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MECHANISM OF CORNEAL VASCULARIZATION*

BY

NORMAN ASHTON AND CHARLES COOK

Department of Pathology, Institute of Ophthalmology, London

THE fundamental biological phenomenon of new vessel formation is one of enormous complexity, and has excited the interest of scientific workers for many years as shown by the extensive bibliography of the physiological and pathological aspects of the problem. The normal cornea, having the great advantages of avascularity, transparency, and ready accessibility to biomicroscopical examination, has been widely employed as an experimental tissue in these investigations. There are, however, several special features in the process of corneal vascularization which, as we shall subsequently show, prevent an exact analogy with vessel growth elsewhere, thus rendering the cornea, in fact, a somewhat unsuitable medium for the study of the general question of capillary growth, and necessitating a specific approach to the problem of corneal vascular invasion. This paper, although drawing upon the relevant findings in extra-ocular experimental work, is concerned in the main with the mechanism of vascularization as seen in corneal tissue.

The many theories which have been advanced to explain the initiation of new vessel formation in the cornea may be considered under two main headings:

(1) *That in disease processes a substance is elaborated in the cornea which stimulates the limbal vessels to grow towards the site of its maximum concentration, i.e., a process combining growth stimulation and positive chemotaxis.*

From experimental evidence it has been assumed for many years that the general biological phenomenon of directed tissue growth, as seen, for example, in the development of the axons of embryonic neuroblasts, may be controlled by rising and falling gradients of chemical concentration. To this class of cell movement the term "chemotropism" has been applied. In the case of capillary penetration, it is possible that the proliferating vascular buds move from a zone where some chemical substance is in low concentration, towards a region of traumatized or necrotic tissue containing this substance in high concentration.

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The release of growth-stimulating substances from tissue cells has been extensively investigated. Thus Fischer (1930) demonstrated the promotion of growth which resulted from the infliction of wounds upon tissue cultures, and Cameron (1935) showed that extracts of inflamed tissue were similarly effective. Such substances, which are particularly active in embryonic tissue, have been demonstrated in extracts of leukocytes and named "trephones" by Carrel (1922). In repair processes Menkin (1941) holds the view that several proliferative factors may be involved; indeed it is known that proteoses (Carrel and Baker, 1926; Carrel and Ebeling, 1928), peptones (Kiaer, 1928), nucleoprotein fractions (Fischer, 1939), glutathione and haemoglobin (Baker, 1929), and the sulphydryl group in general (Hammett, 1929) may all be effective stimuli in certain circumstances. In plants it has been shown that growth-stimulating "wound hormones" are liberated from injured cells (Haberlandt, 1922), and more recently one of the substances has been isolated in pure form and named traumatic acid (English, Bonner, and Haagen-Smit, 1939).

The application of this principle to the problem of corneal vascularization has been particularly emphasized by Campbell and Michaelson (1949), who suggested from their studies on standardized burns in rabbits' corneae that corneal new vessel formation involves a factor released at the site of the lesion whence it diffuses to stimulate and direct new vessel growth from the limbal plexus.

Subsequently Campbell and Ferguson (1950) showed that corneal vascularization occurs more readily in scorbutic than in normal guinea-pigs; they believed this indicated an inability of corneal tissue deficient in ascorbic acid to meet the increased metabolic demands of repair; this led to an accumulation of metabolites which might constitute the factor postulated by Campbell and Michaelson (1949).

Many consider that histamine may be the growth-stimulating factor and its possible role has been fully discussed by Offret and Chauvet (1950). Haessler (1927) suggested that the stimulus came from toxins in combination with blood constituents, and Julianelle and Lamb (1934) have demonstrated that the intracorneal injection of egg albumen and bacterial nucleoproteins can give rise to corneal cloudiness followed by vascularization. If such a diffusing vascularizing substance exists, it is perhaps, surprising that it never extends beyond the limbus to stimulate proliferation in the immediately adjacent scleral vessels. Nor does the theory of such a factor assist in the conversely related problem of why the cornea is normally avascular.

Local anoxia or the sequential accumulation of acid metabolites might be the operative stimulus. In the cornea, Bessey and Wolbach (1939) and Johnson and Eckardt (1940) attributed the marked ingrowth of capillaries which follows riboflavine deficiency to a break-down in the corneal oxidative system. Swindle (1938) believed that vessel ingrowth is attributable to an increase in the concentration of hydrogen ions. Indeed, that oxygen-lack

or an excess of carbon dioxide in the tissues is a potent factor in the production of neovascularization generally, is supported by a considerable amount of experimental evidence.

Thus the presence of such influences is implicit in the important observations of Michaelson (1948) upon the development and anatomy of the normal retinal vessels; his preparations demonstrated a capillary-free zone around the retinal arteries, and he found that the formation of retinal capillaries in the developing embryo is pre-eminently a formation of the retinal veins and that if a vein and artery are close to each other, growth takes place predominantly from the side of the vein remote from the neighbouring artery. Experiments which logically follow from these findings have been carried out by Campbell (1951) who subjected litters of rats to a low oxygen environment and found that the capillary-free zone around the retinal arteries was significantly narrowed, thus further tending to show that oxygen tension is closely related to capillary growth.

