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THE ROLE OF ASCORBIC ACID IN CORNEAL VASCULARIZATION*

BY

F. W. CAMPBELL AND I. D. FERGUSON

Institute of Physiology, University of Glasgow

Introduction

ALTHOUGH the cornea normally has no blood vessels, yet, under certain conditions, new vessels freely enter the substance of the cornea from the limbal plexus.

To explain this invasion many theories have been advanced. In riboflavin deficiency there is corneal vascularization. Since riboflavin is part of an oxidation enzyme system, Bessey and Wolbach (1939), and Johnson and Eckardt (1940), believe that anoxia is a stimulus for corneal vascularization. Campbell and Michaelson (1949) brought forward evidence that injury to the cornea close to the limbus sets free a humoral substance which then diffuses to the limbal vessels to stimulate new vessel formation. Julianelle and Lamb (1934) injected an antigen into the cornea of a sensitized animal. This evoked vascularization of the cornea.

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Mayer and Chaffee (1940) and Bacsich and Riddell (1945) look at the problem from a somewhat different angle. They believe that avascular tissues, cornea, cartilage, and Wharton's jelly, normally contain a substance inhibiting vascularization. One could postulate that injury to the cornea destroys or inhibits formation of this hypothetical substance, thus permitting invasion of blood vessels.

Cogan (1949), on the other hand, believes that vascularization is always preceded by oedema of the cornea involving the tissues of the limbus. The resulting reduction in the corneal tissue compactness allows invasion by blood vessels.

We recently studied the influence of lack of ascorbic acid on the rate of healing of heat injuries to the cornea of guinea-pigs (Campbell, Ferguson, and Garry, 1950). Our findings are summarized in Table I. We also found that there was marked persistence of structural weakness in the corneal stroma at the site of the lesion, presumably due to defective formation of collagen.

TABLE I

Time for Epithelial Healing of Deep Corneal Heat Injuries in Guinea-pigs

No. of wounds	Mean time of healing (hours)	Difference between controls and deficient animals (hours)
32 control 32 scorbutic	94.0 (\pm 6.02)* 125.8 (\pm 7.02)	31.8 (Highly significant)

't' = 3.43 P < 0.01 *Standard Error of the Mean.

During this investigation we took the opportunity to observe the incidence and progress of vascularization in the normal controls and in the scorbutic animals.

Method

Animals.—Female non-pregnant guinea-pigs were used. Their initial weights were between 450 and 650 g.

Diets.—The basal diet was crushed rat-cake cubes (Thomson, 1936) moistened with a little water. These cubes are free from ascorbic acid. To supplement this six drops of cod liver oil were added daily to the diet of each animal.

Ascorbic Acid.—To ensure that the cavies had a uniform initial level of saturation with ascorbic acid, 20 mg. ascorbic acid (Roche) were given orally in 2 ml. water once per day. This intake was given for 21 days in all cavies to obtain tissue equilibrium (Jones, Bartlett, Ryan, and Drummey, 1943).

Control Animals.—Control animals were injured after 21 days of saturation with Vitamin C and the daily intake of 20 mg. was continued thereafter.

Scorbutic Animals.—After a preliminary 21 days of saturation with 20 mg. ascorbic acid per day, the animals were given 0.5 mg. ascorbic acid every second day for a further 21 days. The injuries were then made to the animals, the dosage of 0.5 mg. ascorbic acid every second day being continued thereafter.

Apparatus.—The lesions were produced with a cautery made from a loop of 32 S.W.G. platinum wire. A predetermined constant voltage was fed to the cautery through a relay connected to the one second contacts of a Palmer A.C. time clock. This circuit allowed the current to flow through the cautery for exactly one second when required.

Technique.—The cornea was anaesthetized by instilling into the conjunctival sac two drops of a 2 per cent. pontocaine hydro-chloride solution. The operations did not appear to cause discomfort since the corneal reflex was never elicited and the animals remained quiet. No signs of distress appeared after the operation and in no case did infection occur.

The cold cautery was pressed firmly and vertically on the cornea 2 mm. from the limbus at 12 o'clock. The current was allowed to flow for exactly one second and the cautery was removed one second later. This gave a standard heat injury similar to that used by Campbell and Michaelson (1949). Histological examination showed that the resulting lesion was 1 mm. in diameter, and that the corneal epithelium and the anterior two-thirds of the substantia propria were destroyed.

All thermal injuries were carried out by the one operator, who was unaware whether a control or a scorbutic animal was being injured.

Examination of the injury, including degree of vascularization and of oedema, was made at eight hourly intervals by one observer with the aid of a binocular loupe and focal illumination. Once epithelial healing had occurred observations were continued at 24-hour intervals.

Results

During healing, vascularization of the cornea occurred in nine out of 32 eyes in control guinea-pigs, and in nineteen out of 32 eyes of deficient animals (Table II). The results were analysed by the

TABLE II

Incidence of Vascularization in the Control and Scorbutic Groups

Eyes	No. of vascularized corneae	No. of nonvascularized corneae
Control	9	23
Scorbutic	19	13

$\chi^2 = 5.1 \quad P < 0.05$ (Significant).

