

ETIOLOGY OF THE RETINOPATHY OF PREMATURITY: A PROGRESS REPORT

Since the publication in 1981 of an overview of our previous 15 years' research into the genesis of the retinopathy of prematurity, the incidence of this disease has continued to rise as the survival rates of increasingly premature infants have risen. Since then, we have completed a study of the morphology of the developing retinal vasculature that lends support to the hypothesis described in that overview and have turned our attention to the possible role of the choroidal circulation in the retinopathy of prematurity. This article very briefly summarizes this more recent work.

INTRODUCTION

A 1981 article on the role of oxygen in the retinopathy of prematurity (ROP)¹ described the clinical picture and what was known then of the etiology of this disease, which can seriously impair vision or cause blindness as a result of abnormal retinal vascular development thought to be caused by excessive exposure to oxygen. The focus of the article was on the development of a hypothesis about the physiological behavior of immature retinal blood vessels. The hypothesis, based on experimental work involving animal models of ROP, implicated other causative agents in addition to oxygen in its genesis. Newborn kittens and puppies were used because their developing retinal vasculatures were known to mimic aspects of the behavior exhibited by the developing human retinal vasculature when high concentrations of oxygen were breathed. One unexpected, but significant, result was the production of cicatrices (scar tissue) in the retinas of some puppies. Such an advanced form of ROP had never before been seen in an animal model, and that deficit had been a major criticism of the kitten model.

As the survival rate of increasingly premature infants continues to rise, the spectrum of retinopathies encountered may be expected to broaden and so, also, might the list of potential causes. Serious consideration should be given to the possibility that physiological changes that may occur during the perinatal period are among those causes.

DISCUSSION

The scarcity of human tissues and the enormous obstacles to the direct examination of the premature fundus led to the use of animal models to investigate virtually every other aspect of ROP. The same scarcity and obstacles force us also to use animal models when studying the effects of physiological factors on the pathogenesis of ROP. Unfortunately, the validity of such models has, itself, become a subject of controversy. Better methods of monitoring and examining premature infants have produced much data concerning human

ocular development and ROP. Many widely held concepts derived from animal models about its pathogenesis may not be valid. Consequently, extrapolation of data from animal models to the clinical situation is less readily accepted now than it once was.

Still, the most important criterion applied in determining the validity of animal model data is the faithfulness with which the model mimics that aspect of human ROP of interest to the investigator. An animal model that is used to study the physiology of the developing human vasculature must also meet the requirement that its morphological development is similar to that of the human. It does not seem likely that abnormal developmental processes like those leading to ROP can be recognized—much less understood—in an animal model without first recognizing the normal ones that are present. In light of this, and because of the promise the newborn puppy had shown as the best available ROP model, we undertook an extensive morphological study of the puppy retina.² The study demonstrated that vasculogenesis in the puppy retina is strikingly similar to that in human fetal retinas observed by other investigators.

Vascular precursors, or angioblasts (primitive cells from which embryonic blood cells and vascular endothelium are differentiated), were found in the puppy avascular (without blood vessels or lymphatic vessels) retina. They were observed subsequently to organize into a vascular network by essentially the same sequence of steps described for the human retina by Ashton in 1970,³ but the whole process can be observed in much greater detail in the puppy.

Muller cell processes apparently provide a structural matrix throughout the avascular retina on which differentiated angioblasts organize into a vascular network. Within the matrix, arteries develop in beds of primordial capillaries lying near the edge of the developing vasculature. This process precedes vein formation, which involves the coalescence of embryonic capillaries that, themselves, were derived from primordial capillaries. Then, through a gradual reorganization, the mature vas-

culature emerges. These events occur in a wave-like progression that moves radially across the avascular retina from the optic disc (Fig. 1).

The results of our morphological study were extremely important in that they strongly supported our faith in the validity of the puppy ROP model and, consequently, in the conclusions we had drawn from the use of the model. Specifically, the primordial capillary beds at the periphery of the developing retinal vasculature constituted the weak structural element of the vascular network and clearly could be damaged when subjected to the ex-

cessively high transmural pressures we hypothesized would occur when vasodilation significantly reduced arterial blood flow resistance.

