Histol Histopath (1994) 9: 807-843

Histology and Histopathology

Invited Review

Angiogenesis: an update

L. Díaz-Flores, R. Gutiérrez and H. Varela
Department of Anatomy, Histology and Pathology, Faculty of Medicine, University of La Laguna, Canary Islands, Spain

Summmary. Angiogenesis is the neovascularization or formation of new blood vessels from the established microcirculation. It is particularly important and indispensable in a large number of normal and pathological processes during pre- and post-natal life, including neoplasia, inflammation, wound repair and collaterization in response to ischemic stimuli. The current interest in the role of neovascularization in the transition from hyperplasia to neoplasia, as well as in the tumour growth and metastasis, has brought about a large number of studies on angiogenesis. The complex processes of neovascularization, quiescent in the adult organism, may occur rapidly in several circumstances, with the implication of the following events: a) endothelial cell (EC) and pericyte activation; b) basal lamina degradation; c) migration and proliferation of EC and pericytes; d) formation of a new capillary vessel lumen; e) appearance of pericytes around the new capillaries; f) development of a new basal lamina; g) capillary loop formation; h) persistence or involution, and differentiation of the new vessels; and i) capillary network formation and, eventually, organization into larger microvessels. The use of numerous "in vivo" and "in vitro" systems has facilitated the assessment of angiogenesis control, in which angiogenic (fibroblast growth factors, vascular endothelial growth factor, platelet endothelial growth factor, E series prostaglandin, angiogenin, monobutyrin) and antiangiogenic (cartilage-derived angiogenic inhibitor, thrombospondin, protamine, platelet factor-4, interferon, angiostatic antibiotics, steroids) substances intervene. Heparin and heparin sulphate also play a key role in these mechanisms. A greater knowledge of angiogenesis control may lead to the development of a potential therapy in angiogenesis-related processes.

Key words: Angiogenesis, Endothelium, Pericytes, Tumour angiogenesis, Angiogenic factors, Angiogenic antagonists

Offprint requests to: Prof. Lucio Díaz-Flores, Departamento de Anatomía, Anatomía Patológica e Histología, Facultad de Medicina, Universidad de la Laguna, Tenerife, Islas Canarias, Spain

Processes in the development of new vessels. Vasculogenesis and angiogenesis

In the development of new blood vessels, it is necessary to distinguish between vasculogenesis and angiogenesis (Risau et al., 1988; Risau and Lemmon, 1988; Demir et al., 1989). Vasculogenesis is the process by which some vessels develop in the embryo, such as the dorsal aortae and the posterior cardinal veins. Histogenically, vasculogenesis is defined as capillary development from differentiating endothelial cells (EC) "in situ". This process takes place in blood islands, which originate from the splachnopleuric mesoderm. After their segregation from the mesoderm, some mesenchymal cells transform into nests of isolated cell cords of hemangioblasts, which are the precursors of both EC and blood cells. The peripheric cells of the angioblastic masses differentiate into EC, while the blood cell precursor cells are found in the centre of the blood islands, wherein a lumen is developed. The newly formed lumens soon coalesce (Gonzalez-Crussi, 1971; Pardanaud et al., 1987; Coffin and Poole, 1988). Finally, the smooth muscle cells and pericytes are aggregated from undifferentiated mesenchyme.

Angiogenesis is the process characterized by the formation of new blood vessels from an established microvasculature during the development of the embryonic vascular tree as well as in several normal and pathological conditions during post-natal life. Histogenically, it is defined as a mechanism of neovascularization by the sprouting of capillaries from pre-existing vessels.

In the present article, only the angiogenesis process will be reviewed.

Incidence and importance of angiogenesis

Angiogenesis is indispensable in embryonic and fetal development, as well as in a large number of normal and pathological processes during post-natal life (Warren, 1979a,b; Schor and Schor, 1983; Folkman, 1985; D'Amore and Thompson, 1987; Madri and Pratt, 1988; Paweletz and Knierim, 1989). Therefore, the area

involved in the study of angiogenesis is far-reaching, covering numerous fields and many disciplines (Auerbach et al., 1991).

During early embryonic development, new vessel formation or neovascularization, is an important event (Feinberg et al., 1991). Thus, a wide range of investigations at cellular and molecular levels on embryonic angiogenesis during organogenesis exist (Stewart and Wiley, 1981; Ekblom et al., 1982; Risau, 1986; Risau and Ekblom, 1986; Risau and Lemmon 1988; Risau et al., 1988).

Angiogenesis is generally a quiescent process in the adult organism. Nevertheless it may occur rapidly in several normal circumstances. For example, the need for additional vasculature is imposed on the cyclic evolution of transient structures in the female reproductive system (for review, see Findlay, 1986), including the sequential maturation of ovarian pre-ovulatory follicles (Harris and Eakin, 1949; Richards, 1980; Sato et al., 1982; Koos and LeMaire, 1983; Makris et al., 1984; Frederick et al., 1984; Rone and Goodman, 1985; Koos, 1986) and subsequent development of the corpora lutea (Brambell, 1956; Jakob et al., 1977; Heder et al., 1979; Kanzani et al., 1985; Goodman and Rone, 1985), cyclic extensions and repair of the functional endometrium (Markee, 1940; Abell, 1946; Foley et al., 1978; Christianens et al., 1982), decidual transformation, implantation and placentation (Edwards, 1980; Gospodarowicz et al., 1985; Feinberg et al., 1991), and mammary gland changes associated with lactation.

Furthermore, angiogenesis is an important component of many pathological processes, such as chronic inflammation, regeneration, wound healing, thrombi organization, collateral circulation development, neoplasias, and of several conditions in which the term "angiogenic disease" has been proposed, since an abnormality of capillary growth is their principal pathological feature (Folkman, 1989). Among the "angiogenic diseases" are hemangiomas, psoriasis (Majewski et al., 1987), scleroderma (Kaminski et al., 1984), rheumatoid arthritis, diabetic retinopathy (Davis, 1988) and neovascular glaucoma.

The role of angiogenesis in neoplasias is of great interest, especially in the progressive growth and metastases of solid tumours (Folkman, 1972, Schor and Schor, 1983; Folkman, 1985a,b,c). This has brought about a large number of descriptive studies on angiogenesis, as well as studies on the possible mechanisms involved in this process (Folkman, 1975, 1985a,b,c; D'Amore and Thompson, 1987; Folkman and Klagsbrun, 1987; Zetter, 1988; Paweletz and Knierim, 1989; Blood and Zetter, 1990).

Angiogenic phenomena

The regions of the vascular tree with angiogenic capacity, the timing, pattern and the events of new blood vessel formation, and some of the peculiar forms of angiogenesis, will be considered in this section.

