Choroidal vascular repair: Scanning and transmission electron microscopy

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Abstract. Repair of the choroidal vasculature following laser photocoagulation in the rat was examined with vascular casts and correlated with observations on thin-sections. The regenerative process began at the periphery of the damaged area, starting from the surviving choriocapillaris and venules, and proceeding towards the center by means of recanalization of damaged vessels and growth of new ones. In small healed lesions the capillary bed was re-formed. It resembled the adjacent undamaged choriocapillaris morphologically but appeared to be less dense than the intact choriocapillaris when examined by scanning microscopy. In large lesions the capillary bed was re-formed at the periphery but not at the center. Also present at the edges of the large lesions were groups of new vessels which, when observed by scanning microscopy, appeared to extend in two directions; towards the subretinal space and towards the choroidal network. Another aspect of the repair process was the simultaneous occurrence of new vessel growth and capillary regression, which was observed both at the level of the choriocapillaris and at the foci of new vessels
Key words. Krypton laser photocoagulation; choriocapillaris; neovascularization; vascular casts.

Choroidal subretinal neovascularization is a major cause of blindness in age-related macular degeneration, and an important cause of visual loss in numerous other chorioretinal diseases[1,2]. Numerous studies have investigated the pathogenesis of choroidal subretinal neovascularization in various animal models by using high-intensity laser photocoagulation to induce subretinal neovascularization[3–6]. The present study, however, was designed to examine the vascular remodeling process following laser treatment. We used moderate- to high-intensity laser photocoagulation, and correlated observations of vascular casts with those of thin sections. Using vascular casts and mounting the specimens choroidal side up (unlike previous studies in which specimens were mounted retinal side up), we were able to observe an additional feature of vascular regeneration not previously reported, namely that in addition to the vessels comprising the subretinal neovascularization and oriented towards the retina, other new vessels are oriented in the opposite direction towards the choroidal plexus.

Material and methods

Krypton laser burns causing whitening of the retina were assumed to be comparable with therapeutic lesions of moderate to high intensity. The burns were applied to the posterior pole of the retina in 12 mature pigmented rats (175–200 g b.wt), as previously described[7]. In the upper half of the retina two types of lesions were applied: 1) the upper nasal quadrant received 10–15 small lesions, each consisting of a single burn of 100 μ beam size, at a power level of 55–65 mW and exposure time of 0.05 s; 2) the upper temporal quadrant received four to eight large lesions, each consisting of six confluent small burns, and one larger lesion made up of numerous small burns above the optic nerve. The nonirradiated hemisphere

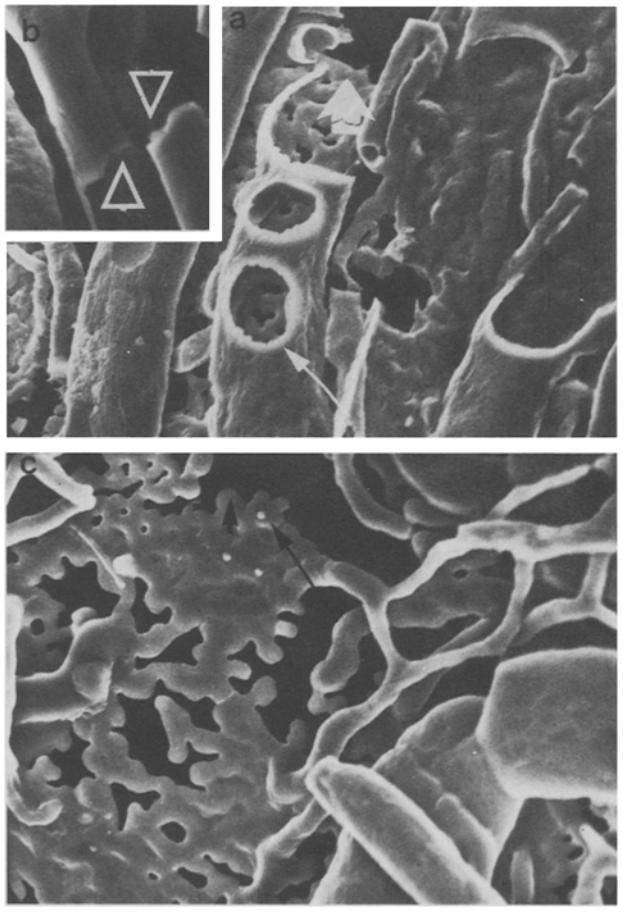

Figure 1. *a* Vascular cast 7 days after lasering shows filling defects with irregular, ruptured capillary edges (wide arrow), and circular defects of the larger vessels (long arrow). (× 320). *b* Inset, an artifactually broken vessel with matching proximal and distal portions (triangles) (× 150). *c* Vascular cast showing regeneration of the choriocapillaris. The capillary edges are rounded and bulbous (short arrow). Short protrusions arising from capillaries (long arrow) are thought to be sprouts. (× 200).

