonperfusion (lack of ADPase-positive blood vessels; Fig. 3). The microaneurysms were often associated with small IRMAs, also adjacent to areas with capillary loss.

In four of the severely diabetic monkeys, large areas of capillary loss were apparent (Fig. 4). In three of these animals, the area of dropout included most of the vasculature between optic disc and fovea. In these most severe cases, the central half of fovea was nonperfused, whereas the temporal fovea had viable blood vessels (Fig. 4B). Two of the four animals with this pattern of vascular loss had apparent cilioretinal vascular systems that were still viable (Fig. 4A, arrow). In sections through the macular region, two patterns of neuronal loss were apparent (Figs. 4C, 4E, 4G). Nasal to fovea, some areas had neurons in the inner nuclear layer appearing normal in number, even though viable retinal vasculature was not present in the area (Figs. 4C, 4G). In adjacent areas, photoreceptor nuclei appeared viable but were reduced slightly in number, whereas the inner nuclear layer neurons were almost eliminated (Fig. 4E). In all these areas, RGCs were not present. Apparent fluid-filled spaces were also present in nasal and inferior macula in three of the four monkeys with severe retinopathy. Most of the spaces were in the outer retina (Fig. 4F).

Macrovascular changes were apparent in retinal arteries near the optic disc. In hypertensive nondiabetic monkeys, there was reduplication of basement membrane, which had an onion-skin appearance, which is characteristic of hypertensive arteries (Fig. 5C). In severe diabetic animals with severe hypertension, the luminal diameter was reduced because of reduplication of the thickened basement membrane (Fig. 5D). The outer adventitia was more positive for PAS than the inner, and the two areas appeared distinct. Occasionally, a nucleus was observed in the vessel wall that had the appearance of a foam cell. We also observed venous loops adjacent to large areas of capillary dropout.

**Elastase Digestion and Counting Pericytes**

Elastase digests from three diabetic and three nondiabetic monkeys were analyzed. If two retinas were available from a monkey, the results from the two retinas were averaged. In diabetic retinas, some major veins appeared dilated, and increased basement membrane staining with PAS was observed, which suggested basement membrane thickening had occurred. There were no specific abnormalities apparent in nondiabetic retinas.

The mean number of pericytes per 1000 endothelial cells was 657.4 in diabetic retinas (range, 581.8–735.3) and 639.8 in

**FIGURE 2.** Areas of capillary loss (no ADPase activity) in 31.2-year-old diabetic monkey 13 (A–C) and a 24.3-year-old diabetic monkey (D). Capillaries lacking ADPase activity (arrow) were present in the superficial retinal vasculature (A, C) and in the deep capillary network (arrowheads), when visualized with focus in two planes. Arteriolar pruning (arrow) was apparent at the edge of this area of capillary dropout. Lead ADPase reaction product with dark-field illumination.
nondiabetic retinas (range, 587.6–676.6). This difference was not statistically significant ($P = 0.75$; Table 3). Even though some pericyte ghosts were observed in diabetic retinas (average, 1.7; range, 0–4.15 per 1000 endothelial cells), it did not result in a significant loss of pericytes in diabetic retinas (the ratio of pericytes to endothelial cells was 0.66 in diabetics and 0.64 in nondiabetic retinas).

**RGC Counts**

In sections, the ADPase reaction product was developed with ammonium sulfide to yield a brown lead sulfide reaction product visible with bright-field microscopy wherever ADPase activity was present. This technique allowed easy differentiation between viable and nonviable blood vessels. The sections were counterstained with thionin to stain cell nuclei blue, allowing for cell counts in the GCL.

RGCs were counted in two perfused retinal areas (nasal to disc and macular areas) in nondiabetic animals and in diabetic animals, with and without retinopathy. There was no significant difference in age between diabetic (26.4 ± 5.7 years) and nondiabetic (22.1 ± 6.8 years, $P = 0.226$) retinas and between diabetic retinas with retinopathy (27 ± 4.4 years) and those without retinopathy (25.6 ± 7.7 years, $P = 0.691$). In the nasal to optic disc area, RGC counts were 26.5 ± 9.8 in diabetic and 31.3 ± 4.3 in nondiabetic ($P = 0.363$) retinas. The RGC numbers in the nasal area were not influenced significantly by severity of diabetes, the presence of retinopathy or hypertension, although there was a trend toward reduction in diabetics with retinopathy compared with diabetic retinas in eyes with no retinopathy. Diabetic eyes with retinopathy had $22.2 ± 11.4$ RGCs in the nasal retina compared with $31.6 ± 4.3$ in diabetic eyes with no retinopathy ($P = 0.116$). The animals with nasal angiopathy had the lowest numbers of RGCs in the nasal retina.

