COMMUNICATIONS

STUDIES ON DEVELOPING RETINAL VESSELS*

I. INFLUENCE OF RETINAL DETACHMENT

BY

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IN the early stages of the normal development of the human retina, the choroidal circulation is apparently responsible for maintaining retinal nutrition, but, as the metabolic requirements of the growing retina increase, this source of supply gradually becomes inadequate and new vessels bud into the retina from the hyaloid artery at the disc. The chorio-capillaris, however, continues throughout life to supply the outer retina to a depth of about 130 microns (Michaelson, 1951) and in health this zone is never invaded by retinal capillaries, which in a remarkably constant way, extend no deeper than the outer limits of the inner nuclear layer, despite the absence of any anatomical barrier.

The sharp demarcation of this avascular area in the outer retina suggests that the chorio-capillaris is capable of supplying nutrition into the retina for only a limited distance; it would, therefore, be of interest to know what effect separation of the retina from the choroid might have upon the growth of retinal vessels.

In this paper experiments are reported which show that detachment of the developing kitten retina, in which the normal pattern of vascular growth is in every way comparable to that in man, leads to a downgrowth of retinal vessels beyond the inner nuclear layer, frequently penetrating the retina completely and forming a network on its outer surface. Vessels may also extend into the vitreous to form glomerular tufts typical of such intravitreal vasoproliferation. In the adult cat, however, retinal detachment had no such influence on the retinal vasculature.

Experimental Findings

Experiment 81.—One mother cat and two kittens (k1, k2) 3 days old.

Plan.—Through a needle withdraw enough vitreous from the right eye of each anaesthetized kitten to induce retinal detachment. Left eye to remain as control. Kill k1 after 8 days and k2 after 18 days, to demonstrate:

(a) Normal retinal vascularization at 11 days old (k1 left) and at 21 days old (k2 left).

(b) Effect of 8 days' retinal detachment at 11 days old (k1 right) and of 18 days' detachment at 21 days old (k2 right).

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Results (both retinae injected with Indian ink)

(a) *k1 left*.—Normal pattern, immature network approaching ora serrata, maximum outgrowth 8 mm.

k2 left.—Normal pattern, immature network at periphery, maximum outgrowth 11 mm.

(b) *k1 right*.—Total retinal detachment. Normal pattern, vessels slightly engorged, retina somewhat shrunken showing a few scattered subretinal haemorrhages. No evidence of abnormal vasoproliferation, maximum outgrowth 6 mm.

k2 right.—Retina widely detached and thrown into folds. In the region of the folds vasoformation was profuse and new vessels extended into the vitreous in the form of loops and glomerular tufts. Elsewhere the vascular pattern was roughly normal but the retinal vessels extended more deeply into the retina and in some areas had completely penetrated it and formed a polygonal network on its under surface. Maximum outgrowth 8 mm. (Figs 1, 2, and 3, opposite).

Conclusions.—This experiment shows that retinal detachment has no apparent influence on retinal vascularization after 8 days, but may considerably affect it after 18 days, when vasoproliferation may occur into the vitreous and outwards through the whole thickness of the retina to form a network on its under surface. In this experiment, however, there were no controls to show the possible influence of needle puncture alone (Cf. Exp. 92).

Experiment 87.—One mother cat and three kittens (k1, k2, k3) 5 days old.

Plan.—Procedure as in Exp. 81; kill k1 after 8 days, k2 after 18 days, and k3 after 29 days, to demonstrate:

(a) Normal retinal vascularization at 13 days old (k1 left), 23 days old (k2 left) and 34 days old (k3 left).

(b) Effect of 8 days' detachment at 13 days old (k1 right), 18 days' detachment at 23 days old (k2 right) and 29 days' detachment at 34 days old (k3 right).

Results (both retinae injected with Indian ink)

(a) *k1 left*.—Normal pattern, immature network at periphery, maximum outgrowth 9 mm.

k2 left.—Normal pattern, few immature vessels at periphery, maximum outgrowth 11 mm.

k3 left.—Normal pattern, fully developed, maximum outgrowth 12 mm.

