

KEY WORDS: capillary; colchicine.

In recent years so-called cytoskeletotropic preparations, such as colchicine, cytochalasin B, etc., have been widely used in experimental morphology and, in particular, to study cultures of epithelium. These substances, on penetrating into a cell, disturb the course of mitosis and thereby exert a cytostatic effect. Most investigations devoted to the study of the mechanism of action of colchicine have been undertaken on cultures of endothelial cells [2, 3, 5, 6]. Parallel studies of the effect of this drug on the state of the endotheliocytes of the large blood vessels have been undertaken in acute experiments.

Because of the absence of data on the mechanism of action of colchicine on vessels of the microcirculatory bed in the intact organism, it was decided to determine the effect of this drug on the vascular endothelium of capillaries of the perilimbal region of the rabbit eye.

EXPERIMENTAL METHOD

Colchicine ointment (0.05%) was applied beneath the palpebral conjunctiva of one eye of a rabbit with intervals of 6 h for 8 days. At the same time the action of the ointment base, including all its components except colchicine, serving as the control material, was studied on the other eye. The animals of a second group received intravenous injections of colchicine solution at intervals of 6 h in a dose of 5 μ g of the drug/100 g body weight. The vascular bed of the perilimbal region was investigated by a histochemical reaction [4] for exogenous peroxidase, which was injected into the vascular system 1 min before the animal's sacrifice. The subsequent study of the vessels began after preliminary light-optical identification, using the topical principle of analysis [1, 8]. Specimens for investigation in the electron microscope were prepared by the usual method.

EXPERIMENTAL RESULTS

Application of the ointment base caused no visible changes in the vessels of the microcirculatory bed, whereas colchicine disturbed the angioarchitectonics of that region. On the other hand, the density of the capillary network was increased and the configuration of the capillaries changed as a result of their considerable dilatation, whereas on the other hand, growing capillaries appeared (Fig. 1a). These newly formed blood vessels, with pointed ends or saccular in shape, arising mainly from the venous rim of the capillary system, had no definite orientation. A rather different picture was observed on examination of the preparations after intravenous injections of colchicine. In this case there were no appreciable changes in the microcirculatory bed itself in the perilimbal region, but the newly appearing capillaries, as a rule with pointed ends, grew toward the center of the cornea (Fig. 1b).

The study of semithin sections and of large areas of the cornea under low power of the electron microscope revealed considerable changes in the structure of the substantia propria of the cornea. These changes took the form not only of the appearance of vessels in the cornea, where they are usually absent, but also accumulation of large numbers of

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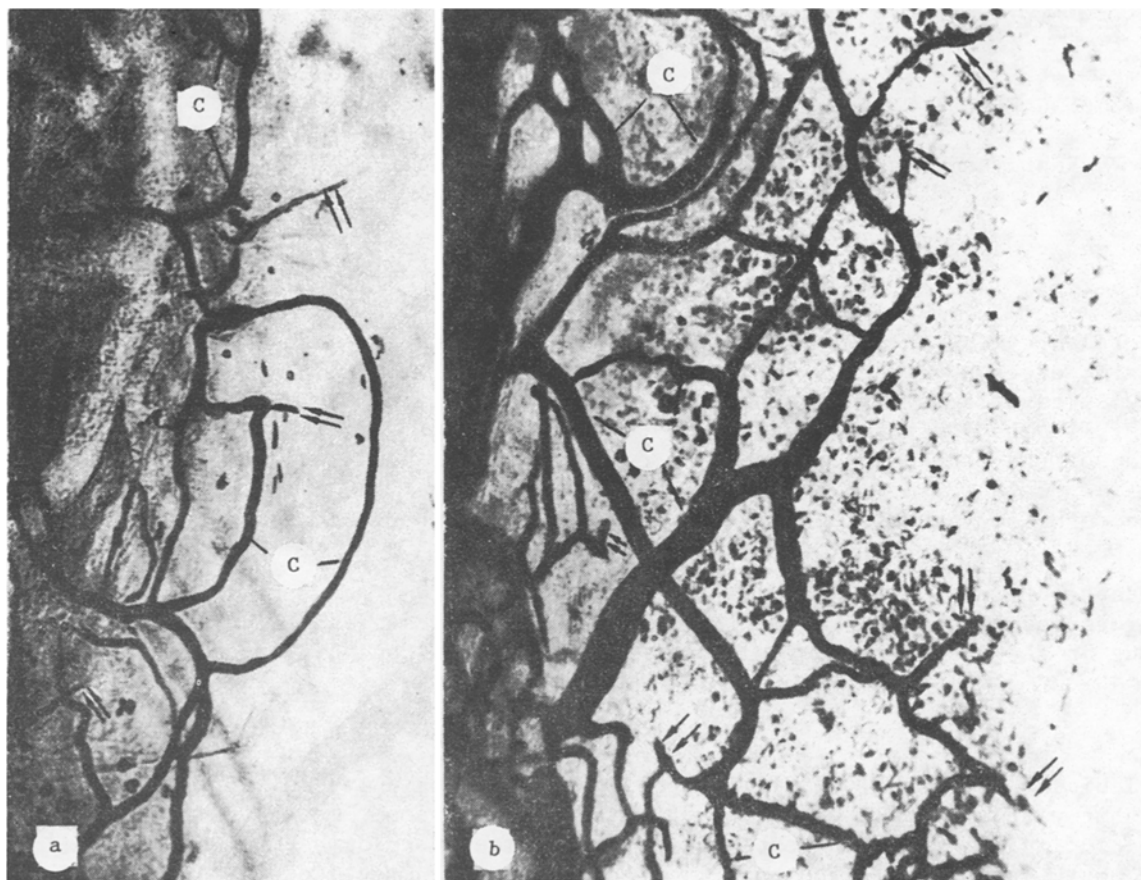