In the macular area, the counts were $64.1 ± 34.2$ in diabetic and $89.3 ± 14.3$ in nondiabetic ($P = 0.187$) retinas. However, the difference was significant between diabetic eyes with retinopathy ($46.1 ± 30.1$) and diabetic eyes without retinopathy ($91 ± 19.6$, $P = 0.031$). Also, there was a significant difference between diabetic eyes with retinopathy and nondiabetic eyes ($P = 0.035$).

**Incidence of Retinopathy in Relation to Hypertension**

The incidence of diabetic retinopathy was significantly related to the presence of hypertension and severity of diabetes (Fig. 6). The diastolic blood pressure was significantly elevated in diabetic animals with retinopathy compared with nondiabetic animals ($P = 0.019$) but was not significantly elevated between

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**FIGURE 3.** IRMAs (A, B) and aneurysms (A, B, D) in 29.1-year-old monkey 9 with severe hypertension and diabetes for 6 years. Small aneurysms (arrow) were present in the vessels of an IRMA (A). (B) A small IRMA near the optic disk with aneurysms and arteriolar pruning (arrowhead). (C) Arteriolar pruning (arrowhead) was present at the edge of a large area of capillary loss. (D) A viable capillary from the lower area of the field shown in (C) that has small aneurysms (arrows). Lead ADPase reaction product with dark-field illumination.
FIGURE 4. (A) En bloc photograph of a large area of capillary dropout extending from the optic disc (✱) to fovea in diabetic monkey 9. An apparent cilioretinal vascular system (arrow) is still viable. At higher magnification (B), it is apparent that blood vessels temporal to the fovea were viable (have ADPase activity), whereas blood vessels central to fovea were not viable. A section through the fovea (C) of the retina in (A) showed fairly normal-appearing retina temporal to fovea (left), whereas retina nasal to the fovea (right) had outer atrophy, disruption, and loss of the
diabetic animals with no retinopathy and nondiabetic animals. The diastolic blood pressure was significantly elevated in the diabetic monkeys with retinopathy compared with diabetic animals without retinopathy \((P < 0.024)\), suggesting that hypertension exacerbates microvascular changes in the diabetic retina. The significance of the effect of hypertension on the incidence of diabetic retinopathy was investigated with exact logistic regression analysis. The odds ratio of 1.67 suggests that the risk of retinopathy is increased with hypertension and severe diabetes but the data are not significantly different because of low power, and the number of individuals is too small to perform further analysis. However, hypertension alone was not sufficient to cause the retinopathy we observed, because four hypertensive animals were included in our nondiabetic group (Table 1), and no retinal changes were observed in those monkeys.

There was a significant difference in the FPG between the diabetic group with retinopathy (mean FPG, 260 mg/dL) and the diabetic group with no retinopathy (mean FPG, 156 mg/dL; \(P = 0.0172\)). This suggests that severity of diabetes also was a risk factor for retinopathy.

**DISCUSSION**

Retinopathy in spontaneously type 2 diabetic monkeys shares many similarities with retinopathy in type 1 and 2 diabetic human subjects. We observed loss of viable capillaries and arteriolar pruning that preceded large areas of capillary loss. It has been documented that vascular basement membrane thickening occurs before other changes in retina in these monkeys. There was a significant difference in the FPG between the diabetic group with retinopathy (mean FPG, 260 mg/dL) and the diabetic group with no retinopathy (mean FPG, 156 mg/dL; \(P = 0.0172\)). This suggests that severity of diabetes also was a risk factor for retinopathy.

**FIGURE 5.** Large arteries near the optic disk. (A) The normal appearance of arteries near the optic disk is shown in nondiabetic monkey 1. (B) PAS staining of the artery wall was more intense in 29.8-year-old normotensive diabetic monkey 10, which had been diabetic for >7 years. (C) The characteristic onion-skin-like appearance of large artery walls was apparent in 27-year-old nondiabetic monkey 5 with severe hypertension. (D) A large artery in 31.5-year-old, severely diabetic and severely hypertensive diabetic monkey 14 had two domains: the onion-skin–like quality inner arterial wall and the extremely PAS positive, sclerotic outer artery wall (PAS and hematoxylin).