Anomalous vascular growth has been produced by Byerly (1926), in developing chick embryos asphyxiated by immersion of the egg in water-glass, and Windle, Becker, and Weil (1944), in studying alterations in the brain structure of guinea-pigs which followed asphyxiation at birth, found vascular proliferations in regions where neurons had been destroyed by anoxia. Champy and Louvel (1938) have induced new vessel formation in the frog's foot by immersing it in carbon dioxide, and in the frog's and guinea-pig's mesentery following intraperitoneal injection of the same gas; these authors concluded that carbon dioxide is not only a vaso-dilating agent but also a vaso-formative one.

In conditions in which ischaemia arises as a result of vascular occlusion (such as Eales's disease, occlusion of the central retinal vein, and diabetic retinopathy), anoxia has been held responsible for the new vessel formation which frequently follows. Anoxia is also said to play a part in the angiomatosis of Lindau's disease (Ingalls, 1948) and in the neovascularization which is a characteristic feature of retrolental fibroplasia (Szewczyk, 1952; Ingalls and others, 1952; Crosse and Evans, 1952).

It will thus be seen that the theory of anoxia as a stimulus to new vessel formation demands serious attention and it will be referred to again in our subsequent discussion. It should be borne in mind, however, that even if tissue anoxia were proved to be a stimulating factor in new vessel formation it may, nevertheless, operate indirectly through the release of growth promoting factors.

(2) *That the limbal vessels are normally prevented from entering the cornea either through its chemical content or through growth-inhibiting substances or through the compactness of its tissue. Vascularization would then arise through destruction of the antagonizing substance or through a reduction in corneal compactness.*

The theory postulating the inhibition of new vessel formation by a factor present in the corneal tissue was first advanced by Meyer and Chaffee (1940

a, b) who isolated a sulphate ester of hyaluronic acid from the substantia propria. By analogy with hyaline cartilage, which contains a very similar carbohydrate (chondroitin sulphuric acid), and like the cornea is also avascular, they advanced the hypothesis that the polysaccharide was intimately connected with corneal transparency and the absence of blood vessels. They showed that these characteristics were abolished by hyaluronidase and suggested that various pathological lesions may produce an increase in such enzyme activity and so lead to corneal vascularization. It was in accordance with this concept that Jones and Meyer (1950) considered that the inhibitory effect of cortisone on corneal vascularization might be due to its ability to prevent such a break-down of corneal mucopolysaccharide.

Considerable doubt, however, as to the importance of the role played by corneal mucopolysaccharide has been cast by the work of Wislocki and others (1947) who demonstrated that hyaluronidase does not abolish the metachromatic staining reaction of the cornea, of Woodin (1950), who found that hyaluronidase is inactive as a spreading factor in the cornea, and of Wise (1943), who showed that hyaluronic acid ester was not diminished in quantity in vascularizing corneae.

Although growth inhibiting substances have been demonstrated in normal serum in concentrations increasing with age, and in tumours and liver extracts (Heaton, 1926; Drew, 1927; Brues, Subbarow, Jackson, and Aub, 1940), no inhibiting factors have as yet been demonstrated in corneal extracts.

The thesis that the degree of compactness of the corneal tissue is the determining factor in the initiation of neovasculogenesis was originated by Cogan (1949 a). He rightly pointed out that tissues such as cartilage and finger nails cannot be vascularized because they have no invadable intercellular substance and he believes that these conditions are equally applicable to the cornea. In a series of experiments he produced small corneal lesions by burning with a cautery and by injection with hydrochloric acid or sodium hydroxide and he has observed a regular sequence of events leading to new vessel formation. Engorgement of the limbal vessels was followed by the formation of saccular aneurysms which burst, and new vessels then proliferated into the resulting haemorrhage between the corneal lamellae. The one event which constantly preceded these vascular changes was swelling of the corneal stroma, and he therefore concluded that these vascular developments were attributable to a reduction in the compactness of the corneal tissue.*

Cogan's hypothesis is attractive in that it explains with nicety why vessels are prevented from entering the healthy cornea and how it becomes possible for them to invade it in disease. A point against the argument, however, is that new vessel formation must necessarily involve a proliferation of endothelial cells and although the stimulus for cellular regeneration is unknown, the available evidence is against its being dependent upon alterations in tension; indeed, there has been a general tendency to abandon Ribbert's tissue-tension theory.

*The terms "corneal swelling" and "reduction of tissue compactness" are used synonymously in this paper and that of Cogan (1949a) because they cannot be distinguished by simple observation. It is theoretically possible, however, that corneal swelling may occur without opening of the intercellular spaces, e.g., from intracellular fluid inhibition; the term "reduction in tissue compactness" implies a stromal separation due to increased extra-cellular fluid and is the more accurate term when referring to a removal of obstruction to ingrowing vessels.

