PROLIFERATIVE ACTIVITY OF ENDOTHELIOCYTES OF GROWING CAPILLARIES OF THE RABBIT CORNEA

O. Yu. Gurina, S. G. Mamontov, and V. V. Banin

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With the aid of parameters of intracellular DNA activity, such as incorporation of ^{3}H-thymidine into the nuclei, it is possible to assess reasonably accurately the kinetics of cell proliferation [3, 5]. In endothelial cells, these parameters are usually small [7]. Ausprunk and co-workers [6] demonstrated a change in the proliferative activity of endotheliocytes of growing blood capillaries. However, it is not yet clear how the relatively high mototic activity of the endothelial cell of newly formed capillaries correlates with the intensity of their growth, especially under the influence of substances of the colchicine type.

The writers have studied the intensity of DNA synthesis by cells of newly formed capillarites, growing in the rabbit cornea, after infliction of a silver nitrate ($AgNO_3$) burn and local application of colchicine. The intensity of capillary growth was investigated during stimulation and a combination of the burn with colchicine, and also determined changes in activity of DNA synthesis by the endotheliocytes of newly formed capillaries during exposure throughout growth to these procedures.

EXPERIMENTAL METHOD

Experiments were carried out on 15 rabbits weighing 2.5-3 kg, divided into three groups: 1 control and 2 experimental. The intensity of cell proliferation in animals of all groups was studied by measuring incorporation of ^{3}H-thymidine into the endotheliocyte nuclei. A single injection of ^{3}H-thymidine into the animals in a dose of 3 μCi/g body weight was given 40 min before fixation of the tissue. The frequency of incorporation of ^{3}H-thymidine into endotheliocyte nuclei in capillaries of the limbus was studied in control animals. Animals of experimental group 1 were anesthetized with pentobarbital and a burn was inflicted on the center of the cornea with an $AgNO_3$ crystal for 20 sec [9, 10]. The animals of experimental group 2, immediately after burning, received an application of 0.05% colchicine ointment beneath the palpebral conjuctiva at 6-hourly intervals for 8 days. Before removal of the material and 1-3 min before fixation of the tissue, the rabbits received an intravenous injection of peroxidase of plant origin, in a dose of 25 mg/100 g body weight, after which a

TABLE 1. Quantitative Characteristics of Blood Vessels of the Limbus and Newly Formed Corneal Capillaries

Experimental conditions	Number of vessels in limbus per 100 μ² area (definitive+growing capillaries)	Number of growing capillaries in cornea per 10 μ length of circumference of limbus
Control	13,8±0,04	0
$AgNO_3$ burn	15,1±0,11	9,0±0,08
$AgNO_3$ burn + colchicine ointment	17,8±0,15	18,0±0,16

Legend. For all groups of animals $p < 0.05$.

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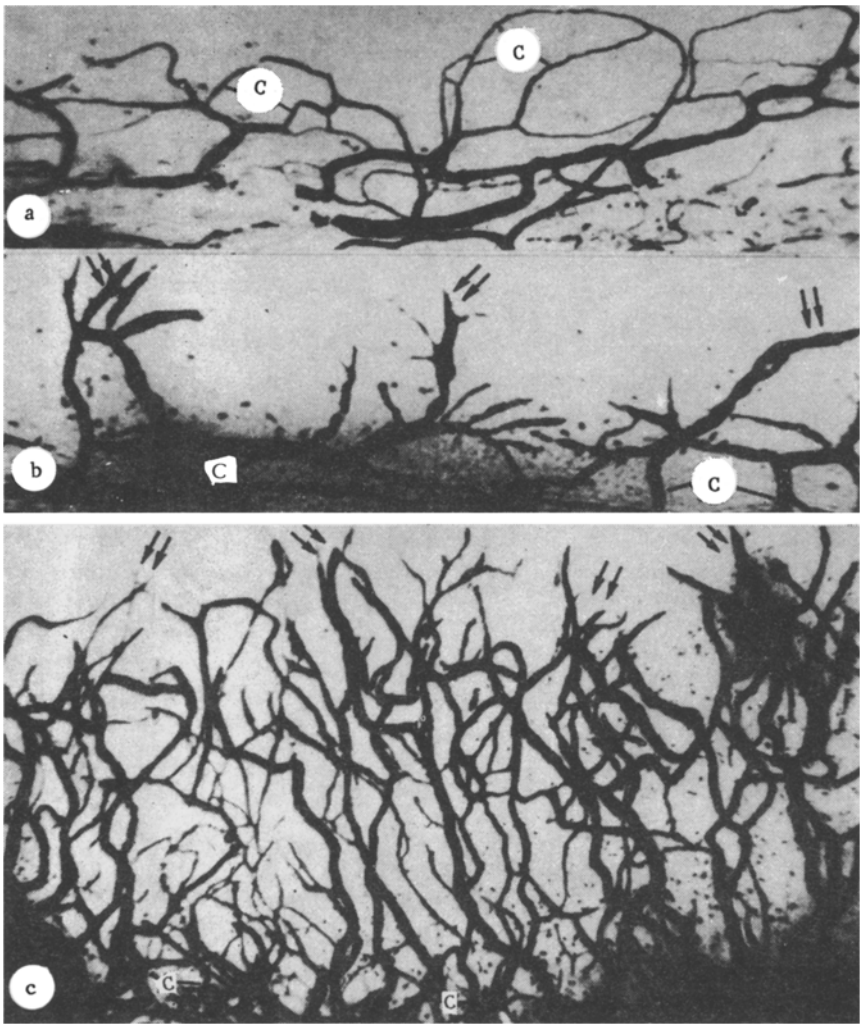

