Experimental Retinal Vein Occlusion: Effect of Acetazolamide and Carbogen (95% O₂/5% CO₂) on Preretinal PO₂

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**PURPOSE.** To evaluate the variations of preretinal oxygen partial pressure (PO₂) in normal and in ischemic postexperimental branch retinal vein occlusion (BRVO) areas, during normoxia, hyperoxia (100% O₂), and carbogen (95% O₂, 5% CO₂) breathing before and after intravenous injection of acetazolamide.

**METHODS.** Preretinal PO₂ measurements were obtained in intervascular retinal areas, distant from the retinal vessels of 13 anesthetized mini-pigs with oxygen-sensitive microelectrodes (<10 μm tip diameter) introduced through the vitreous cavity by a micromanipulator. The microelectrode tip was placed <50 μm from the vitreoretinal interface in the preretinal vitreous. PO₂ was measured continuously for 10 minutes under systemic normoxia, hyperoxia, and carbogen breathing. A BRVO was induced with an argon green laser, and oxygen measurements were repeated under normoxia, hyperoxia, and carbogen breathing, before and after intravenous injection of acetazolamide (500 mg bolus).

**RESULTS.** In hyperoxia, a moderate nonsignificant preretinal PO₂ increase in both normal (∆PO₂ = 2.20 ± 4.16 mm Hg; n = 25) and ischemic retinas (∆PO₂ = 4.30 ± 3.57 mm Hg; n = 16) was measured in spite of a substantial increase in systemic PaO₂. Carbogen breathing induced a significant increase in systemic PaO₂ and a higher systemic PaO₂ than hyperoxia. Furthermore, it significantly increased the preretinal PO₂ in normal areas (∆PO₂ = 19.57 ± 16.41 mm Hg; n = 26), and in ischemic areas (∆PO₂ = 14.94 ± 8.53 mm Hg; n = 14). Intravenous acetazolamide did not affect the preretinal PO₂. Acetazolamide induced an increase of the preretinal PO₂, to a greater extent when it was associated with carbogen breathing (∆PO₂ = 15.15 ± 9.15 mm Hg; n = 7) than when it was combined with hyperoxia (∆PO₂ = 6.96 ± 4.49 mm Hg; n = 7).

**CONCLUSIONS.** Carbogen breathing significantly increased preretinal PO₂ in normal and in ischemic postexperimental BRVO areas of mini-pigs. The concomitant use of acetazolamide injection and carbogen breathing or hyperoxia could restore an appropriate oxygenation of BRVO areas. (Invest Ophthalmol Vis Sci. 2004;45:3669–3677) DOI:10.1167/iovs.04-0086

Branch retinal vein occlusion (BRVO) is the second most common retinal vascular disease leading to visual loss in developed countries, the most frequent cause being diabetic retinopathy. Patients in the fifth and sixth decade of life are most usually affected, and only 5% of the patients are younger than 45.³

The hemodynamic modifications on the vasculature of the affected areas in acute BRVO include venous vasodilation, as well as the reduction of arteriolar blood flow.²⁻⁴ Visual acuity is often decreased due to the development of intraretinal hemorrhages, macular edema, capillary nonperfusion, and vitreous hemorrhage secondary to retinal neovascularization. Retinal neovascularization appears in approximately 25%,⁵ while persistent macular edema affects almost 60% of patients with BRVO.⁶⁻⁷

Therefore, both the physiopathogenic mechanisms and the various treatment modalities of BRVO are important, having been in the center of clinical and experimental research. In BRVO, venous stasis induces changes in the blood-retinal barrier⁴⁻¹⁰ and leads to extravasation and formation of extracellular retinal edema and hemorrhages.

Arteriolar vasoconstriction, which settles in the hours after the occlusion, occurs as a result of either changes in retinal metabolism, or reduction of nitric oxide (NO) release,¹¹ which plays a major role in retinal arteriolar tone,¹² or myogenic vasoconstriction secondary to the intravascular pressure increase in the affected vascular bed. Reduction of arteriolar blood flow leads to tissue hypoxia in the inner retinal layers,¹³ Na/K-ATPase pump dysfunction, formation of intracellular retinal edema, and neuronal cell destruction by necrosis and apoptosis.