As we have already indicated, it is more probable that the proliferation is a response either to metabolic demands or to growth-promoting substances liberated by injured cells (Davidson, 1943). It would seem, therefore, that some factor in addition to corneal swelling is likely to be involved in the vascularizing process.

It may thus be concluded that despite the attention the subject has attracted, the mechanism of corneal vascularization remains in doubt, and that none of the many theories advanced appears to merit complete acceptance. In an endeavour to clarify the present position, we have carried out further experiments on the problem, repeating some of those of other workers, particularly those of Cogan, and devising methods of our own. The findings are discussed in relation to the above concepts, and an attempt is made to assess their bearing upon clinical appearances and upon the therapeutic action of cortisone.

Experiments

The experiments were designed to investigate the two most important questions in corneal vascularization, namely, the likely roles that may be played by a vascularizing substance and alterations in tissue compactness. Although the two problems overlap to a considerable extent, it will be convenient to consider them under two headings.

(1) To investigate the presence of a vascularizing substance.—The methods employed were those of injecting saline extracts and grafting discs from vascularizing corneae into the corneae of normal eyes. Control experiments were carried out with extracts and grafts from normal corneae; the effects of needle puncture and saline injection were separately controlled. Rabbits anaesthetized with nembutal were used throughout the experiments.

Preparation of Extracts.—Dilute extracts were first employed to avoid the complicating factor of viscous solutions; a more concentrated extract was used later.

(a) Dilute Extracts.—The control and test extracts were prepared respectively by grinding six normal corneae and six vascularizing corneae in physiological saline in a pestle and mortar. The resulting solution was centrifuged and the supernatant fluid freeze-dried; the deposit was then weighed and made up to a 5 per cent. solution. (Vascularization was induced by Langham's alloxan technique reported by Ashton, Cook, and Langham, 1951; none of the vessels was included in the corneal discs which were taken for extraction on the 7th to 10th day, when marginal vascularization was established).

(b) Concentrated Extract.—Thirteen control and eight vascularizing corneae were treated as above and reconstituted to a fully saturated solution (30–40 per cent.).

EXPERIMENT (i).—A series of four animals received an intracorneal injection of 0.02 ml. physiological saline 4 mm. from the limbus in the left eye, and a sterile needle track was made in the right eye 4 mm. from the limbus.

Result.—There was no vascularization in either eye in any of the four animals.

EXPERIMENT (ii).—A series of six rabbits received an intracorneal injection of 0.02 ml. dilute corneal extract 4 mm. from the limbus. The right eye was used for the test extract and the left for the control.

Result.—In three animals both corneae became vascularized, but the ingrowth of vessels in the control eyes was less marked and appeared later than in the test eyes. In one animal both corneae became vascularized but the control eye vascularized earlier than the test eye. In one animal only the test eye vascularized, the control eye remaining normal. In the sixth animal neither eye vascularized. Corneal vascularization was thus inconstant but could

be induced by the extracts of both normal and vascularized cornea (Figs 1 and 2).

FIG. 1.—Experiment (ii). Control rabbit, 6 days after intracorneal injection of control corneal extract (5 per cent. solution). Note deep marginal vascularization extending into area of corneal haze.

FIG. 2.—Experiment (ii). Test rabbit, 6 days after intracorneal injection of extract of vascularized corneae (5 per cent. solution). Note well-marked marginal vascularization of cornea.

EXPERIMENT (iii).—The experiment was exactly similar to that described above except that concentrated extract was used.

Result.—In two animals both corneae vascularized. In one animal only the control cornea vascularized. In one animal only the test cornea vascularized. No difference was, therefore, demonstrated between the test and control experiments.

EXPERIMENT (iv).—In a further series of six animals, 0.02 ml. concentrated corneal extract was injected into the centre of the cornea. The right eye was used for the test extract and the left for the control.

Result.—In both eyes there was a dense central corneal haze but in no case did this extend to the limbus. No vascularization occurred in either test or control eyes.

EXPERIMENT (v).—A series of four rabbits received a central full-thickness corneal graft. In two the grafts consisted of normal corneae, while in the other two the grafts were taken from corneae in which vascularization had been induced by the intracameral injection of alloxan (Figs 3 and 4).

FIG. 3.—Experiment (v). Control rabbit, showing extensive corneal vascularization 21 days after insertion of control graft.

FIG. 4.—Experiment (v).—Test rabbit, showing corneal vascularization 21 days after insertion of graft from a vascularized cornea.

Result.—Corneal vascularization occurred in all four animals.

(2) To evaluate the role played by alterations in tissue compactness.—In these experiments attempts were made to decrease the tissue compactness by opening up the lamellae with a fine flattened probe, by injecting nitrogen, saline, and serum, and by inducing corneal oedema through traumatising the corneal endothelium.

EXPERIMENT (vi).—In two rabbits a plane of cleavage was made through the middle thickness of the cornea by means of a fine flattened probe. The apex of the triangle was 5 mm. from the limbus and the base extended along 15 mm. of the corneo-scleral junction.