Regions of vascular tree with angiogenic capacity

In general, it is accepted that vascular sprouts originate from the walls of pre-existing capillaries and from small venules (Mc Cracken et al., 1979; Tano et al., 1981; Sholley et al., 1984), but mainly from the latter (Schoefl, 1963; Ausprunk and Folkman, 1977). Some authors restrict the origin and point out that new vessels arise only from the venous side of the circulation (Phillips et al., 1991), specifically from small venules which lack smooth muscle cells (Ausprunk and

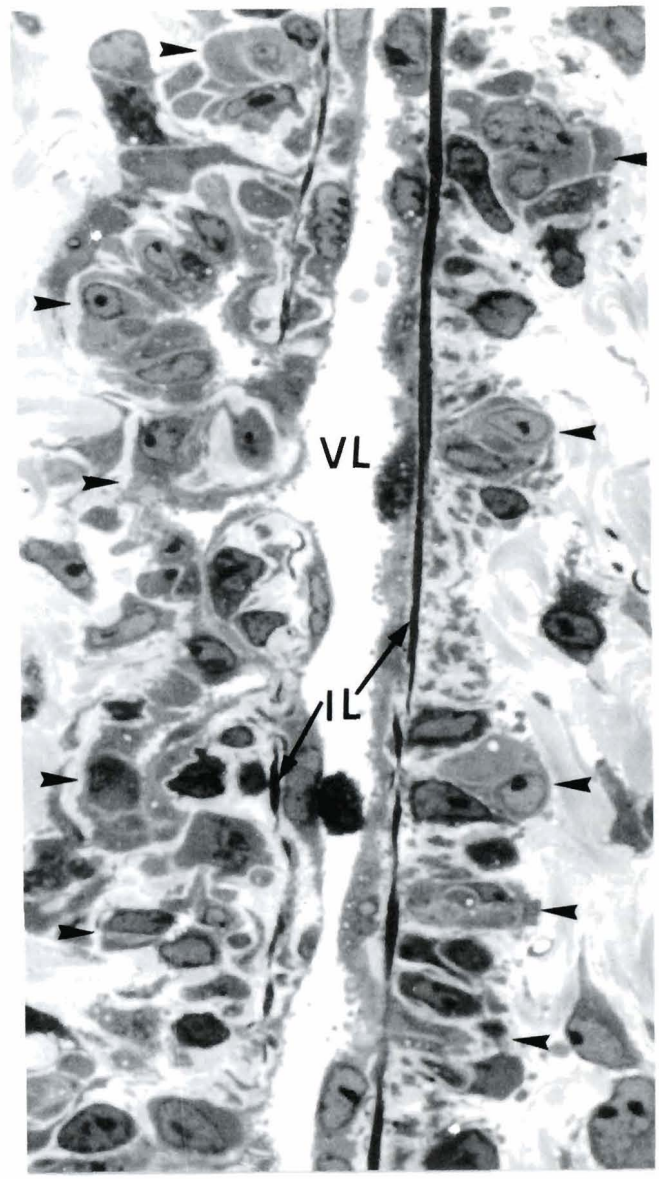

Fig. 1. Femoral vein of the rat 5 days after PGE2 and triacetin administration into the soft tissue surrounding it. Numerous vascular buds arising from the vein are present (arrowheads). VL: Vein lumen; IL: discontinuous internal elastic lamina. Semithin section; toluidine blue. x 900

Folkman, 1977). For others, the fact that the venules were the predominant source, perhaps only reflects the larger surface area of the venular plexus in certain explored areas (Burger et al., 1983). Recently, it has been demonstrated that vessels of greater calibre in the venous side of the circulation, such as the rat femoral vein, with a discontinuous internal elastic lamina and smooth muscle cells in their media layer, are capable of contributing to angiogenesis, on occasions with an intense neovascularization (Fig. 1). Indeed, a single application of prostaglandins E1 and E2 in triacetin solution into the soft tissue surrounding the rat femoral vein induces a sudden and intense angiogenesis, with the vascular sprouts arising from the EC in the intima of the vein (Diaz-Flores et al., 1994).

Although few ^{3}H-labelled EC in small arteries have been demonstrated during angiogenesis (Burger and Klintworth, 1981), new vessels do not seem to originate from the arterial side of the circulation.

Timing and pattern of capillary formation. Vision of angiogenesis using scanning electron microscopy

Scanning electron microscopy of vascular casts has been utilized to study the early changes in vasculature responding to angiogenic stimuli (Burger et al., 1983; Garbett and Gibbins, 1987; Forsman and McCormak, 1992). During the first hours, preformed capillaries and postcapillary venules became widely distended and tortuous, the postcapillary vein being affected more extensively. In the walls of the venules, numerous impressions occur, corresponding to marginating leukocytes. Vascular sprouts originate from both venules and capillaries, although predominantly from venules. The sprouts appear rapidly, being seen as early as 27 hours after angiogenic stimuli (Burger et al., 1983). Between three and five days, the number of sprouts is intensely increased to produce a rich anastomosing plexus. In some conditions, the process of neovascularization may be extremely fast. For example, throughout days 1 and 2 of the cycle, the hamster corpus luteum vasculature grows from the existing theca vessels. Flat veins, characteristic of the external surface of a mature corpus luteum, appear at day 2. Involution of the corpus luteum vasculature takes place throughout day 3 of the cycle (Forsman and McCormack, 1992).

Events of new blood vessel formation

Angiogenesis is a multistep complex process which has been considered in several studies under various pathological conditions (Cliff, 1963; Schoefl, 1963; Yamagami, 1970; Bar and Wolff, 1972; Cavallo et al., 1973; Ausprunk and Folkman, 1977; Warren, 1979; Sholley et al., 1984; Dvorak et al., 1987; Wakui et al., 1988; Paku and Paweletz, 1991). Besides the inflammatory phenomena which occur prior to and during angiogenesis, the events essentially involved in capillary growth in vivo include (Brenk, 1955; Cliff, 1963; Crocker et al., 1970; Yamagami, 1970; Inomata et al., 1971; Ausprunk and Folkman, 1977; Schor and Schor, 1983, Sholley et al., 1984; Folkman, 1985a.b.c; Freemont and Ford, 1985; Furcht, 1986; D'Amore and Thompson, 1987; Madri and Pratt, 1988; Paweletz and Knierim, 1989; Paku and Paveletz, 1991; Diaz-Flores et al., 1992): a) EC and pericyte activation; b) degradation of the basal lamina of pre-existing vessels by EC (proteolytic destruction of the extracellular matrix); c) EC migration from pre-existing vessels towards the angiogenic stimulus; d) EC proliferation; e) migration and proliferation of pericytes from pre-existing vessels; f) formation of a new capillary vessel lumen (vascular