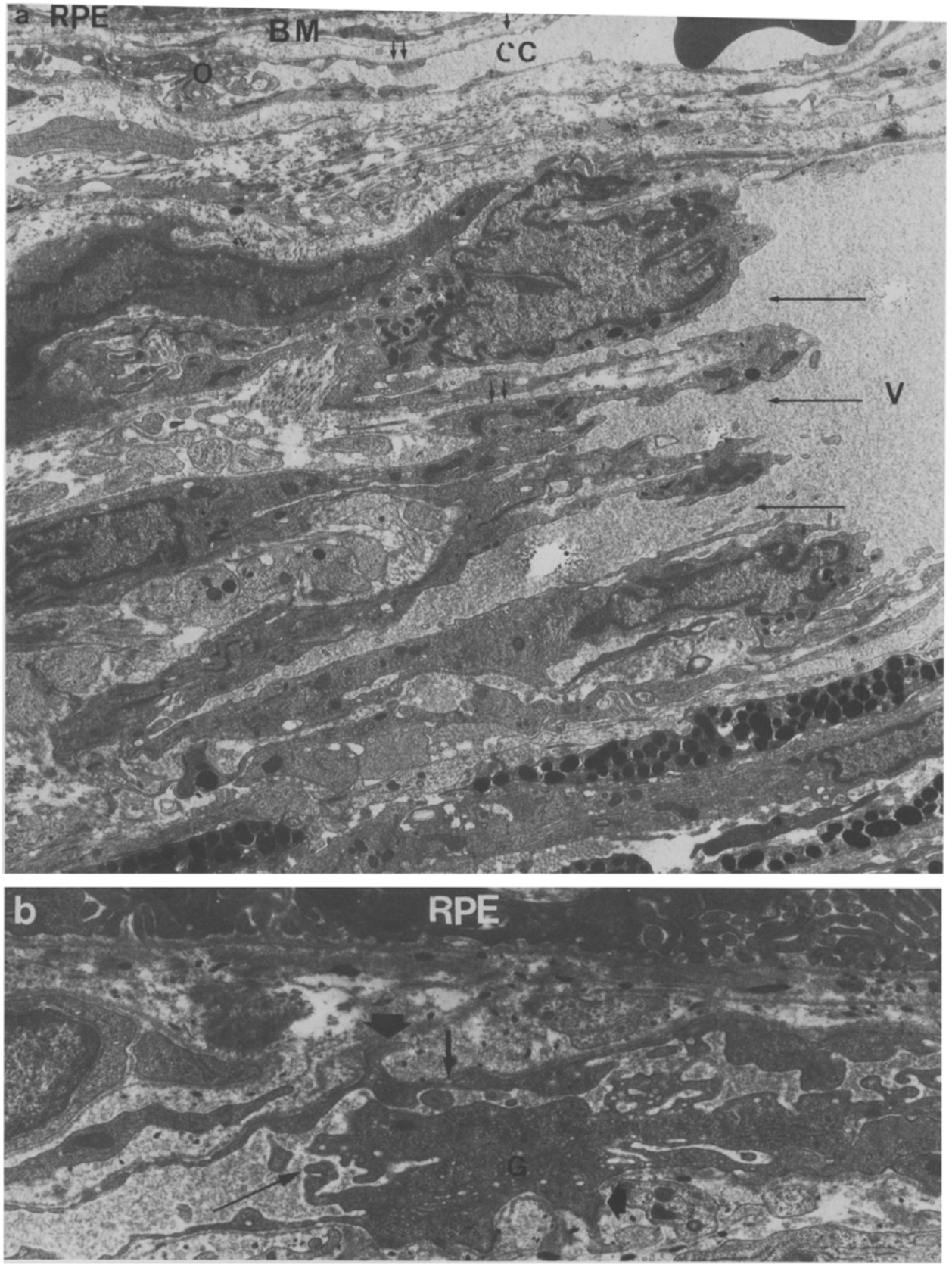

Figure 2. TEM of a sprouting choriocapillary and venule. *a* A normal-looking choriocapillary (CC) with fenestrae (short arrow) and basement membrane (double short arrows) is seen at the top right, with a sprout protruding to the top left (circle, see *b*) for details). Three narrow lumina (long arrows) branching from a venule (V) are surrounded by active endothelial cells embedded in basement membrane (short double arrow) and forming new capillaries. BM, Bruch's membrane; RPE, edge of a retinal pigment epithelial cell. (× 6500). *b* Serial section of part of the capillary denoted by the circle in *a*). An active endothelial cell with a Golgi apparatus (G) and free ribosomes projects sprouts towards the lumen (long thin arrow) and towards the basement membrane (wide arrow). The basement is irregular and discontinuous adjacent to the foci of sprouts (short arrow). RPE, retinal pigment epithelium. (× 18,100).

