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Reduced Severity of Oxygen-Induced Retinopathy in Kittens Recovered in 28% Oxygen[1]

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ABSTRACT. Chronic hypoxemia adversely affects the retinopathy observed in kittens after a hyperoxic exposure (80% oxygen) in the 1st wk of life. To test the converse hypothesis, 55 kittens were randomly assigned to recover in room air (21%) or 28% inspired oxygen after a 65-h hyperoxic exposure. At 4 wk of age, the retinopathy was found to be less severe in the 28% recovered kittens, severity score 3.0 ± 3.3 (mean ± SD), than in the room air recovered animals, severity score 5.7 ± 3.3, $p < 0.01$. This finding suggests that the clinical practice of restricted oxygenation in premature infants warrants reevaluation. (*Pediatr Res* 24: 106–109, 1988)

Abbreviation

ROP, retinopathy of prematurity

ROP has been linked with supplemental oxygen administration since the 1950s when it was determined that the prolonged (more than 4 wk) use of more than 50% inspired oxygen in human premature infants without regard to cyanosis caused a 10-fold increase in severe ROP (1). Animal models of this disorder further reinforced the association with oxygen because a similar retinopathy can be produced in kittens, puppies, mice, and some other mammals by exposing them to high inspired oxygen during the 1st wk of life (2–4). This oxygen-induced retinopathy, however, does not progress to full retinal detachment and blindness, as ROP does in some infants. However, major clinical and research efforts have been aimed at preventing vision loss in infants through ever tightening control of oxygen administration (5, 6). Despite this, the disorder remains a significant problem in the 1980s, even with the most stringent oxygen monitoring efforts (7).

While immaturity of the retinal vasculature at the time of birth is clearly the dominant determinant of risk for ROP, case control studies have found that the degree of illness and number of complications after delivery significantly affect the risk of severe disease after adjusting for the infant's level of immaturity (8–12). The observation that among the most premature infants, the sickest and most unstable are at greater risk for vision loss than those whose clinical course goes smoothly led to the investigation of variable oxygenation after a retinal vascular injury. The sickest infants have more unstable oxygenation status, and on average lower arterial oxygen levels than stable infants (10). Therefore the effects of chronic hypoxia and extremely variable oxygenation were studied in the kitten model of oxygen-induced retinopathy. In this model, an initial injury to the retinal vasculature was caused by exposing the kittens to 80% oxygen for 65 h followed by a 3-wk recovery period. Quite clearly, hypoxia during the recovery period adversely affected the retinopathy (13), but surprisingly, a variable oxygenation status appeared to reduce its severity (14). In reporting the results of that study, we speculated that the wide but rapid (18 min) swings of oxygen from 8 to 43% where experienced by the retina as a single average effect. By integrating the area under the inspired oxygen curve in that study, we found that the mean oxygen exposure during the recovery period in these animals was 28%.

The current study was undertaken to explore this unusual finding. The specific question asked was whether increasing the arterial partial pressure of oxygen mildly (28% oxygen) and continuously during the recovery process would result in less retinopathy and more orderly healing. If so, this finding would have potential clinical implications.

METHODS

Kittens were obtained from 16 specific pathogen-free pregnant queens conditioned to laboratory conditions from birth and fed standard cat lab food *ad libitum*. The queens were observed daily, and the day kittens were discovered was defined as day 1. On day 3, the queen and her kittens were placed in an infant incubator (Air Shields Isolette, Hatboro, PA) at 26° C with the oxygen concentration adjusted to 80 ± 1%. The kittens were given identification numbers at birth, and after 65 h, half of each litter was randomly selected (by drawing numbered cards from a box) to return to room air for recovery or to remain in the incubator at 28 ± 1% oxygen (Fig. 1). The flow rate for the added oxygen exceeded 4 liter/min and no accumulation of carbon dioxide occurred because of the combined flow of oxygen and the circulation system of the incubators. Those kittens that went through the recovery period in the incubator were continuously exposed to 28% oxygen. Oxygen concentrations were measured at least twice daily with a Beckman Oxygen analyzer and were consistently stable.

The recovery period continued until 28 ± 2 days of age when the kittens were killed, the retinal vasculature perfused via the aorta with india ink, and the retinal flat mounts prepared as previously described (15). The permanently mounted retinae were coded and scored by the investigator without knowledge of the recovery assignment. Randomly selected retinae that had been scored once were mixed back in subsequent batches and rescored to confirm consistency in scoring. Four categories of