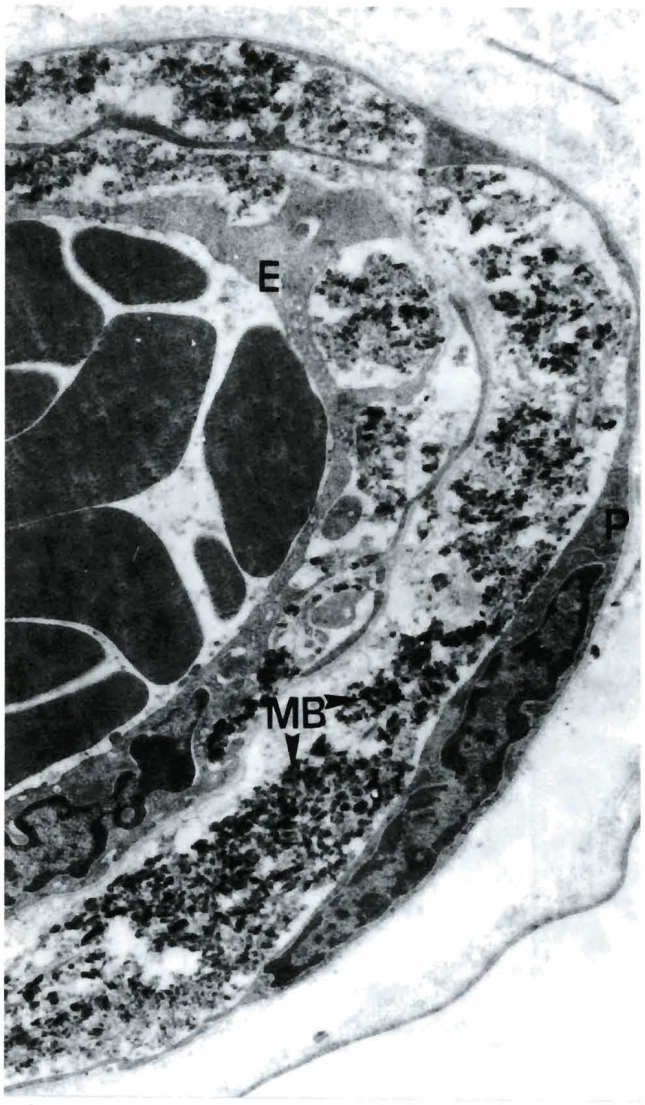

Fig. 2. Preformed postcapillary venule of rat surrounding epineural microcirculation after angiogenesis stimulus and intravenous administration of Monastral Blue (MB). MB particles have leaked through interendothelial gaps and are present between the endothelial cells (EC) and the pericytes (P), within the basal lamina. Ultrathin section; uranyl acetate and lead citrate. x 8,000

tube formation); g) appearance of pericytes around the new capillaries (pericytes in angiogenesis); h) changes in extracellular matrix with development of a new basal lamina; i) capillary loop formation; j) early changes in the newly formed vessels (persistence, involution and differentiation); and k) capillary network formation and eventually organization of larger microvessels.

Although, stepwise, the current model of angiogenesis is controversial, needing reconsideration, (Schlingemann et al., 1991), our review will follow the latter, referring to the doubts therein and including the associated inflammatory phenomena.

Inflammatory phenomena associated with angiogenesis:

New blood vessel formation is an orderly process which is coordinated with inflammation, immunological activity, debridement and fibroplasia (Jennings and Florey, 1970; Auerbach, 1981; Peacock, 1984; West et al., 1985). Thus, an inflammatory response, with vascular dilation, increased vascular permeability, and diapedesis of leukocytes may precede and accompany the angiogenic phenomena (Mc Cracken et al., 1979).

Dilation of the blood venules and capillaries occurs rapidly. For example, in corneal vascularization induced

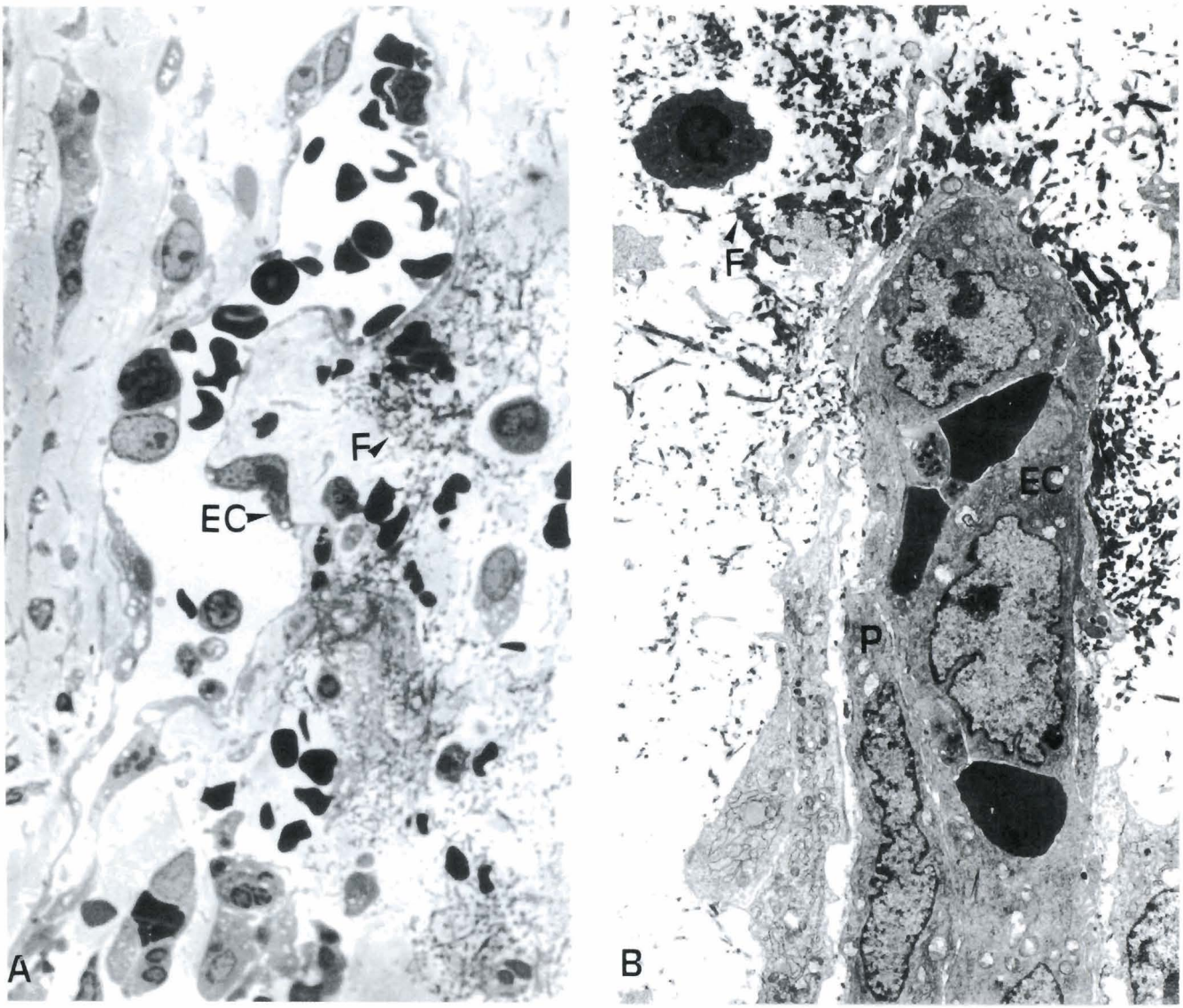

Fig. 3. The increased vascular permeability during angiogenesis is demonstrated by the presence of extravascular fibrin (F) around both a parent venule **(A)** and an advanced border of a new vessel **(B)** (arrowheads). EC: Endothelial cell; P: Pericyte. A) Semithin section. x 1,100. B) Ultrathin section; uranyl acetate and lead citrate. x 12,000

by silver nitrate, the dilation takes place within 1 hour (Mc Cracken et al., 1979), intensifying progressively. The connective tissue becomes edematous and intercellular contacts between EC have been described as opened (Ausprunk and Folkman, 1977; Dvorak et al., 1988; Paku and Paweletz, 1991).

