The Effect of Regional Retinal Photocoagulation on Vitreal Oxygen Tension

Valerie A. Alder, Stephen J. Cringle, and Michael Brown

PO2 measurements have been made for the first time within the retina and vitreous of cat eyes to compare normal and photocoagulated areas. This was done to test the hypothesis that the observed beneficial effects of pan-retinal photocoagulation therapy in the treatment of retinal vascular diseases with an ischemic origin, may be due to more oxygen becoming available to the remaining functioning retina. A xenon arc photocoagulator was used to photocoagulate large areas of cat retinas served by one major set of vessels while leaving the remaining retina untouched. After 6 months an acute experiment was performed in which retinal and vitreal oxygen tension profiles were measured using oxygen-sensitive microelectrodes to compare PO2 profiles in normal and photocoagulated regions, for two ventilation conditions: air and 100% O2. The only differences in PO2 values were found for the 100% O2 breathing condition, where values within the retina and in the overlying vitreous were larger in photocoagulated areas. It is proposed that any differences in PO2 distribution which occur for air breathing are masked by the autoregulatory capacity of the retinal circulation and the PO2 buffering capacity of hemoglobin. Invest Ophthalmol Vis Sci 28:1078-1085, 1987

Retinal photocoagulation has been used since 1959 to treat diabetic retinopathy and other retinal vascular diseases which cause large areas of inner retina to become ischemic. Initially, focal photocoagulation of the new, leaky retinal vessels which grow into the ischemic inner retinal areas was used, in the hope of preventing the development of retinal edema and the subsequent retinal detachment. More recently, however, pan-retinal photocoagulation, either of peripheral retina or of large areas of ischemic retinal tissue, has been shown to be more effective, both in hindering the further progress of the retinopathy, and also in causing some remission of optic disk neovascularization. The effectiveness of photocoagulation has been shown to be independent of the type of photocoagulator used and appears primarily related to the area of retinal tissue ablated. Successful pan-retinal photocoagulation destroys or damages only the outer retina while leaving the inner retina histologically normal.

Diabetic retinopathy is a disorder of the retinal circulation which nourishes and drains only the inner retina. Why then should destruction of the outer retina by photocoagulation protect the diabetic retina from further damage? Increased availability of oxygen to the remaining functioning retina has been suggested as one possible explanation. It can be argued that in photocoagulated regions the consumption of oxygen is reduced in the damaged outer retina. This would allow some oxygen from the choroidal circulation to diffuse further into the inner retina, thus reducing the demand for oxygen from the vulnerable retinal circulation. Moreover, it is possible that the diffusion and solubility coefficients for oxygen in the retinal tissue are altered by photocoagulation in such a way that the diffusion range of choroidal oxygen is increased.

There have been four other studies to determine the effect of retinal photocoagulation on retinal and vitreal oxygenation; they present apparently conflicting results. Stefansson, Landers et al and Diddie and Ernests, using monkeys, made point measurements of PO2 close to the retina and found no difference in air breathing between normal and photocoagulated regions. In contrast, the results of Molnar et al show that for miniature pigs there is a greater vitreal PO2 in photocoagulated regions than in normal regions.

In order to resolve this question we have measured for the first time PO2 profiles within the retina and also in the vitreous of pan-retinal photocoagulated cat eyes in order to determine whether more oxygen is available to the remaining normal retina. Experiments have been performed for two conditions of...
ventilation, air and 100% O₂, in an attempt to control for the masking effect of retinal circulation autoregulation.

Materials and Methods

Pilot Study

In order to ascertain the optimum settings for intensity, time and aperture size for the Xenon Photocoagulator which would produce burns equivalent to those produced in human pan-retinal photocoagulation, a pilot study was necessary.

Four cats were exposed to a variety of photocoagulation burns of the retina of varying intensities, times and aperture sizes. Individual photocoagulation burns were performed at different times so that each retina carried a time sequence of burns. Frequent fundus photography provided documentation of the time course followed by the lesions before stabilization occurred. These cats were subsequently killed by anaesthetic overdose. Light microscopy of the retina in photocoagulated areas was performed in order to trace the photocoagulator settings required to mimic human photocoagulator lesions. An example of this is shown in the retinal cross section of Figure 1, which demonstrates that in the photocoagulated region the outer retina is destroyed or disorganized while the inner retina appears undamaged. Three months appeared to be more than adequate for the damaged retina to stabilize morphologically.