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**photoreceptor nuclei in the outer nuclear layer (arrow).** Higher magnification of retina temporal to fovea (D) demonstrated normal stratification of the retina and viable nuclei in both nuclear layers, but there was a paucity of ganglion cells. Inner retinal neuronal atrophy in a section nasal to fovea (E) demonstrated loss of ganglion cells and inner nuclear layer neurons, whereas many photoreceptor nuclei appeared viable. PAS-positive tubes in the inner retina (arrows) are the vestiges of blood vessels. (F) Area in macula of diabetic monkey 14 shows large central nonperfused region with serous-filled cysts in the outer retina (✱). Section from a large nonperfused area in monkey 9 (G) from an area adjacent to the area in (E) shows outer retinal atrophy (arrow), loss of photoreceptor nuclei, and disruption of the outer nuclear layer. (A, B) Lead ADPase reaction product with dark-field illumination; (C–G) sections stained with PAS and hematoxylin.
and IRMAs associated with areas of vascular dropout. In three animals, we observed histologically fluid-filled spaces in the retina that resembled the cystoid macular edema observed in human diabetic subjects. We did not observe preretinal neo-vascularization or macroaneurysms.

Retinopathy has been induced in monkeys using STZ, but has not been reported in spontaneously diabetic monkeys. Büchi et al. and Tso et al. divided the development of the microangiopathy into three stages in STZ-induced diabetic hypertensive monkeys. Early background retinopathy was characterized by IRMA and capillary dropout. In the second stage, exudative retinopathy was featured with massive vascular leakage, intraretinal exudates and hemorrhages, cystoid degeneration, and cotton-wool spots. In the final stage, chronic ischemic retinopathy was characterized by vascular occlusions and areas of retinal atrophy. In the studies of Büchi et al. and Tso et al., no clinical sign of diabetic retinopathy was detected in monkeys with spontaneous or STZ-induced diabetes for 4 to 15 years, if the monkeys were not hypertensive—a trend we observed in spontaneously diabetic animals.

One of the earliest changes we observed was cotton-wool spots that were usually near the optic nerve. As in humans, the lesion had cystoid bodies and axonal death was apparent. It has long been recognized that these lesions represent disruption of axoplasmic transport and are associated with a loss of vasculature. Dollery et al. observed cotton-wool spot-like lesions after occluding vascular segments. In the diabetic monkeys, there was a loss of capillaries around the lesions but it is not possible for us to say whether the adjacent loss in capillaries is a result of axonal transport disruption and not a cause of cotton-wool patch formation. In malignant hypertension, Hayreh et al. claimed that cotton-wool spots are due to occlusion of the terminal retinal arterioles, resulting in acute focal inner retinal ischemia.

Capillary loss was observed early in the monkey retinopathy as well. Subsequent arteriolar pruning appeared to precede the formation of large areas of vascular loss in the posterior pole. Tso et al. in streptozotocin-induced diabetic monkeys observed capillary dropout that was focal in the early phase of the disease. As the disease progressed, the retinal arterioles and arteries were occluded, resulting in the extensive atrophy of the retina in monkeys. The pattern of loss in the posterior pole of the spontaneously diabetic monkeys is noteworthy because the capillary network on the nasal side of the fovea is lost while capillaries on the temporal side of the fovea remain viable. In two of the four monkeys with this pattern of vascular loss, there was a cilioretinal vascular system on the temporal side of the disc that remained viable, whereas the surrounding vasculature was atrophic. Because this system originates from the ciliary artery and not the central retinal artery, the viability of the cilioretinal vascular system in the presence of complete loss of adjacent vessels may lie in the differences between the vascular sources. The central retinal artery and its first-order retinal vessels have high pressure and vascular resistance, whereas the unusual cilioretinal vasculature may have reduced resistance. Intravascular pressure may be elevated in the area between the vascular arcades and optic disc and macula. Hypertension provides additional intravascular pressure in diabetic retinas, providing an explanation for why seven of eight of the monkeys in which diabetic retinopathy developed also were hypertensive. It is noteworthy that Hatchell et al. observed a similar large central area of nonperfusion in pancreatectomized cats. Niki et al. observed capillary nonperfusion particularly within 1.5 to 2.5 disc diameters (DD) nasal to the optic disc, and this zone had a tendency for the nonperfused area to proceed rapidly to involve the farther periphery. The loss of capillaries is likely to have a cellular origin like polymorphonuclear neutrophils (PMN) plugging or endothelial cell dysfunction, whereas the large areas of vascular loss may have their origin in the hemodynamics of the retinal vascular system.