χ -squared method ($\chi^2=5.1$). Such a result could occur by chance only less than 1 in 40 times, and we may therefore presume that the greater incidence of vascularization in the scorbutic animals is significant.

This higher incidence might be due to the greater time required for healing in the group of deficient animals (Table I) for it is a common clinical observation in man that a corneal ulcer of long duration is more likely to induce corneal vascularization than one of short duration. If this explanation be true, then the eyes with vascularization will be associated with the injuries taking longer to heal. Table III shows the mean time of healing for the vascularized and nonvascularized eyes in the scorbutic group. The

TABLE III

Comparison of the Mean Time of Wound Healing in the Vascular and Non-vascular Cornea in the Scorbutic Group

No. of Eyes	Mean time of healing (hours)	Difference in Means (hours)
19 vascular	127.6 (± 9.3)*	4.5 (Not significant)
13 nonvascular	123.1 (± 11.5)	

't' = 0.79 P > 0.4 *Standard Error of the Mean.

difference of 4.5 hours between the two groups is not significant. A greater difference in the means exists in the control group (Table IV). The difference of 17.7 hours is again, however, not significant. There is thus no conclusive evidence to suggest that, under

TABLE IV

Comparison of the Mean Time of Wound Healing in the Vascular and Non-vascular Corneae in the Control Group

No. of Eyes	Mean time of healing (hours)	Difference in Means (hours)
9 vascular	106.7 (± 13.8)*	17.7 (Not significant)
23 nonvascular	89.0 (± 6.3)	

't' = 1.33 P > 0.1 *Standard Error of the Mean.

the conditions of this experiment, the greater incidence of vascularization in the scorbutic group is due to the longer time required for epithelial healing.

The onset of vascularization, the time of its maximum extent, and the time of disappearance all tended to be delayed in the scorbutic group compared with the control group (Table V).

TABLE V

Comparison of the Progress of Corneal Vascularization in Control and Scorbutic Groups

Vascularization	Mean Time (hours)		Difference in Means (hours)
	Control (9 eyes)	Scorbutic (19 eyes)	
Onset	33.7 (± 8.30)*	52.6 (± 8.79)*	18.9
Maximum	64.9 (± 6.99)	79.6 (± 9.31)	14.7
Disappearance	134.2 (± 33.44)	146.5 (± 21.56)	12.3

*Standard Error of the Mean.

The time of onset and the time of maximum oedema are shown in Table VI. The difference between the means of the times of onset is not significant. However, the scorbutic group did take

TABLE VI
Comparison of the Progress of Corneal Oedema in Control and Scorbutic Groups

Oedema	Mean Time (hours)		Difference in Means (hours)
	Control (32 eyes)	Scorbutic (32 eyes)	
Onset	17.3 (±2.53)*	16.5 (±1.83)*	0.8
Maximum	25.8 (±2.78)	42.8 (±6.22)	17.0

*Standard Error of the Mean.

significantly longer to reach the time of maximum oedema. The figures could be not be analysed for the time of disappearance of the oedema as some of the animals were killed for histological examination before the oedema had disappeared.

Discussion

The cornea is known to contain high concentrations of ascorbic acid, although there is some disagreement concerning the partition of ascorbic acid between the various layers of the cornea (Schmid and Bürki, 1943; Henkes, 1946; Pirie, 1946).

In experimental scurvy in guinea-pigs, ascorbic acid is known to disappear from the cornea in from 2 to 3 weeks (Henkes, 1946). Nevertheless, the cornea shows no obvious change even in severe and prolonged scurvy in human beings or in guinea-pigs. On the other hand, ascorbic acid is known to be necessary for formation of collagenous tissue in the repair of wounds in the cornea. Thus, the injuries we inflicted to the cornea possibly unmasked a deficiency not otherwise apparent. Presumably new formation of collagen makes additional metabolic demands which cannot be adequately met in a state of scurvy. Just as ariboflavinosis evokes spontaneous vascularization of the cornea, avitaminosis C may lead to vascularization when there is the extra metabolic demand following on injury.

Presumably failure to satisfy fully a metabolic need, however caused, leads to accumulation of metabolites. These metabolites may act as a direct stimulus to new blood-vessel formation. On the other hand, the metabolites may lead to oedema and opening up of the lamellae of the substantia propria, and thus permit invasion of blood vessels. Is it too much to suggest that the

accumulation of metabolites is the humoral factor postulated by Campbell and Michaelson (1949)?

This hypothesis is, of course, in the highest degree speculative, but our new finding that ascorbic acid deficiency significantly increases the incidence of vascularization of the cornea after injury does seem to warrant an attempt to find a common causal factor to explain the invasion of the avascular cornea by blood vessels in the conditions where such invasion is known to occur.

Summary

(1) A method is described for producing small standard heat injuries to the cornea.

(2) These injuries were inflicted on control and scorbutic guinea-pigs.

(3) New vessel invasion of the cornea following the injury occurred with significantly greater frequency in the scorbutic group than in the control group.

(4) The causal factor in new vessel formation in the cornea is discussed in the light of these findings. It may be that repair of an injury makes additional metabolic demands which cannot be met in a state of ascorbic acid deficiency. As a result, metabolites accumulate and evoke by some means, not yet fully understood, vascular invasion of the corneal substance.

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