It was interesting, also, to note that the puppy retina is much more completely vascularized at birth than that of the newborn kitten (Fig. 2). Nevertheless, by postnatal day 14, vascularization reaches the retinal periphery in both kitten and puppy. This might explain why more severe ROP had not been produced in kittens. During oxygen exposure, the more rapid cellular differentiation that apparently occurs in the kitten retina might permit

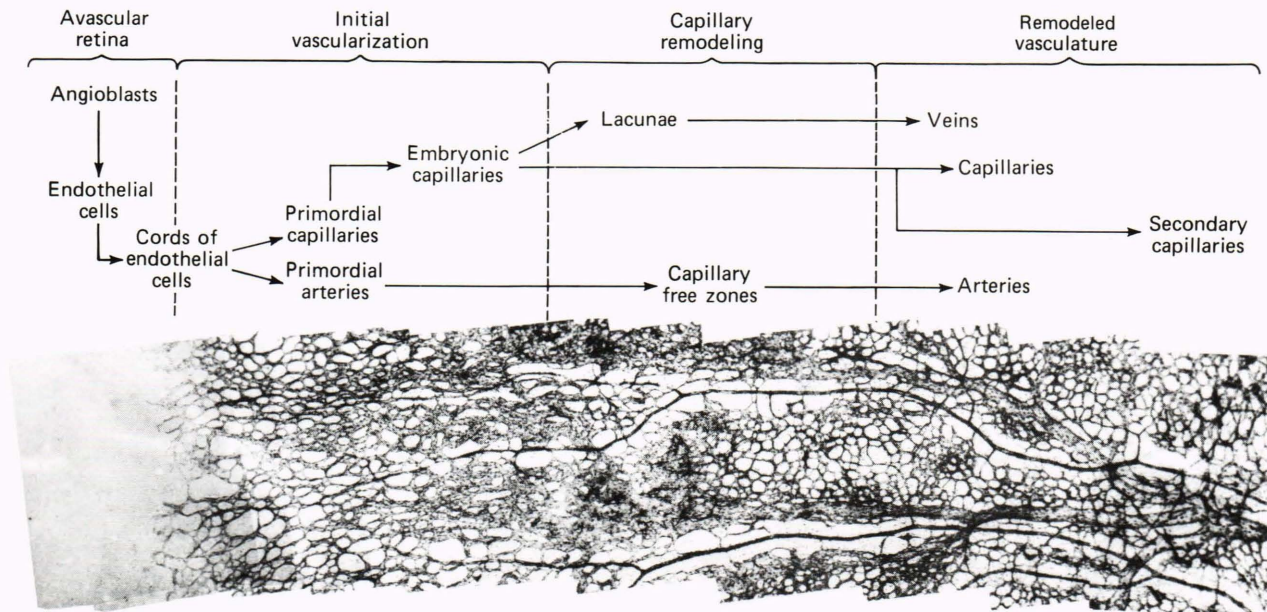


Figure 1. Summary of the vasculogenesis of a flat-mounted retina from a 14-day-old puppy (original magnification, $\times 60$).