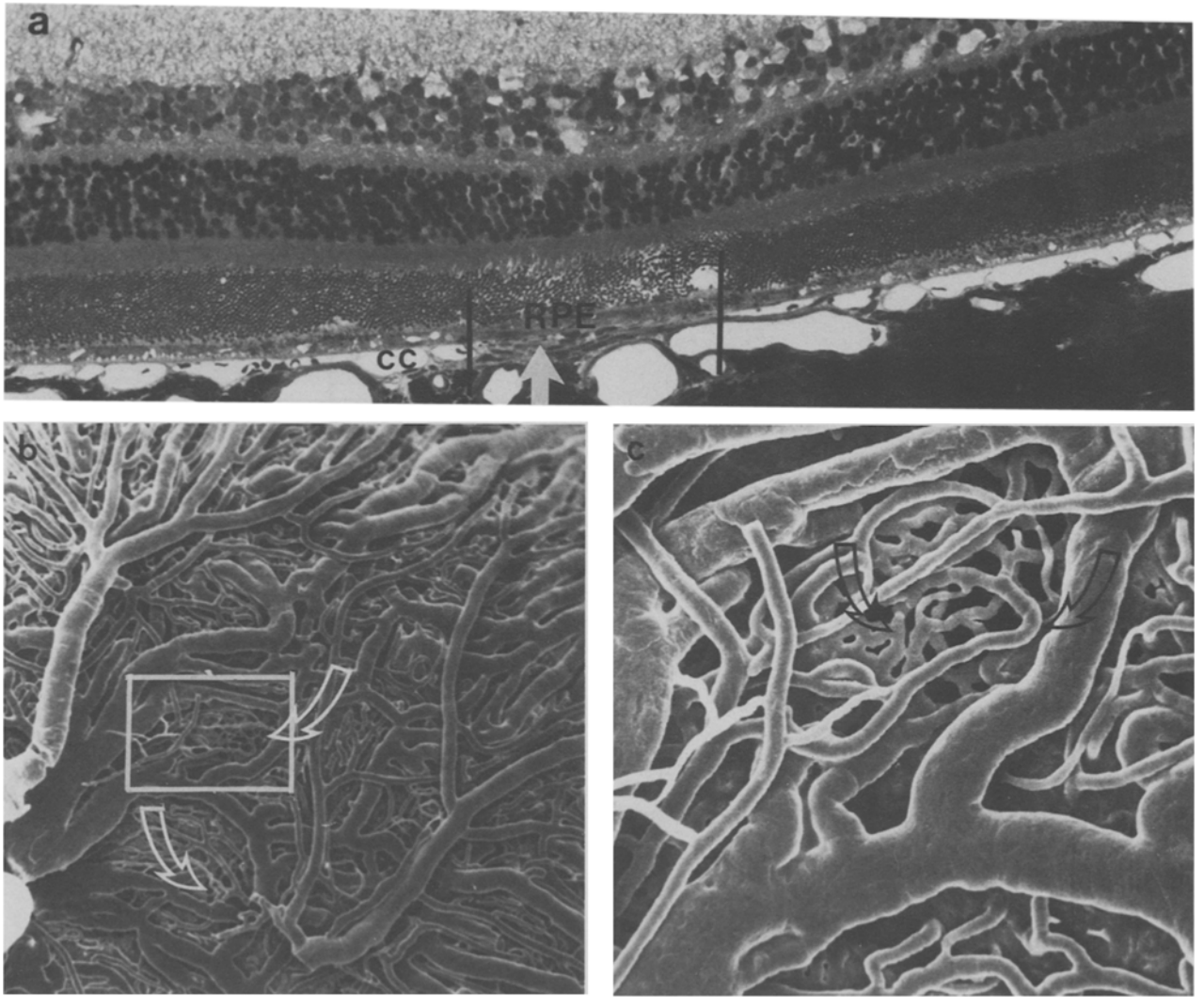

Figure 3. Re-formation of the choriocapillary bed 35 days after lasering. *a* LM of a healed lasered lesion (between bars) with areas of capillary drop-out (arrow) at its center (×100). RPE, retinal pigment epithelial cells; CC, choriocapillaris. *b–c* Low and high magnification of vascular casts of a small lasered lesion. The capillary bed has been re-formed, but its density is lower at the lasered sites (arrows) than that of the adjacent normal capillaries. Details of boxed area in *a*) are seen in *b*). (*a*) × 70, (*b*) × 250.

served as a control. On days 7, 14, 21, 28, 35 and 50 after lasering two of the rats were anesthetized with sodium pentobarbital (40 mg/kg b.wt, i.v.) and perfused through the heart with saline followed by Mercox (Ladd Research Industries, Burlington, VT). Procedures used were those recommended by Burger et al.[8], except that the tissue was digested away by immersion of the eye in Clorox brand bleach (Clorox Corp., Oakland, CA) instead of in sodium or potassium hydroxide[9]. The casts, which were cleaned within hours using this procedure, were rinsed in water, air-dried, and mounted choroidal side up on copper stubs, coated with gold and examined on a Joel JSM-335 scanning electron microscope (SEM). Correlative light and transmission electron microscopy (TEM) were carried out with material obtained 7 to 50 days after lasering and used in a previous study[10].

Results

First week after lasering. In the vascular casts the laser lesions appeared initially as plastic filling defects surrounded by vessels with irregular tips when the choriocapillaris was damaged and as circular defects when the larger vessels were injured (fig. 1 a). Vessels that had been artifactually broken during preparation of casts, unlike laser-damaged vessels, had straight cut edges, and it is possible to match the proximal and distal portions (fig. 1 b).

Second and third weeks after lasering. Seven days after lasering, SEM revealed evidence of vascular regeneration, bulbous vessel endings and short protuberances arising from the surviving choriocapillaris at the periphery and pointing towards the center (fig. 1 c). Capillary and venular sprouts were also seen by TEM (fig. 2). Re-