Current treatment of acute BRVO aims to restore venous circulation. Isovolemic hemodilution,¹⁴,¹⁵ that leads to an increase in ocular blood flow¹⁶ and regression of tissue hypoxia,¹⁷ and troxerutine,¹⁸ an erythrocyte antiaggregant, constitute currently available modalities. Their efficacy to limit visual loss has been demonstrated with varying success by randomized trials.¹⁴,¹⁵,¹⁸ Grid photocoagulation improves visual prognosis in eyes with macular edema after BRVO and decreases the risk of neovascularization and vitreous hemorrhage in eyes with ischemic retinal areas larger than five disc diameters.¹⁹,²⁰

Pilot studies have evaluated the efficacy of fibrinolytic treatment with tissue plasminogen activator delivered by intravitreal injection,²¹ retinal vein intravascular injection,²² intravenous systemic injection,²³ or superselective ophthalmic artery catheterization.²⁴ Surgical decompression and separation of the artery and the vein by adventicectomy is an interesting approach currently under evaluation.²⁵

An alternative treatment aims to restore tissue normoxia by inhalation of 100% O₂ or carbogen (95% O₂, 5% CO₂). The systemic hypoxemia, thus induced, could effectively increase the oxygen partial pressure (PO₂) of the inner retina through diffusion of oxygen from the choroid.²⁶ Systemic hypoxemia increases the inner retinal PO₂ to normal in retinal areas with venous stasis retinopathy as presented 48 hours after an ex-
Experimental BRVO in mini-pigs.\textsuperscript{13} Carboxen, through CO\textsubscript{2}-induced\textsuperscript{27} retinal arteriolar vasodilatation, might potentially be effective in increasing the diffusion of oxygen and the normalization of PO\textsubscript{2} in the inner retina. Previous studies, and our preliminary results previously published,\textsuperscript{28} provided data showing that breathing carboxen induced higher preretal PO\textsubscript{2} than 100% oxygen.\textsuperscript{29,30} Preretal PO\textsubscript{2} reflects oxygen diffusing from the retinal circulation.\textsuperscript{31} Furthermore, the addition of intravenous administration of acetazolamide increases the PO\textsubscript{2} over the optic disc in domestic pigs\textsuperscript{32} through an increase in systemic CO\textsubscript{2}.\textsuperscript{33} This effect should probably enhance the capability of hyperoxia and carboxen to induce an increase in preretal PO\textsubscript{2}.

The aim of this study was to evaluate the variations of preretal PO\textsubscript{2}, in normal and in ischemic post-BRVO areas during normoxia, hyperoxia, or carboxen breathing, before and after acetazolamide administration.

\textbf{MATERIAL AND METHODS}

Experiments were performed on one eye of 13 miniature pigs (10 to 12 kg; Arare Animal Facility, Geneva, Switzerland), whose retinas closely resemble human retina in both neuroanatomic and vascular aspects.\textsuperscript{34,35} All experiments were conducted in compliance with the ARVO Statement on the Use of Animals in Research.

\textbf{Animal Preparation}

Mini-pigs were prepared for experiments as previously described.\textsuperscript{31} In brief, after intramuscular injection of 3 mL azaperone (Stresnil, 5 mg; Janssen Pharmaceutica, Beersse, Belgium), 2 mL of the tranquilizer midazolam maleate (Dormicum; Roche Pharma, Reinach, Switzerland; 10 mg) and 1 mL (0.5 mg) atropine, anesthesia was induced with 2-3 mg sodium thiopental (Pentothal; Abbott AG, Baar, Switzerland) injected into the ear vein. After arterial, venous, and bladder catheterization, the animal was curarized with 4 mg pancuronium bromide (Pavulon; Organon SA, Paffikon, Switzerland), intubated, and artificially ventilated. During the experiment, anesthesia and myorelaxation were maintained by continuous perfusion of Pentothal and Pavulon, respectively.