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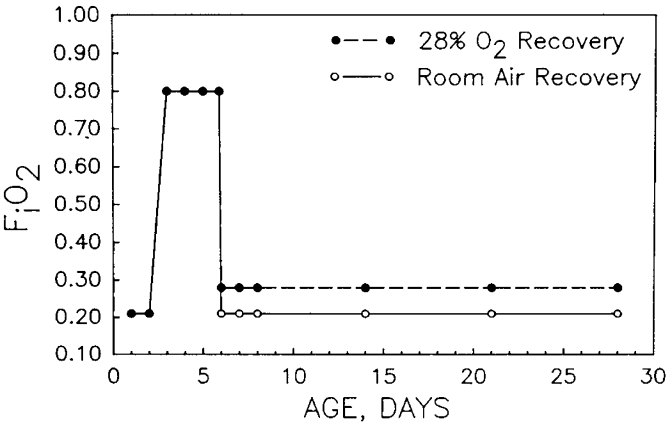

Fig. 1. Study design. The time course over 4 wk for each litter is shown. After the initial 65-h-exposure to 80% oxygen, half of each litter recovered in room air and the other half in the incubator in 28% oxygen.

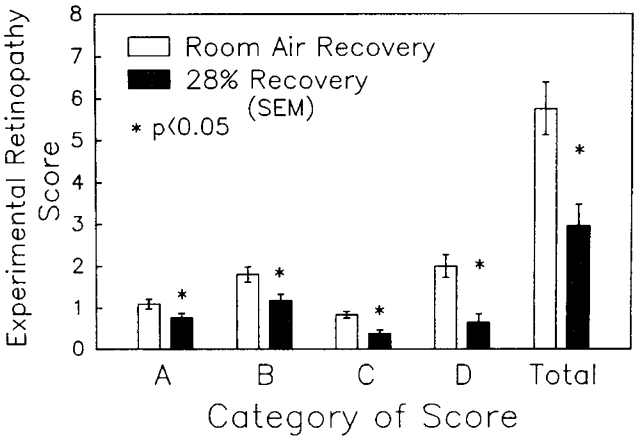

Fig. 2. Oxygen-induced retinopathy score results for 28% oxygen recovery. The *bars* show the mean ± 1 SEM for each of the four categories of the scoring system and the total score. Sample size = 15 because room air recovery kittens from each litter were averaged as a single control value for that litter compared to the average of the 28% recovered kittens from the same litter.

the retinopathy were scored separately, the total yielding a score of 13 for the worst cases, and a normal score of 0–1 (13, 15). Category A measured the extent of vascularization of the retina, category B the number and pattern of retinal vessels, category C the loss of periarteriolar capillary-free zone, and category D the amount of extraretinal neovascularization. The retinae were scored using a binocular dissecting microscope at 0.7–3×. The final score for each kitten was taken as the arithmetic mean of the scores from the right and left eyes. There were two final scores for each litter, the average of the room air kitten scores, and the average of the 28% oxygen recovery scores.

The sample size was based on prior experience with this model (13–15) where the predicted retinopathy scores after room air recovery to 4 wk after 65 h of 80% oxygen have been 5.3 ± 2.5 (mean ± SD). Assuming that we wished to be 80% certain (type II error = 0.20) that we would detect a difference of at least two points in the retinal score (type I error = 0.05, two-tailed paired *t* test), just over 15 litters would be needed. Therefore, 16 litters were enrolled in the study. The paired *t* test was chosen to permit comparison of experimental to control kittens in the same litter because of the known litter to litter variability in retinal scores of kittens recovered in room air. Although Student's *t* tests are not usually applied to data that have an upper and lower boundary, the experimental retinopathy scores fell in the midzone of the scoring system and were approximately normally distributed, making its use reasonable in this case. To be certain, however, the Sign test was also applied to the results.

RESULTS

Fifteen litters (28 room air kittens and twenty-seven, 28% oxygen kittens) completed the study (one litter stillborn). All appeared healthy, and both halves of the litters grew equally well (no statistically significant differences between their weights at weekly intervals from birth to 4 wk). Arterial blood gases obtained via cardiac puncture in selected animals in this and previous studies showed that the PaO_2 in 80% oxygen is 305 ± 91 (mean ± SD), $n = 21$; in 28% oxygen is 126 ± 18, $n = 4$; and in room air is 96 ± 26, $n = 8$.

The kittens recovered in 28% oxygen had significantly less retinopathy than their litter mates who recovered in room air. Figure 2 shows the retinopathy scores for each of the four categories, A–D, and the total scores. The paired data are shown in Table 1 and were compared using the paired *t* test on data within litters ($p < 0.01$). Table 2 shows the raw scores for the total retinopathy by litter, and analysis using the Sign test on the direction of difference in each litter confirms the findings on the paired analysis ($p < 0.01$).

Finally, linear regression analysis was also used to estimate the

Table 1. *Experimental retinopathy scores—28% oxygen recovery*

	Score component				Total score
	A	B	C	D	
Mean difference*	0.45	0.72	0.51	1.44	3.10
SD of differences	0.45	0.72	0.41	1.02	2.29
p†	<0.01	<0.01	<0.001	<0.001	<0.001

* The mean difference is the average of each of the differences for the 15 litters. Each individual litter difference is the average of the 28% oxygen recovery scores from the one to three kittens in one litter subtracted from the average of the one to three room air-recovery kittens from the same litter.