Result.—In one eye a 2 mm. zone of vascularization developed after 3 days and by the 6th day it had extended to 3 mm. with cloudiness and swelling of the adjacent cornea. The cloudiness disappeared slowly, and after 10 days the vascularization had disappeared. The plane of cleavage was then re-opened with the probe and well-marked vascularization recurred with marked surrounding corneal swelling, both of which had disappeared by the 12th day.

In the second eye a 2 mm. zone of vascularization appeared by the 3rd day, but by the 6th day there was no corneal oedema and the vascularization had almost disappeared. The plane of cleavage was then re-opened and a well-marked vascularization re-appeared in 3 days, but again both corneal oedema and vascularization had disappeared by the 6th day (Fig. 5).

EXPERIMENT (vii).—Nitrogen was injected into the cornea, towards and 5 mm. from the limbus. The corneal swelling had disappeared within 24 hrs and no vascularization occurred. Two weeks later a further nitrogen injection was made *via* the original site of injection and into the same area.

Result.—In one eye the needle entered the anterior chamber and in 3 days a 2 mm. zone of vascularization occurred. No vascularization developed in the second eye in which the anterior chamber was not entered.

EXPERIMENT (viii).—In three rabbits physiological saline was injected into the cornea.

Result.—The bleb had almost disappeared in 24 hours and no vascularization ensued.

EXPERIMENT (ix).—A daily intracorneal injection of 0.15 ml. physiological saline was given in two eyes. The injection was made approximately 8 mm. from the corneo-scleral junction, and the area of corneal turgescence extended to the limbus on each occasion.

Result.—The corneal opacity was only transient in nature, transparency being regained within 12 hours. After the fifth injection, however, an incipient vascular invasion of the corneal stroma was apparent and after the sixth injection a well-marked intracorneal vascular ingrowth could be seen.

EXPERIMENT (x).—In two eyes 0.02 ml. rabbit serum was injected into the cornea towards and 5 mm. from the limbus.

Result.—The bleb had almost disappeared within 24 hours and no vascularization occurred in either eye. A further injection of serum was then given, at the same site and in the same direction. In one eye a faint corneal haze persisted between the limbus and the needle puncture and two small vascular twigs, 1 mm. and 2 mm. in length, appeared by the 3rd day, and persisted for one week. In the other eye no haze persisted and no vascularization developed.

EXPERIMENT (xi).—Two non-penetrating needle tracks were made into one cornea, one entering 3 mm. from the limbus and the other 5 mm. from the limbus.

Result.—No vascularization occurred in either site.

FIG. 5.—Experiment (vi) Marginal corneal vascularization 3 days after producing plane of cleavage with fine probe.

EXPERIMENT (xii).—Two needle tracks penetrating into the anterior chamber were made, one at 10 o'clock 5 mm. from the limbus and the other at 2 o'clock 3 mm. from the limbus.

Result.—In 3 days one small vessel 1 mm. in length had invaded the cornea opposite the 2 o'clock perforation, and in 6 days two small vessels 2 mm. in length were present. There was, however, no vascularization opposite the needle track 5 mm. from the limbus, the intervening corneal stroma being clear.

EXPERIMENT (xiii).—In three eyes the endothelium was abraded by means of a metallic foreign body previously introduced into the anterior chamber, according to the technique of Cogan (1949b).

Result.—In one eye the abraded area was immediately adjacent to the limbus. A localized area of faint to moderate central haze resulted, but it did not extend to the limbus and no vascularization ensued (Fig. 6). In a second eye the abraded area was 4 mm. from the limbus; again a localized central area of opacity developed, which did not reach the limbus, and no vascularization ensued. In the third eye the central endothelium was abraded, and the result was the same. In all three cases localized corneal vascularization occurred in the region of the original keratome incision.

EXPERIMENT (xiv).—The endothelium and Descemet's membrane in three eyes were removed with a blunt curette from a small area 5 mm. from the limbus.

Result.—In 3 days there was a diffuse corneal haze extending up to the limbus, and a dense 2-mm. zone of corneal vascularization was present. By the 6th day the haze was absorbing rapidly, but the vascularization was 3 mm. in area and increased to 4.5 mm. by the 11th day (Fig. 7)

EXPERIMENT (xv).—The endothelium was removed from a similar area in three eyes with a bent wire covered with polythene tubing.

Result.—In all three eyes a localized area of faint to moderate corneal haze followed; this did not extend to the limbus and no vascularization ensued.

FIG. 6.—Experiment (xiii). Residual central corneal opacity resulting from abrasion of small area of endothelium immediately adjacent to upper limbus.

FIG. 7.—Experiment (xiv). Marked corneal haze and zone of peripheral vascularization resulting from removal of small circular area of endothelium and Descemet's membrane 5 mm. from limbus (4th day).

Although the corneal epithelium was not damaged, a marked pigment slide has occurred.