The changes in vascular permeability during angiogenesis were studied using the intravenous injection of different markers (Schoefl, 1963; Sugiura and Matsuda, 1969; Yamagami, 1970; Garbett and Gibbins, 1987), such as colloidal carbon or monastral blue to label leaky vessels (Garbett and Gibbins, 1987; Diaz-Flores et al., 1992) (Fig. 2). Preformed capillaries and post-capillary venules become variably permeable to the introduced colloid marker, the observed pattern being different from the characteristic labelling pattern produced by the inflammatory mediator, histamine (Garbett and Gibbins, 1987). During angiogenesis, the interconnecting networks of newly-formed vessels often label strongly, especially along the advancing border of new vessels, until the basal lamina is laid down and pericytes emerge along the length of the sprout (Schoefl, 1963). The increased vascular permeability is also demonstrated by the presence of extravascular fibrin (Fig. 3) and dilated lymphatics. Extravascular fibrin deposits, which are found during inflammation, wound healing (Madri and Pratt, 1988) and in the periphery of tumours (Nagy et al., 1988), are important during angiogenesis. Thus, neovascularization is induced by fibrin gels in vitro (Nicosia et al., 1982) and in vivo (Dvorak et al., 1987). Also, it has been demonstrated that cultured EC synthesize fibronectin (Jaffe and Mosher, 1978; Birdwell et al., 1978) and that the growing capillaries produce fibronectin in situ (Clark, 1982a,b). During EC growth, the fibronectin may

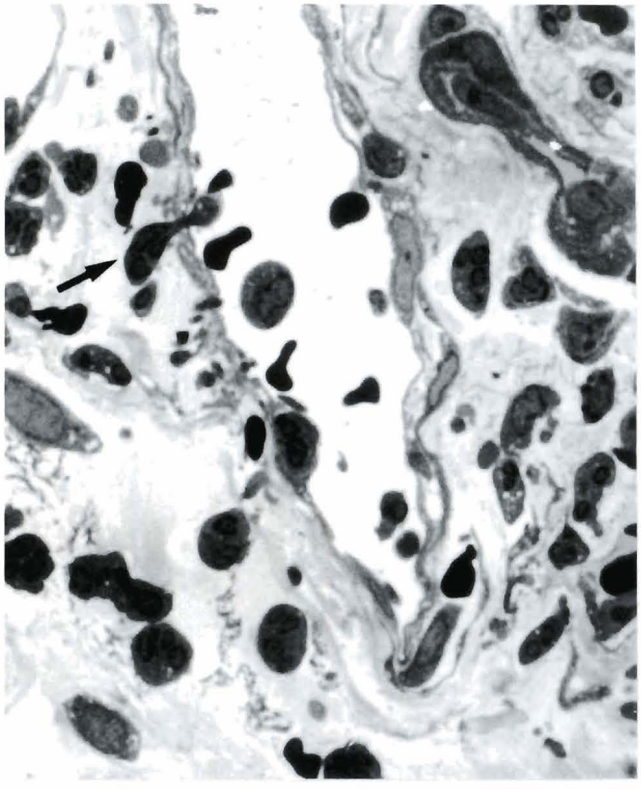

Fig. 4. Dilated perilimbal venule in the first stage of corneal vascularization induced by silver nitrate in the rat. Inflammatory cells (predominantly polymorphonuclear leukocytes) are seen passing through the endothelial junction (arrow) and in the interstititum. Semithin section; toluidine blue. x 700

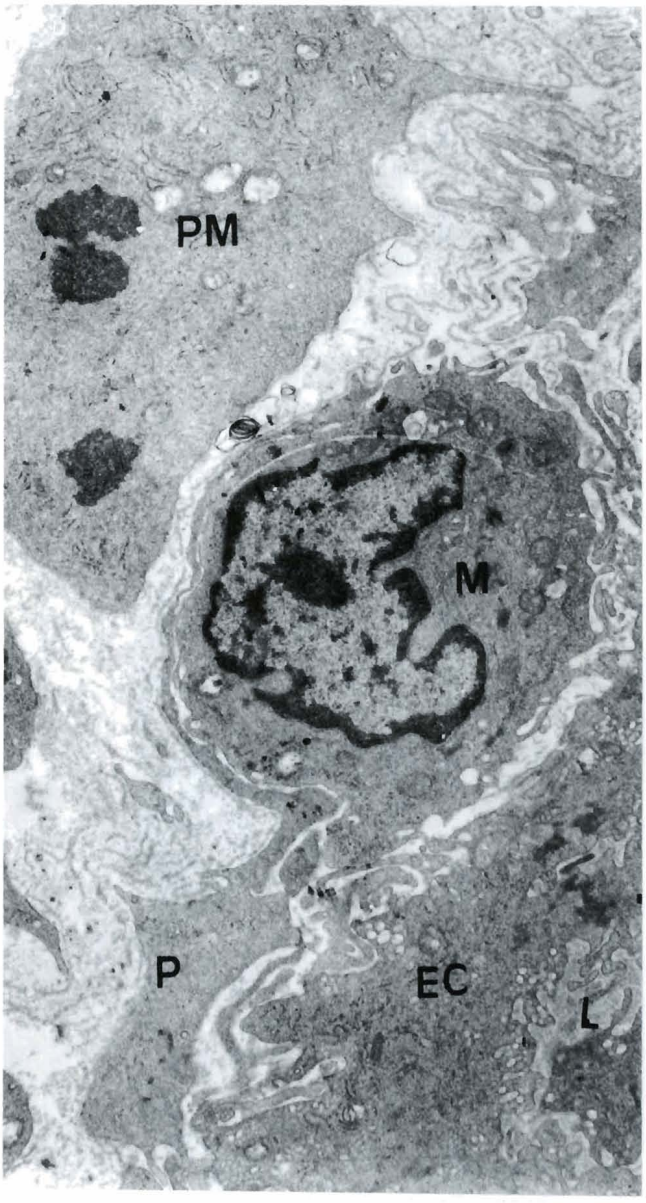

Fig. 5. Preformed postcapillary venule after angiogenic stimulus. A macrophage (M) appears trapped between endothelial cells (EC) and pericytes (P), within the basal lamina. A pericyte in mitosis (PM) is observed. L: Postcapillary venule lumen. Ultrathin section; uranyl acetate and lead citrate. x 13,500

mediate EC adherence (Clark, 1985) and chemotaxis (Bowersox and Sorgente, 1982).

