BLOOD-VESSEL FORMATION IN THE CORNEA*

BY

F. W. CAMPBELL *and* I. C. MICHAELSON

UNIVERSITY OF GLASGOW

INTRODUCTION

THE experimental production of corneal vascularisation has been performed by various workers. Ehlers (1927) made daily applications of ethyl alcohol to rabbits' corneae resulting in a loss of corneal transparency and an ingrowth of vessels from all round the limbus into the anterior two-thirds of the cornea. Julianelle (1933) produced " pannus " formation by intra-corneal injection of proteins after sensitisation of rabbits and monkeys. Mann (1943) described new-vessel formation in the cornea after application of mustard gas and related substances. In none of these

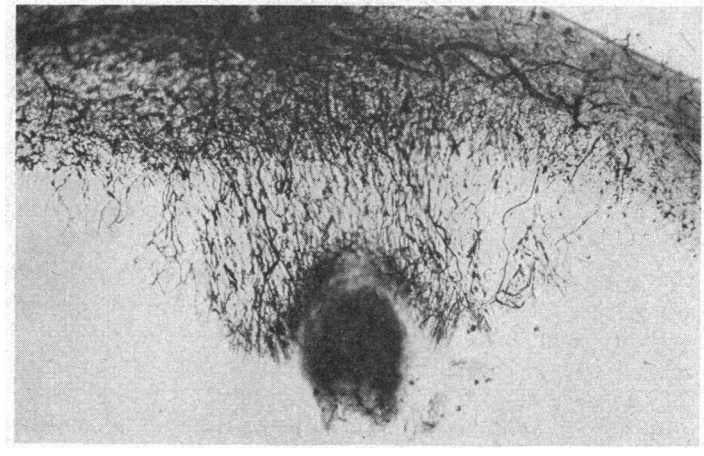

FIG. 1.

Note the isosceles triangular area of vascular infiltration from the limbal plexus above.

methods was the injury sufficiently localised or of an intensity constant enough for our purpose.

From clinical observation it is known that, if a localised lesion of the cornea is accompanied by vascularisation, the vessel formation usually begins at the part of the limbus nearest to the lesion, and that it often tends to take a triangular form as indicated in Fig. 1. We decided to study this phenomenon in rabbits under

* Received for publication, August 11, 1948

standard conditions unattainable in clinical work, and this series of experiments was designed to discover if the site of a localised injury such as a burn had any effect on the formation of these new vessels from the limbus.

METHOD

A " standard lesion " was produced by applying a platinum wire cautery to a rabbit's cornea for approximately two seconds. The cautery was kept at a uniform temperature throughout the experiment by supplying a constant voltage. Two minutes before cauterisation two drops of 1 per cent. pontocain hydrochloride

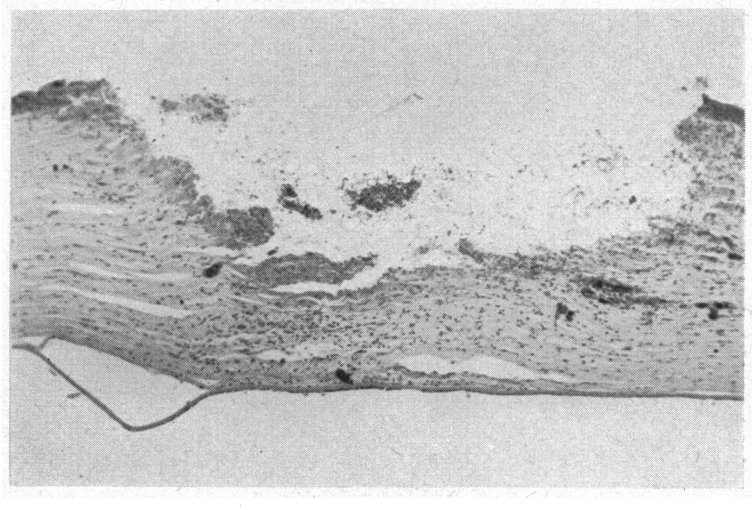

FIG. 2.

The lesion involves the epithelium and the anterior two-thirds of the stroma.

were placed in the conjunctival sac. The burn to each cornea was repeated daily on the same site for the duration of the experiment, usually 10-14 days. The lesion was about 1 mm. in diameter and involved the epithelium and the superficial two-thirds of the substantia propria (Fig. 2). With two exceptions infection of the wound did not occur. The distance of the injury from the limbus varied from rabbit to rabbit, although the injuries were placed along the same meridian—usually 12 o'clock.