Fig. 1. Growth of capillaries in intact cornea of rabbit's eye under the influence of colchicine. a) Appearance of new sprouting capillaries after application of colchicine ointment, most of them located among blood vessels of the limbus; b) growth of capillaries after intravenous injection of colchicine. C) closed capillaries of the perilimbal region. Arrows indicate sprouting new capillaries. Total preparations. 100 \times .

cells in the substantia propria: erythrocytes, mast cells, macrophages, and so on (Fig. 2a). A characteristic feature of the action of colchicine was the formation of extensive areas of rarefaction of the matrix, due to lysis of the substantia propria of the cornea. The subsequent study both of vessels of the microcirculatory bed in the perilimbal region and of growing capillaries revealed numerous changes in the ultrastructural organization of the vascular wall and of the endothelial cells forming it. Polymorphism of the picture observed was expressed by the appearance of vessels with a dilated lumen, unconnected with the pericytes, or the presence of capillaries with an obliterated lumen, completely surrounded by a "cuff" of pericytes. Numerous vacuoles and giant vesicles could be seen in the cytoplasm of the endotheliocytes (Fig. 2b); on fusion the latter formed transendothelial canals. Disaggregation of the lamellar complex and destruction of the dictyosomes into small cisterns and vesicles could be observed, whereas the rough endoplasmic reticulum was hypertrophied: greatly dilated cisterns appeared with numerous ribosomes. It was virtually impossible to find any microtubules in the cells although structuralization of the microfilaments into bundles, located in the perinuclear and perijunctional zones of the endotheliocytes could be clearly seen (Fig. 2b). Changes in the ultrastructural organization of the interendothelial spaces were observed, in connection with an increase in the number of open ("flowing") junctions and with partial opening of the vacuoles in them.

The results are evidence that in the intact organism during chronic application of colchicine or its administration by any other method, it exerts angiogenic activity relative to the endothelial cells of blood vessels in the perilimbal region. In response to

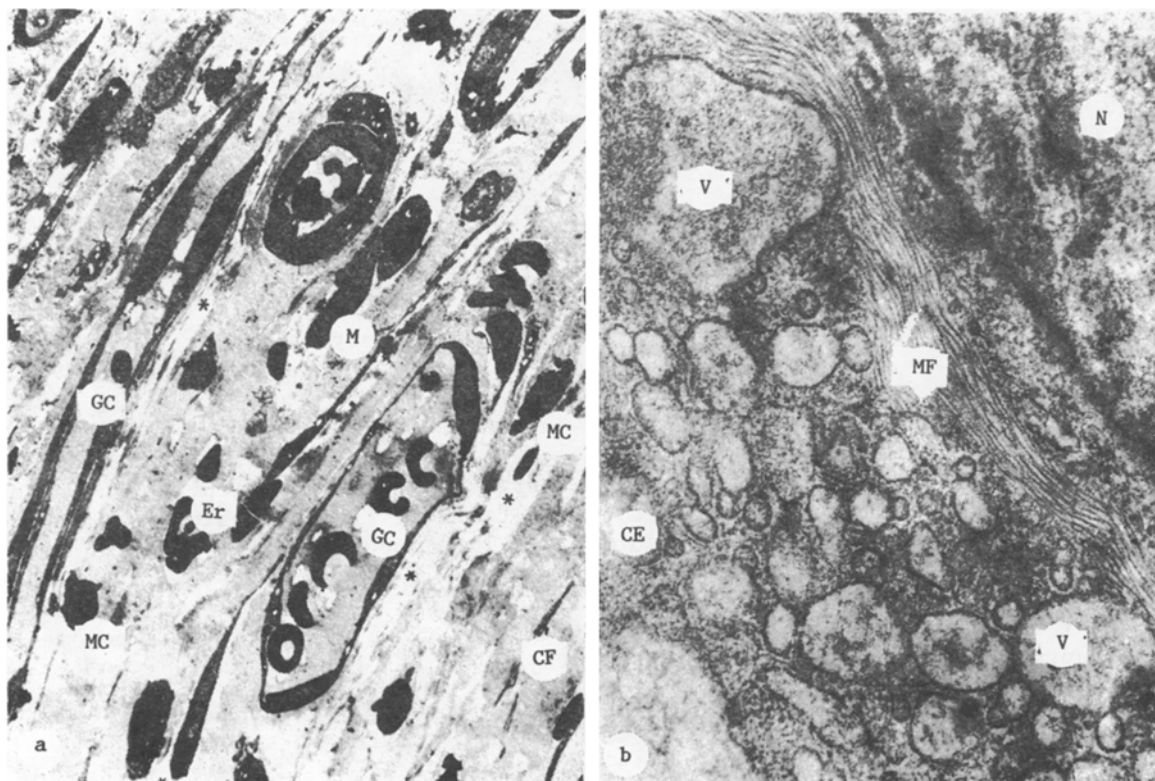


Fig. 2. State of substantia propria of cornea and intracellular organoids in endotheliocytes of newly formed capillaries under the influence of colchicine. a) Substantia propria of cornea with numerous growing capillaries (GC), mast cells (MC), macrophages (M), and extravasated erythrocytes (Er). CF) Collagen fibrils. Asterisk indicates site of rarefaction of substantia propria of cornea. 1200 \times ; b) fragment of endothelial cell whose cytoplasm contains numerous vacuoles (V) and bundles of microfilaments (MF) in perinuclear zone. N) Nucleus of endotheliocyte, CE) cytoplasm of endotheliocyte. 63,000 \times .

the general and local action of colchicine on the blood vessels and surrounding tissues in vivo this substance induces more varied changes in the endothelial cells of the capillaries than when it acts on endotheliocytes in tissue culture.

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