Fig. 1. Vascular bed of the limbus and newly formed capillaries growing into the rabbit cornea. a) Looped capillary formations of the limbus, filled with peroxidase reaction product; b) growing capillaries 8 days after burning; c) newly formed capillaries growing after combination of burning and application of colchicine ointment for 8 days. Total preparations, photograph in buffer. Method of Graham and Karnovsky. 50 ×.

histochemical reaction developed [8]. By injecting the marker into the vascular bed it was possible to identify the vascular network of the limbus and the capillaries growing in the cornea. Subsequent treatment of the material was by the usual methods of electron microscopy. The topical principle of analysis [4] enabled individual growing capillaries to be studied in detail and the proliferative activity of the endotheliocytes of the newly formed capillary to be demonstrated in its various parts. For this purpose serial semithin sections were obtained at 10 to 12 levels of the growing capillary, starting from its apex and ending with the point of origin from the intact capillaries of the limbus. Endotheliocyte nuclei containing ^{3}H-thymidine were counted in sections 0.5-1 μ thick. In each case 1000 nucleated endotheliocytes were counted. The number of growing capillaries was determined in total preparations of the cornea and calculated per unit area of the preparation and per unit length of the circumference of the limbus. The numerical results were subjected to statistical analysis after Student's test.

EXPERIMENTAL RESULTS

The light-optical study of total preparations of the rabbit cornea showed that the vascular network of the limbus consists of looped formations, and that in regions of the cornea