Each animal was ventilated at approximately 18 strokes/min, with a continuous flow of 20% O\textsubscript{2} and 80% N\textsubscript{2}O, using a variable volume respirator. Systolic and diastolic blood pressures were monitored via the femoral artery using a transducer. PaCO\textsubscript{2}, PaO\textsubscript{2}, and pH were measured intermittently from the same artery with a blood gas analyzer (Labor-system, Flukiger AG, Menziken, Switzerland) and controlled by adjusting ventilatory rate, stroke volume, and composition of the inhaled gas.

A head-holder was used to avoid respiratory movements; upper and lower eyelids were removed as well as a rectangular area of skin surrounding the eye; the bulbar conjunctiva was detached; the sclera was carefully cleaned to 5 mm from the limbus; the superficial scleral vessels were thermo-cauterized; and an incision at the pars plana was performed.

\textbf{PO\textsubscript{2} Measurements}

Measurements of preretal PO\textsubscript{2} were made by double-barrel O\textsubscript{2}-sensitive microelectrodes with a tip diameter of 10 \(\mu\)m as previously described.\textsuperscript{31} The microelectrodes were inserted in the vitreous cavity through a sclerotomy placed 4 mm posterior to the limbus, aided by a micromanipulator\textsuperscript{36} (Fig. 1a) and positioned at a distance <50 \(\mu\)m from the vitreoretinal interface (Fig. 1b). The analyzed territories were intervascular areas at a distance of at least five vessel diameters from the arterioles and far from the optic disc. In all animals, measurements were repeated in several retinal areas.

The timeline of measurements was as follows: A baseline measurement under normoxia and a stable continuous recording for at least 10 minutes preceded inhalation of 100% of oxygen for 10 minutes. Then normoxia was induced, aiming to obtain a stable recording for at least 10 minutes; this recording was considered a baseline before inhalation of carboxen for 10 minutes. After a recovery to normoxia, a branch vein occlusion was performed by argon green laser,\textsuperscript{2,3,13} inducing an ischemic microangiopathy in the studied retinal territory. In this ischemic condition, the same timeline of measurements was performed before and after intravenous injection of acetazolamide (bolus of 500 mg).

The mean and the standard deviations (SD) of preretal PO\textsubscript{2}, and systemic PaO\textsubscript{2}, PaCO\textsubscript{2}, and pH were calculated at baseline and 7 minutes after starting hyperoxia or carboxen breathing.

\textbf{Statistics}

A two-tailed paired Student's t-test was used to detect differences between groups. A value of \(P < 0.05\) was used to define statistically significant differences. For extremely small values, a conventional format of \(P < 0.0001\) was used. A Friedman test was performed to attest the respective effect of hyperoxia and carboxen breathing in the same territory at four predetermined times (2, 5, 7, and 10 minutes). A box plot representation was used to provide an excellent visual summary of the median values and the 5%, 25%, 75%, and 95% percentiles. Moreover, a Wilcoxon signed-rank test was used to compare the effect of carboxen breathing and hyperoxia. In addition, the Bonferroni correction allowed more precise statistical analysis.

For every presented value, the \(n\) parameter represents the number of territories where measurements were done. An \(n\) value greater than the number of mini-pigs means that more than one retinal area was analyzed in the same eye.

\textbf{RESULTS}

Under systemic normoxia (PaO\textsubscript{2} = 108.43 ± 10.19 mm Hg; PaCO\textsubscript{2} = 35.63 ± 2.54 mm Hg; pH = 7.44 ± 0.07; \(n = 51\)), the mean preretal PO\textsubscript{2} recorded at the normal retinal intervascular...
lar areas of 13 eyes was 23.30/5.26 mm Hg (n = 51), a value similar to that previously described. Figure 2 shows a typical recording of preretinal PO2 in a normal retinal area in conditions of systemic normoxia followed by systemic hyperoxia, a return to baseline (i.e., normoxia) and finally carbogen inhalation.

