COMMUNICATIONS

ROLE OF OXYGEN IN THE GENESIS OF RETROLENTAL FIBROPLASIA

A PRELIMINARY REPORT

BY

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IN view of the present controversy centring in the role of oxygen in the aetiology of retrolental fibroplasia, we have thought it essential to investigate the influence of varying concentrations of oxygen upon the immature retina. Since it is now well established that the earliest stages of this disease are angioblastic in nature and present as an overgrowth of the developing retinal vessels, it was necessary to select a laboratory animal in which the anatomy and embryology of the retinal vessels were comparable with those in man. In the cat, embryo vascular budding commences at the disc between the 35th and 45th day of intra-uterine life, but the vessels do not reach the retinal periphery until about 3 weeks after birth, whereas, in man, the retinal vasculature is complete at birth. The degree of retinal vascularization in the full-term kitten at birth and in the ensuing 3 weeks is therefore comparable in extent to that in the premature baby. Since, with minor variations, the retinal vascular development is in all other respects very similar to that in man, the kitten appeared to be ideal for our purpose.

Our experiments have, therefore, been designed to investigate the immediate and remote effects of high, moderate, and low concentrations of oxygen upon the process of retinal vascularization during the first 3 weeks of the kitten's life. Not all of these experiments have been completed and several others are now in progress, but the findings so far obtained appear to be sufficiently striking and significant to warrant a preliminary communication.

Full experimental details and a discussion of the results will be given in a subsequent paper; this report will be confined to a description of the effect of high concentrations of oxygen upon growing retinal vessels, and the subsequent developments which follow the transfer of the animal to ordinary atmospheric conditions.

Material and Methods

Standard bacteriological incubators were converted to gas chambers into which oxygen was passed through an inlet tube and the flow so adjusted as to provide a concentration of 75–80 per cent. of oxygen at normal ambient pressures. The

oxygen levels were measured with a B.O.C. oxygen analyser at half-hourly intervals throughout the day and night. CO₂ readings were made with a Fry's gas analyser, and temperature and hygrometer readings were taken at hourly intervals.

The mother cat and test kittens were placed on a shelf in the chamber and maintained in the experimental conditions for a period of some days; at the end of each experiment the kittens were anaesthetized and, after one eye had been removed for section, the left ventricle was injected with Indian ink. The remaining injected eye was then removed and placed in fixative for 12 hrs; after freezing, it was opened transversely, and the retina was removed and mounted flat for study under the stereoscopic microscope.

In some of our more recent experiments it has been possible, by using foster mothers, to obtain control animals from the same litter, of the same age, and of similar weight; this is a desirable precaution in view of the variability in development of the kitten's retinal vessels. The results described in this paper, however, are those obtained in high concentrations of oxygen and are far beyond the limits of normal variation.

Experimental Findings

Two typical experiments will be described.

Experiment 1.—Cat and three kittens (K₁, K₂, K₃) 3 days old.

Plan.—Put mother and litter (K₁, K₂, K₃) in incubator maintained at 60–70 per cent. oxygen for 4 days (K₁, K₂) and 6 days (K₃) to demonstrate:

- (a) Effect of 4 days' hyperoxia at 7 days old (K₁, K₂).
- (b) Effect of 6 days' hyperoxia at 9 days old (K₃).

Results (both retinae were injected with Indian ink):

(a) Only a few vessels were seen around the disc. Maximum outgrowth (MO) 2mm. No other injected vessels apparent in retina. High power microscopical examination showed constricted vessels containing blood. Tunica vasculosa lentis and hyaloid artery (TVL and HA) normal. Both eyes from both kittens showed the same picture.

(b) Both retinae showed only one or two spicules of injected vessels around the disc. MO 1 mm. No other vessels visible. TVL and HA normal and fully injected.

Conclusion.—This experiment shows that conditions of hyperoxia were able to obliterate developing retinal vessels. In the normal animal of corresponding age the retinal network is dense and the maximum outgrowth over 6 mm. (Figs 1 and 2, opposite). In this experiment, however, there were no controls of the same age and same litter.

Experiment 4.—Cat and six kittens (K₁₋₆) 1 day old. One foster mother.

Plan.—Kill and examine K₁. Put K₂ and K₃ with foster mother in air. Put K₄, K₅, and K₆ with mother in incubator at 75–80 per cent. oxygen for 4 days. Then kill K₂ and K₄, transfer K₆ to foster mother in air for 3 days, and change mother and K₅ to 10–15 per cent. oxygen for 3 days. Then kill K₃, K₆, and K₅ to demonstrate:

- (a) Normal retina at 1 day (K₁).
- (b) Normal retina at 5 days (K₂).
- (c) Normal retina at 8 days (K₃).
- (d) Effect of 4 days' hyperoxia at 5 days old (K₄).
- (e) Effect of 4 days' hyperoxia followed by 3 days' air at 8 days old (K₆).
- (f) Effect of 4 days' hyperoxia followed by 3 days' hypoxia at 8 days old (K₅).