Discussion

So far as the production of evidence in favour of a vascularizing substance is concerned, it will be seen that the above experiments failed to demonstrate

any significant difference between normal and vascularized corneal extracts, or between normal corneal grafts and vascularized corneal grafts. Thus normal corneal extracts (Figs 1 and 2) or grafts (Figs 3 and 4) were equally successful in stimulating new vessel formation, as were the extracts and grafts from the vascularizing corneae. The great disadvantage of the experimental technique, however, is that tissue-trauma, which has been held responsible for the release of a vascularizing factor, was involved in the preparation of both control and test extracts and in the control and test grafting experiments. For this reason the grafting experiments could only have been of value if a difference was to have been demonstrated between the test and control grafts. The fact that we obtained vascularization in all grafts is completely inconclusive, but the fact that both the normal and the vascularized corneal extracts failed to produce vascularization when injected into the centre of the cornea, is evidence against the existence of a diffusible vascularizing factor extractable by the methods employed. Nor was there evidence of a directional influence towards the lesion (Fig. 8), the vascularization occurring principally in areas of greatest swelling, even when these were not adjacent to the site of injury; we have also frequently observed new vessels growing beyond the exciting lesion, at which site a diffusible factor might be expected to be at its maximum concentration. There is, of course, a directional influence towards the centre of the cornea. Although we have neither succeeded in devising experiments sufficiently delicate or uncomplicated to prove or disprove the existence of a vascularizing factor, nor excluded the possibility of such a factor being masked by growth-inhibiting substances, we have, nevertheless, found no evidence to support the concept that vascularization occurs in response to the release of a specific "vascularizing substance" in the diseased cornea.

FIG. 8.—Marginal vascularization of cornea induced by corneal laceration re-opened every 3rd day over a period of 17 days. Note that vascularization shows no evidence of directional influence towards the lesion.

On the other hand, the second group of experiments indicates that a reduction in the compactness of the corneal tissue, whether produced mechanically or by the induction of corneal oedema, may give rise to vascularization, providing the potential pathways resulting from the stromal separation communicate with the limbal vessels and are maintained for a sufficient period of time. In no case in either group of experiments was vascularization produced which was not preceded by a zone of corneal swelling extending up to the limbus. Rapidly diffusible substances such as

saline produced no vascularization unless the cornea was repeatedly injected, whereas the viscous and slowly absorbable corneal extract was followed in a high proportion of cases by a well-marked ingrowth of new vessels, provided that the associated corneal swelling itself reached the limbal region.

Our experiments involving the induction of corneal oedema should ideally be considered in relation to normal function. Unfortunately the physiological mechanism whereby the cornea retains its transparency and compactness has been a subject of much controversy for many years, and, although the factors involved have been very thoroughly investigated (Kinsey and Cogan, 1942; Pirie, Schmidt, and Waters, 1948; Davson, 1949; Potts and Johnson, 1950; Maurice, 1951), their mode of operation is as yet unknown. It is certain, however, that the transparency of the cornea is closely connected with its water content (Fischer, 1933; Kinsey and Cogan, 1942), which in turn may be determined by the osmotic forces operating between it and the surrounding fluids. The cornea normally contains 76 per cent. of water, and an addition of 10 per cent. causes it to swell and turn cloudy (Fischer, 1927). Kinsey and Cogan (1942) suggested that the cornea was maintained in a deturgescient and compact state by the hypertonicity of the tears and aqueous fluid, but Davson (1949) and Maurice (1951) thought this theory required modification, and advanced reasons for supposing that the fluid is extruded out of the cornea by some active process. In any event it is clear that, to maintain respiration and control of fluid transference, there must be no impairment of the continuity or functional integrity of the corneal epithelium and endothelium (Rones, 1940). Maurice and Giardini (1951) have shown that after removal of the corneal epithelium the corneal stroma swells to double its previous thickness, whereas the removal of the endothelium is followed by a much greater degree of stromal swelling; this swelling is still further enhanced when Descemet's membrane is ruptured, in which case it may be some six times greater than that produced by epithelial removal alone.

That these facts have a close bearing upon vascularization is shown in our experiments, and in those of Cogan, involving the removal of corneal endothelium.

It was found that the degree of trauma to the endothelium, the extent of the resulting oedema, and the incidence of vascularization were directly related. Thus, when an area of endothelium was completely removed and Descemet's membrane was ruptured, a diffuse corneal thickening and opacification ensued which was followed by a profuse ingrowth of new vessels. When, however, the endothelium alone was abraded, a faint diffuse haze with slight corneal swelling followed and there was no accompanying vascularization. This latter failure to vascularize is attributable, at least in part, to peripheral clearing of the cornea (Fig. 6); a point to be immediately discussed.

The role played by the limbal plexus in the control of corneal hydration has received considerably less attention than those of the epithelium and

endothelium. It has been shown, however, that destruction of the limbal vessels does not give rise to swelling or opacity in the cornea (Gruber, 1894), from which it follows that the factors operating through the epithelium and endothelium are themselves sufficient to maintain normal deturgescence. When, however, corneal swelling arises as a result of minimal endothelial damage, and gives rise to a faint corneal haze, the part played by the limbal vasculature may become apparent. Thus, in our experiments in which this technique was employed, it was noted that the corneal haze and swelling cleared rapidly at the periphery and more slowly centrally, irrespective of the site of endothelial injury. Throughout the endothelial abrasion experiments this central opacity was consistently present.