Within a few hours, intravascular accumulation of platelets and polymorphonuclear leukocytes (PMNs) occurs, with early diapedesis of leukocytes outside the vessel lumen. This association of inflammatory cells with the neovascularization in several processes is a recognised observation (Clark and Clark, 1939; Mc Donald, 1959; Grillo, 1963; Jennings and Florey, 1970; Ross et al., 1970, Ryan and Spector, 1970). Indeed, before and during vascular sprouting, inflammatory cells are observed adhering to the endothelium of the parent vessels, as well as passing through the endothelial junctions and the pericyte-endothelial space. Between 1 and 6 h after angiogenic stimuli, the PMNs predominate (Fig. 4). Thereafter, the number of monocytes/ macrophages increases, while the number of PMNs decreases dramatically. Frequently, the monocytes/ macrophages, either individually or in small clusters of two or three, simultaneously appear trapped between the EC and the pericytes, within the basal lamina (Fig. 5). The new vessels arise following the margination and diapedesis of the leukocytes. In an immunohistochemical study of the cellular events after chemical cauterization of the murine cornea, it has been demonstrated that the infiltrating cells which preceded the ingrowth of new blood vessels are granulocytes and inflammatory monocytes. On the contrary, macrophages, T lymphocytes, eosinophils, or mast cells were not part of the infiltrate before the appearance of new capillaries (Sunderkötter et al., 1991). Leukocytes do not seem to be essential for the initiation and continuation of angiogenesis. For example, corneal vascularization has been produced in the absence of leukocytes in rats and rabbits (Sholley et al., 1978). Nevertheless, leukocytes may have a facilitatory or augmentative role in vascularization (Fromer and Klintworth, 1975a,b, 1976; Polverini, 1977a,b; Sholley et al., 1978). Progressively, fibrin material, erithrocytes, macrophages and dividing fibroblasts appear in the interstitium. Usually, angiogenesis is also accompanied, to a variable extent, by fibroblast proliferation which participates in reparative processes.

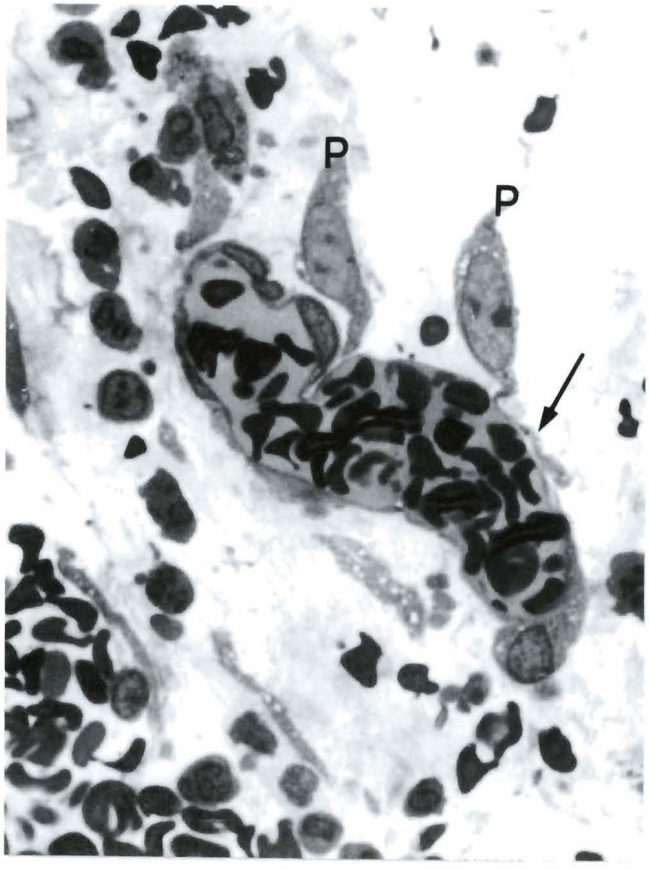

Fig. 6. Bulging pericytes (P) from a limbal postcapillary venule with shortening of their processes (arrows). Semithin section; toluidine blue. x 900

Endothelial cell and pericyte activation:

The earliest morphological changes in the normally quiescent EC consist of hypertrophy with bulging in the vascular lumen, nuclear enlargement, nucleolar prominence, dispersal of the ribosomes into their free form, increase in the number of organelles and the formation of projections from their surfaces (Schoefl, 1963; Yamagami, 1970; Ausprunk and Folkman 1977; McCracken et al., 1979; Burger and Klintworth, 1981; D'Amore and Thompson, 1987). Increased endothelial DNA-synthesis beginning at the onset of angiogenesis has been described (Burger and Klinworth, 1981; Burger et al., 1983). Proteases such as metalloproteases and plasminogen activators are secreted from sprouting EC (Pepper et al., 1990).

The pericytes also display modifications of both their morphological characteristics and topographic relationship (Diaz-Flores et al., 1992) (Fig. 6). The first ultrastructural changes in the enlarged pericytes from post-capillary venules include the shortening of their processes and an increase in the number of cytoplasmic polyribosomes (Fig. 7) (McCracken et al., 1979) (see pericytes in angiogenesis).

Basal lamina degradation:

The fragmentation and disappearance of the basal lamina is a necessary step for the EC migration from the mother vessels (Cliff, 1963; Schoefl, 1963; Ausprunk and Folkman, 1977). Complete disintegration occurs on the side closest to the angiogenic stimulus, coinciding with those areas wherein the EC start to grow outwards, while subtle alterations seem to appear around the whole circumference of the parent vessel. Therefore, the EC sprouts do not have basal lamina, but a homogeneous provisional substratum with altered proteoglycans (Clark et al., 1982a,b). The changes in the original basal lamina

are due to proteolytic enzymes synthesized and secreted by the activated EC (Rifkin et al., 1982; Montesano et al., 1986; Moscatelli and Rifkin, 1988). Indeed, the release of plasminogen activator and collagenase, in response to angiogenic factors, has been demonstrated in EC "in vitro" (Rifkin et al., 1982).

Endothelial cell migration:

Traditionally, it is considered that blood vessels grow by means of a movement of EC (His, 1868). This fact of EC migration is currently considered an important step during angiogenesis (Ausprunk and Folkman, 1977). In the initial phase of neo-vascularization, the EC degrade the vascular basement membrane of the parent vessel, protrude through its wall and begin to migrate into the interstitial space towards the angiogenic stimulus. Most researchers agree that these changes precede endothelial replication in such a way that migration and mitoses are independent phenomena (Sholley et al., 1977a,b; Wall et al., 1978). In other words, angiogenesis begins with pseudopodia of migrating EC and progresses to the proliferation of these cells (Matsuhashi, 1961, 1962; Sugiura and Matsuda, 1969; Yamagami, 1970; McCracken et al., 1979). Therefore, angiogenic stimuli may operate through chemotaxis and EC mitosis may be a secondary event (Sholley et al., 1977a,b; Folkman, 1982). When the entire EC migrates into the interstitium,