Table 3. Pericyte and Endothelial Cells in Retinal Capillaries

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Diabetes Duration (y)</th>
<th>Pericytes/1000 Endothelial Cells</th>
<th>Pericyte Ghosts/1000 Endothelial Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>13.6</td>
<td>No</td>
<td>655.2</td>
<td>—</td>
</tr>
<tr>
<td>18</td>
<td>20.6</td>
<td>No</td>
<td>587.6</td>
<td>—</td>
</tr>
<tr>
<td>19</td>
<td>21.3</td>
<td>No</td>
<td>676.6</td>
<td>—</td>
</tr>
<tr>
<td>20</td>
<td>24.2</td>
<td>4.5</td>
<td>581.8</td>
<td>4.2</td>
</tr>
<tr>
<td>21</td>
<td>25.9</td>
<td>1.0</td>
<td>753.5</td>
<td>—</td>
</tr>
<tr>
<td>22</td>
<td>26.8</td>
<td>8.2</td>
<td>655.5</td>
<td>0.8i</td>
</tr>
</tbody>
</table>

Microaneurysms

Microaneurysms, focal outpouchings of capillary walls in the region of vascular occlusions, are a clinicopathologic hallmark of human diabetic retinopathy. Stitt et al. studied human type 2 diabetic eyes by the trypsin digest technique and observed small saccular and fusiform microaneurysms in the peripheral retina. In the central retina, microaneurysms ranged in morphology from thin-walled, cellular forms to dense, acellular, hyalinized forms. Genuine microaneurysms, such as those present in the early phase of human diabetic retinopathy, were uncommon in the monkeys with STZ-induced diabetic with ischemic retinopathy and appeared only late in the disease. We observed aneurysms in spontaneously diabetic monkeys, but they were small and associated with areas of nonperfusion and IRMAs. The reason that aneurysms are small in the diabetic monkeys may be that pericyte loss is not significant, so expansion of the outpouching may be limited by the pericytes. Stitt et al. observed an extensive accumulation of PMNs in the lumen of microaneurysms in type 1 diabetes in which the endothelium remained intact, but pericytes were variably absent. We often observed PMNs in aneurysms.

Loss of Neurons in Diabetic Retina

Neuronal loss was apparent in both inner and outer nuclear layers in adjacent areas near the macula in cases of advanced retinopathy. The loss of photoreceptor nuclei may be related to the choriocapillaris insufficiency that we have documented in these monkeys. Axonal transport was disrupted early in diabetic retinopathy, causing cotton-wool spots. RGC loss occurred in diabetic monkeys with retinopathy. Ganglion cell death by apoptosis was observed in the KKAY mice with type 2 diabetes. Kawai et al. observed that the number of RGCs in the diabetic rats was not different from the control animals in the central and peripheral retinas. There was no significant RGC loss in monkeys, unless retinopathy was present in our study. Thus, the diabetic condition does not cause loss of RGCs. However, when diabetic rats underwent retinal ischemia–reperfusion, there was a small but significantly greater percentage of RGC loss, suggesting that diabetes causes increased susceptibility to ischemia–reperfusion injury. Barber et al. have observed neuronal death by apoptosis early in human and rat diabetes as well.

Hypertension and Diabetic Retinopathy

There was a significant correlation between the severity of retinopathy and hypertension in the monkeys with spontaneous type 2 diabetes. Tso et al. found that hyperglycemia alone was not sufficient to bring about retinopathy in young monkeys. They observed microangiopathic retinopathy in six monkeys with experimentally induced diabetes after the ap-
pearance of spontaneous or induced mild hypertension. They could see that the retinopathy had distinct ischemic features in STZ-induced and spontaneous diabetic animals with hypertension similar to the findings reported herein.\textsuperscript{16} However, these retinal findings were not characteristic of diabetic retinopathy in nonhypertensive monkeys, nor were they features of hypertensive retinopathy. There were four hypertensive nondiabetic monkeys in our study (Table 1) and none had pathologic changes in their retinal vasculature, suggesting that hypertension alone did not cause the retinopathic changes observed in hypertensive diabetic monkeys.