It would appear, therefore, that in cases of slight endothelial damage the normal fluid exchange between the limbal vessels and the peripheral cornea is sufficient to cope rapidly with excess fluid in this part of the cornea but is insufficient to deal with a central swelling; the latter disappears only after a considerably longer period, and that only after the endothelium has regenerated.

Thus far our investigations fully substantiate Cogan's conclusion that a reduction in the tissue-compactness of the cornea in the region of the pre-existing vessels is an essential precursor of the process of corneal vascularization (Cogan, 1949a). On general principles, however, it is difficult to accept his thesis that new vessel formation in the cornea is solely initiated by a loosening of the tissue spaces at the limbal margin, for this would confer upon the limbal vessels a property which is not apparently possessed by the general vascular system. As we are aware, there is no evidence that a reduction of tissue compactness by chronic oedema, even of long duration, has any influence upon new vessel formation outside the cornea. In the retina, for instance, new vessel growth is certainly not necessarily so associated. The compactness of the corneal tissue, like the compactness of cartilage, may be regarded merely as an obstruction to invading vessels; the fact that a reduction in this density allows newly formed vessels to enter the tissue does not necessarily imply a stimulus to neovascularization. As has been indicated earlier in this paper, new vessel formation is more likely to arise as a response to some growth-promoting substance resulting from injury to the tissues of which anoxia may be an example; it is even possible that anoxia may directly stimulate growth.

Our experimental findings do not entitle us to draw conclusions beyond this point, but since our experiments and those of Cogan have failed to demonstrate any extractable or diffusible vascularizing factor, it may be helpful in planning future research work, to speculate upon the presence of other stimulating influences, which can be accepted as being generally applicable to the vascular system as a whole.

Since corneal vascularization can be produced by simple separation of the corneal lamellae (*e.g.*, with saline) it would appear likely that the vascular-

izing stimulus, the existence of which seems both logical and necessary, is constantly present in an active form in the normal cornea. It is, therefore, instructive to refer to the recent researches of Langham (1952b) who has shown that the cornea in the living animal does not utilize oxygen to its maximal capacity; in experiments *in vitro*, however, this deficit was made good owing to the more ready availability of the atmospheric oxygen. In other words, the cornea *in vivo* is constantly and normally in a state analogous to that pertaining in anoxia, which, as we have already shown, is strongly supported experimentally as a potent stimulus to vascularization throughout the body. If this condition of "suboxidation" can give rise to neovascularization, then such a stimulus permanently resides in normal corneal tissue.*

This finding of Langham's would, therefore, complete a possible hypothesis for the mechanism of corneal vascularization, namely: vascularization is normally prevented by the compactness of the tissue and when this is decreased, vascularization will readily occur, in response to a stimulus which is constantly present in the normal cornea, provided the potential pathways resulting from the stromal separation are adjacent to the limbus and maintained open for a sufficiently long time.

The vast majority of the experimental findings in the literature can be satisfactorily explained on the basis of the above mechanism, but we shall turn now to a consideration of those reports which would appear to be opposed to it. Although the idea of "suboxidation" as the vasoformative factor in the cornea has many points in common with the concept of Campbell and Michaelson (1949), it differs in some respects. It does not embrace their idea of a substance released in injured tissue and diffusing to the limbus, but their experimental results may be explained otherwise than by postulating such a factor. The small standard burns which they produced in the cornea would have given rise, through epithelial damage and through inflammation, to a local corneal oedema, distributed in a circular fashion around the lesion. As Cogan (1949 a) and our own experiments have shown, vascularization would then only occur providing the oedematous tissue was in contact with the limbal vessels, and, in the case of a small lesion, this in turn would be dependent upon its distance from the limbus. Such a process would explain both their findings of a "critical distance" and the triangular segmental zone of vascularization. Corneal oedema, therefore, together with the metabolic conditions prevailing in the normal corneal tissue, provides an equally satisfactory explanation of their results.

A much more serious challenge to the above hypothesis is that of Bessey and Wolbach (1939), who found that the vascular ingrowth induced by riboflavin deficiency was unaccompanied by any change in corneal transparency (and, therefore, presumably in corneal thickness), until many days and often

*The term suboxidation used in this context is not strictly accurate in that it connotes a pathological state in which the oxygen supply is deficient for the tissues normal requirements, whereas the cornea normally consumes less oxygen than it is capable of utilizing in abnormal circumstances. Nevertheless, the normal corneal metabolism is comparable to that found in anoxic tissues, and the word "suboxidation", in the absence of a better term, is used in this sense throughout the discussion.