(b) *k1 right*.—Extensive retinal detachment, vitreous haemorrhage. The retinal vessels were markedly engorged showing a uniform density, but there was no abnormal vasoproliferation either into the vitreous or within the retina. Maximum outgrowth 10 mm.

k2 right.—Total detachment with distortion of normal pattern of vessels. There was no intravitreal vasoproliferation, but the retinal vessels extended more deeply into the retina and had penetrated it in all areas to form a polygonal network on its under surface. Maximum outgrowth 9 mm. (Figs 4 and 5, overleaf).

k3 right.—Total detachment, vessels irregular and engorged. On the temporal side there was a laceration (needle track) around which a brushwork of vessels extended into the vitreous, but elsewhere there was no evidence of intravitreal growth. Vessels had extended through the retina to form a plexus on its under surface; this occurred mainly at the periphery but there were a few tufts within retinal grooves. Maximum outgrowth 12 mm.

Conclusions.—This experiment confirms Exp. 81 in that detachment had no influence on vascularization after 8 days. The two kittens retained for a longer period (18 and 29 days) both showed extension of vessels through to the outer surface of the retina. Intravitreal vessels occurred in one animal only (k3 right) and these were around the needle track. No new vessels, however, occurred around the needle track in k2 and see also Exp. 92.

Experiment 92.—One mother cat and three kittens (k1, k2, k3) 4 days old.

Plan.—Procedure as in Exp. 81 for k1 and k2, but in k3 only a needle puncture to be done as a control. Kill all kittens after 18 days to demonstrate:

(a) Normal retinal vascularization at 22 days old (k1, k2, k3 left).

(b) Effect of control needle puncture after 18 days at 22 days old (k3 right).

(c) Effect of 18 days' detachment at 22 days old (k1, k2 right).

FIG. 1.—Exp. 81, k2 right, 21 days old. Shows effect of 18 days' detachment on developing retinal vessels. The retina is widely detached and thrown into folds, in the region of which vasoformation is profuse and new vessels extend into the vitreous in loops and glomerular tufts (see also Fig. 2). Elsewhere the vascular pattern is roughly normal, but vessels have penetrated the retina (cf. Fig. 3). $\times 9$.

FIG. 2.—High-power view of area indicated in Fig. 1, showing glomerular tufts of vessels extending into the vitreous. $\times 35$.

FIG. 3.—Posterior view of retina shown in Fig. 1. Vessels have penetrated the retina to form a polygonal plexus on its under surface. $\times 15$.

FIG. 4.—Exp. 87, k2 right, 23 days old. Shows effect of 18 days' detachment. In this case there were no intravitreal proliferations from the retinal surface or around the needle track (arrow), but vessels penetrated the retina (cf. Fig. 5). $\times 6.75$.

FIG. 5.—Posterior view of retina shown in Fig. 4. Vessels have penetrated the retina to form a plexus on its under surface. $\times 19$.

Results (both retinae injected with Indian ink)

(a) *k1, k2, k3 left*.—Normal pattern but not yet fully developed, hence less advanced than average for this age. Maximum outgrowth 12 mm.

(b) *k3 right*.—Normal pattern, nearly complete. On the temporal side there was a clean hole at the periphery (needle track). There was no evidence of new vessels in the vitreous in this region or elsewhere. No haemorrhages and no penetrating vessels were seen.

(c) *k1 right*.—Ruptured lens, total folded detachment, subretinal exudate, vessels irregular. At numerous places but particularly on the inward crests of the folds there were tufts and delicate vascular plexuses extending into the vitreous. In many areas the retinal vessels had grown through the retina to form small loops and tufts on its outer surface.

k2 right.—Total folded detachment, vascularization of retina incomplete and three complexes consisting of irregular vessels still seen. On the temporal side there was a hole (needle track) and in this half of the retina there were extensive tuft-like proliferations into the vitreous, some of which appeared to be related to a haemorrhage while others did not. On the nasal side there was a vitreous haemorrhage without intravitreal neovascularization. Only in one or two areas had the retinal vessels extended through to the outer side; much less than in *k1* (Figs 6 and 7, opposite).

FIG. 6.—Exp. 92, k2 right, 22 days old. Shows effect of 18 days' detachment on developing retinal vessels. Around the needle track there is extensive intravitreal vasoproliferation which appears to be associated with haemorrhage (A), but there are also areas of intravitreal vessels without haemorrhage (B), and a haemorrhage without new-vessel formation (C). (See also Fig. 7). $\times 9$.