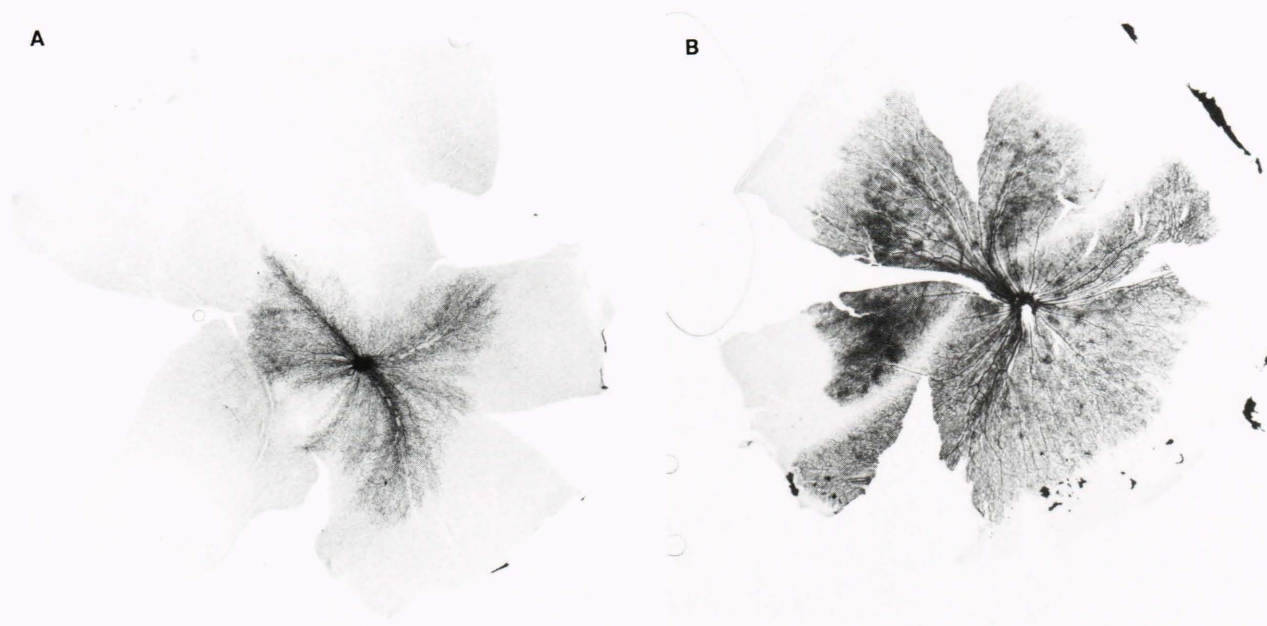


Figure 2. Flat-mounted retinas from the right eyes of (A) a 2-day-old kitten and (B) a 2-day-old puppy (original magnification, $\times 3.7$).

it to cope more effectively with whatever vascular damage is induced by breathing oxygen, while the less rapidly differentiating puppy vasculature cannot—further evidence that our decision to concentrate on the puppy rather than the kitten model of ROP was a good one.

Beyond studying details of the morphology of puppy retinal vascular development, we did no further work directly related to the retinal vasculature. Instead, our attention has been directed toward the choroidal vasculature. The choroid lies beneath the retina, and its system