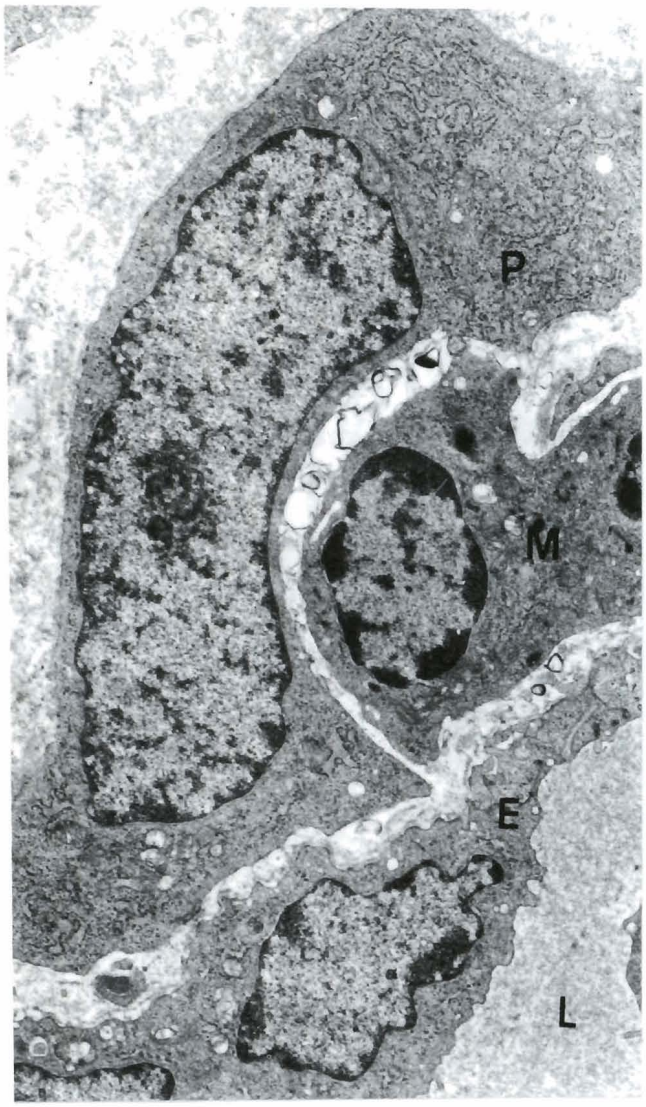

Fig. 7. Preformed postcapillary venule after angiogenic stimulus. A bulging pericyte (P) with increase in its size and in the number of cytoplasmic polyribosomes is seen. A macrophage (M) appears between the pericyte and endothelium (E). L: postcapillary venule lumen. Ultrathin section; uranyl acetate and lead citrate. x 13,500

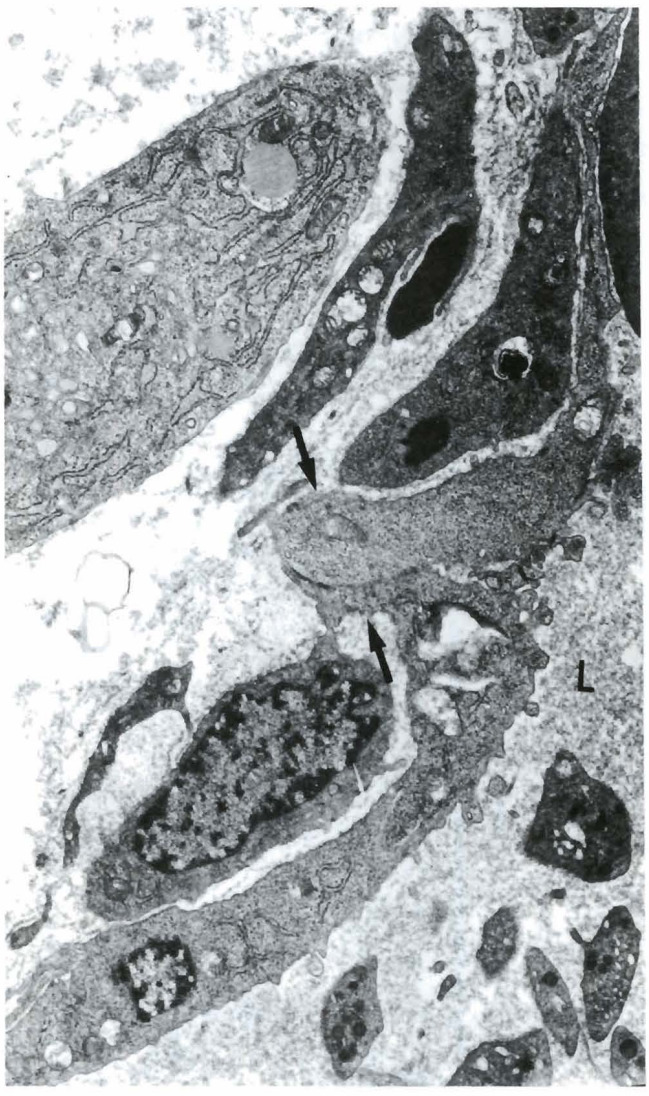

Fig. 8. Endothelial cell migration during angiogenesis. Bicellular or bipolar configuration. Two endothelial cells (arrows) migrate from the wall of the parent vessel towards the perivascular space. L: Parent vessel lumen; ultrathin section. Uranyl acetate and lead citrate. x 13,500

other EC follow and loose EC sprouts or cords are formed in the perivascular stroma. Two different types of EC migration have been described (Paku y Paweletz, 1991): a) bicellular or bipolar configuration (Burger et al., 1983; Folkman, 1984; Wakui, 1988), also termed as telescoping formation (Sholley et al., 1984); and b) linear formation with a single cell type (Folkman, 1986). In the bicellular configuration (Fig. 8), two or more EC migrate from the wall of the parent vessel towards the perivascular space, forming nearly parallel processes (Sholley et al., 1984; Wakui, 1988). The pair of EC, attached to each other, with numerous free polyribosomes and abundant intermediate filaments, may appear in an area embedded within the wall of the parent vessel, while their processes extend outwards in unison to form the endothelial sprout with a narrow slit-like lumen (Wakui, 1988). A giant dense body, has been found in the cells forming the endothelial sprouts (Furusato et al., 1984; Wakui, 1988). In the linear formation (Fig. 9), a single EC projection and/or pseudopod migrates into the surrounding connective tissue from the parent capillaries (Ausprunk and Folkman, 1977; Folkman et al., 1979; Furusato et al., 1984, 1985; Folkman, 1984, 1986). In both cases of migration, the EC aligned with one another to create a solid sprout, or with intercellular slit-like lumina. The elongation and proliferation of the EC progressively lengthens the sprout.

During the process in which the EC protrude and the vascular basement membrane is degraded, microscopic bleeding may occur.