At the end of the serial cauterisation the rabbit was anaesthetised with ether, a carotid artery exposed, and a cannula inserted directed cranially. Five to ten c.c. of a 50 per cent. dilution of Indian ink (Reeves) in water was then run in under a pressure of 100 to 150 mm. Hg until the vascularised area of the cornea was filled

with ink particles. The animal was killed, the eye excised and placed in 10 per cent. neutral formalin. For examination the whole corneal thickness, including the limbus, was mounted in glycerine. The vascularised area was measured with a microscope eyepiece-micrometer and with a Vernier moving stage.

RESULTS

Experiment 1. The right corneae of five young adult rabbits were cauterised daily for 13 days. In four of the corneae a typical triangular vascular area was produced. The results are presented in Table I. In these corneae, as in the others subsequently examined, the vascular triangle was isosceles (Fig. 1).

TABLE I

Rabbit	Distance of lesion from limbus in mm.	Size of isosceles sides in mm.
A	1·7	3·3
B	1·7	3·3
C	2·1	3·2
D	2·1	3·2
E	4·2	No vascularisation

In rabbits A and B with a lesion 1·7 mm. from the limbus, the length of " d " (Fig. 3) was the same—3·3 mm. That is, vessels grew from all parts of the limbus within 3·3 mm. of the centre of the lesion. Again, in rabbits C and D, with a lesion 2·1 mm.

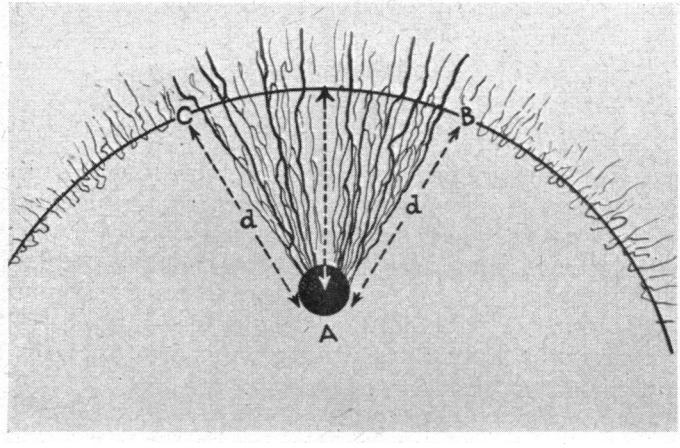

FIG. 3.

A diagramatic representation of the vascular triangle. The vessels do not all grow towards the lesion as shown, but tend to grow towards the centre of the cornea (Fig. 1).

from the limbus, vessels grew from all parts of the limbus within 3·1 mm. of the lesion. On the other hand, when the lesion was placed 4·2 mm. from the limbus as in rabbit E, the limbal vessels were not affected, and no vascularisation was produced.

The approximation of the measurements for " d " in these four corneae (3·3, 3·3, 3·1 and 3·1 mm.) stimulated us to repeat the experiment on a larger scale in order to investigate further the degree of constancy of " d " for a standard lesion.

Experiment 2. This experiment was carried out along similar lines, but both corneae were injured in each rabbit. In this group, only 10 successive daily cauterisations were done. Moreover the cautery point was larger and the temperature was slightly higher. The standard lesion, although constant within this group, was therefore different from that used in experiment 1.

The results in the second series of 8 rabbits involving 16 corneae are shown in Table II. Triangular areas of corneal vascularisation were obtained in all the eyes. The experiment on the left eye of rabbit G was spoiled by an error in the technique. In rabbit F the wounds became infected, as shown by general redness of the conjunctiva and a discharge from the eyes. All the other eyes were white and without discharge. We therefore excluded the results from these animals.

TABLE II

Rabbit	Eye	Distance of lesion from limbus in mm.	Size of isosceles sides "d" in mm.
A	Rt.	1·4	4·0
E	Lt.	1·9	4·4
C	Lt.	2·1	4·3
D	Lt.	2·1	4·4
H	Lt.	2·1	3·9
B	Rt.	2·2	4·4
D	Rt.	2·2	4·4
E	Rt.	2·2	4·2
A	Lt.	2·3	4·3
H	Rt.	2.4	3·8
G	Rt.	2·5	3·9
C	Rt.	2·7	4·2
B	Lt.	3·3	4·3

In the remaining 13 corneae the distance from the centre of the " standard lesion " to the basal angle of the vascular triangle is fairly constant. The mean of the measurement of " d " in these 13 corneae is 4·2 mm. with a standard deviation of 0·21 mm. The range is from 3·8 mm. to 4·4 mm.

In corneae H rt. and H lt. a triangle of pigment was noted to occupy the same position as the vascular area (Fig. 4). In the remaining rabbits limbal pigmentation was very slight or absent.