Pan-Retinal Photocoagulation

Eight adult domestic cats weighing 2.5 to 5.5 kg were used. Anaesthesia for restraint was induced by an intravenous injection of 2 ml Saffan (alphadalone acetate 3 mg/ml, alphaxalone 9 mg/ml (Glaxo Australia, Boronia, Victoria, Australia). The pupil of the right eye was dilated with Mydriacyl (tropicamide 1%) (Alcon [Australia], Brookdale, New South Wales, Australia) eye drops and the nictitating membrane retracted with topical Neo-Synephrine (phenylephrine HCl 10%) (Sterling Pharmaceuticals, Sydney, New South Wales, Australia). A xenon arc photocoagulator (O'Malley Clinitex LOG2, Clinitex Inc., Danvers, MA) was used to produce up to 300 photocoagulator burns within a wedge-shaped area supplied by one retinal artery.
Most of the lesions were within 30° of the optic disk, as more peripheral areas were difficult to damage in a reproducible fashion. This meant that a large proportion (but not all) of the area supplied by one artery was photocoagulated. The progress of the photocoagulated area was documented by fundus photography. An acute experiment was performed on the photocoagulated eye at least 6 months after photocoagulation.

**Acute Experiment**

**General surgery:** Anaesthesia was induced as detailed above. The trachea was cannulated, the vago-sympathetic trunk was severed bilaterally to reduce eye movements, and the femoral artery was cannulated to allow the continuous monitoring of blood pressure (Hewlett Packard transducer and monitor 78205B, Doncaster East, Victoria, Australia) and the discrete sampling of arterial blood gases (Corning blood gas analyser 166 micro). After an initial dose of 2 ml Flaxedil (gallamine triethiodide 40 mg/ml) to achieve paralysis, the cats were ventilated with either 21% O2 and 79% N2 or 100% O2. Long term stable anaesthesia and paralysis were achieved with an intravenous infusion through the cephalic vein of Flaxedil (May & Baker Australia, West Footscray, Victoria, Australia) (5 mg/kg/hr) and Saffan (2 ml/hr.)

Rectal temperature was maintained at 38°C and the heart rate and pupil size of the unoperated eye were monitored continuously. Experiments lasted up to 14 hr, after which the cat was killed with a barbiturate overdose and the eyes removed for histological preparation.

**Ocular surgery and microelectrode placement:** After dilating the pupil and retracting the nictitating membrane, a gas-permeable hard contact lens of zero power was placed on the cornea for protection. The conjunctiva was removed from the temporal sclera, which was then punctured with a hypodermic needle through the pars plana. This hypodermic needle was connected to a hydraulic microdrive system so that the oxygen-sensitive microelectrode could be inserted through the needle to enter the vitreous at the chosen location.

Microelectrode manufacture, response and calibration: Membranised oxygen-sensitive microelectrodes of tip size 1–5 μm were constructed and calibrated in saline as described in detail by Alder and Cringle. As oxygen is reduced at the microelectrode surface a current is produced which is proportional to the oxygen tension of the region surrounding the microelectrode tip. Most PO2 values are stated in terms of this microelectrode current rather than as absolute PO2 values, because the membrane of the microelectrode does not set up a sufficiently large diffusion barrier to oxygen to render the electrode totally insensitive to alterations in the diffusion and solubility coefficient of the medium in which it is measuring. In a few cases the microelectrode was also calibrated in aspirated vitreous so that absolute PO2 values could be determined. However, even this calibration may not be accurate for intraretinal measurements.

**Experimental Regime**

We have already established that considerable PO2 gradients exist in the vitreous for distances of up to 1000 μm from the internal limiting membrane. The exact form of these gradients is determined by the local vascular geometry in the region. This means that point measurements of PO2 close to the retina are of limited value. Therefore, in this study PO2 profiles were measured, initially in 10-μm steps and later in 50-μm and 500-μm steps, as the microelectrode was progressively withdrawn from just touching the internal limiting membrane into "mid vitreous," where the PO2 gradient is very shallow. These PO2 profiles were measured commencing at retinal arterioles, veins, and intermediate locations (ones in which no retinal vessels were visible with an ophthalmoscope) in both the normal retinal areas and in those areas which had been photocoagulated. For all profiles the microelectrode orientation was chosen to be as perpendicular to the retina as possible. Histology showed the retina to be thinner in photocoagulated areas. However, the profile technique, whereby the zero position is set by the microelectrode contacting the internal limiting membrane before the PO2 values are measured as a function of distance from the internal limiting membrane, made comparison between normal and photocoagulated regions valid. In two animals the considerably more difficult task of entering the retina with the microelectrode and measuring the intraretinal PO2 as a function of distance through the retina was achieved. Only profiles which gave reproducible results on withdrawing the microelectrode through the retina were used, as these were assumed to have caused no tissue damage. Measurements were performed at two ventilation conditions, an air-equivalent composition of nitrogen and oxygen, and pure oxygen.

