Intraretinal oxygen distribution in urethan-induced retinopathy in rats

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Yu, Dao-Yi, Valerie A. Alder, Stephen J. Cringle, Er-Ning Su, and Margaret Burns. Intraretinal oxygen distribution in urethan-induced retinopathy in rats. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H2009–H2017, 1998.—This study was performed to explore the interaction between chronic neural degeneration and the subsequent vascular remodeling. Weekly urethan administration in rats from birth produces a retinopathy model characterized by photoreceptor degeneration, retinal vascular regression, and retinal pigment epithelium (RPE) neovascularization. We investigated the hypothesis that altered oxygen distribution across the retina and choroid could be involved in the vascular changes seen in this retinopathy. We compared measurements of vitreal, intraretinal, and choroidal oxygen tension (PO2) distribution in anesthetized and ventilated control and urethan-treated rats at 8 and 16 wk of age with the use of oxygen-sensitive microelectrodes. Striking differences were observed in both choroidal and intraretinal PO2 distribution in urethan-treated rats compared with controls. At both ages, intrachoroidal PO2 was much lower in the urethan-treated rats. In addition, the intraretinal PO2 distribution across the inner retinal layers was more uniform than normal. A small elevation in PO2 was present at 8 wk in the inner nuclear layer where subsequent vascular regression occurred, and a small reduction in PO2 was present at the RPE, which recovered to normal values by 16 wk in regions where RPE vessel networks were first evident. Although the retinal arteries were considerably thinner at both ages in the urethan-treated rats, the vitreal PO2 profiles and superficial retinal PO2 remained normal. The unexpected and large reduction in the oxygen delivery from the choroid in the urethan-treated rats may account for the lack of major hyperoxia within the normally developing retina and the lower oxygen tension in the RPE before the vascular proliferation in this region. We propose that tissue PO2 is an important determinant of the vascular remodeling, which is seen in this model of neural degeneration and that the PO2 distribution changes described in this study help provide a new view of the pathogenesis of this model.

Oxygen tension; retina; urethan retinopathy; neovascularization; vessel regression; oxygen microelectrodes

THE INTERACTION among pathological neurons, oxygen metabolism, and the consequent long-term vascular remodeling in surrounding tissue is an important issue in many diseases, with the role played by local tissue oxygen tension in this remodeling remaining obscure. The well-characterized urethan-induced retinopathy is a useful example of such a model within the developing retina, where photoreceptor degeneration is believed to be the primary effect of urethan toxicity (5, 7–9) and may have relevance to retinal disorders such as retinoid pigmentosa, retinal dystrophies, and macular degeneration. As the photoreceptor cells degenerate they set in train a sequence of vascular modifications involving both vessel regression in the retina and neovascularization of the retinal pigment epithelium (RPE). We set out to test the hypothesis that changes in local retinal PO2 caused by photoreceptor degeneration drive the subsequent remodeling of the vasculature.

The neural and vascular tissue of the normal rat retina pass through their final developmental stages to reach the layered adult form during the first 2 wk of life. The avascular photoreceptor layer located in the outer retina forms approximately one-half of the retinal thickness, abuts onto the retinal pigment epithelial cells, and is predominantly supplied by the choroidal circulation, which lies outside the retina. The neurons in the inner retina further process the visual signal generated by the photoreceptors. This region is supplied by the retinal circulation, which originates from a central retinal artery at the disk, forming two or three capillary layers at different depths within the inner retina (9).

Photoreceptors are normally high consumers of oxygen (1, 6, 14), so their gradual degeneration can be assumed to significantly reduce oxygen demand of the outer retina. This may lead to hyperoxia within the remaining retina as the retina is “flooded” with oxygen from the lavish choroidal circulation. This belief has generated the hypothesis that the period of vessel regression, which begins in the deep retinal vascular bed, is triggered by retinal hyperoxia (9). Certainly there is strong circumstantial evidence that oxygen tension in the normally developing retina drives the development of the retinal vascular pattern (4, 10, 15). Although the sequence of anatomic changes to the developing retina in the urethan model has been definitively described, there are no direct complementary physiological measurements of retinal tissue PO2 to test these hypotheses.

