DIRECT OBSERVATION OF THE EFFECT OF OXYGEN ON DEVELOPING VESSELS
PRELIMINARY REPORT

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In previous papers it has been shown that high concentrations of oxygen are able to exert on growing retinal vessels a peculiar type of constrictor action which progresses gradually to complete obliteration of the ingrowing complexes (Ashton and others, 1953, 1954). The possible factors involved in this mechanism have already been fully discussed; it is the purpose of this paper to report preliminary experiments designed to elucidate the problem further.

In order to study the process of vaso-obliteration and to correlate the findings in injected retinae with events in life, we have utilized a new limbal window technique, devised by one of us (C.C.), which permits direct observation of the living retinal vessels. To determine whether oxygen has a similar action on growing vessels in the adult animal we have investigated the effect of hyperoxia on vascularization of the rabbit cornea and rabbit ear chamber; indirect evidence for its action on vessels in the embryo has been sought in a few experiments on hens’ eggs. The methods employed together with the results obtained will be reported under the appropriate headings.

(1) Oxygen and Corneal Vascularization

The rabbit was used because a method of inducing corneal vascularization by the intracameral injection of alloxan was available (Langham, 1953), and it had been shown that the vaso-obliterative phenomenon occurs in the developing retinal vessels of this animal (Ashton and others, 1954). All the animals in two series of experiments were injected with alloxan according to the above technique.

In the first series of six animals, three served as controls, whilst the remaining three, on the 6th day after the alloxan injection, by which time a well marked peripheral zone of corneal vascularization was present, were placed in an oxygen chamber maintained continuously at 70–80 per cent. oxygen.

In the second series of six animals, three served as controls, whilst the remaining three animals were placed in the oxygen chamber immediately after the alloxan injection.

The extent and density of the new vessel formation was examined daily and compared with that present in the control animals over a period of 6 to 7 days. There was no difference in either experiment between the control and test animals; thus oxygen in concentrations of 70–80 per cent. apparently had no vaso-obliterative effect on growing vessels in the rabbit cornea, and did not noticeably affect the rate of vascular growth.

(2) Oxygen and New Vessel Formation in the Rabbit Ear Chamber

Perspex chambers were inserted into one ear of five rabbits by a technique similar
to that of Sandison (1928). A well-marked capillary ingrowth was present at the periphery of the chamber 14 days after they were inserted. A series of photo-micrographs was taken of selected areas of the vascular ingrowth, and the rabbits were then placed in a gas chamber maintained continuously at 70–80 per cent. oxygen. The ear chambers were re-examined daily and serial photomicrographs were taken of the chosen areas over a period of 4 to 5 days. All the animals died suddenly at the end of this time. During microscopical examination pure oxygen was administered to the animals through a closely fitting mask; in one animal the microscopical appearance of the ear vessels was observed continuously over a period of 10 hrs from the commencement of oxygen administration.

There was no evidence of vaso-constriction or vaso-obliteration, or of any significant alteration of the rate of vascular growth.

(3) Direct Observation of the Developing Retinal Vessels of the Kitten

To observe the effect of oxygen on the developing retinal vessels in the living animal and to determine the time relationships of the vaso-obliterative phenomenon, a new limbal window technique was employed. The method is as follows:

The animal is anaesthetized with intraperitoneal Nembutal. Both the upper and lower lids and the nictitating membrane are then removed and the conjunctiva is incised around the limbus and reflected to expose a 3 mm. ring of sclera. Four equidistant sutures are inserted into the sclera 2 mm. behind the limbus and the ends are left free. A metal ring 9–10 mm. in diameter, which encircles the limbus, is then attached to the globe by means of the sutures which pass through four perforations in the rim. The entire cornea is then removed either by cautery or scissors and the iris is completely abscised. It has been found that haemorrhage can be almost completely prevented by application of the cautery to the base of the iris prior to its removal.

The anterior capsule of the lens is then excised with scissors and the soft lens matter gently removed by curette and irrigation. The posterior capsule and posterior portion of the tunica vasculosa lentis should be left intact, for if they are broken a retinal detachment rapidly occurs. A circular glass cover-slip of the appropriate size is then inserted within the ring so that it rests upon a narrow ledge ground into its rim; it is held firmly in place by means of a circular wire clip which is similarly inserted into the ring to rest upon the cover slip (Figs 1 and 2).