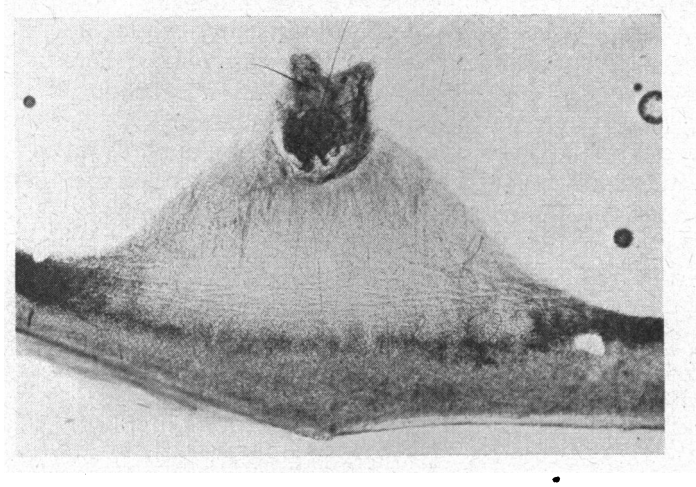

FIG. 4.

Note the migration of pigment from the limbal pigment ring below.

Experiment 3. Six rabbit corneae were injured under conditions identical with experiment 2. The " standard lesions " were made at 4·5, 4·6, 4·8, 5·0, 5·1, 5·3 mm. from the limbus. In no case was ingrowth of limbal vessels observed.

DISCUSSION

A localised lesion, in the rabbit's cornea, placed within a certain distance from the limbus, can produce an area of vascularisation which has the form of an isosceles triangle. If, however, the lesion is placed beyond this distance no vascularisation is observed. Further, there is a relatively constant distance between the site of the lesion and the basal angles of the vascular triangle (the distance " d " Fig. 3). These findings could be accounted for by assuming that a factor, which is produced by the corneal lesion at A (Fig. 3), diffuses from that situation equally in all directions, and in doing so becomes less effective the further away it is from A. The amount of this factor present at the angles B and C at the base of the vascular triangle would represent the minimum amount of the factor capable of stimulating limbal vessels to bud. Beyond B and C the concentration of the factor is apparently too low to stimulate budding.

The shape of the resulting vascular area can be readily appreciated by studying Fig. 5. Each large black circle represents a standard lesion, and the distance between the concentric lines is 1 mm. A factor diffusing from the lesion will become weaker,

the further it is from the source. Let us assume that, for this particular lesion, the factor is able to stimulate vascularisation up to the 3 mm. ring, while beyond this distance the factor is too weak to produce new vessels. It can be seen from Fig. 5 that, although the shape of the vascular triangle varies according to the distance of the lesion from the limbus, the distance from the lesion to the basal angles of the triangular area remains unchanged.

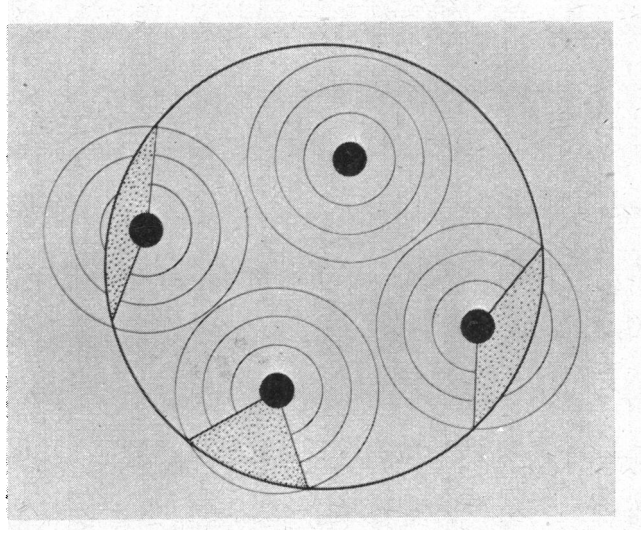

FIG. 5.

The black circle represents the standard lesion. The concentric lines are placed at 1mm. intervals. The shaded area indicates the theoretical shape of the area of infiltration.

The distance " d " (Fig. 3) is the radius of the zone in which the concentration of the " factor " is sufficient to produce vascularisation from the limbus.

The findings would then appear to offer evidence that new-vessel formation in the cornea, in the circumstances described, depends on a factor released by the primary lesion and diffusing from the site of the lesion.

Studies of the retina have also shown evidence of the presence in that tissue, in certain circumstances, of a factor or factors able to influence the budding of new vessels (Michaelson, 1948).