The histological retinal changes in urethan retinopathy are well documented. Urethanol is given as 8 weekly subcutaneous doses from birth. By 8 wk there is a complete loss of the outer and inner segments of the photoreceptors and most of the rows of photoreceptor nuclei (the outer nuclear layer, ONL). Following this, by 12 wk, progressive degeneration of retinal vessels in the outer plexiform layer (OPL) begins. In regions where there is complete photoreceptor loss, the few remaining retinal vessels in the OPL are left apposed to the RPE, which can enfold surrounding these vessels (9). New vessels in the RPE (16) begin to form at some
retinal locations by 16 wk, by which time the retinal vessel regression has spread to vessels in the inner nuclear layer (INL). By 24 wk photoreceptor loss is virtually complete, retinal vessel regression still continues, and there are increasingly large numbers of new vessels present in the RPE. Thus neural degeneration, vessel regression, and neovascularization occur simultaneously in different retinal layers. Without the photoreceptor layer, the retina is considerably thinner, but at 16 wk the inner retinal layers appear morphologically normal (9). Measurements of vitreal, intraretinal, and choroidal PO2 at defined times and locations during the progress of the retinopathy are expected to reveal any relationship between vascular remodeling and local tissue PO2. The major questions addressed are threefold. First, does the relatively remote retinal circulation compensate for the degeneration occurring in the outer retina? To answer this question, we measured PO2 preretinally (in the vitreous) at retinal arterial, venous, and intervacular locations. Second, could retinal tissue hyperoxia be the stimulus for the subsequent retinal vessel regression, and third, does the oxygen level play a role in the later RPE neovascularization? To answer these questions, detailed intraretinal and vitreal PO2 profiles were measured in control and urethan-treated rats at two relevant stages and correlated with fundus appearance and retinal histology: These experiments utilized the techniques developed and reported for measurements of PO2 in rat and guinea pig eyes (3, 11, 12, 19–21).

Methods

Animal preparation and surgery. Standard urethan induction was performed in the laboratory of M. Burns in California. Sixteen Long-Evans rats were injected subcutaneously with urethan (ethyl carbamate, Aldrich Chemical), 1 mg/g body wt at birth and then at weekly intervals for 8 wk. Eleven rats were sham injected with phosphate-buffered saline. All animal procedures conformed to the Australian Code of Practice for the use of animals in medical research. The acute experiments were performed at 8 wk (6 urethan-treated and 5 control rats) and 16 wk (10 urethan-treated and 6 control rats).

For the acute experiments the rats were anesthetized with an intraperitoneal injection of 5-ethyl-5-(1’-methyl-propyl)-2-thiobarbiturate (Inactin, 100 mg/kg, Byk Gulden, Constance, Germany) and atropine sulfate (20 µg). Artificial ventilation was performed (air, 90 breaths/min, model 683, Harvard rodent respirator) with tidal volume determined by body weight. The femoral artery was cannulated for continuous arterial blood pressure and heart rate monitoring and periodic blood gas sampling (Corning pH/blood gas analyzer, 166 micro). Rectal temperature was maintained between 36.5 and 37.5°C using a homeothermic blanket (Harvard Apparatus). The rat was positioned prone in a modified Stellar stereotaxic instrument (model 51400, Stoelting), with the head clamped to the stereotaxic frame, and we immobilized the eye by suturing it to an eye ring. Pupils were dilated with 1% Mydriacyl (tropicamide, Alcon, Australia).

The lingual artery was cannulated in four of the 16-wk urethan-treated animals to allow a 50-µl saline bolus to be injected to determine the source of blood flow to the proliferative vessels at the RPE.

Manufacture and calibration of oxygen-sensitive microelectrodes. Recessed oxygen-sensitive microelectrodes (1-µm tip) were manufactured and calibrated in our own laboratory, as described previously (20). Typical calibration currents were 6–10 pA in air-equilibrated saline solutions. Microelectrodes were repeatedly tested to ensure that calibration remained stable during experimentation. The reference electrode was Ag/AgCl placed on subcutaneous tissue overlying the skull.