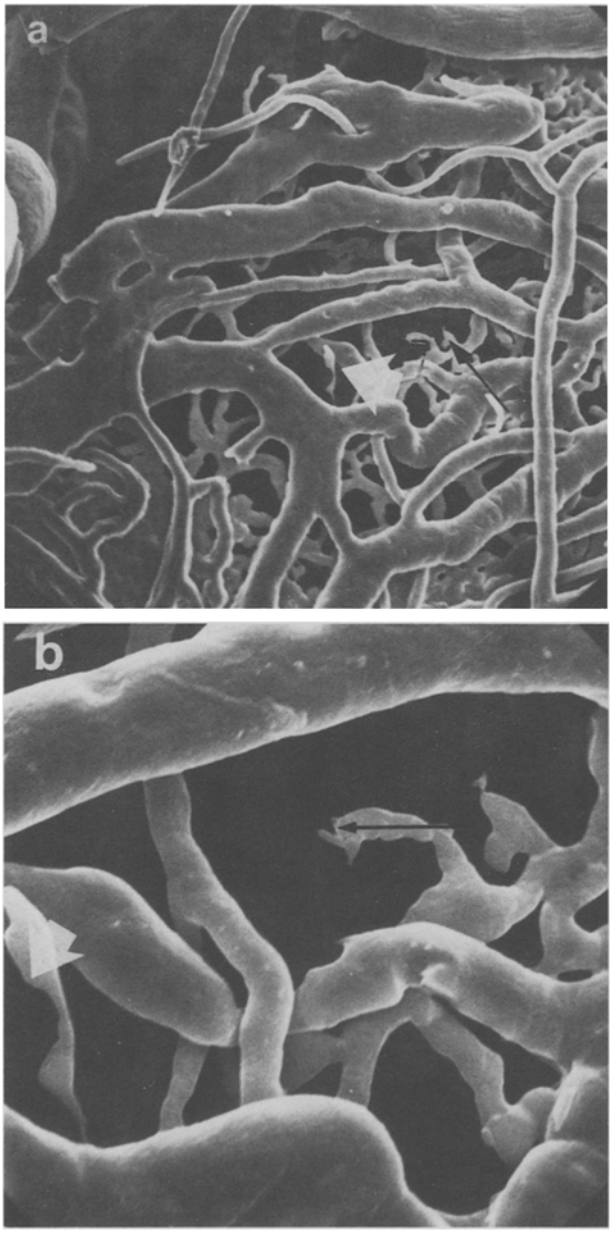

Figure 4. *a–b* Low and high magnification of vascular casts of a large lasered lesion shows non-formation of the capillary bed at the center. A dense network of normal capillaries is seen to the bottom left. A sparse network of regenerated capillaries can be seen at the junctional zone between the center of the lasered area and the normal choriocapillaris. Some of these capillaries have an irregular luminal caliber (short arrow) and irregularities on the surface (thin arrow). (*a*) × 150, (*b*) × 780.

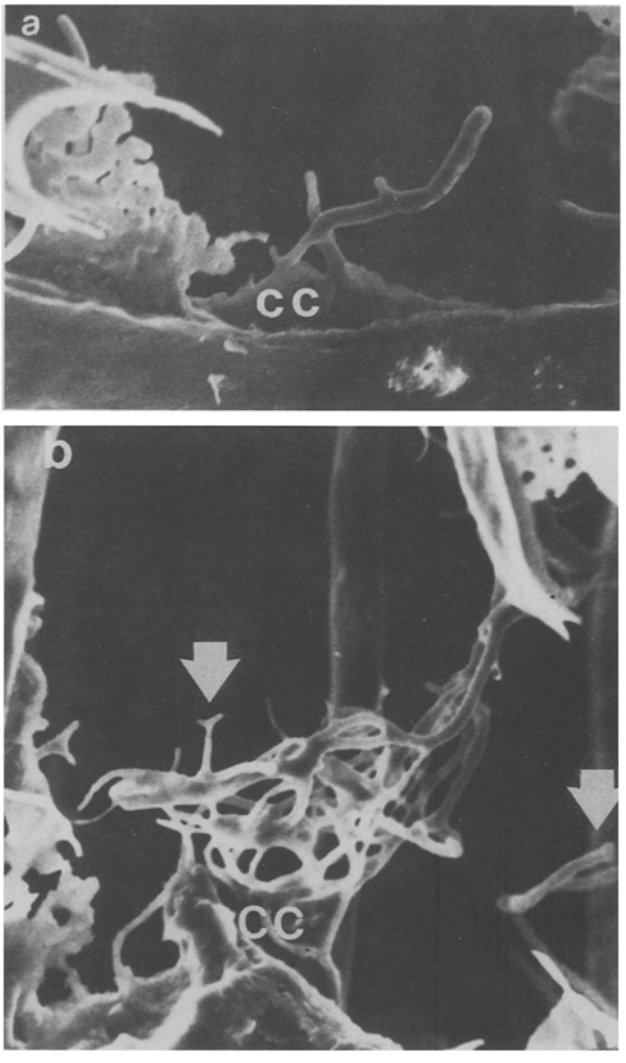

Figure 5. Vascular casts of new vessel growth. *a* New vessels arising from the edges of surviving choriocapillaries (CC) at the periphery of a large lesion, 14 days after lasering. (× 320). *b* A more developed new vessel frond, at the edge of a large lesion 28 days after lasering, arising from the choriocapillaries (CC), showing growing vessels with bifurcating tips (arrows) (× 150).

generation of the choriocapillaris was manifested by endothelial sprouting in two directions, towards the lumen and towards the basement membrane, the latter causing discontinuity of the basement membrane (fig. 2b). A similar sprouting pattern was observed in the venules, whereas the growing capillaries had irregular, plump endothelial cells and cellular processes projecting towards the basement membrane. These features were observed in lesions of all sizes.

From the fourth week after lasering. At this stage differences were noted between the repair of small and large lesions.

Small lesions: One month after lasering, in most small lesions the regenerated capillaries formed a bridging network of capillaries over the filling defect. However, the network of re-formed capillaries was less dense than that of the adjacent normal capillaries (fig. 3). Light microscopy revealed loss of choriocapillaries at the center of the laser lesions (fig. 3a).

Large lesions: In the large lesions capillaries were reformed at the periphery of the lesion but not at its center. As in small lesions, the restored capillary network was less dense than the normal choriocapillaris. In some foci the re-formed capillaries were slender vessels, with irregular wall thickness and vessel tips (fig. 4).