The nature of the factor liberated at the site of the burn might possibly be a substance similar to histamine directly affecting the limbal vessels. On the other hand there is evidence accumulating which suggests that there may be present in the normal corneae a

substance, or substances, which prevent the invasion of limbal vessels (Wise, 1943; Bacsich and Riddell, 1945; Bacsich and Wyburn, 1947). Destruction of this substance around the site of a thermal burn would allow new-vessel invasion of the cornea in a manner similar to our findings.

According to Mann (1944), pigmentation of corneal epithelium is evidence of healing by " sliding " of epithelial cells from the limbus on to the cornea. In the two corneae in our second series where pigmentation was present, the pigment was distributed in

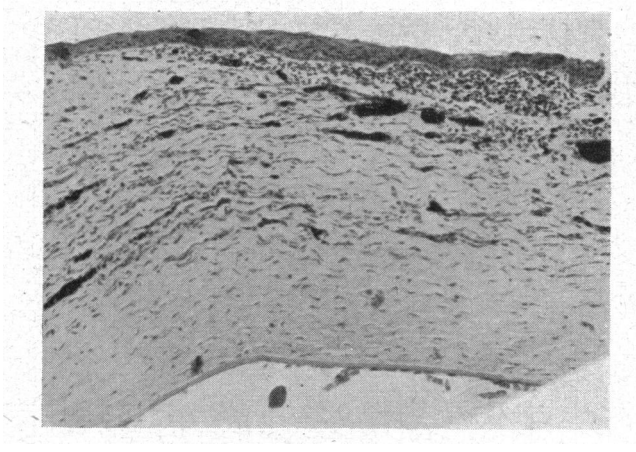

FIG. 6.

The epithelium is above. The vessels occupy the anterior two-thirds of the cornea. There is also considerable cellular infiltration.

a triangular form and co-extensive with the underlying new vessel formation. Furthermore, the vessels occupy the anterior half of the substantia propria and epithelium (Fig. 6). To us this raises the possibility that there may be a common mechanism influencing epithelial sliding and new-vessel formation.

This work has raised a number of interesting problems about the nature of the new-vessel stimulus, and it is hoped that further investigation will provide a clue to its structure.

SUMMARY

(1) A method is described for producing a relatively standard corneal lesion in rabbits and for studying the resulting vascularisation of the cornea.

(2) If the lesion is greater than a certain critical distance from the limbus no vascular response occurs. At less than this distance

from the limbus, vascularisation occurs, and the vascular area has the form of an isosceles triangle.

(3) If a series of "standard lesions" be placed at different distances from the limbus, but within the critical distance for that lesion, the distances between the sites of the lesions and the basal angles of the triangular vascular areas are fairly constant.

(4) The results suggest that the new-vessel formation in the cornea, in the circumstances described, involves a factor released by the lesion and diffusing from the site of that lesion.

We are indebted to Prof. R. C. Garry for helpful criticism and encouragement.

The cost of this investigation was defrayed by the Rankin Medical Research Fund.

REFERENCES

BACSICH and RIDDELL (1945).—*Nature, Lond.*, **155,** 271.
BACSICH and WYBURN (1947).—*Proc. Roy. Soc. Edin.*, **62,** 321.
EHLERS (1927).—*Acta Ophthal.*, **5,** 99.
JULIANELLE, *et alii* (1933).—*Amer. J. Ophthal.*, **16,** 962.
MANN and PULLINGER (1943).—*Amer. J. Ophthal.*, **26,** 1253.
MANN (1944).—*Brit. J. Ophthal.*, **128,** 26.
MICHAELSON (1948).—*J. Anat.*, **82,** 167.
WISE (1943).—*Amer. J. Ophthal.*, **26,** 591.

BOOK NOTICES

Principles and Practice of Ophthalmic Surgery. By E. B. SPAETH. Fourth edition. Pp. 1044. 8 colour plates, 649 figs. References in text. Henry Kimpton, London. 1948. Price, £3 15s. 0d.

This edition follows the lines of its predecessors, and like them will be warmly welcomed. In the writing of text-books on ophthalmic surgery, it is difficult to find the mean between, on the one hand, the author's personal practice, which is often so individualistic as to be of little value except to himself and those who are psychologically in relation to him, and, on the other, a compilation of the views of the many distinguished exponents of ophthalmic surgery as an art, a compilation which would be so compendious as to be useless and to appal even the stoutest-hearted bibliophile. The work under review has long been recognised to be as happy a compromise as may perhaps be expected in an imperfect part of the world where scientific doctrine and practice are as yet independent of the state, although, even in it, some may have considered that an undue amount of space has been devoted to the principles of plastic surgery and reconstruction; this criticism, however, is hardly valid in the aftermath of the war.