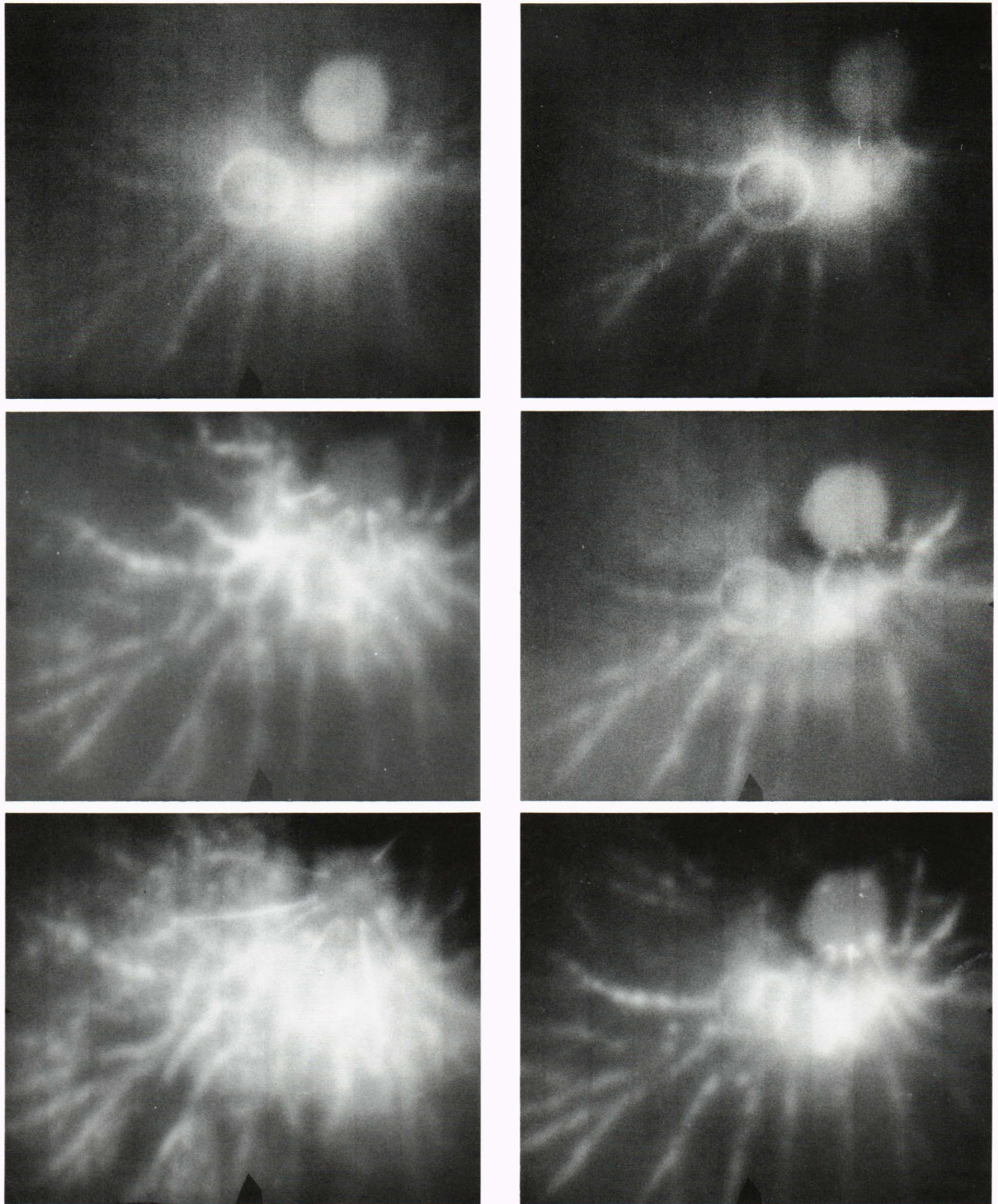


Figure 3. Comparison of three consecutive indocyanine green dye angiogram frames of a puppy after breathing air (left column) with those from the same puppy after breathing oxygen (right column).

of vessels is at least as important to the maintenance of the retina as the retinal vasculature itself, especially when the retinal vasculature is not fully developed and functional. In my view, the most troubling assumptions often made when designing animal experiments and interpreting the data from them are those related to the role of the choroid, both in normal development of the retina and in ROP pathogenesis.

The ROP literature contains little of significance regarding the choroid. Most reports imply or simply assume that at birth the choroidal circulation is comparable to that of the adult in terms of development and function. In general, what little is known or, more often, believed to be true of the adult choroid is assumed

also to be true of the choroid of the immature eye. It is usually accepted that, under normal circumstances, the choroidal circulation maintains approximately the outer two-thirds of the retina, but under some conditions of hyperoxia it can supply the entire oxygen needs. Several investigators have hypothesized that the choroidal vasculature of the mature eye can autoregulate its blood flow, but choroidal autoregulation has not been rigorously demonstrated. The fact that the choroidal circulation of the immature eye may be affected by oxygen exposure is virtually never considered.