† *p* value, Student's paired *t* test.

Table 2. *Mean total retinopathy score values for 28%- and room air-recovered animals in each litter*

	Score	
Litter	28% recovery	Room air recovery
1	7.00	8.12
2	3.33	5.75
3	3.20	7.08
4	4.62	6.00
5	2.25	5.25
6	7.62	9.50
7	5.87	8.75
8	0.5	5.62
9	0.0	7.50
10	2.87	7.37
11	1.25	0.83
12	2.25	2.50
13	1.37	6.62
14	1.62	8.00
15	0.25	1.67
Mean ± SD*	3.0 ± 3.3	5.7 ± 3.3

* Mean and SD are of all animals rather than a mean of the average for each litter (two to four animals/litter). Sign test, 14/15 litters have a positive difference, $p < 0.01$.

effects of oxygen recovery, holding constant any litter-specific effects. The results provide very similar estimates to those of the simple paired t tests in Table 1, as should be the case with a randomized trial design. In this analysis the estimated SE of the treatment effect become even smaller when controlling for litter specific outcome effects. (Regression tables for the total score and each category are available from the author on request.)

Figure 3 is a photograph of representative retinae from two kittens in the same litter, one who recovered in room air and one in 28% oxygen. The better progress of vessels toward the ora serrata, and the near normal fine delicate vessels are apparent in the retina of the kitten recovered in 28% oxygen.

DISCUSSION

The degree of retinopathy in the kittens recovered in room air was consistent with our observations in other studies with a mean value of 5.7 ± 0.6, mean ± SEM, confirming the consistency of the model and the scoring system (Fig. 4). We have found that a 65-h exposure to 80% oxygen in kittens reliably produces some degree of retinopathy, which on average gives a severity score of 5.7. However, we have no explanation for the variability from litter to litter so evident in the data in Table 2. The differences could be nutritional or genetic based on variations in retinal vasculature maturity at the time of birth or other factors. It is clear that studies using this model will continue to require the use of litter mate controls.

In the variably oxygenated recovery study we speculated that the retinal vasculature had responded to a more averaged effect of oxygenation rather than to the extremes in the abrupt 6 to 18-min cyclical changes. The lower scores observed after 28% oxygen recovery (3.0 ± 0.5, mean ± SEM) support this hypothesis and are of a magnitude similar to those seen in the variable recovery study (2.0 ± 0.5) (Fig. 4). It is logically consistent that tissue growth, disease, and repair should respond to environmental conditions presented over hours rather than minutes.

These results have significant implications for our understanding of vascular retinopathies such as ROP, diabetic, or sickle cell retinopathy. In ROP, we have previously thought only of the degree of injury as the determinant of severity, but this study, combined with the previous reports already described (13, 14) strongly supports the hypothesis that oxygenation status during recovery from a retinal vascular injury influences the course of that recovery. It has long been believed that diabetic and sickle cell retinopathies develop as an overgrowth of the retinal vessels in an attempt to revascularize hypoxic retina that has lost capillary supply due to microinfarcts and microaneurysms. It would appear that oxygen-induced retinopathy is also a response to hypoxemic retina in the remaining nonvascularized areas.

The findings in this report are consistent with the increasing body of knowledge on the molecular control of retinal vascularization. There are present in the retina of both kittens (16, 17) and humans (18) potent growth factors that stimulate endothelial proliferation, migration, and angiogenesis. It would be consistent for oxygen to play an important role in affecting the release of

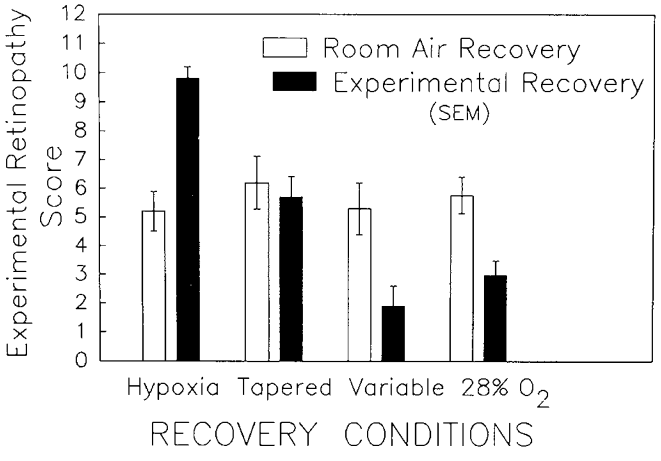

Fig. 4. Comparison of recovery conditions from previous studies. The *bars* show the mean ±1 SEM in four studies of differing recovery conditions after a 65-h, 80% oxygen exposure. The hypoxia group recovered in 13% oxygen (13), the tapered group were slowly withdrawn from oxygen over 3.5 wk (14), and the variable kittens were recovered in an incubator cycling between 8 and 43%, oxygen (14).