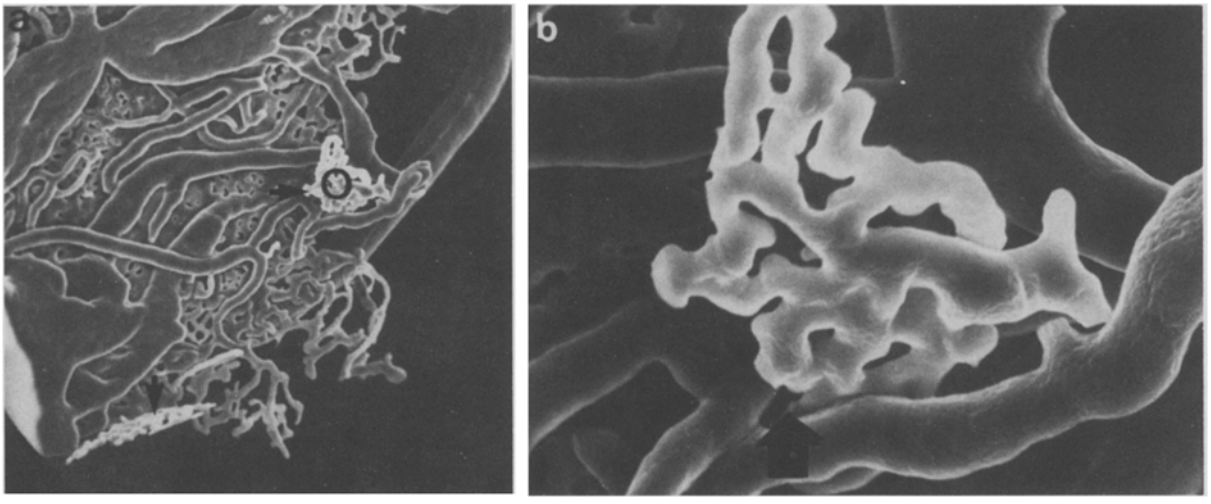

Figure 6. Vascular casts of choroidal neovascularization 50 days after lasering. *a* Low magnification of two foci of new vessels lying on the choroidal side. O, detailed in (*b*). (×130). *b* High magnification of the upper foci of new vessels seen in (*a*), shows that the new vessels originate from the choriocapillaris (arrow) (×660).