Oxygen measurements. The microelectrode was inserted into the rat eye through a small hole posterior to the limbus. A high-quality stereoscopic view of the fundus and the electrode was achieved by combining a planoconvex corneal contact lens with an operating microscope (Zeiss OPMI 1-FC). This enabled the observer to determine the relationship between the retina and the electrode tip and provided a clear view of both the retinal and choroidal vasculature by appropriate focussing. This permitted clear identification of three important retinal locations: where the microelectrode touched the internal limiting membrane (the vitreal surface of the retina), the retinal pigment epithelium, and the choroid. The retinal locations used for data collection were in the inferior retina 2 or 3 disk diameters from the optic disk margin. In the 16-wk urethan-treated rats where localized networks of vessels at the RPE were visualized, we deliberately aimed the microelectrode to record from these areas. Vitreal PO2 profiles were measured, withdrawing from (for a distance of 1,000 µm) and returning to a retinal artery, vein, and intervacular location. Intraretinal PO2 profiles were then measured in 10-µm steps from the retinal surface through to the deep choroid, and measurements were repeated during microelectrode withdrawal. We performed all measurements in photopic light conditions.

Histology. In the postacute experiment, both eyes were enucleated and used for histological studies to characterize the retinal structure for comparison with the oxygen measurements. The whole eyes were placed in 2.5% glutaraldehyde for 24 h. All histology presented is from the region in which microelectrode measurements were performed. The eyes were dehydrated in graded ethanols and embedded in paraffin for further dehydration. The whole eyes were placed in 2.5% glutaraldehyde for 24 h. All histology presented is from the region in which microelectrode measurements were performed. The eyes were dehydrated in graded ethanols and embedded in paraffin for further dehydration.

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Artery diameter is less than vein diameter. At 8 wk in the urethan-induced rats, the retinal arteries were narrowed, and the retina appeared thinner, bringing some larger choroidal vessels into focus, particularly in the region of the posterior pole (not shown).

By 16 wk (Fig. 1B) these urethan-induced fundus changes were more evident. The microelectrode (asterisk) penetrates the inferior retina. Retinal arteries were clearly narrowed. The retina was thinner bringing choroidal vessels (thick solid arrow) more in focus. The vascular annulus surrounding the optic disk (open arrow) was also more in focus extending out to ~1 disk diameter distance from the disk. A network of deep fine vessels at the RPE was also visible close to the microelectrode tip (thin arrow). These vessels are more evident in Fig. 1C (thin arrow), in which a blue filter was inserted into the light path. They were observed only at few retinal locations, and the microelectrode was specifically directed to record from these locations.

It was established that the blood flowing in this fine network of RPE vessels originated from the disk. This was demonstrated by observing the passage of an arterially delivered saline bolus through the ocular vasculature after retrograde injection into the lingual artery (18). This transparent bolus was clearly seen as it moved through choroidal and retinal circulations with choroidal blanching occurring before retinal blanching. The network of RPE vessels blanched simultaneously with the retinal circulation. To further explore this observation, we reduced blood flow in the ocular circulations in two animals using exsanguination to lower systemic blood pressure to <40 mmHg (20), thus slowing the blood flow. This procedure clearly demonstrated a functional connection between the disk vessels and network of fine vessels at the RPE, with individual red blood cells easily visualized as they passed from the disk vessels radially to the RPE vessels.