Choroidal circulation can be observed routinely using an infrared fluorescent dye technique,⁴ but its successful use in the newborn puppy has been infrequent

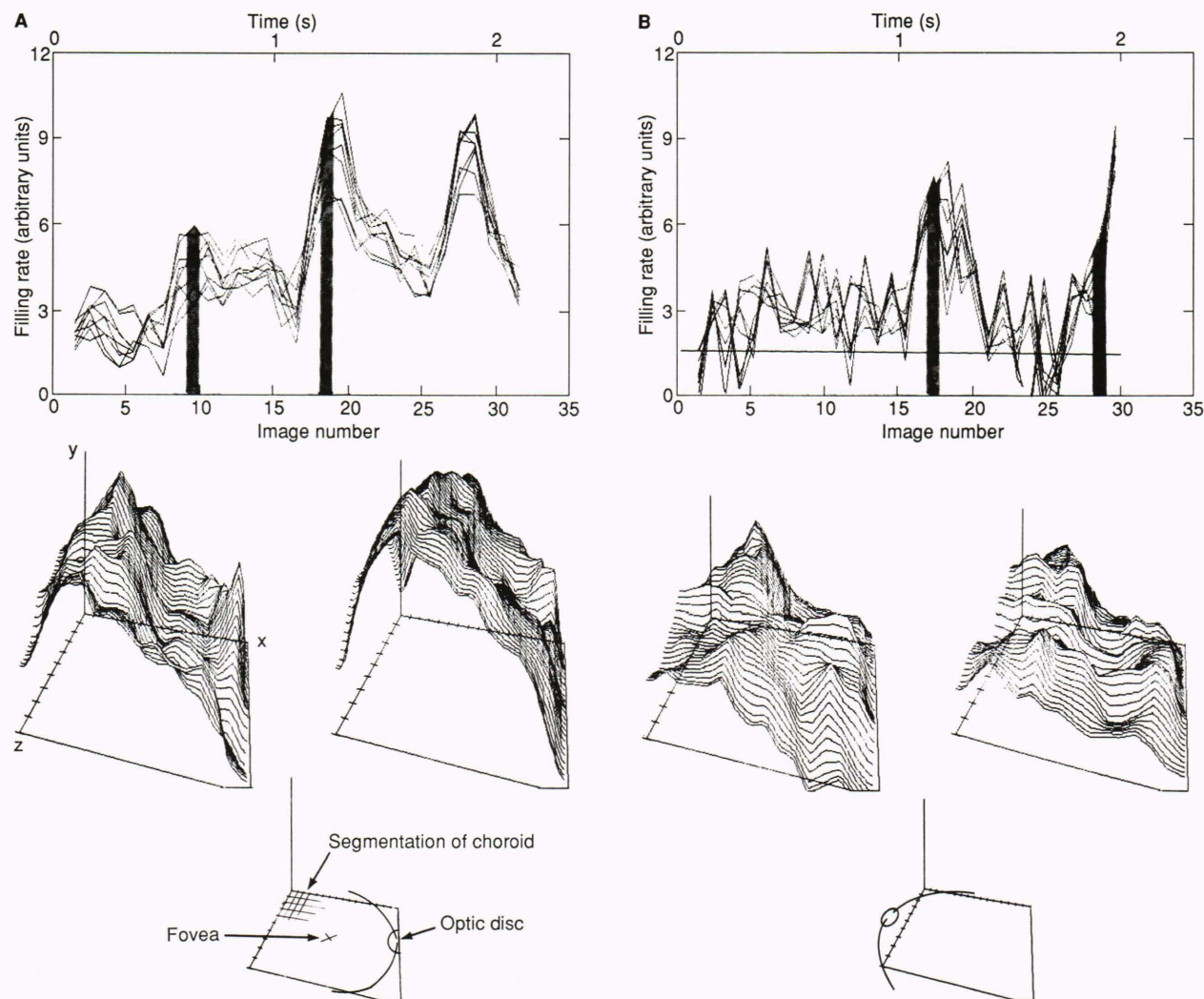


Figure 4. Interim results used to generate characteristic three-dimensional surfaces for two human choroidal angiogram sequences. For each subject, the schematic fundus representation at the bottom of the figure provides orientation by indicating the location of the optic disc, fovea, and segmentation of the choroid used in the application of the algorithm. The top of each figure shows the first derivatives of the time-varying average gray levels of the choroidal segments. They represent the instantaneous rate of indocyanine green dye filling of each choroidal segment as a function of time. Note the cyclic variations of filling rates, which are synchronous with the cyclic variations in heart rate and, hence, with ocular blood pressure pulse. The shaded areas indicate the systolic periods (the periods for which choroidal segment filling rates are represented by the three-dimensional surfaces below). The filling rates that occur during each systolic period are represented by three-dimensional surfaces (middle figures) for each entire choroidal area (lying in the x-y plane). The z-axes are the magnitudes of the filling rates during systole. There is a similarity between all surfaces from the same choroidal angiographic sequence, but there are distinct differences between those from different choroids.