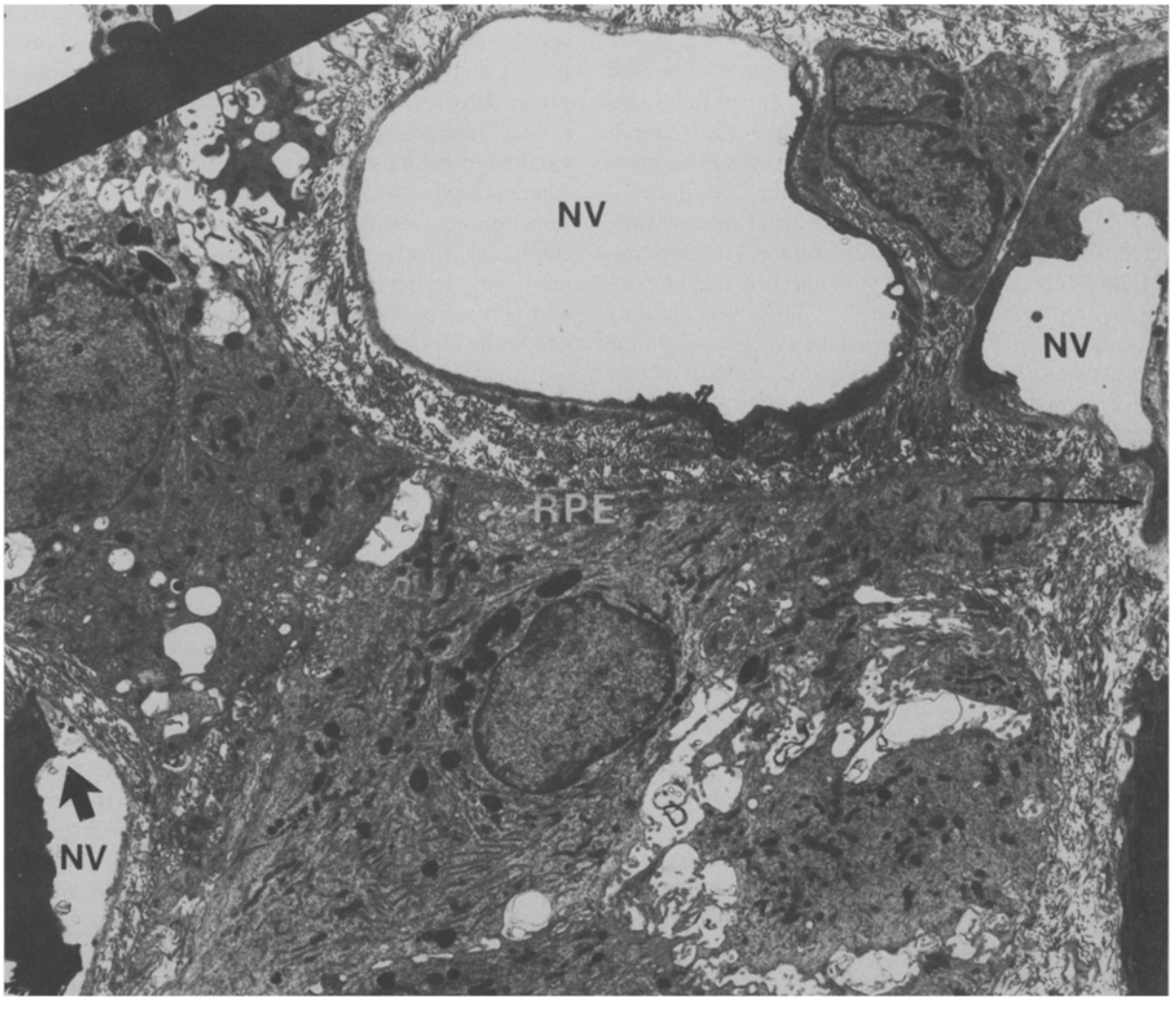

Figure 7. TEM of a plexus of choroidal new vessels embedded in retinal pigment epithelial cells (RPE) showing signs of both growth and degeneration. The vessels at the top right contain endothelial sprouts (long arrow) and the vessel at the bottom left contains a degenerating endothelial cell detaching from its basement membrane (short arrow) (×6500).

Another finding in large lesions only, which was apparent from the beginning of this period, was the sprouting of vessels in a glomerular configuration, which was consistent with previous descriptions of new vessels (fig. 5). Groups of new vessels were seen on both the retinal and the choroidal surfaces (fig. 6a,b). Growth and regression of new vessels occurred at the same time (fig. 7).

Discussion

A number of observations in this study illustrate the fact that the overall result of a biological process represents a balance between different influences. Firstly, the direction of endothelial sprouting in the early phase of vascular regeneration was not only inwards towards the lumen but also outwards towards the basement membrane. Secondly, new choroidal vessels grow not only in the direction of the subretinal space but also – a finding not previously noted – in the opposite direction, i.e., towards the choroidal vascular plexus. Thirdly, vascular repair following lasering consists of not only growth but also regression of new vessels.

A week after lasering, the disrupted edges of damaged capillaries, which at first appeared to be ruptured, became rounded and displayed short protuberances similar to those described as sprouts in other studies of regeneration and neovascularization of the choriocapillaris[11–14]. In