The presence of abundant contractile intermediate filaments in the endothelium of the sprouts might be important for the extension and migration of these sprouts (Furusato et al, 1985; Wakui, 1988). Some authors have reported the loosening of intercellular contacts between migrating EC with other EC of the mother vessels, suggesting that the disrupted cell junctions may release EC from contact inhibition and allow proliferation, while producing an increased vascular permeability (Ausprunk and Folkman, 1977). On the contrary, other investigators have never observed opened intercellular contacts in the neighbourhood of new capillaries, and are of the opinion that pre-existing intercellular junctions contribute to a parallel movement of the EC and preserve the inside-outside polarity of the

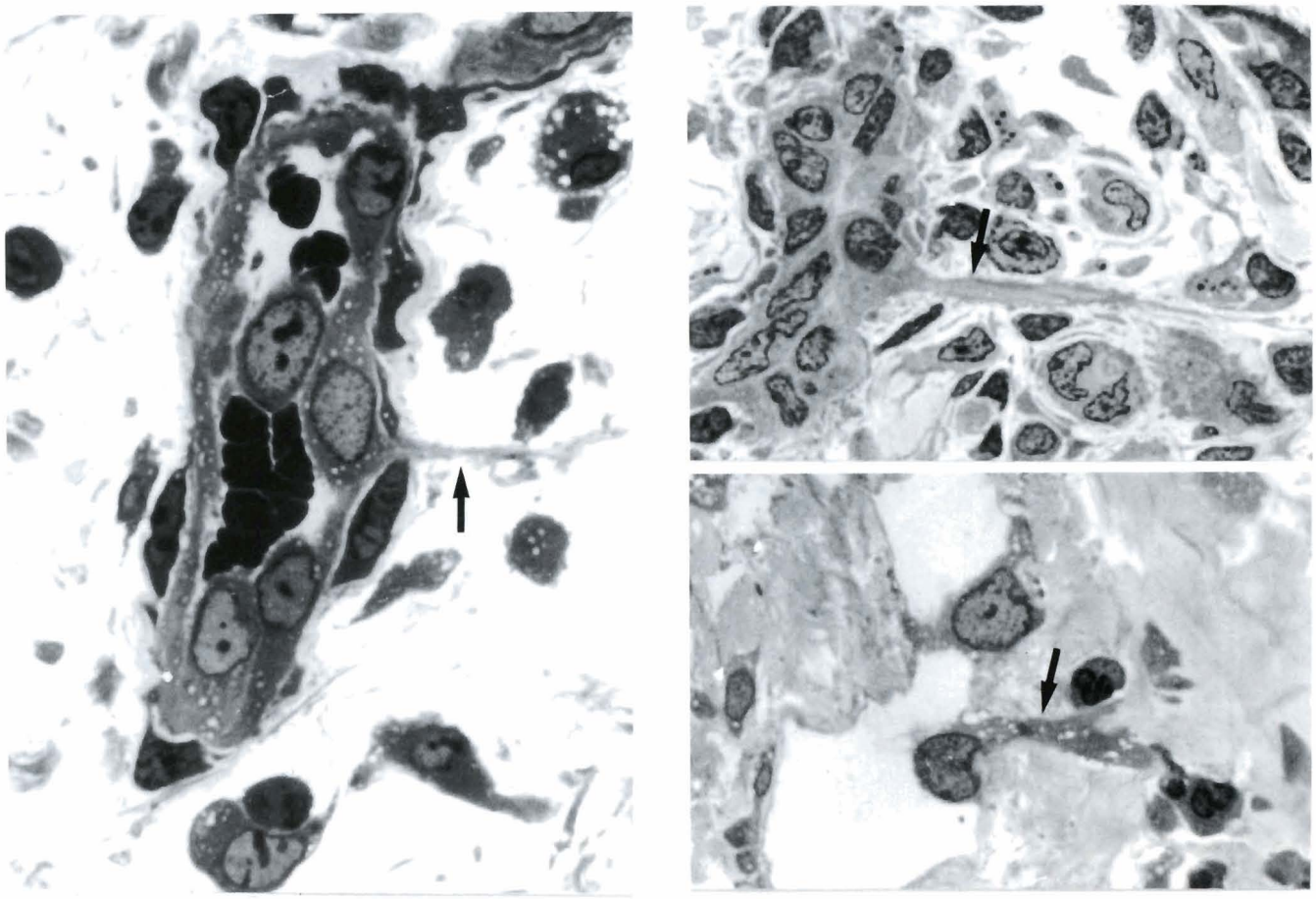

Fig. 9. Endothelial cell migration during angiogenesis. Linear formation. A single EC projection or pseudopod (arrows) migrates into the interstitium from the parent vessel. Semithin sections; toluidine blue. x 900

sprouting EC (Paku and Paweletz, 1991).

The chemotactic behaviour of EC at the tips of growing vessels is facilitated by the secretion of plasminogen activator and collagenases (Moscatelli et al., 1981).

The EC migration, in response to the extracellular matrix, depends on the integrin family of cell adhesion receptors (Leavesley, 1993). Indeed, attachment, spreading and migration of EC are mediated by integrins alfa2B1 and alfa4B3 (Leavesley, 1993). EC migration on collagen and vitronectin is mediated by alfa4B3 and it occurs in a calcium-dependent manner, while collagen recognition by alfa2B1 promotes EC migration in the absence of calcium (Leavesley, 1993).

Endothelial cell proliferation:

Mature endothelial cells, normally in a resting state, show an extremely slow turnover rate (Algire et al., 1945; Altschul, 1954; Sparagen et al., 1962; Folkman and Cotran, 1976) of 2 months or more. Thus, using ^{3}H-Thymidine, the labelling index is lower than 1% in normal capillary and venular EC of the retina, liver (Tannock and Hayashi, 1972), myocardium, stomach (Tannock and Hayashi 1972), striated muscle (Tannock and Hagashi, 1972) and skin (Cavallo et al., 1972, 1973; Tannock and Hayashi, 1972; Polverini et al., 1977b). For example, it is 0.01% in capillary EC in the adult rat retina. Since the turnover rates of EC are extremely low, angiogenesis is generally a quiescent process in the healthy adult organism (Shweiki et al., 1993). Nevertheless, the EC can quickly convert to a proliferative state during angiogenesis and in several related processes, such as endothelium repopulation in organ transplants, repair of large vessel defects and thrombi recanalization (Cavallo et al., 1973; Folkman, 1984). However, EC proliferation is not absolutely essential, since angiogenesis has been shown to take place even in the absence of EC replication (Sholley et al., 1984).

During angiogenesis, endothelial DNA synthesis occurs in parent vessels before sprouting (Fig. 10), and according to some authors as early as 6 to 8 hours after an angiogenic stimulus is applied (Cavallo et al., 1973). The increase of the turnover rate of EC can be considerable. For example, the ^{3}H-Thymidine labelling index of EC increases to 9% in tumours (Denekamp and Hobson, 1982). The time and the exact site of EC division are controversial. For some investigators, EC mitosis appear concomitant with sprouting (Sholley et al., 1984), while most authors are of the opinion that EC begin in mitosis after they start to migrate. The EC mitosis appear in both the parent vessels (Fig. 11) and the newly formed vessels. In the latter, it has been pointed out that they occur at the tip (Clark and Clark, 1939, Hadfield, 1951), but it is accepted that when capillary sprout budding begins, endothelial proliferation takes place in cells following the "leader EC", but not