The findings in diabetic monkeys mirror the relationship between diabetic retinopathy and hypertension in humans. The risk of retinopathy in type 2 diabetes is strongly associated with elevated blood pressure.\textsuperscript{28} Tight blood pressure control in patients with hypertension and type 2 diabetes achieves a clinically significant reduction in retinopathy and risk of death.\textsuperscript{29} Moreover, hyperglycemia, hypertension, and obesity are determinants for retinopathy in a general population of humans\textsuperscript{30} as they were in the colony of monkeys at the University of Maryland. Diabetes is thought to contribute to development of hypertension and its complications, either directly by increasing capillary vascular resistance or indirectly by releasing proteolytic enzymes that act on the angiotensin system or free radicals that interfere with endothelium-derived relaxing factor.\textsuperscript{31,33}

Thickening and vacuolization of the basement membrane in retinal capillaries\textsuperscript{14} and large retinal arteries (Fig. 5) of the spontaneously diabetic monkeys may have been multifactorial, representing interactions and sequelae of diabetes, hypertension, and aging. Hayreh et al.\textsuperscript{21} produced malignant arterial hypertension in rhesus monkeys and observed focal intraretinal periaxial transudates, cotton-wool spots, and retinal hemorrhages as the usual retinal lesions associated with hypertensive retinopathy. Therefore, hypertension alone in monkeys can induce some of the changes observed in diabetic, hypertensive monkey retina.

**Differences between Human and Monkey Diabetic Retinopathy**

Several pathologic changes that occur in human diabetic retina were not observed in the monkeys. Davis et al.\textsuperscript{34} reported in the Airlie House Symposium on the Treatment of Diabetic Retinopathy that the occlusions occur first temporal to the macula, whereas, in our monkeys, the occlusions were pre-
dominantly nasal to fovea. Cogan and Kuwabara documented pericyte loss as an early change in human diabetic retina. However, there was no significant difference in the ratio of pericyte to endothelial cells between diabetic and nondiabetic monkeys. It is noteworthy that the pericyte-to-endothelial cell ratio in monkeys (0.64) was substantially lower than the 1:1 ratio observed by Cogan and Kuwabara in human retina. The aneurysms we observed were small and infrequent. Perhaps, having a normal number of pericytes prevented expansion or ballooning of the vessel wall, keeping aneurysms small and infrequent compared with human diabetic subjects. Because pericytes are the contractile cells of capillaries and venules, saccular aneurysms may never have formed because of the physical constraint of the pericytes. Kern and Engerman found a poor correlation between pericyte loss and formation of microaneurysms in the dog. Orlidge and D’Amore have demonstrated that pericytes produce TGF-β, which can inhibit endothelial cell proliferation. This cytokine may have prevented proliferation of the endothelial cell population in the aneurysms, thus limiting angiogenesis in IRMAs and even preventing preretinal neovascularization. Veins also have pericytes, and venous abnormalities were limited to a few venous loops, but no venous beading as observed in human subjects. Overall, monkeys had small aneurysms and IRMAs compared with humans, and no beaded veins, few venous loops, and no preretinal neovascularization. It seems contradictory to have large nonperfused areas and no preretinal neovascularization. One possibility is that endogenous inhibitors such as endostatin, angiostatin, and pigment epithelial-derived factor are higher in monkeys than in humans. Another possibility is that the angiogenic factors are low as well, perhaps due to rapid occlusion and death of vessels in the central retina (i.e., retina progressing from viable to necrotic rapidly, with limited periods of ischemia). These questions await further analysis of growth factors in this tissue and careful clinical documentation of the retinal vasculature after cataract surgery.

**Importance of Spontaneously Diabetic Monkeys**

The spontaneously diabetic monkeys are important, because they are the only animal model for diabetic retinopathy with a macula. Apparent macular edema was observed histologically, but fluorescein angiographic confirmation has not been achieved yet because of the presence of cataracts. If confirmed, these monkeys would be the only animal model for diabetic macular edema, the leading cause of blindness in patients with type 2 diabetes. The monkeys had many other changes in the retina that are characteristic of human diabetic retinopathy. There were some differences from human disease as well, such as small aneurysms and lack of preretinal neovascularization. Careful longitudinal clinical observations of these monkeys may shed light on the origins and progression of these pathologic structures and the duration and severity of diabetes essential for the manifestation of these ocular changes. Also, the monkeys live in a controlled environment, and their metabolic history is well documented, and so long-term relationships between retinopathic changes and metabolic status can be studied without the confounding lifestyle factors encountered in humans. The monkey also permits evaluation of hemorheologic and hemodynamic parameters that could contribute to the unique central nonperfused area observed in the diabetic monkey retina.

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**References**