Histology. Retinal cross-sections are shown in Fig. 2 from control (Fig. 2A), 8-wk (Fig. 2B), and 16-wk (Fig. 2C) urethan-treated animals. The general retinal patholgy at 8 and 16 wk in the urethan-treated animals was similar to that reported previously for this model (9). The control retina (Fig. 2A) shows the clearly demarcated cell and plexiform layers, including the photoreceptor outer and inner segments (OS, IS), their nuclei (ONL), the outer plexiform layer (OPL), the inner nuclear layer (INL), the inner plexiform layer (IPL), and the nerve fiber and ganglion cell layer (NF/GC). Beyond the outer retina are the RPE layer,
Fig. 2. Retinal micrographs from control retina (A), urethan-treated retina at 8 wk (B), and urethan-treated retina at 16 wk (C). Locations of these micrographs are taken from a region very close to oxygen electrode recording location. A: note organized layered retinal structure in control retina, with choroid, retinal pigment epithelial cell layer (RPE), Bruch's membrane (BM), photoreceptor outer and inner segments (OS, IS), photoreceptor nuclei (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), and nerve fiber and ganglion cell layers (NF/GC). Note deep blood vessels in OPL (arrows) and larger vessel close to retinal surface. B: note loss of IS/OS and most of ONL. Remaining retina is approximately normal. Deep vessels in OPL are still present (arrows), although they appear larger. C: note almost complete loss of IS/OS and ONL, disrupted INL, and loss of ONL vessels, as well as new vessel formation at RPE (arrow). Magnifications: ×260 (A), ×315 (B), ×315 (C).
Bruch’s membrane (BM), and the choroid. The larger blood vessels at the retinal surface and the finer ones forming the deep capillary layer within the retina (arrows) are evident.

In urethan-induced rats at 8 wk (Fig. 2B), the retina is thinner than in the control animal mainly due to the loss of photoreceptors, with very few OS and IS and only two rows of photoreceptor nuclei remaining (ONL). The inner retinal layers and the RPE appear normal at this stage, although the deep retinal vessels (arrows) appear larger than in controls. At 16 wk (Fig. 2C) the retina is thinner and more disorganized. Almost all photoreceptors have been lost (ONL/IS/OS), and the INL is thinner and less ordered, whereas the IPL and NF/GC appear unchanged. The deep vessels are missing, and in this micrograph vessels (arrow) are visible within the RPE, which has thickened and extended out into the remaining inner retina in some locations.

Oxygen distribution. The preretinal oxygen distribution in the vitreous adjacent to retinal arteries, veins, and intervascular regions away from the visible retinal vessels was similar in the treated and control groups and not significantly different from that already published for Sprague-Dawley rats (3, 19). The mean values of the oxygen tension at the retinal surface adjacent to a retinal artery, vein, and intervascular location are shown in Table 1, along with a value representative of the vitreous (500 µm back from the retinal vein, where Po2 gradients are negligible). The data sets from the 8- and 16-wk controls are combined because there was no significant difference between them. Figure 3 compares average vitreal Po2 (±SE) as a function of distance from the retina (in µm) starting from arterial (circles), venous (triangles), and intervascular (squares) locations for control (Fig. 3A), 8-wk urethan (Fig. 3B) - and 16-wk urethan (Fig. 3C)-treated rats. In each case, Po2 falls with distance from the artery, whereas the profiles from the retinal vein and intervascular locations are relatively flat. There was no significant difference in the vitreal profiles from each group.

The intraretinal Po2 profiles however are very different in the urethan-treated and control groups. The profiles are presented as a function of track depth from the retinal/vitreal surface [internal limiting membrane (ILM)]. No correction is necessary for the slightly nonperpendicular angle of penetration because this angle was similar for all animals. Figures 4–6 present Po2 profiles from the control, 8-, and 16-wk urethan-treated animals, respectively. A single Po2 profile is shown (Fig. 4A) together with the average Po2 profile (Fig. 4B) for all control animals (n = 11). Again the control data for the 8- and 16-wk are combined because there are no significant differences between them. The control group (Fig. 4) has an intraretinal oxygen distribution very similar to that demonstrated previously in Sprague-Dawley rats (11, 20). The mean Po2 at the retinal surface (ILM) is 18.8 ± 0.9 mmHg (n = 11), which then falls to a minimum of 5.0 ± 1.3 mmHg (n = 11) between the superficial and deep capillary beds of the retina.