FIG. 7.—High-power view of intravitreal proliferations (zone A) in the detached retina shown in Fig. 6. $\times 19$.

Conclusions.—This experiment confirms Exps 81 and 87, in that detachment after 18 days gives rise to an extension of vessels through to the outer surface of the retina and may produce intravitreal proliferation, which is apparently not attributable to the trauma of the needle puncture alone, since the control animal (k3 right) showed no abnormality.

Experiment 85.—Two adult cats (C1, C2).

Plan.—Procedure as in Exp. 81. Kill C1 after 18 days and C2 after 36 days to demonstrate the effect of detachment on the retinal vessels of the adult cat.

Results (both retinae injected with Indian ink)

C1 left (control).—Normal retina of adult cat.

C1 right.—Extensive retinal detachment with subretinal exudate, no abnormal vasoproliferation either into the vitreous or through the retina.

C2 left (control).—Normal retina of adult cat.

C2 right.—Extensive detachment of retina on temporal side, subretinal exudate and vitreous haemorrhage. No abnormal vasoproliferation either into the vitreous or through the retina.

Conclusions.—This experiment shows that retinal detachment does not give rise, after periods of 18 and 36 days, to abnormal vasoproliferation in the retina of the adult cat.

Discussion

The foregoing experiments show that, while retinal detachment has no apparent influence on the growth of retinal vessels of the adult cat (Exp. 85), it has a profound effect on the developing retinal vessels of the kitten (Exps 81, 87, 92). After artificial detachment of the retina, these vascular changes take over 8 days to develop and are well established after 18 days. The changes are twofold. First, the developing vessels in the detached retina extend more deeply, growing beyond the normal outer limit of the inner nuclear layer, and eventually penetrate the whole thickness of the retina to form a capillary plexus on its outer surface. Secondly, new-formed vessels may extend inwards to form loops and glomerular tufts within the vitreous; this, however, did not occur in every case of detachment (positive in four out of five kittens), and it may be interpreted as a result of trauma from the needle puncture itself or from associated haemorrhage. Several findings, however, indicate that this is probably not the cause of the intravitreal proliferations: a simple needle puncture without retinal detachment did not result in such proliferations (Exp. 92, k3 right), nor did they occur in one animal with detachment (Exp. 87, k2 right). Furthermore, although intravitreal vessels were seen in association with haemorrhages, others were not so related (Exp. 92, k2 right). It would therefore appear that extension of the growing retinal vessels outward through the retina and inwards into the vitreous is attributable solely to separation of the retina from the choroid.

These findings raise questions of importance. Since it has been shown that the vaso-obliterative effect of oxygen may be prevented by retinal detachment (Ashton and Cook, 1954) it would seem that effective levels of oxygen can no longer reach the retina in these circumstances. It is, therefore, reasonable to assume that the abnormal growth of vessels in the detached developing retina may similarly be related to a fall in oxygen tension resulting from choroido-retinal separation. Thus the growth of vessels towards the outer

surface of the retina is in accord with the hypothesis that a vasoformative stimulus is elaborated in the retina in low oxygen tensions and, conversely, that such a stimulus is normally opposed or destroyed by higher oxygen tensions (Ashton and others, 1954).

The growth of vessels into the vitreous is likewise consistent with this concept. The delicate balance normally existing between the stimulus and vascular response ensures that neither shall become excessive. It seems possible that a lowered oxygen tension in the detached outer retina may considerably disturb this mechanism, leading to so great an accumulation of the vasoformative factor that it seeps into the adjacent vitreous and is thus responsible for the growth of vessels inwards.

Indeed, the effects of retinal detachment are very closely analogous to those following oxygen vaso-oblation. In the latter case the inner layers of the retina are rendered hypoxic by closure of the retinal vessels while the outer layers continue to be adequately served by the choroidal vessels, whereas in the former case the outer layers are rendered hypoxic through detachment while the inner layers continue to be supplied by the retinal vessels. (It is to be remembered, however, that in the developing retina the oxygen supply to the inner layer is presumably less than the demand since the vessels are growing into it).