thin sections the endothelial sprouts were directed towards the lumen, which supports the assumption that reformation of the vascular bed includes recanalization of damaged vessels[4,15]. However, budding of endothelial cells towards their basement membrane and protrusion through it, representing a phase of new capillary growth, was also observed. Degradation of basement membrane has been described as the first step in neovascularization[16]. The penetration of vessels through Bruch's membrane, called subretinal neovascularization, may therefore be regarded as a more advanced stage of the regenerative process.

Both the re-formation of the capillary bed and the beginnings of neovascularization can be seen more clearly in the vascular casts than in thin sections. The centripetal direction of vascular growth in small lesions was clearly seen, with regenerating capillaries proceeding from the periphery to the center of the lesion, bridging the area devoid of capillaries and forming a network resembling that of the adjacent undamaged choriocapillaries. In large lesions the re-formed capillary bed did not cover the center of the lesion. At the periphery of the lasered area foci were seen with an elongated profile with bifurcating or bulbous tips, and foci of blood vessels in a glomerular configuration, consistent with other descriptions of neovascularization[17]. The venular origin of the new vessels, as described by Ohkuma and Korte[17,18], was also demonstrated more clearly in the vascular casts than in thin sections. It was possible to see that the new vessels originating from the choroidal plexus were growing in two directions, towards the subretinal space and towards the choroidal network.