The inhalation of 100% O2 induced a mean increase of preretinal PO2 of ΔPO2 = 2.20 ± 4.16 mm Hg, 13 eyes, n = 25. Under systemic hyperoxia, the mean preretinal PO2 increased from a mean value of 23.73 ± 5.08 mm Hg to 25.93 ± 6.26 mm Hg and that difference, although moderate, was statistically significant (P = 0.0142), yet disproportional to a substantial increase in systemic PaO2 (ΔPaO2 = 299.77 ± 89.39 mm Hg).

The inhalation of carbogen induced a mean increase of preretinal PO2 of ΔPO2 = 19.37 ± 16.41 mm Hg, 13 eyes, n = 26. The preretinal PO2 significantly increased from a mean value of 22.88 ± 5.50 mm Hg to 42.25 ± 16.93 mm Hg (P < 0.0001; n = 26). Under this condition, systemic PaO2 (ΔPaO2 = 382.85 ± 88.12 mm Hg) and systemic PaCO2 (ΔPaCO2 = 13.74 ± 5.72 mm Hg) significantly increased. The CO2 increase induced a respiratory acidosis from a mean pH value of 7.44 ± 0.07 to 7.33 ± 0.07.

Linear regression analysis showed the variation of preretinal PO2 increase with time during hyperoxia and carbogen breathing (Fig. 3). The figure reveals the statistically significant effect of carbogen breathing with time (R² = 0.21; 13 eyes; n = 26), in contrast to hyperoxia (R² = 0.0003; 13 eyes; n = 25).

In addition, considering 22 retinal territories of 13 mini-pigs submitted to the same physiological conditions at four different times (2, 5, 7, and 10 minutes), the Friedman test revealed the more statistically significant effect of carbogen inhalation on the variations of preretinal PO2 with time (P < 0.0001; n = 22; Fig. 4). In contrast, during hyperoxia, the preretinal PO2 remained within nearly stable values, although the Friedman test revealed a moderate significant increase with time (P = 0.013; n = 22). However with the Bonferroni correction, all the tests performed for hyperoxia remained nonsignificant.

At each of the four analyzed times, there was a significantly greater effect of carbogen inhalation on preretinal PO2 variations compared with systemic hyperoxia (Wilcoxon signed-rank test, P < 0.0001, n = 22). Even with the Bonferroni correction, all the tests performed for carbogen breathing remained significant, which was not the case for hyperoxia.
In nine eyes, a branch vein occlusion was performed. Under systemic normoxia (PaO₂ = 106.29 ± 9.11 mm Hg; PaCO₂ = 36.36 ± 2.19 mm Hg; pH = 7.46 ± 0.07; n = 25), the mean preretinal PO₂ recorded at the affected intervascular areas was 19.41 ± 4.82 mm Hg, n = 25, a value significantly lower than that recorded before the vein occlusion in the same territories (P < 0.0001; n = 25).

A typical recording of preretinal PO₂ in ischemic territories under normoxia, hyperoxia, and carbogen breathing is shown in Figure 5.

Systemic hyperoxia induced a moderate, statistically significant elevation of preretinal PO₂ (ΔPO₂ = 4.30 ± 3.57 mm Hg; 9 eyes; n = 16) from a mean value of 21.51 ± 5.86 mm Hg to 25.81 ± 6.03 mm Hg (P = 0.0002; n = 16). Hyperoxia induced a similar systemic PaO₂ change (ΔPaO₂ = 282.16 ± 94.76 mm Hg) to that reached before the BRVO.

Carbogen breathing induced a statistically significant increase in preretinal PO₂ (ΔPO₂ = 14.94 ± 8.53 mm Hg; 9 eyes; n = 14) from a mean value of 20.75 ± 6.32 mm Hg to 35.69 ± 11.07 mm Hg (P < 0.0001; n = 14). The systemic gazometric values during carbogen breathing changed in a similar way as before the BRVO (mean ΔPaO₂ = 349.36 ± 64.94 mm Hg, mean ΔPaCO₂ = 13.26 ± 6.63 mm Hg), leading to respiratory acidosis from a mean pH value of 7.43 ± 0.08 to 7.31 ± 0.08; n = 14.