### Table 1. Vitreal Po2 values at 3 preretinal locations and in vitreous for control, 8-, and 16-wk urethan-treated rats

<table>
<thead>
<tr>
<th>Location</th>
<th>Control (n=11)</th>
<th>8 wk (n=6)</th>
<th>16 wk (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinal artery</td>
<td>38.56 ± 0.82</td>
<td>41.17 ± 2.72</td>
<td>38.21 ± 2.02</td>
</tr>
<tr>
<td>Intervascular location</td>
<td>21.32 ± 1.03</td>
<td>19.45 ± 1.54</td>
<td>20.86 ± 1.49</td>
</tr>
<tr>
<td>Retinal vein</td>
<td>19.33 ± 0.68</td>
<td>20.48 ± 2.37</td>
<td>20.96 ± 1.70</td>
</tr>
<tr>
<td>Vitreous*</td>
<td>21.50 ± 1.80</td>
<td>20.17 ± 1.23</td>
<td>20.63 ± 0.84</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. *Vitreous was 500 µm back from retinal vein. None of the values are significantly different (P > 0.05) among the 3 groups of rats.
before climbing again to a small peak 11.3 ± 2.2 mmHg (n = 11) at the level of the DB, 220 µm. The PO2 falls again on further penetration before rising steeply, passing through the RPE to a peak in the deep choroid 41.6 ± 0.9 mmHg (n = 11) at a depth of 420 µm (labeled as C in Fig. 4B).

Oxygen profiles from the urethan-treated animals at the 8-wk stage are shown in Fig. 5, A–C, with A representing a single PO2 profile, B showing individual PO2 profiles from each animal, and C giving the average PO2 profile (±SE), (DB), before climbing again to a small peak 11.3 ± 2.2 mmHg (n = 11) at the level of the DB, 220 µm. The PO2 falls again on further penetration before rising steeply, passing through the RPE to a peak in the deep choroid 41.6 ± 0.9 mmHg (n = 11) at a depth of 420 µm (labeled as C in Fig. 4B).

Profiles from the 16-wk urethan-treated group are shown in Fig. 6, A–C, with A representing a single PO2 profile, B showing individual PO2 profiles from each animal, and C giving the average PO2 profile (±SE).
respectively. It should be remembered that these profiles were recorded specifically from the few locations in the inferior retina where areas of RPE vessel networks were visible. Oxygen distribution is similar to that in the 8-wk group with the ILM $P_{O_2}$ being not significantly different ($18.7 \pm 0.3$ mmHg, $n = 10$) from controls and the 8-wk urethan-treated group, and a relatively flat $P_{O_2}$ profile in the reduced thickness retina ($166 \pm 3 \mu m$, $n = 10$). The peak $P_{O_2}$ now occurs not at the DB but at the RPE where $P_{O_2}$ is now not significantly different from control values. The peak $P_{O_2}$ values are more pronounced in this group, especially in some profiles, compared with the 8-wk urethan-treated group (compare Figs. 5, A and B, and 6, A and B); again these peaks are smoothed by the averaging process (Fig. 6C). In several instances, particularly those in which a large $P_{O_2}$ peak was observed, it was clear from visual observation that passage of the electrode through the localized vascular network caused transient "blanching" for a few minutes, presumably due to closure of the vessels. If, during this blanching time, the microelectrode was withdrawn and subsequently reinserted, $P_{O_2}$ profiles were devoid of the RPE peak in the deep retina with all oxygen tensions in this region reduced. This indicates that the vascular network is responsible for the $P_{O_2}$ peak. Choroidal $P_{O_2}$ distribution is dramatically different from control eyes, with a dip in $P_{O_2}$ during further penetration into the choroid to $7.3 \pm 0.8$ mmHg after which oxygen tension rises to $40.2 \pm 2.0$ ($n = 10$) at $320 \mu m$ (labeled C in Fig. 6).