several weeks after the invading vessels became visible. Furthermore, neither the corneal endothelium, Descemet's membrane, nor the corneal epithelium showed any demonstrable changes until late in the deficiency. Their thorough experimental technique provides no obvious escape to the conclusion that the vessels were able to invade a compact corneal tissue—a faculty which is denied by the bulk of experimental work on corneal vascularization. There may, however, be many ways in which corneal vascularization can arise, the mechanism we have suggested being but one, and it may be that special unknown factors come into play in riboflavin deficiency. Alternatively, it is possible that some diminution in compactness may occur without a readily recognizable change in transparency. Indeed, if vital factors are involved in the maintenance of corneal deturgescence, as suggested by Davson (1949) and Maurice (1951), one would expect them to be affected in a deficiency which undermines the very foundations of cellular respiration. It would be interesting to repeat the experiments of Bessey and Wolbach (1939) with accurate measurements of corneal thickness; their suggestion that the vascular obliteration which follows correction of the riboflavin deficiency, is due to a compression between the corneal lamellae, certainly implies a reversal of corneal swelling. Furthermore, other workers (Totter and Day, 1942) have stated that in riboflavin deficiency vascularization occurs only after a diffuse corneal opacity becomes apparent.

There is also some difficulty in understanding the mechanism of corneal vascularization in tryptophane deficiency (Totter and Day, 1942; Albanese and Buschke, 1942; Buschke, 1943; Albanese, 1945), lysine deficiency (Hock, Hall, Pund, and Sydenstricker, 1945), and methionine and protein deficiency (Sydenstricker, Hall, Hock, and Pund, 1946). In all these conditions, however, the vascularization is superficial and conjunctival oedema has been noted in association; it is possible that the deficiencies lead, through some common metabolic path, to epithelial dystrophy. This would result in an interference with deturgescence, particularly anteriorly, and a superficial vascular ingrowth would follow.

Clinical Considerations and the Role of Cortisone.—If the hypothesis that vascularization results from decreased corneal compactness and “suboxidation” were correct, new vessel formation would depend upon fluctuations in corneal thickness, since the factor of “suboxidation” is relatively constant. This in turn will depend to a great extent upon pathological changes at the limbus and in the corneal epithelium and endothelium. In some cases of corneal vascularization, only one of these factors may be involved, but in others, two, or all three, may be incriminated, the final extent and pattern of vascularization being the result of their interplay.

For instance, the clinical counterpart of the experiments in which endothelial abrasion gave rise to a corneal haze with peripheral clearing and no vascularization is seen in cases of disciform keratitis, buphthalmos, and corneal injury leading to epithelial or endothelial damage. The experiments involving more severe endothelial injury with diffuse opacification and vascularization are paralleled in keratitis profunda (uveo-keratitis) wherein severe toxic endothelial degeneration occurs.

It is doubtful whether limbal congestion alone can sufficiently interfere with the mechanism of deturgescence to give rise to vascular ingrowth. Although Wise (1943) believes that the formation of new vessels in the cornea in acne rosacea arises in response to limbal engorgement, as part of a general facial vaso-dilatation, it is probable that the process is in fact a less simple one. It is well recognized that ciliary congestion *per se*, even if of considerable duration, is not usually associated with corneal vascularization. The limbal vessels, however, may be primarily responsible for vascularization in those intense inflammatory reactions which lead to such a gross outpouring of exudate into the corneal stroma that the dehydrating mechanism is overwhelmed.

It would thus appear that the vascularization induced primarily by an inflammatory reaction at the limbus and that induced by the loss of integrity of the corneal limiting membranes, subserve two entirely different functions:

(1) In the former, the new vessel invasion represents an extension of the inflammatory process into the corneal tissue, and only in the later stages, when the aetiological factors underlying the inflammation are on the wane, will the intra-corneal vessels take up a reparative role. In such cases, therefore, an inhibition of the vascular invasion is essential if the cornea is to be spared. The beneficial result following such an inhibition is strikingly shown by the results of cortisone therapy in cases of deep keratitis. A rapid reduction of the inflammatory phenomena is followed by both a decrease in corneal swelling and a clearing of the associated corneal infiltration.

(2) In the case of vascularization induced by injury of the corneal limiting membranes, however, the role played by any resulting vascularization has a beneficial and reparative effect, for it apparently assists in draining away the corneal oedema. It is well recognized that clearing of the cornea is especially rapid in those cases which show vascularization. Any success gained by cortisone therapy, therefore, is rather to be attributed to its suppression of the primary intra-ocular inflammation which allows endothelial regeneration and restoration of normal deturgescence.

The close relationship between the degree of corneal swelling and vascularization in man, has been demonstrated by Cook and Langham (1953), who measured the corneal thickness in a variety of corneal diseases, according to the technique of Maurice and Giardini (1951); their findings are illustrated elsewhere in this issue.

In conclusion, it may be of value to review the possible factors involved in the suppressive effect which cortisone is able to exert on corneal vascularization. It may achieve this result:

- (i) by inhibiting new vessel growth.
- (ii) by preventing a decrease in corneal compactness.