FIG. 1.—Control kitten, 1 day old (Exp. 4, K₁, L). Normal developing retina. There are three vascular complexes, maximum outgrowth 6 mm. Proliferation most dense at periphery. (Compare Fig. 2). Injected Indian ink. Mounted flat. $\times 7$.

FIG. 2.—Experimental kitten, 9 days old (Exp. 1, K₃, R). Shows effect of 6 days' continuous hyperoxia (60–70 per cent. oxygen). Apart from a few attenuated vessels around the disc, the vascular complexes, which should be even more advanced than those shown in Fig. 1, have been completely obliterated. No other vessels are visible in the retina. Hyaloid artery and tunica vasculosa lentis normal. Injected Indian ink. Mounted flat. $\times 7$.

Results.—In every case one eye was removed for section and the results in the other (injected) eye were as follows:

- (a) Normal retina showing most dense proliferations at periphery. MO 7 mm. TVL and HA normal.
- (b) Normal retina showing most dense proliferations midway between disc and periphery. MO 6 mm. TVL and HA normal.
- (c) Normal retina showing most dense proliferations at periphery. MO 9 mm. Vessels had reached ora on nasal side. TVL and HA normal.

FIG. 3.—Experimental kitten, 5 days old (Exp. 4, K₄, R). Shows effect of 4 days' continuous hyperoxia (75–80 per cent. oxygen). Only nine or ten small spikes of injected vessels remain around the disc. Elsewhere the vascular bed is obliterated and in some areas it contains trapped blood. A portion of the normal hyaloid artery may be seen in the centre of the disc. There is a small leak of Indian ink below the disc. Injected Indian ink. Mounted flat. 19.

FIG. 4.—Experimental kitten, 8 days old (Exp. 4, K₈, R). Shows effect of 4 days' hyperoxia (75–80 per cent. oxygen) followed by 3 days' air. The pre-existing vascular complexes have partially re-opened but the capillary network is of a grossly abnormal architecture, being cobweb in type without definition of arteries and veins. In the periphery of the retina the vessels tail off into a thrombosed network from which many haemorrhages have occurred. Vascularization appears to have recommenced in the region of the disc, and in this area there is a circular zone 4 mm. in diameter showing a more normal architecture but a richer plexus of vessels. Injected Indian ink. Mounted flat. $\times 9$.

(d) Only nine or ten small spikes of injected vessels present around disc. MO (injected) 1 mm. Elsewhere vascular bed obliterated except for islands of thrombosed vessels. No haemorrhages. TVL and HA normal (Fig. 3).

(e) Showed roughly three complexes. MO 5 mm., but network irregular and of cobweb type with no differentiation of arteries and veins. No increase in density at periphery where capillaries tailed off into a thrombosed network from which many haemorrhages had occurred. In region of disc vascularization appeared to have recommenced; in this area a circular zone, 4 mm. in diameter, showed a more normal architecture but with a richer plexus of vessels (Figs 4, 5, and 6). TVL and HA normal.

FIG. 5.—Control kitten, 8 days old (Exp. 4, K₈, R). Shows normal architecture of developing vascular bed. Arteries and veins may be distinguished and there is a well marked capillary-free peri-arterial zone (Compare Fig. 6). Injected Indian ink. Mounted flat. $\times 50$.

FIG. 6.—Experimental kitten, 8 days old (Exp. 4, K₈, R). Shows effect upon capillary network of 4 days' hyperoxia (75–80 per cent. oxygen) followed by 3 days' air. The transfer to air has led to the re-opening of the capillaries which had been obliterated by the period in oxygen. The network is grossly abnormal, no arteries and veins can be discerned, and the picture resembles that of the histological pattern of pulmonary emphysema (Compare Fig. 5). Injected Indian ink. Mounted flat. $\times 50$.

(f) No injected vessels seen except on nasal side where a fine cobweb network of vessels extended 4 mm. from disc. Beyond this point thrombosed capillaries could be seen and there were a few haemorrhages at capillary tips. No evidence of

normal vascular architecture, but vessels around disc obscured by leak of Indian ink. TVL and HA normal.

Conclusions.—This experiment was completely satisfactory in that the degree of retinal development before the test was known and each experimental animal was paired with a control litter-mate of the same age and comparable weight. The findings completely confirm the conclusions reached in Experiment 1—that high concentrations of oxygen are able to obliterate developing retinal vessels—and they further show that some of the collapsed vessels contain trapped coagulated blood (K_4). After transfer to air for 3 days, such vessels as remain patent appear to re-open, giving rise to a grossly abnormal cobweb network of capillaries. The normal architecture is not restored. This re-established blood supply would seem to be inadequate and the process of vascularization recommences from the disc (K_6). Transfer to hypoxia instead of air did not appear to make any difference and the greater growth of vessels that might have been expected did not occur. The area of the disc, however, was obscured in this case by a leak of ink.