On the basis of the above hypothesis, the abnormal growth of vessels developing in each of these sets of circumstances is, therefore, exactly as might be expected, but that retinal detachment should have no vasoformative effect on the retinal vessels of the adult animal is more surprising and a finding of great potential significance. If the working hypothesis we have adopted is correct, a series of possibilities follow:

- (a) that the adult retinal vessels have lost the power of regeneration,
- (b) that detachment no longer results in retinal hypoxia,
- (c) that in the adult retina the power of elaborating a vasoformative factor is lost or modified in the degree of hypoxia resulting from detachment.

Since it is known that adult retinal vessels are able to regenerate, and that detachment leads to degeneration of the outer layers of the retina, it would appear that the most likely explanation is the last, namely that the vasoformative factor is less readily produced in the fully mature retina.

It is becoming clear that the whole mechanism of vaso-oblation and of vasoproliferation may well be related to the rate of anaerobic glycolysis and to the effects of oxygen upon it as described by Warburg and other (1924). In this connexion it is interesting to note that the human foetus and young infant can exist by means of a totally anaerobic metabolism for as long as 30 minutes, and the increased toleration for anoxia of new-born infants as compared with adults is partly due to this capacity of anaerobiosis (Brinkman, 1953). Lactic acid levels in the blood are higher in premature than in full-term infants and higher in full-term babies than in older infants (György and others, 1927, 1928; Brehme and others, 1928). Studies on brain tissue from

stillborn infants have shown a high degree of anaerobic metabolism (Himwich, 1953), while the rate of anaerobic glycolysis in the kidneys of suckling rats is higher than in adult rats (Brehme, 1955). In other words, here is a metabolic process which apparently diminishes as the tissues mature. If similar changes occur in the retina, the different responses we have here demonstrated in the cat and kitten retina may be related to them. Until more is known of the metabolism of the developing and adult retina it would be unrewarding to speculate further.

What relation our experiments bear to ocular disease cannot at present be assessed, but it should be pointed out that the intravitreal proliferations obtained by detachment are very similar to those which follow oxygen exposure and, therefore, to those seen in Stage I retrolental fibroplasia. The possibility that some cases of Stage I retrolental fibroplasia, which have not been exposed to high oxygen concentration, may be secondary to some degree of retinal detachment must therefore be taken into consideration in future studies of this disease.

Summary

(1) Experiments are reported which show that detachment of the developing kitten retina leads to:

(a) an outward proliferation of vessels to form a plexus on the outer retinal surface and

(b) an inward proliferation of vessels to form glomerular tufts in the vitreous.

(2) In the adult cat, retinal detachment had no such influence on the retinal vasculature.

(3) The significance of these new findings is discussed in the light of cognate work.

(4) It is pointed out that there is a great similarity between the intravitreal proliferations obtained by retinal detachment and those seen in Stage I retrolental fibroplasia. The possibility that some degree of retinal detachment may play a role in cases of retrolental fibroplasia in which supplemental oxygen has not been given, should be taken into consideration in future studies of this disease.

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REFERENCES

- ASHTON, N., and COOK, C. (1954). *British Journal of Ophthalmology*, **38**, 433.
 ———, WARD, B., and SERPELL, G. (1954). *Ibid.*, **38**, 397.
 BREHME, T. (1955). *Lancet*, **1**, 671.
 ———, GYÖRGY, P., and KELLER, W. (1928). *Jb. Kinderheilk.*, **120**, 42.
 BRINKMAN, R. (1953). In "Anoxia of the Newborn Infant": Symposium organized by the Council for International Organizations of Medical Sciences, 1951, p. 123. Blackwell, Oxford.
 GYÖRGY, P., BREHME, T., and BRAHDY, M. B. (1927). *Jb. Kinderheilk.*, **118**, 178.
 ———, KELLER, W., and BREHME, T. (1928). *Biochem. Z.*, **200**, 356.
 HIMWICH, H. E. (1953). In "Prematurity, Congenital Malformation, and Birth Injury". Proceedings of Conference, New York, 1952, p. 59. Ass. for the Aid of Crippled Children, New York.
 MICHAELSON, I. C. (1951). "XVI Conc. ophthal., 1950, Britannia Acta", vol. 1, p. 605. B.M.A. London.
 WARBURG, O., POSENER, K., and NEGELEIN, E. (1924). *Biochem. Z.*, **152**, 309.