because of their very small eyes. Nevertheless, the few successes demonstrated that breathing high concentrations of oxygen significantly reduces choroidal blood flow. On the left of Figure 3 are frames from a 40-frames-per-second angiographic sequence made of a puppy breathing air. The middle frame shows dye just entering the retinal vessels at the disc. Note also the large number of dye-filled choroidal vessels. On the right are angiogram frames from the same eye after 60 minutes of breathing oxygen; these frames were taken at the same times following dye injection as those on the left. Clearly, the amount of dye in the choroid and the number of filled choroidal vessels are greatly reduced as a result of breathing oxygen.

Although choroidal angiography has not yet been applied frequently to the newborn animal model, some details that may apply to the premature eye have been learned about the physiology of choroidal blood flow using monkeys and adult human subjects. For example, using data from high-speed angiograms (15 to 30 frames per second), a subject's choroidal circulation can be characterized uniquely in terms of the dye-filling rates in different choroidal areas during each systole of the intraocular pulse pressure. Dye-filling rates are extracted from data obtained from a frame-by-frame analysis of angiograms using a recently developed computer-aided image analysis technique.⁵

At the bottom of Figures 4A and 4B are small schematic representations of the 30° fundus fields of the human eyes used in these examples; they indicate the orientation within the area over which the three-dimensional surfaces in the middle of the figures were constructed. Each three-dimensional surface represents the distribution of dye-filling rates over the entire 30° choroidal area during one systolic period, as indicated by the shaded areas in the ocular pulse pressure curves at the top of the figures. The higher the surface above any given position on the fundus reference plane, the greater the dye-filling rate in the choroid at that position. The three-dimensional surfaces are nearly identi-

cal from pulse to pulse for each subject, but there is a significant dissimilarity between subjects (compare Figs. 4A and 4B). These surfaces have proved to be reproducible from dye injection to dye injection and from day to day.

This method was used to investigate the effects of breathing different gases on choroidal blood flow in rhesus monkeys. Each anesthetized monkey, monitored to ensure a relatively steady heart rate and blood pressure, breathed the indicated humidified gas mixtures for 8 to 10 minutes before the angiogram was made. To be sure the results were consistent, the experiments and angiograms were repeated for two of the four monkeys.

Figure 5 shows that, relative to the air control, breathing high concentrations of oxygen generally reduced choroidal blood flow, while 10% carbon dioxide generally increased it, particularly at the periphery. (The fundus areas depicted in Fig. 5 are 50° fields centered on the macular areas.) The most surprising result was that 10% carbon dioxide mixed with oxygen produced the same general increase in choroidal blood flow as was produced by breathing 10% carbon dioxide and air, rather than the decrease produced by oxygen alone. This is precisely the same response that was described in the 1981 article¹ as occurring in the immature retinal vasculature as a result of breathing the same gas mixtures.

Of course, these experiments must be repeated in the puppy model once the technical problems of using such small eyes are resolved. In the meantime, the results at least indicate that the choroid is not the totally passive vascular bed it is often thought to be. Indeed, if the choroidal vasculature of the immature eye behaves as the adult one appears to behave, one might be persuaded that oxygen-induced ROP is more likely to result from ischemia than from oxygen cytotoxicity.