General Conclusions

The findings from our experiments, of which two examples are quoted above in this preliminary communication, indicate that high concentrations of oxygen (60–80 per cent.) at atmospheric pressure are able to obliterate the ingrowing vessels in the developing retina of the kitten. Since the tunica vasculosa lentis, hyaloid artery, and other vessels examined in the body showed no structural alteration, and the fully mature retina of the kitten was not affected in this way, it would appear that this process represents a specific effect upon growing vessels or a fundamental interference with the process of vascularization. In the latter respect it is possible, for instance, that conditions of hyperoxia extend the nutritional range of the choroid to include the inner layers of the retina, so that the developing retinal vessels lose their growth stimulus, become redundant, and atrophy. The well known vasoconstrictive effect of oxygen may also play a part in this process. Further experiments, to be reported later, have shown that the extent of vascular obliteration induced by oxygen is directly proportional to the degree of immaturity of the retinal vascularization, to the duration of exposure to oxygen, and to the degree of the oxygen concentrations.

Obliteration of the vascular channels is irreversible in many vessels and this appears to be due to some extent to the coagulation of entrapped blood. After transferring the animal to air, such vessels as remain patent refill with blood, but the resulting vascular architecture is grossly abnormal, consisting of an irregular cobweb of vessels without definition into arteries or veins, and extensive haemorrhages may appear at the periphery of the re-opened network. The picture of the injected vessels resembles that of the histological pattern of an emphysematous lung. This re-established blood supply appears to be totally inadequate for the requirements of the retina when the animal is breathing air, and vessel growth into the ischaemic retina recommences from the disc region.

At the present time we do not know the ultimate outcome of this severe interference with the development of the retinal vasculature, but our most recent experiments have shown new vessels growing into the vitreous 18

FIG. 7.—Experimental kitten. The animal was subjected to 5 days' hyperoxia (75-80 per cent. oxygen) followed by 18 days in air. Vitreous removed from surface of revascularizing retina shows glomerules of blood vessels and proliferating endothelial buds extending into it. Injected Indian ink. $\times 27$.

days after exposure to high concentrations of oxygen (Fig. 7), and this was accompanied by retinal detachment 30 days after such exposure. It is clear, therefore, that high ambient concentrations of oxygen can destroy the normal process of vascularization in the retina of the kitten, resulting in abnormal vascular proliferation and retinal detachment when the animal is subsequently transferred to air.

Since the development of the retinal vessels in the kitten is so closely comparable to that in man, it is probably justifiable to draw analogies between our experiments and the conditions to which the premature baby is subjected in the nursery incubator. Indeed, we feel that it is highly probable that a similar obliteration or vasoconstriction of the retinal vessels may occur in the premature infant, and that this effect may be the underlying cause of retrolental fibroplasia. Such a theory would certainly explain many of the clinical findings and conflicting opinions regarding the aetiology of the disease. Thus while the basic injury of vascular obliteration is inflicted in high oxygen concentrations, the effect of this action is not evident until the animal returns to ordinary atmospheric conditions. Consequently, in a sense, those who hold that the disease is due to high oxygen concentrations and those who believe relative anoxia to be the important factor would both, if analogy with the kitten retina is valid, be partly correct.

Many questions remain to be answered and the elucidation of these problems must await further experimental investigation; meanwhile, it is hoped that this demonstration of the profound influence of oxygen on the process of retinal vascularization may assist in narrowing the field of inquiry.

Summary

(1) The development of retinal vessels in the retina of the kitten at birth and for the following 3 weeks is closely comparable to that in the human foetus during the terminal months of intra-uterine life, and, therefore, to that in the premature baby.

(2) In an attempt to assess the role of oxygen in the genesis of retrolental fibroplasia, kittens of a few days old were subjected to a high concentration of oxygen (60–80 per cent.) for several days.

(3) It was found that oxygen in these concentrations has a profound influence on the process of retinal vascularization, and that it may, in certain circumstances, completely obliterate the ingrowing retinal complexes.

(4) Transfer of the animal to air led to a re-opening of the vessels, but many were permanently obstructed, either through collapse or blood clot, so that the normal architecture was not restored. The reformed network was grossly abnormal, haemorrhages occurred, retinal re-vascularization recommenced from the disc, blood vessels grew into the vitreous, and retinal detachment developed.

(5) These phenomena are regarded as significant in the genesis of retrolental fibroplasia in man.

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