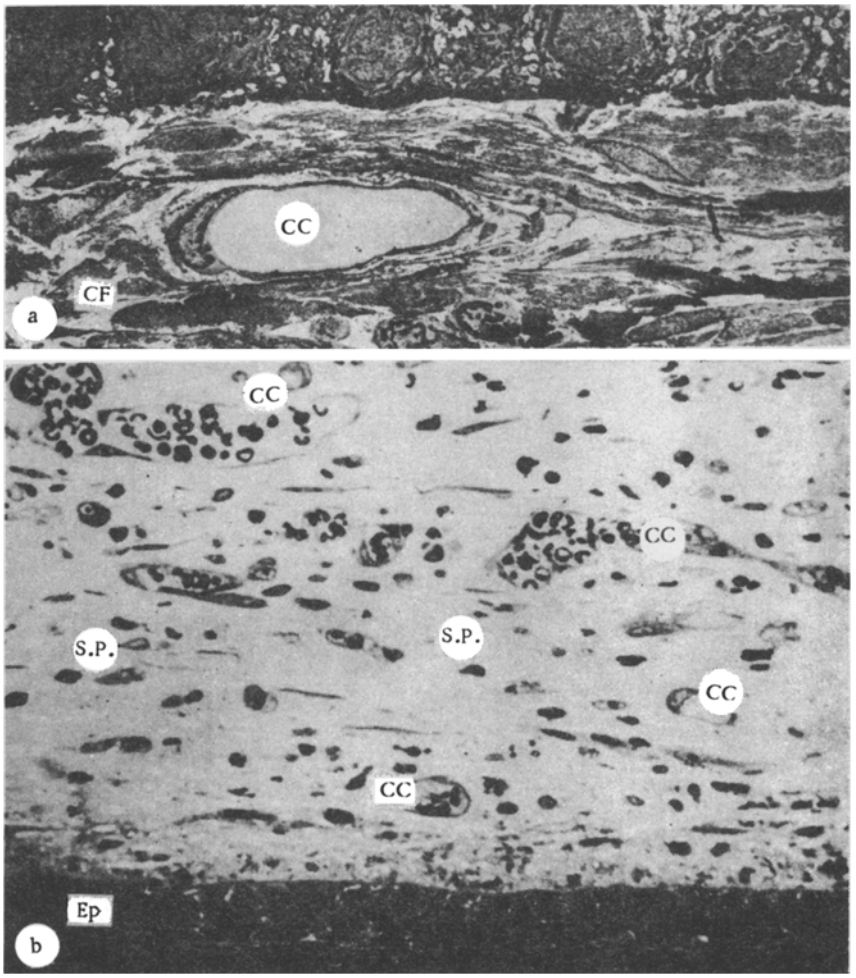

Fig. 2. Profile of newly formed vessels growing in the tunica propria of the cornea. a) Capillary growing in cornea after burning. 1800 ×. b) Semithin sections through vessels growing in cornea after combined action of burn and colchicine. 200 ×. Legend: CC) Corneal corpuscles: CF) collagen fibrils; SP) substantia propria; EP) epithelium.

adjacent to it there are no blood vessels (Fig. 1). A silver nitrate burn with or without application of colchicine led to intensive growth of capillaries toward the center of the cornea. The rate of growth of the capillaries in response to a combination of the burn and colchicine was more than twice that observed after the burn alone: 0.3-0.4 and 0.07-0.15 mm/day, respectively. Counting the number of growing capillaries showed that after both types of procedure the density of the perilimbal vascular network was increased by only a very little. The number of newly formed capillaries in the cornea after a combination of burning and colchicine was twice that observed after the burn alone (Table 1). In our opinion these results indicate that colchicine has an inducing action on the endothelium of the newly formed blood vessels and that summation of the growth-stimulating action of the burn and colchicine takes place.

Examination of semithin sections revealed an important fact: after burn stimulation growth of the capillaries took place in the surface layers of the cornea in the immediate vicinity of the epithelium, whereas after combined exposure to the burn and colchicine, most of the growing capillaries were located in the middle layer of the cornea (Fig. 2).

The histoautoradiographic study of intact vessels of the limbus revealed their low proliferative activity: the number of endotheliocytes containing ^{3}H-thymidine was only 0.075 ± 0.003% of the total number of cells. In response to stimulation of growth the intensity of thymidine incorporation by nuclei of the endothelial cells of the newly formed capillaries was significantly higher: 6.5% following burning and 35% following a combination of burning and colchicine (Table 2). Investigation of the distribution of labeled endotheliocytes along the