CONCLUSION

The choroid already is generally credited with playing a major role—albeit as a passive vasculature—in maintaining the retina until the retinal vasculature fully

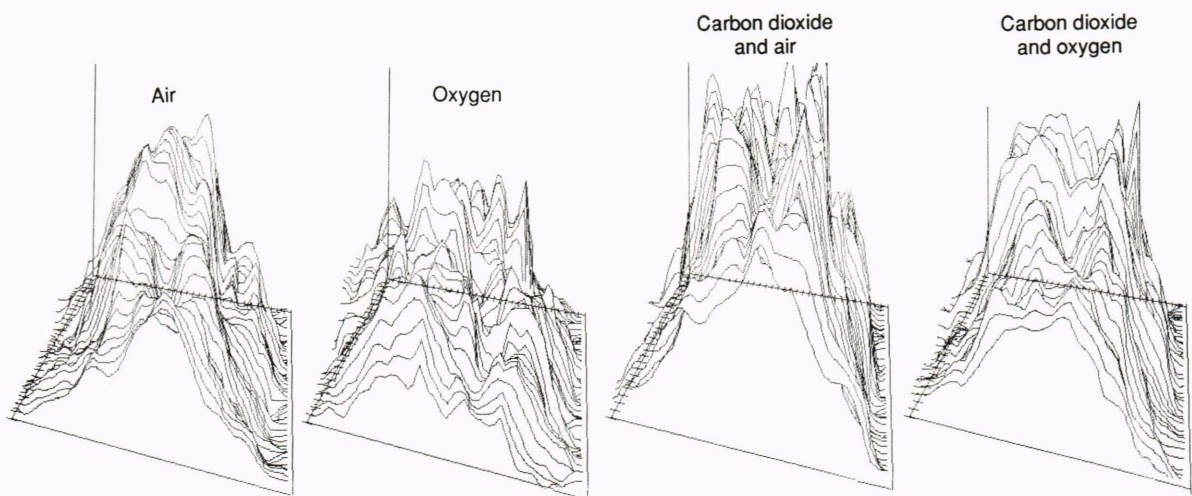


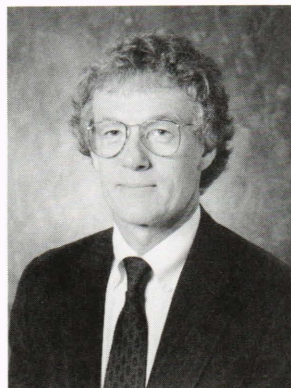
Figure 5. Characteristic three-dimensional surfaces demonstrating the relative choroidal blood flow rates of the same monkey eye as a result of breathing the indicated gas mixtures.

develops. It may be, however, that the choroid accomplishes its purpose instead as a functionally active vasculature. Therefore, the potential role of the choroid in ROP pathogenesis deserves serious consideration. Although I certainly do not recommend yet another name change for ROP, for purposes of reminding ourselves that the retina is, in fact, supported by two vasculatures, even though one of them is difficult to observe, it might be useful at least to *think* in terms of the retino-choroidopathy of prematurity.

REFERENCES

- ¹Flower, R. W., "The Role of Oxygen in the Retinopathy of Prematurity," *Johns Hopkins APL Tech. Dig.* **2**, 143-152 (1981).
- ²Flower, R. W., McLeod, D. S., Luty, G. A., Goldberg, B., and Wager, S. D., "Postnatal Retinal Vascular Development of the Puppy," *Invest. Ophthalmol. Vis. Sci.* **26**, 957-968 (1985).
- ³Ashton, N., "Retinal Angiogenesis in the Human Embryo," *Br. Med. Bull.* **26**, 103-106 (1970).
- ⁴Flower, R. W., "High Speed Human Choroidal Angiography Using Indocyanine Green Dye and a Continuous Light Source," in *International Symposium on Fluorescence Angiography*, Documentation Ophthalmologica Proceedings Series 9, DeLaey, J. J., ed., Dr. W. Junk b.v., The Hague, p. 59 (1976).
- ⁵Klein, G. J., Baumgart, R. H., and Flower, R. W., "Characterization and Classification of Choroidal Blood Flow," *Invest. Ophthalmol. Vis. Sci.* **31** (to be published, 1990).

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