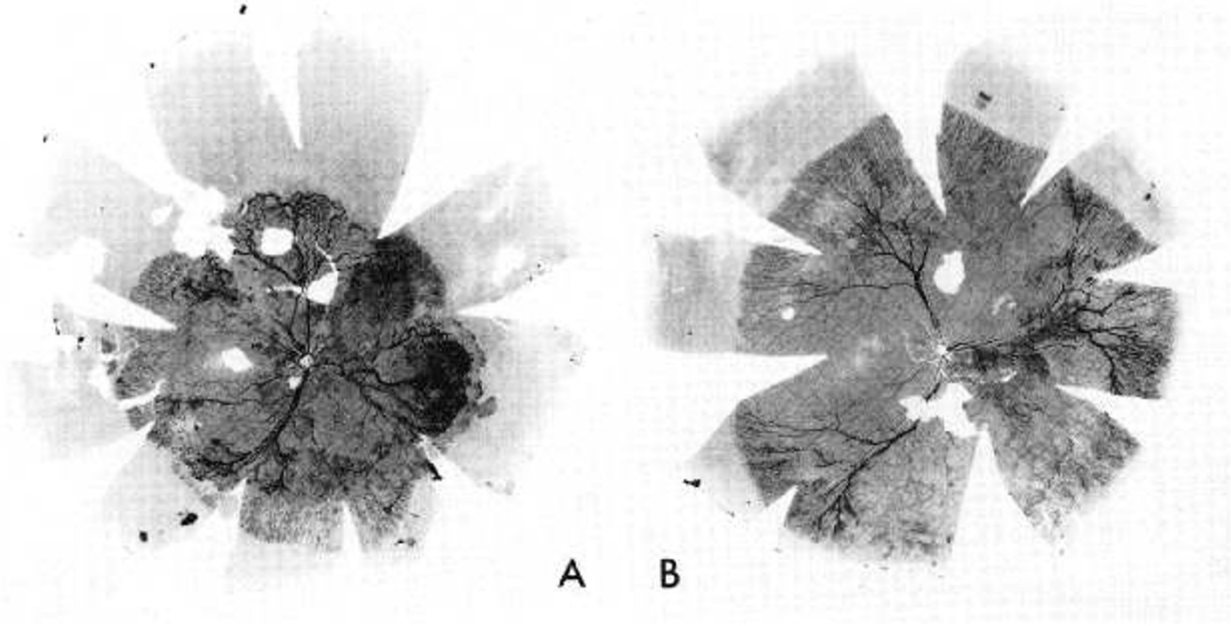

Fig. 3. Retinae from 28% and room air recovery. These two retina are from litter mates. The kitten recovered in room air (*A*) shows a typical wide avascular zone with an excessive number of large vessels leaving the disk, plus extraretinal neovascularization. The other retina from a kitten in the same litter, but recovered in 28% oxygen (*B*) shows that vessels have reached the ora serrata on the nasal side and are much closer to the temporal side than in its litter mate. There is minimal extraretinal neovascularization and the near normal delicacy of the three major pairs of retinal vessels in the kitten has been preserved.

these growth factors and thus promoting the ingrowth of vessels to hypoxic tissue and regression of vessels from tissue that is adequately supplied. Preliminary work in the kitten retina using rearing conditions of 13 to 30% oxygen has shown that the distance between arterioles and the nearest capillaries is directly proportional to the arterial oxygen concentrations (19). If one considers that blood samples with a PaO_2 of 90, 190, or even 390 torr are all 100% saturated, it must be concluded that the modulators of capillary growth respond to partial pressures of oxygen rather than to saturation of the arterial blood. Inasmuch as the tissues in need of capillary ingrowth receive their oxygen only by diffusion, the partial pressure of oxygen as a driving force for that diffusion seems to be a reasonable biochemical reactant for the modulators of vessel growth to measure and respond to.

Clinicians now face a clear need to test the implications of this hypothesis in human infants who have ROP. Remembering that the animal models do not develop retinal detachments as a complication of oxygen-induced retinopathy, no matter how severe, leaves serious doubts in the minds of some investigators as to whether the results can be applied to the human disorder at all. However, it is extremely attractive to envision the "turning off" of proangiogenic growth factors by increasing the arterial PaO_2. These data suggest that it would not require a great increase above the usual oxygen levels to have an effect. The 28%-recovered kittens had a PaO_2 of only 30 torr higher than the room air-recovered animals. Carefully designed clinical trials will be required before we can learn about the risks and benefits of such an approach in the premature population with severe ROP.

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