Linear regression analysis demonstrated a statistically significant increase of preretinal PO₂ with time during carbogen breathing (R² = 0.29; 9 eyes; n = 14), in contrast to hyperoxia (R² = 0.024; 9 eyes; n = 16; Fig. 6).

Considering 14 retinal territories of nine mini-pigs submitted to the same ischemic conditions, where PO₂ measurements were obtained at four different times (2, 5, 7, and 10 minutes), the Friedman test revealed, as in normal retinal areas, the statistically significant effect of carbogen inhalation on the variations of preretinal PO₂ with time (P < 0.0001; n = 14) compared to that of hyperoxia (P = 0.10; n = 14; Fig. 7). At each of the four analyzed times, there was a significantly greater effect of carbogen inhalation on preretinal PO₂ variations compared with systemic hyperoxia (Wilcoxon signed-rank test, P < 0.05, n = 14).

In ischemic retinal territories, after intravenous injection of 500 mg of acetazolamide and during normoxia, the preretinal PO₂ values measured 7 minutes after the injection did not change significantly (ΔPO₂ = 0.88 ± 3.14 mm Hg; 8 eyes; n = 8), from a mean value of 20.68 ± 6.73 mm Hg to 21.56 ± 6.99 mm Hg (P = 0.452; n = 8; Fig. 8a). Sixty minutes later, the preretinal PO₂ remained almost stable (ΔPO₂ = 3.75 ± 4.42 mm Hg; 6 eyes; n = 6), from a mean value of 18.07 ± 5.56 mm Hg to 21.83 ± 6.33 mm Hg (P = 0.09; n = 6), although PaCO₂ increased significantly from a mean value of 36.53 ± 2.40 mm Hg to 47.74 ± 3.60 mm Hg (P = 0.0007; n = 6), simultaneously to the decrease of pH from a mean value of 7.45 ± 0.05 to 7.32 ± 0.06 (P = 0.0001; n = 6; Fig. 8b). PaO₂...
remained within the physiological range ($\text{PaO}_2 = 107.05 \pm 11.71 \text{ mm Hg}; n = 6$).

The inhalation of 100% oxygen led to a moderately significant increase in preretinal PO$_2$ ($\Delta \text{PaO}_2 = 6.96 \pm 4.49 \text{ mm Hg}; 7$ eyes; $n = 7$), from a mean value of $22.71 \pm 0.08 \text{ mm Hg}$ to $29.67 \pm 10.25 \text{ mm Hg}$ ($P = 0.006; n = 7$). The $\text{PaCO}_2$ increase confirmed that the experiment was correctly performed ($\Delta \text{PaCO}_2 = 329.60 \pm 67.04 \text{ mm Hg}$). In hyperoxic conditions, $\text{pH}$ and $\text{PaCO}_2$ remained practically stable ($\text{pH} = 7.32 \pm 0.12$; $\Delta \text{PaCO}_2 = 44.00 \pm 4.35 \text{ mm Hg}; n = 7$).

During carbogen inhalation, a significant increase in preretinal PO$_2$ was recorded ($\Delta \text{PaO}_2 = 15.15 \pm 9.15 \text{ mm Hg}; 7$ eyes; $n = 7$) from a mean value of $21.96 \pm 6.36 \text{ mm Hg}$ to $37.11 \pm 12.52 \text{ mm Hg}$ ($P = 0.005; n = 7$). As demonstrated in previous experiments, carbogen breathing induced a significant increase in systemic PO$_2$ ($\Delta \text{PaO}_2 = 376.20 \pm 56.29 \text{ mm Hg}$), and $\text{PaCO}_2$ ($\Delta \text{PaCO}_2 = 12.01 \pm 2.80 \text{ mm Hg}$), leading to a deeper systemic acidosis from a pH of $7.33 \pm 0.05$ to a pH of $7.24 \pm 0.06$, $n = 7$.