The stability of the microelectrode during the long recording time was checked by frequent removal from the eye and recalibration. The physiological stability of the preparation was confirmed by a frequently checked, stable "mid vitreous" PO2 value. Arterial blood gases were sampled at discrete intervals during the course of the experiment. Vitreal PO2, blood pressure, rectal temperature, arterial PO2, arte-
Fig. 2. Vitreal oxygen tension profiles measured from a retinal artery (circles), vein (triangles) and intermediate region (squares) for a normal (full lines) and photocoagulated area of retina (dashed lines), with the cat respired on an air-equivalent gas mixture. The oxygen tension current is given in nA and distances are measured in μm from the internal limiting membrane.

Results

Vitreal Profiles

In Figure 2 the PO₂ vitreal profiles from an artery (circles), vein (triangles), and intermediate region (squares) are compared for a normal retinal region (full line) and a photocoagulated retinal region (dashed line), with the animal ventilated on an air equivalent gas mixture. Although there are differences between the two sets of profiles within 600 μm of the retina, the values are remarkably similar for distances further than that. The differences between the photocoagulated and normal PO₂ values close to the retina are a consequence of the local vascular topography at the individual recording locations, and are therefore of little interest for this investigation. In no case were there significant differences in the PO₂ values at distances further than 600 μm from the retina with air breathing. In this case the photocoagulation was performed on the temporal retina and normal retinal recordings were measured in the nasal retina. Other experiments in which the nasal retina received the photocoagulation gave equivalent results.

A similarly obtained set of PO₂ profiles for 100% O₂ breathing are shown in Figure 3. In this case two features of the results are evident: there is marked variation between the normal (full line) and photocoagulated (dashed line) PO₂ profiles for artery (circles), vein (triangles), and intermediate (squares) locations for distances of up to 500 μm from the retina, again caused by the differences in local vascular topography. However, at 100% O₂ the plateau value that all curves asymptote to at distances of greater than 100 μm from the retina is higher for the photocoagulated area than the normal area.

This difference, which always occurred for 100% O₂ ventilation, is displayed more clearly in the data of Figure 4, where two profiles are compared over a longer vitreal distance from intermediate retinal locations in the normal (full line) and photocoagulated (dashed line) area. It can be seen that this difference in plateau value extends out to 8000 μm and only in “mid vitreous,” which is loosely defined to be of the order of 10,000 μm away from the retina, do the PO₂ values approach each other.

The one reproducible difference which was evident close to the retinal surface was for intermediate locations at 100% O₂ breathing, where the PO₂ in the center of a photocoagulation burn was often markedly higher than in a normal intermediate location. This effect is demonstrated in Figure 4 but is supported statistically by the data of Table 1. This data summarizes the results of measurements made at the internal limiting membrane for those few experiments where absolute calibrations of the microelectrode were obtained in vitreous. This allowed the absolute PO₂ values of the starting point of the profiles.

arterial PCO₂ and arterial pH were recorded on a chart recorder.

Experiments were performed in unchanging photopic luminance conditions. Complete sets of data were obtained from four cats. Only results from these experiments were used in the analysis.

At the completion of the acute experiment, the animal was killed with a barbiturate overdose and the eyes removed for histology. All experiments performed in this investigation conformed to the ARVO Resolution on the Use of Animals in Research.
close to the retina to be calculated in mmHg. These data show that the only significant difference (at the 5% level) between normal and photocoagulated PO$_2$ values close to the internal limiting membrane is for the case of the intermediate location at 100% O$_2$ ventilation where PO$_2$ values were higher.

Intraretinal Profiles

When recording intraretinal PO$_2$ profiles, it is important to be sure that the microelectrode properties remain unchanged, that the retina is undamaged and the physiological state of the eye remains constant. This means that satisfactory profiles are only rarely obtained. Even more difficult is the attempt to compare profiles in different retinal regions. We therefore measured three kinds of profiles, all at intermediate locations. The first was in a normal area of retina, the second in the center of a photocoagulation burn and the third in an apparently "normal" area of retina adjacent to a photocoagulated region. The latter "normal" region close to a burn was chosen to facilitate stability testing. As we have already demon-

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**Fig. 3.** Vitreal oxygen tension profiles measured from a retinal artery (circles), vein (triangles) and intermediate region (squares) for normal (full lines) and photocoagulated (dashed lines) retinal areas with the cat respired on 100% O$_2$. The oxygen tension current is given in nA and distances are measured in $\mu$m from the internal limiting membrane.