(i) Regarding the first possibility, it has been suggested that cortisone may suppress endothelial proliferation (Duke-Elder and Ashton, 1951); this view received some indirect support from our experiments with cortisone in the healing of corneal wounds, that there was a marked inhibition in regeneration of the corneal endothelium (Ashton and Cook, 1951). It is to be remembered, however, that the endothelial cells of the cornea are peculiar in that they secrete Descemet's membrane, a function which no other endothelium appears to possess, and it has been suggested on other grounds that they are not truly endothelial cells (Sondermann, 1930, 1949). Whatever be the cause of the inhibition in this instance,

it may not be justifiable to relate it to that of the vascular endothelium. In order to elucidate this point we have studied the effect of cortisone upon the ingrowth of new vessels into transparent rabbit-ear chambers (Ashton and Cook, 1952). The most striking features noted in the vascularizing test chambers were generalized vascular constriction, increased translucency of the vessel walls, and an exaggerated tonic response to extraneous stimuli. In the animals treated with cortisone there was a moderate delay in vascularization, to some extent more apparent than real, since the majority of the capillary buds and the vessels from which they arose were exsanguinated. Endothelial proliferation and canalization of the newly-formed buds occurred as in the control animals. We concluded that cortisone was able to inhibit vascularization in the rabbit-ear chamber, but that this influence was more probably due to its profound effect upon the circulation than to a direct inhibition of endothelial growth. The site and mode of the action of cortisone in the production of these vascular changes are as yet unknown. Reasons were given for suggesting that future enquiry might profitably be directed towards a study of the influence of cortisone upon vasomotor mechanisms. Applying these observations to the suppressive action of cortisone upon corneal vascularization, it will be seen that a direct inhibition of new vessel growth, through the mechanism described above, is probably an important factor. Indeed, the vasoconstrictive effect of cortisone on corneal vessels can be readily demonstrated clinically (Figs 9 and 10). The vasoconstrictive effect is not,

FIG. 9.—Superficial keratitis. Cornea shows marked superficial vascularization. Compare with Fig. 10.

FIG. 10.—Same cornea as shown in Fig. 9 after six subconjunctival injections of cortisone over a period of 3 weeks. Note marked vasoconstriction.

however, an immediate one; it gradually develops and increases as the period of cortisone administration extends. Langham (1952 a) holds the view that cortisone has no direct effect upon capillary proliferation, but this influence might have become apparent had his test rabbits been subjected to cortisone for a longer period.

(ii) Turning to the second possibility, we may quote the experimental work of Langham (1952a), who studied the effect of cortisone upon the increase in corneal thickness which follows the intracameral injection of alloxan, and demonstrated the ability of cortisone to restrain a swelling of the cornea. It is not known how cortisone achieves this effect, but, in view of the close association between corneal swelling and vascularization, there can be little doubt that this action is a potent factor, if not the most significant one, in the inhibition of corneal vascularization by cortisone. Furthermore, it is known that

cortisone is able to prevent an increase in capillary permeability (Cook and MacDonald, 1951), and this effect would play an important part in preventing the extension of exudate from the limbal vessels into the corneal stroma. Whether cortisone similarly influences the permeability of the corneal endothelium is a question which has not yet been investigated. Clearly, if it had such an action, it could affect fluid exchanges through this membrane and so alter the degree of thickness. It might further be argued that cortisone modifies corneal vascularization by damping down the severity of the inflammatory reaction within the corneal tissue, but it is not yet clear what factors, besides vasoconstriction and depression of capillary permeability, are involved in this process.

Summary

The theories which have been advanced to explain the mechanism of corneal vascularization are critically reviewed, and it is concluded that not one is wholly satisfactory. Further experiments have been carried out, in an endeavour to clarify the present position, and the findings are discussed in relation to current concepts, clinical implications, and the therapeutic action of cortisone.

No evidence has been found to support the idea that vascularization occurs in response to the release of a specific vascularizing substance in the diseased cornea, but the findings do not exclude this possibility. On the other hand, the results fully support the hypothesis of Cogan (1949a) that a reduction in the corneal compactness in the region of the limbus is a necessary stage in the process of corneal vascularization. We do not, however, agree with the view that an increase in corneal thickness is the stimulus to new vessel formation, but regard it merely as a removal of the compactness which normally obstructs their ingrowth. It is possible that a number of different mechanisms may be involved in corneal vascularization, and it is suggested as a possible hypothesis that the stimulus to new vessel growth in the cornea may be attributable to its peculiar metabolism, which results in a constant state of "sub-oxidation" in the normal corneal tissue; so that when there is a decrease in tissue compactness, vascularization will regularly occur, providing the swelling is adjacent to the limbus and is maintained for a sufficiently long period.

Since the factor of "suboxidation" is relatively constant, new vessel formation will depend upon the fluctuations in corneal thickness, which result to a great extent from the interplay of pathological changes at the limbus and in the corneal epithelium and endothelium.

The significance of vascularization induced primarily by an inflammatory reaction at the limbus is contrasted with the significance of that arising through loss of integrity of the corneal limiting membranes, and analogies are made between experimental results and clinical appearances.

The role of cortisone in the inhibition of corneal vascularization is discussed. Reasons are advanced for believing that this effect is attributable partly to its ability to promote vasomotor tone and prevent an increase in capillary permeability, and partly to its inhibitory effect on corneal swelling.

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