Linear regression analysis revealed the variation of preretinal PO$_2$ increase with time during hyperoxia or carbogen breathing (Fig. 9). In those ischemic retinas and after acetazolamide injection, this test showed a statistically significant effect of carbogen breathing (Fig. 10). This test revealed the greater statistically significant effect of carbogen inhalation on the variations of preretinal PO$_2$ with time ($P = 0.0002; n = 7$) than hyperoxia ($P = 0.003; n = 7$). Otherwise, the Wilcoxon signed-rank test demonstrated the systematically greater effect of carbogen inhalation on preretinal PO$_2$ variations at all four analyzed times than in systemic hyperoxic conditions (Fig. 11), with $P < 0.05$ ($n = 7$), except at 2 minutes ($P = 0.34; n = 7$).

**DISCUSSION**

In mini-pigs, an acute BRVO induces a significant decrease of preretinal PO$_2$ recorded at the affected intervascular areas, a value significantly lower than that recorded before the vein occlusion in the same territories. As tissue hypoxia is established early after the occlusion as a result of the blood flow decrease, an early improvement of oxygen delivery toward the ischemic/hypoxic retinal territory has to be attempted.

The results of our study indicated that the inhalation of carbogen could improve the delivery of oxygen to an ischemic/hypoxic retinal territory post acute BRVO, reversing tissue hypoxia. Furthermore, carbogen induced a progressive significant increase of the preretinal PO$_2$ with time in normal areas (Fig. 4).
In normal retinas, our results confirmed previous findings in mini-pigs, indicating a regulation of the retinal blood flow during hyperoxia maintaining the preretinal \( \text{PO}_2 \) at constant values in spite of the elevation of the systemic \( \text{PaO}_2 \). In the present series, hyperoxia induced a moderate significant increase of the preretinal \( \text{PO}_2 \), as revealed by the Friedman test \((P = 0.013; n = 22)\). However, after the application of the Bonferroni correction, the results obtained under hyperoxia were not statistically significant.

Indeed, in mini-pigs and most mammals, the retinal vascularization is heterogeneous; the vascular bed of the inner retina is composed of intercommunicating capillary layers from the retinal surface to the inner nuclear layer. The outer retina is not vascularized; its oxygenation is ensured by oxygen diffusion from the choroid. Hyperoxia leads to vasoconstriction of the retinal arterioles and to a decrease of the retinal blood flow of approximately 60%. In spite of the considerable increase in \( \text{PaO}_2 \), the decrease of the arteriolar retinal blood flow and the increase of the oxygen consumption of the retina during hyperoxia do not allow a supplementary contribution of oxygen delivery from the choroid to the inner retina. Thus the preretinal \( \text{PO}_2 \) measurements reflect the oxygen diffusing from the retinal circulation.

The preretinal \( \text{PO}_2 \) increase, in normal areas, under carbogen breathing is due to either the effect of carbogen on the retinal circulation or the modified ability of hemoglobin (Hb) to bind oxygen as the CO\(_2\) increase induces a rightward shift of the oxyhemoglobin dissociation curve.

Carbogen breathing induces a simultaneous increase of the systemic \( \text{PaO}_2 \) and \( \text{PaCO}_2 \), affecting the retinal arteriolar reactiv-
The elevation of PaO2 induces an arteriolar vasoconstriction, whereas the increase in PaCO2 induces a vasodilation similar to that observed in cerebral arterioles. As a result of the vasodilatory effect on retinal arterioles secondary to the increase in PaCO2, the inhalation of carbogen leads to a lesser reduction of retinal blood flow than that of systemic hyperoxia.

In addition to blood flow changes induced by carbogen, the CO2 increase and the resulting pH decrease should affect the ability of Hb to bind oxygen. The blood CO2 increase induces a rightward shift of the oxyhemoglobin dissociation curve. A rightward shift of the oxyhemoglobin dissociation curve reflects decreased affinity of Hb for oxygen, meaning that oxygen is released from Hb more readily, increasing the PaO2 and the oxygen availability in the tissue.