**Fig. 4.** A long vitreal oxygen tension profile measured from intermediate regions in normal (full line) and photocoagulated (dashed line) areas of the retina with the cat respired on 100% O$_2$. The oxygen tension current is given in nA and the distances are measured in $\mu$m from the internal limiting membrane.
strated, the PO₂ in the preretinal vitreous in front of a photocoagulated region is higher than in a normal region, whereas the preretinal PO₂ in the photocoagulated and "normal" regions are the same. This means that frequent checks could be made for stability of vitreal PO₂ and electrode for the "normal"/photocoagulated combination, whereas this is not possible for the normal/photocoagulated combination. Although we recorded intraretinal profiles that we were satisfied with in all three locations, in no case were we successful in recording the normal and photocoagulated regions sequentially so that a quantitative comparison could be made. We did, however, successfully record photocoagulated and "normal" profiles regions for quantitative comparison. As there was no qualitative difference apparent between the profiles measured within normal and "normal" retina, we have chosen to present the latter profiles in Figure 5. Zero on the abscissa of Figure 5 corresponds to the internal limiting membrane, negative values are measured within the retina and positive values are measured in vitreous. The "normal" retinal profile shows a fairly flat minimum within the inner retina before PO₂ values rise as the electrode passes through the outer retina and reaches the choroidal circulation. The retinal profile measured in the center of a photocoagulated region, however, displays a gradually increasing gradient with distance through the retina before entering the choriocapillaris at the same PO₂ as in the normal profile. The PO₂ value at the internal limiting membrane in the photocoagulated region is more than twice that measured in the normal region (cf Table 1).

Table 1. Comparison of PO₂ values measured immediately adjacent to the internal limiting membrane for normal and photocoagulated retinal regions for two ventilation conditions

<table>
<thead>
<tr>
<th>Position</th>
<th>Normal</th>
<th>Photocoagulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artery 20%</td>
<td>84 ± 27, N = 6</td>
<td>84 ± 19, N = 5*</td>
</tr>
<tr>
<td>Artery 100%</td>
<td>256 ± 108, N = 5</td>
<td>206 ± 98, N = 6*</td>
</tr>
<tr>
<td>Vein 20%</td>
<td>49 ± 15, N = 15</td>
<td>42 ± 7, N = 7*</td>
</tr>
<tr>
<td>Vein 100%</td>
<td>76 ± 18, N = 5</td>
<td>71 ± 49, N = 4*</td>
</tr>
<tr>
<td>Int 20%</td>
<td>36 ± 21, N = 6</td>
<td>55 ± 34, N = 5*</td>
</tr>
<tr>
<td>Int 100%</td>
<td>44 ± 31, N = 8</td>
<td>191 ± 80, N = 8†</td>
</tr>
</tbody>
</table>

* Not significant at the 5% level.
† Significant at the 1% level.

Discussion

Results show that photocoagulation causes an increase in both retinal and vitreal PO₂ values for 100% oxygen breathing (Figs. 3, 4, 5). The outer retina receives its oxygen supply by diffusion from the choroidal circulation.17 Because of the high choroidal blood flow, the arteriovenous oxygen difference is small, indicating a high choriocapillaris oxygen pressure driving oxygen into the avascular outer retina. The form of the PO₂ retinal profile in the avascular region depends on the variation of three parameters with distance within the retina: the oxygen consumption (C), the oxygen diffusion coefficient (D), and the oxygen solubility coefficient (S).18 It can be shown mathematically that an increase in PO₂ values in the outer retina will occur if C/DS decreases. Thus our intraretinal data provides evidence for either a decreased oxygen consumption, and/or an increased
oxygen diffusion or solubility coefficient as a consequence of photocoagulation. This is to be expected if the outer retinal cells are not functioning, so that oxygen consumption is less than in a normal area. The fact that there is a broad minimum in the "normal" profile at about −150 μm implies that in this situation the retinal circulation is still contributing to inner retinal oxygenation. There is no evidence for this in the photocoagulated region profile where the PO2 falls smoothly to the internal limiting membrane. Thus we can conclude that for 100% O2 breathing, where one would expect that the retinal circulation autoregulates to reduce flow, the inner retina is supplied by oxygen from the retinal circulation in the "normal" regions and by choroidal oxygen in photocoagulated regions. In a photocoagulated eye, therefore, the retinal circulation would only offload oxygen in normal retinal regions. This would mean that more oxygen would be available to the normal areas.