Fig. 1.—Components of limbal window: recessed metal ring, glass disk, and spring clip. ×1·5

Fig. 2.—Limbal window in situ. Even at this magnification the retinal vessels may be seen in the fundus. ×5
VASO-OBLITERATION BY OXYGEN

The kitten is now placed in a rectangular Perspex box in which an inlet and outlet tube has been inserted for the purpose of administering oxygen. At one end of the top of the box is drilled a small hole, so situated as to correspond with the position of the ring attached to the eye when the animal’s head is held in close apposition to the top of the Perspex box by means of a linen sling. Through this aperture the retinal vessels can now be observed microscopically via the limbal window whilst the oxygen is being administered.

The illuminating system consists of a mercury vapour lamp and a condensing system which directs a convergent beam of light onto a small, plane reflecting mirror placed within the focal length of the condensing system. A divergent beam is thus reflected into the eye, the area of retina illuminated being controlled both by the position of the reflecting mirror and by a small iris diaphragm, which, together with a plate of heat absorbing glass (Chance ON 20), is situated between the source of light and the condensing lens.

The technique has the great merit of permitting examination and photographic record of the living retinal circulation, the smallest vessels of which may be seen in their finest detail. It has the disadvantage of seriously interfering with the structure of the eye so that retinal detachment may occur; the possible effects this may have on normal function must be borne in mind in interpreting the experimental findings.

Results.—Seventeen experiments have been carried out, involving 22 kittens and one cat. All showed the same general changes. Four representative experiments are quoted below.

Experiment 1 (52).—An adult cat maintained in 80 per cent. oxygen showed no changes in the retinal vessels as observed through a limbal window over a period of 1 ½ hrs (Fig. 3).

Fig. 3.—Experiment 1, fundus of adult cat photographed through limbal window. Oxygen had no observable effect on the retinal vessels. ×17
EXPERIMENT 2 (51).—In a kitten (11 days old) after 3-5 minutes exposure to 80 per cent. oxygen the capillaries completely closed and the main vessels were severely constricted (Figs 4 and 5). This picture did not progress to total vaso-obliteration and the vessels began to re-open after about 10 minutes, although the oxygen concentrations were maintained.

On return to air the main vessels dilated and the capillary bed re-opened in 5-10 minutes.

On return to oxygen the same effect as that produced initially occurred more promptly but in the same manner; again, however, the main vessels were not obliterated and continued to carry an active circulation for as long as 4 hrs.

EXPERIMENT 3 (53).—A kitten (12 days old) was pre-treated in a gas chamber (70-80 per cent. oxygen) for 4 hrs before a limbal window was inserted, oxygen being maintained during operation. When the retina was examined microscopically it was found that the whole vascular bed was open although the arteries were constricted. After a total exposure to oxygen of 5 hrs the capillary bed began to close, and it had disappeared in 6½ hrs. In 7½ hrs the arterial side of the circulation had disappeared; the veins were still visible but constricted.

After 8 hrs' exposure the entire retinal circulation had completely closed.

Amyl nitrite administered with the oxygen at this stage had no effect on the closed circulation.

On return to air the circulation began to re-open within 5 minutes in the order of veins, arteries, and capillaries, reaching a maximum within about 30 minutes; this was not as complete as when the animal was first examined, which suggests that the vaso-obliteration was now not entirely reversible.

On return to oxygen a complete closure developed in 7 minutes.

On return to air the vessels re-opened as previously in 5 minutes.
A second return to oxygen again led to complete closure within 5 minutes. (At this stage it was noted that a further injection of Nembutal did not affect the picture).

On return to air the vessels opened as before.

A mixture of oxygen and 5 per cent. carbon dioxide was now given, and this led to an identical closure within 6 minutes.

Raising the temperature of the box from 27° to 37° C. had no effect.

After reopening in air, vaso-obliteration was again induced by returning the animal to oxygen, and Priscol (15 mg.) was given intra-peritoneally; the vessels then slowly re-opened to the maximum degree obtained on the first transfer to air.

Transfer to air showed no further opening up of the capillary bed (Fig. 6a, b, c, d).