The regenerative process involved not only vascular growth but also regression of the re-formed vessels. In small lesions the restored choriocapillary network resembled the normal adjacent choriocapillaris in its general morphology, but was less dense. In large lesions the ends of the capillaries, either in the plane of the choriocapillaris, located more centrally, or at some foci of new vessel fronds, became slender, in particular at their tips, and the lumens had an irregular caliber. These vessels were assumed to be in the process of regression, as described by Ohkuma and Ryan[17], as well as in observations in thin sections, where atrophy was evident on both sides of Bruch's membrane, i.e. both in regenerated capillaries and in newly-formed vessels[10].

It thus appears that regeneration of the choroidal vasculature following laser injury is a multivalent dynamic process involving the bidirectional growth of new vessels and also their regression. The question then arises: what factors determine whether the new vessels will grow towards the subretinal space or towards the choroidal plexus, and whether a new vessel will continue to grow or proceed to atrophy? One possible assumption is that cell-cell interactions occur, in this case between the vascular endothelium and the retinal pigment epithelial cells. There is both structural and functional evidence for interaction between these two tissues. For example retinal capillaries, which are normally unfenestrated and are impermeable to horseradish peroxidase, develop fenestrae and become permeable[19–21] when they become embedded in the retinal pigment epithelial cells. Moreover, retinal pigment epithelial cells can release diffusible substances capable of both stimulating and inhibiting endothelial proliferation[22–24]. However, the vascular endothelium may in turn affect the RPE cells: for example, RPE cells become polarized in response to proximity to a capillary bed, following exposure to fluorescent light, injection of urethane, or laser photocoagulation[19–21,25].

The bidirectional orientation of new vessel growth and the persistent vascular sprouting, seen here in the rat retina, may occur also in humans and might partially explain the persistence and/or recurrence of subretinal neovascularization. In a patient undergoing laser treatment for a disease associated with subretinal neovascularization, neovascular fronds growing towards the choroid may remain unidentified, so that laser applications will not be aimed towards those fronds. This could lead to inadequate treatment and result in persistence of the new vessels. Furthermore, the continuing development of new vessels after lasering may partially account for the recurrence of neovascularization. On the other hand, capillary atrophy may contribute to permanent vascular obliteration and thus partially explain the beneficial effect of laser photocoagulation, despite recurrent

neovascularization, in diseases associated with choroidal subretinal neovascularization.

To summarize, vascular repair of the choroid after laser treatment is characterized by recanalization of damaged pre-existing vessels and the bidirectional growth of new ones, as well as by capillary regression.

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Cerebral ischemia is the main cause for the onset of heat stroke syndrome in rabbits [1]

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Abstract. During the onset of heat stroke, rabbits displayed hyperthermia (42.8 °C), and decreased cerebral perfusion pressure and decreased cerebral blood flow (as reflected by a prolonged cerebral circulation time) compared to those of normothermic rabbits. On the other hand febrile rabbits, during the fever plateau did not show the above responses, although they had a similar level of hyperthermia (42.4 °C). The data support the concept that cerebral ischemia is the main cause for the onset of the heat stroke syndrome.
Key words. Heat stroke; fever; cerebral blood flow; cerebral perfusion pressure.

It has frequently been stated that the tissue damage and the multiple systemic effects associated with heat stroke are caused by a combination of elevated body temperature and exposure duration [2]. The question of whether cerebral blood flow could be an operative factor in heat stroke was raised by Wyndham [3]. The possibility was rejected by Shibolet et al. [4] on the basis of experiments on anesthetized dogs, in which there was no evidence of changes in cerebral blood flow at rectal temperatures up to 42 °C. However, our previous results [5] showed that in unanesthetized rabbits, at the onset of heat stroke both a decrease in mean arterial blood pressure and an increase in intracranial pressure occurred, resulting in a reduction in cerebral perfusion pressure. This indicated that cerebral ischemia is indeed a factor which determines the severity of heat stroke. Therefore, in the present study, we sought to ascertain whether the main cause for the onset of heat stroke is thermal injury, cerebral ischemia, or both. Experiments were carried out to assess alterations in rectal temperature, arterial blood pressure, intracranial pressure, cerebral perfusion pressure and cerebral circulation time parameters in nor-