It might be expected that the failure of the retinal circulation to offload oxygen in the photocoagulated region would be reflected in a reduced arteriovenous oxygen difference. This is not borne out by the data in Table 1, possibly due to the fact that only a fraction of the area served by an artery and vein pair was photocoagulated.

The increased retinal PO2 which occurs as a consequence of photocoagulation is reflected by raised vitreal PO2 values. They are evident close to the retina in intermediate profiles (Fig. 4, Table 1), and the effect extends some 8000 μm into the vitreous (Fig. 4), which means that an extensive volume of vitreous has a raised PO2.

In contrast, for air breathing, any decrease in C/DS caused by the photocoagulation is not reflected in higher vitreal PO2 values (Figs. 2, 3). The PO2 of the bulk of the vitreous is unaffected by the photocoagulation. The only observed differences in PO2 appear within the band of vitreous 500 μm-wide which lies adjacent to the internal limiting membrane. These are due to the considerable oxygen tension gradients which have been shown to exist close to the retina because of the local effect of the superficial retinal circulation. As the actual PO2 currents measured at any one location depend on these gradients and also on the particular spatial integrating properties of the individual microelectrode used to make the measurements, considerable variation is expected to exist between readings made from one artery to another, one vein to another and one intermediate location to another, although the overall shape of the curves from one location type is similar.

It is presumed that the raised retinal tissue PO2 values which occur as a consequence of the photocoagulation cause the retinal circulation to autoregulate, thus returning inner retinal PO2 values to normal. Therefore, in this case the effects of photocoagulation are masked by recording only in the vitreous and not in the retina. The considerable PO2 buffering capabilities of hemoglobin would also come into effect for air breathing, which may also mask any differences in oxygen availability between the normal and photocoagulated regions of the retina.

Our results for air breathing are therefore essentially in agreement with those of Stefansson and Landers et al for cat and monkey10-12 and Diddie and Ernest for monkey,13 although they all used point measurements close to the retina. Our results disagree with those of Molner et al14 who measured vitreal PO2 profiles in miniature pig 2–3 weeks after photocoagulation when, as they stated, their retinas were still edematous, and found a higher PO2 close to the retina in photocoagulated areas. Diddie and Ernest13 also mentioned that they observed larger preretinal values for PO2 immediately after photocoagulation. There may thus be a time course of change in parameters within the retina after photocoagulation which is responsible for the disagreement between the results from the different studies. The measurements of Molner et al were made 2–3 weeks post-photocoagulation when the retina was still edematous, whereas our measurements were made after 6 months when retinal stability had occurred, as determined histologically. Thus, one must postulate that after photocoagulation during the preequilibrium period an increased vitreous oxygenation is detectable for air breathing conditions. After the retina has reached equilibrium, measurements made in the vitreous are unable to detect any difference in retinal oxygen consumption or distribution. However, if measurements are made at 100% O2 ventilation, the different oxygen distribution in the retina, which we have shown occurs after photocoagulation, is reflected in the vitreal oxygen values (Figs. 3, 4, 5).

What relevance do these results have for the clinical cases of retinal vascular diseases such as diabetic retinopathy, where pan-retinal photocoagulation is usually employed? It could be argued that as patients naturally breathe air, there is no evidence to suggest that extra oxygen is available to the remaining functional retina after photocoagulation. However, the increase in PO2 at 100% O2 demonstrates that the altered oxygen distribution in the retina is detectable in the vitreous if the masking effects of retinal circulation autoregulation are avoided. Kohnen19 has shown that in the early stages of diabetic retinopathy the retina is still able to autoregulate and that dilatation of the retinal circulation occurs because of the progressive tissue ischemia. The autoregulatory con-
strictive effect of photocoagulation will increase the dilatation capacity of the circulation supplying the photocoagulated area. This may benefit adjacent, still-functioning retinal areas. It has been suggested\(^1\) that in the later stages of diabetic retinopathy the autoregulatory capacity of the retinal circulation is lost. Photocoagulation in this situation would definitely cause raised \(\text{PO}_2\) values in vitreous which would benefit ischemic areas.

In summary, we have shown that photocoagulation increases the range of diffusion for choroidal oxygen in the retina. Whether this is due to reduced consumption of oxygen by the outer retina or due to alterations in the diffusion or solubility coefficients is unknown. If the retinal circulation is capable of autoregulation, this increased flow of oxygen is not reflected in the vitreal \(\text{PO}_2\) profiles.

**Key words:** photocoagulation, retina, oxygen tension, vitreous, oxygen microelectrodes, cat

### References