**DISCUSSION**

This detailed study of intraretinal and vitreal oxygen distribution at two discrete stages of the urethan model of photoreceptor degeneration/vascular remodeling has extended and altered our understanding of retinal and choroidal events in this model. We have demonstrated dramatic changes in intraretinal and intrachoroidal oxygen distribution in the urethan-treated rats where retinal oxygen consumption is decreased, yet we found normal oxygen levels in the superficial retina and vitreous. The fact that the systemic parameters, blood pressure, and blood gases were not significantly different in the control and urethan-treated groups indicates an ocular origin for all differences between control and urethan-treated rats. The observed histology corroborated previous histological results described by Burns and Tyler (9).

At the 16-wk stage with neural degeneration, retinal vessel regression, and the appearance of localized vascular networks at the RPE evident, there was no evidence for major hyperoxia in the remaining retina. The major reason was that choroidal oxygen supply to the retina was eliminated (Fig. 6C) even though systemic $P_{O_2}$ input to the deep choroid (labeled as C) was normal. The choroid is clearly thicker than that in control rats, as measured by microelectrode track length (compare Figs. 4B and 6C).

Within the retina, at 16 wk there is a small peak in $P_{O_2}$ (Fig. 6C) at the RPE, which was confirmed to be due to the visible network of vessels, resulting in RPE $P_{O_2}$ values not significantly different from control values. The $P_{O_2}$ gradients adjacent to these new RPE vessels demonstrate that they supply oxygen to the adjacent choroid as well as to the immediate retina. The remaining retina has a flat $P_{O_2}$ distribution with values close to 20 mmHg and is 44% thinner than control retinas. The flat featureless intraretinal $P_{O_2}$ distribution clearly differs from that found in equivalent regions of control retinas where $P_{O_2}$ has a U-shaped distribution. This flat $P_{O_2}$ profile may be a consequence of reduced oxygen consumption of the remaining inner retina with a neural function that must be assumed abnormal,
considering the almost complete absence of visual input from the photoreceptors. This view is supported by the narrowed retinal arteries and by the regression of the many retinal capillaries, suggesting a compensated reduced oxygen delivery from this circulation despite a normal arteriovenous oxygen difference.

Thus at 16 wk there is no support for the original hypothesis that the retina is “flooded” with oxygen from the choroid.

At the earlier time of 8 wk, which is before both vessel regression in the deep vascular bed (DB) and the commencement of the formation of localized vascular networks at the RPE, the urethan-treated rat retina again showed dramatic choroidal Po2 distribution differences when compared with those in control rats. There is no delivery of oxygen from the choroid to the retina because the Po2 gradients are in the wrong direction. In fact the retina delivers oxygen to the choroid, with Po2 falling from the DB into the choroid. Po2 at the DB is significantly higher than in control retinas. The thinned remaining retina has a relatively flat Po2 profile with a mean Po2 close to 20 mmHg. What does differ between the 8- and 16-wk stages is the Po2 of the RPE, which is significantly lower at 8 wk than in control and 16-wk retinas.

Thus again there is no supportive evidence for major hyperoxia within the retina, although the small elevation of Po2 in the region of the DB may contribute to vessel regression. Mild RPE hypoxia is present and may be responsible for the later appearance of the RPE vascular networks, which return RPE Po2 to control values by 16 wk in these regions.

What is the origin of these localized networks of vessels at the RPE that are so clearly visible during the acute experiments? One interpretation is that they are the remnants of the deep capillary bed that is regressing and that they are now visible because they are dilated. We can definitively state from our experiments that the major blood flow to these vessels comes radially through vessels that originate at the disk. However, there has never been any histology to support such a direct supply to the deep capillary bed. Alternatively one could argue that these are the same neovascularized vessels that develop at the RPE as described in previous histological studies and that were also present in our histological data. It may be already suggested (9) that originally some of the RPE new vessels bud from the local retinal circulation, but later they must form functional anastomoses with vessels near the disk.