Fig. 6.—Experiment 53, showing delayed effect of oxygen in retinal vessels of kitten continuously exposed to 80 per cent. oxygen. ×17

(a) After 4½ hrs' exposure all vessels still patent.
(b) After 6½ hrs main vessels constricted and capillary bed not visible.
(c) At a later stage main arteries began to close. Horizontal main arterial branches still faintly seen.
(d) After 8 hrs' exposure only one main vein could still be discerned. Soon this too disappeared, leaving a completely avascular white retina.
EXPERIMENT 4 (45).—A kitten (12 days old) pre-treated with oxygen in a gas chamber (70–80 per cent. oxygen) for 5½ hrs, was found on examination through the limbal window to have total vaso-obliteration. Retinal detachment, however, developed in one area, and the vessels in the detached portion re-opened while in the attached retina they remained closed.

*Return to air* re-opened all the capillaries.

*Return to oxygen* closed only the vessels in the attached portion of the retina (Figs 7 and 8). Eventually the whole retina became detached and all the vessels re-opened and remained fully patent for 7 hrs in 90 per cent. oxygen.

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**Fig. 7.**—Experiment 4, kitten retina in air. The white reflex on the right of the picture is at the peak of a developing localized detachment. ×25

**Fig. 8.**—Same retina as that shown in Fig. 7 photographed after exposure to 80 per cent. oxygen. Note that the capillary bed has disappeared only in the attached retina on the left, the vessels being unaffected in the detached area on the right. ×25
Summary of Findings.—It must be emphasized that any conclusions presented at this early stage of our experiments must of necessity be provisional, but on the basis of present observations the following impressions have been gained.

Oxygen has no observable effect on the retinal vessels of the adult cat. On developing retinal vessels it appears to produce immediate and delayed effects. The immediate effect is one of constriction of the large vessels and obliteration of the capillaries, which develops 5 minutes after exposure to hyperoxia. After about 10 minutes the vessels dilate again, and the capillaries re-open and remain approximately in this state for about 5 hours, when the delayed effect comes into operation. The capillary bed again begins to close, the main vessels constrict, and after about 8 hours' hyperoxia the whole retinal circulation is completely obliterated. Once this has been achieved the circulation may be either opened or closed, in each case in about 5 minutes, by alternating air and oxygen.

Total vaso-obliteration is apparently not affected by amyl nitrite, 5 per cent. carbon dioxide, or small rises in temperature.

Vaso-obliteration cannot be induced in the detached retina, and if detachment develops in an area of the retina in which vaso-obliteration has been induced, the vessels re-open, that is, providing that the obliteration is still reversible.

(4) Oxygen and the Developing Chick Embryo

Our experiments studying the effect of oxygen on the developing chick are as yet too inconclusive to give more than a preliminary note. So far three chicks have hatched out alive after being subjected to 80–90 per cent. oxygen during the last 7 days of incubation. They were weaker than normal but gradually recovered and were as vigorous as normal chicks in 24 hours. Vision was apparently unimpaired and there were no defects which would point to any abnormality in the nervous, respiratory, or cardio-vascular systems. The chicks developed normally and remained healthy during a period of 6 weeks' observation.

The only conclusion permissible at this stage is that chick embryos may survive high concentrations of oxygen, hatch out alive, and develop normally in air for at least 6 weeks.

Summary

(1) Preliminary reports are given of experiments designed to elucidate the problem of vaso-obliteration by oxygen.

(2) It was found that hyperoxia has no effect on growing vessels in the rabbit cornea or in the rabbit ear chamber.

(3) A new limbal window technique is described and an account is given of direct observation of the effect of oxygen on the retinal vessels by this method. It was found that:

(a) Oxygen has no observable effect on the retinal vessels of the adult cat.

(b) In the kitten immediate and delayed reactions are seen. The former consists of vaso-constriction and capillary obliteration developing after 5 minutes.
exposure to oxygen. This does not progress to total obliteration and the vessels re-open after about 10 minutes and remain in this state for about 5½ hrs when the delayed effect comes into operation. The vessels then again constrict and total vaso-obliteration gradually develops, becoming complete in about 8 hrs. Once this is achieved the circulation may be opened or closed in about 5 minutes by alternating air and oxygen.

(c) Vaso-obliteration cannot be induced in the detached retina, and obliterated vessels re-open when detachment develops.

(4) Chick embryos may survive 80–90 per cent. oxygen during the last 7 days of incubation, hatch out alive, and develop normally in air.

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REFERENCES