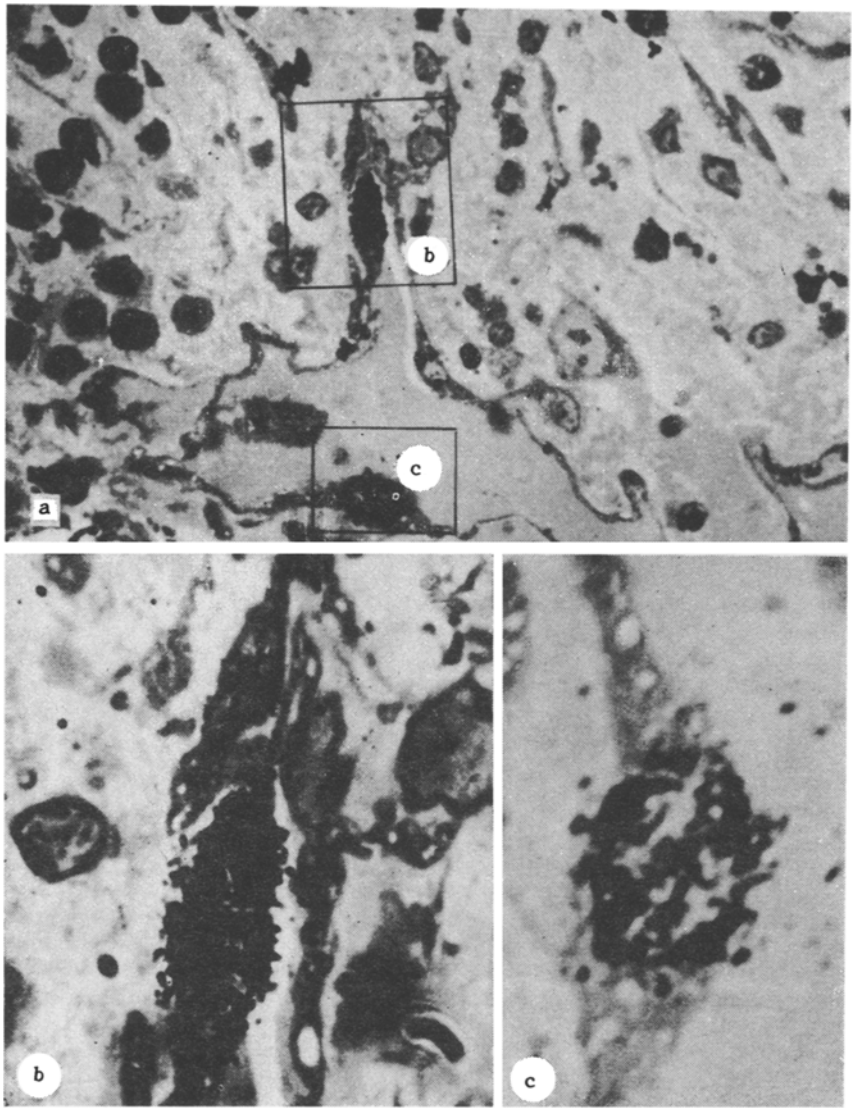

Fig. 3. DNA synthesis in endotheliocyte nuclei of newly formed capillary, growing in response to a combination of the burn and colchicine. a) General view of growing capillary at point of its origin from a definitive vessel of the limbus. 600 ×; b, c) fragments of Fig. 1a showing nuclei of endotheliocytes containing label. 2250 ×.

length of the growing capillaries revealed definite gradients of their distribution: the most actively proliferating cells were those where the young capillaries originated from intact vessels of the limbus (Fig. 3). Toward the apex of the newly formed vessels the number of endothelial cells containing ^{3}H-thymidine decreased. This pattern of distribution of the proliferating endotheliocytes was observed both after stimulation by the burn and after the combined action of burn and colchicine.

The discovery of endotheliocytes containing ^{3}H-thymidine at the apex of the growing capillaries, following combined exposure to the burn and colchicine, may be evidence either that the proliferating endotheliocytes are more clearly revealed by the action of colchicine or of increased ability of the endotheliocytes to proliferate. In our view this latter suggestion seems more reasonable, for it explains not only the more rapid growth of the capillaries following combined exposure, but also the very large number of newly growing capillaries in the cornea.

The results of this investigation thus show that the proliferative activity of the endotheliocytes is increased in growing corneal capillaries. Endothelial cells divide most intensively in the zone of the growing capillary which is adjacent to the intact capillary of

TABLE 2. Distribution of Proliferating Endotheliocytes in Capillaries of Limbus and Newly Formed Corneal Capillaries in Rabbits

Vessels	Number of endotheliocytes containing ^{3}H-thymidine, %
Intact capillaries of limbus	0,075±0,003
Newly formed capillaries growing under the influence of burn:	
Base of growing capillary	6,5±0,38
Middle	0,8±0,04
Apex	0
Newly formed capillaries growing under combined influence of burn and colchicine:	
Base of growing capillary	35±1,25
Middle	6,0±0,36
Apex	0,7±0,028

the limbus. The site of branching of the growing vessel is evidently the true zone of growth. The process of new capillary formation and growth depends on migration of endotheliocytes from this zone in the direction of the growth-stimulating factors. Administration of colchicine *in vivo* has no inhibitory effect on growth of the vessels but, on the contrary, leads to the formation of new capillaries, due in all probability to the acceleration of the course of proliferative processes in the endotheliocytes of the growing vessels. The reaction of the endotheliocytes to colchicine distinguishes them from other epithelial cells, which can form a firm layer. The movement of such a layer and the character of migration of the cells are unchanged by the action of agents which destroy microtubules [1]. Meanwhile, oriented migration of the fibroblast-like cells is blocked by antitubulins on account of disorganization of the cell surface [2]. That is why colcemid stimulates DNA synthesis in fibroblasts but has no such action in epithelium. Consequently, endotheliocytes exhibit a dual reaction to antitubulins: they remain capable of oriented migration as an epithelium and they stimulate DNA synthesis as fibroblasts. The fact of the mitosis blocked by colchicine was not considered in this investigation and calls for special study.

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