The cause of the dramatic changes to Po2 distribution in the choroid and its thickness is unknown. Currently, we can only speculate whether the failure of the choroid to supply oxygen to the retina is due to an increased choroidal oxygen consumption, to an increased choroidal oxygen diffusion barrier, or to a change in the way that choroidal blood flow and oxygen delivery occur. There is suggestive evidence that urethan can produce melanomas in the choroid of the rat (13), which could certainly account for a high oxygen consumption in the choroid. We found no clear evidence of melanoma cells, but there was evidence of choroidal stroma cell proliferation (Fig. 2). Alternatively, we could speculate that there is a direct shunting of choroidal blood flow from arteries to veins, bypassing the smaller vessels and the choriocapillaris. Previous light and electron microscopy studies, including the data from this study, have not reported major morphological changes in the choroid (9) or reported the presence of choroidal melanomas to account for this altered Po2 distribution in urethan-treated rats. However, most choroidal morphological changes we measure.

It may be significant that the oxygen tension in the retina measured in both groups of urethan-treated rats is very similar to that maintained in the innermost retinal region of the control animals. This implies that despite the major structural alterations occurring in the retinas of these urethan-treated animals and the consequent oxygen consumption and vascular changes, the retina adjusts to maintain a tissue Po2 close to 20 mmHg. This supports the notion of an oxygen-controlling system that senses innermost retinal Po2 and sets in motion a long-term feedback system to the retinal circulation, regulating superficial retinal Po2 in this developing and remodeling retina.

We have previously postulated such a Po2-controlling system in acute experiments in normal rat retina where blood pressure was manipulated over a wide range (20). We demonstrated that the inner retina acted to maintain inner retinal Po2 at close to 20 mmHg over a wide range of blood pressures. The same control of inner retinal Po2 has also been demonstrated in cat Po2 profiles in response to acute increases in intraocular pressure (2, 17). The relationship between the signaling processes that produce a short-term regulatory response to maintain inner retinal Po2 constant in the normal retina and the long-term developmental and pathological modifications of the retinal vasculature demonstrated in this study, which are also directed toward maintaining a Po2 of 20 mmHg, is an important issue for future study.

The absence of major retinal hyperoxia at 8 wk in the urethan-treated rats might naively be assumed to indicate that oxygen changes initiated by the degenerating photoreceptors do not trigger the subsequent vessel regression. However, it must be remembered that the measurements performed in this study were in steady-state conditions. It is possible that the stimulus-response mechanism to hyperoxic retinal tissue is very
sensitive to small PO₂ perturbations and that rapid and continuous adjustments are made to retain normal PO₂ values. Perhaps only when the physiological perturbations are too extreme to be compensated for does a failure of the PO₂-controlling feedback system result in abnormal PO₂ values in the retina. Thus the increased PO₂ values in the 8-wk urethan-treated retina at the DB may be sufficient to induce the subsequent vessel regression in this region, especially in a model in which development and neural degeneration are intermixed. We have previously observed a situation in which the retina could not immediately compensate when very low blood pressures were induced in the normal rat (20). If the retina is fully able to maintain inner retinal PO₂ at 20 mmHg in the urethan-treated rats, we can conclude that the events set in motion by the urethan induction are adequately compensated for over time by regression of the deeper capillary bed and new vessel formation at the RPE and by alterations in choroidal oxygen supply.

Could the neovascularization of the RPE be in response to local tissue hypoxia? Comparison of the RPE PO₂ in the 8-wk control group reveals that it is significantly lower than in the control group. This relative RPE hypoxia present at 8 wk is largely compensated by the neovascularized vessels at 16 wk, which increase the PO₂ to normal values. Thus RPE hypoxia may be the stimulus that induces RPE neovascularization.

In summary, we have provided the first measurements of vitreal, retinal, and choroidal PO₂ in this complex model where long-term vascular remodeling in response to gradual and continuous neural degeneration occurs. Normal development and pathology occur simultaneously, and long-term compensations are involved. We have shown that unexpected choroidal changes stop the retina from becoming significantly hypoxic as the photoreceptors degenerate and that the subsequent vascular remodeling may be in response to small changes in local tissue PO₂. Moreover, the tendency for inner retinal PO₂ to be maintained at ~20 mmHg implies the existence of an oxygen-regulating mechanism. These data may be relevant to other tissues where neural degeneration and simultaneous vascular remodeling occur.

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