As a result of these described effects, carbogen induced a higher increase of the PaO2 and preretinal PO2 in normal retinal areas of mini-pigs. In agreement with our findings, the contributive effect of CO2, induces an increase in retinal juxta-arteriolar PaO2 in rats and in normal retinas of newborn and adults rats. In addition, intraretinal PaO2 profiles during carbogen breathing demonstrated that, although the oxygen supply from the choroid increases fourfold, the retinal circulation continues to provide oxygen delivery to the inner retina. These results indicate that during carbogen breathing, as in hyperoxia, the outer retinal layers’ oxygen consumption increases and does not allow a supplementary contribution of oxygen delivery from the choroid to the inner retina. Thus the preretinal PO2 increase under carbogen breathing is related to oxygen diffusing from the retinal circulation.

In ischemic postexperimental branch vein occlusion retinas, intraretinal PO2 gradients preserve their direction under normoxia. Thus, the inner retina is not supplied by the O2 diffusing from the choroid and therefore remains hypoxic. The results of our study indicated that, in contrast to the inhalation of 100% of oxygen, carbogen could also improve the delivery of oxygen to an ischemic/hypoxic retinal territory after acute BRVO, leading to the restoration of appropriate oxygenation of the inner retina, reversing tissue hypoxia.

Intravenous administration of acetazolamide increases cerebral blood flow, probably by increasing PaCO2. This PaCO2 increase is due to significant bicarbonate losses in the renal tubules, resulting in hyperchloremic metabolic acidosis. The CO2 produced by cells cannot be eliminated by carbonic anhydrase and so increases PaCO2 by diffusing through the basement membrane.

In our study, in ischemic retinal territories, after intravenous injection of 500 mg of acetazolamide and during normoxia, the preretinal PO2 values measured continuously during 60 minutes remained almost stable, although PaCO2 increased, concomitantly with a pH decrease (Fig. 8). In addition, our results illustrated that concomitant administration of acetazol-
amide and carbogen breathing can increase preretinal PO2 more significantly than in association with hyperoxia. This effect is probably due to the additive effect of acetazolamid and carbogen on the elevation of PaCO2. Indeed the systemic PaCO2 was higher when acetazolamid was associated with carbogen than with hyperoxia (Fig. 9). As previously mentioned, the PaCO2 increase counterbalances the vasoconstrictive effect of hyperoxia, and decreases the ability of Hb to bind oxygen. The rightward shift of the oxyhemoglobin dissociation curve, under the effect of acetazolamid, increases the oxygen availability in the tissue. Consequently acetazolamid has been used to modify the affinity of Hb for oxygen in the treatment of myocardial ischemia.

Recovery to adequate oxygenation of the retina would reflect an improvement of the retinal function. However, an increase in PaCO2 depresses the neuronal activity of the retina leading to a shape reduction of the b wave of the ERG. This effect is due to a decrease in either saturated rod response or the b-wave amplitude as a result of the extracellular K+ reduction. Some recent studies indicate either an absence of effect or a reduction of contrast sensitivity under the influence of CO2.

Taking into account those findings, the beneficial effect of an improved retinal oxygenation by carbogen inhalation with concomitant intravenous acetazolamid injection on the course of an acute BRVO remains to be demonstrated by clinical findings.

In conclusion, our study demonstrated the efficacy of carbogen breathing to increase the preretinal oxygenation in normal retinas and to restore sufficient oxygenation of the ischemic/hypoxic retinas after BRVO. The addition of acetazolamid inducing an important elevation of PaCO2, enhanced the effect of hyperoxia and carbogen breathing, leading to a more efficient increase in preretinal PO2. The PaCO2 increase affected both the retinal circulation and the ability of Hb to bind oxygen, thus oxygen is more readily released. The beneficial effect of the concomitant administration of carbogen breathing and intravenous acetazolamid on the course of an acute BRVO remains to be demonstrated by clinical findings and functional studies.

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