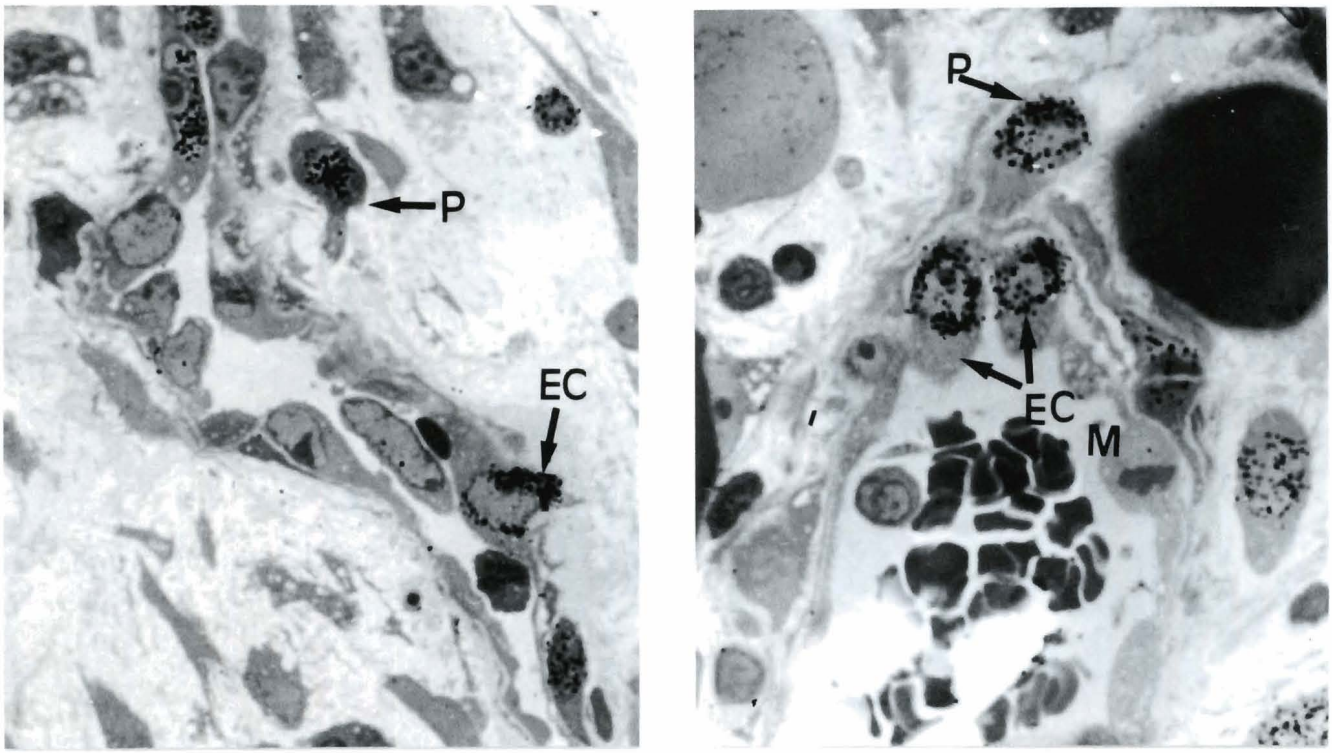

Fig. 10. Autoradiograph of a preformed postcapillary venule after angiogenic stimulus. ^{3}H-labelled endothelial cells (EC) and pericytes (P) are present. An EC in mitosis (M) is observed. Semithin sections; toluidine blue. x 1,150

usually at their tips. In other words, the zone of replication is closer to the parent vessel (Cliff, 1965; Ausprunk and Folkman, 1977; Folkman, 1982, 1986; Clark, 1985).

The ability of angiogenic stimuli to induce replication in confluent EC is associated with disruption of cell-cell contacts (Bavisotto et al., 1990). Likewise, the replicative state and its ability to respond to endogenous mitogens may depend on cytoskeletal organization, such as microtubule destabilization or changes in the cell shape (Liaw and Schwartz, 1993). Finally, the collagen in the interstitium seems to have an influence on EC proliferation (Madri and Stenn, 1982; Schor et al., 1983).

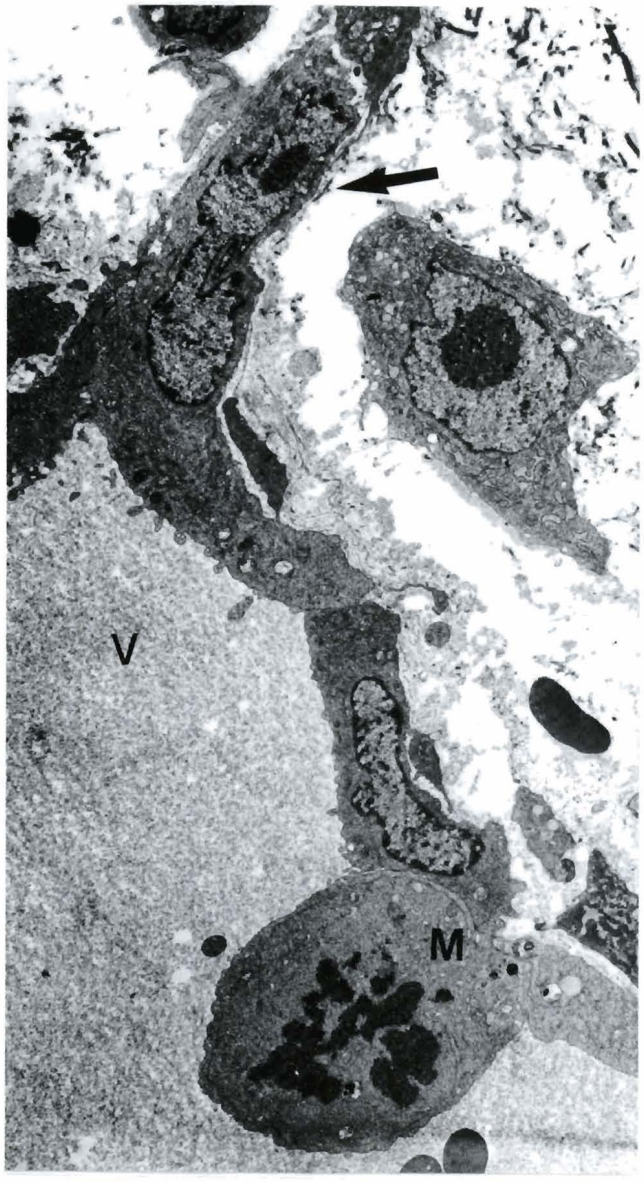

Fig. 11. A venule (V) and its capillary sprout (arrow) are shown. An endothelial cell in mitosis (M) is observed in the parent vessel. Ultrathin section; uranyl acetate and lead citrate. x 13,500

Migration and proliferation of pericytes from pre-existing vessels (see Pericytes in angiogenesis):

Formation of the new capillary vessel lumen. During angiogenesis, the endothelial cells form tubular channels capable of carrying blood. Two distinct types have been considered in the formation of the new capillary vessel lumen (Wagner, 1980): a) previous intracellular vacuolization in the endothelial cytoplasm of contiguous cells which leads to intercellular canalization by connection of the vacuoles (Sabin, 1920; Folkman and Haudenschild, 1980; Furusato et al., 1984, 1985); and b) initial intercellular canalization of adjacent endothelial processes (Figs. 12 and 13), by curvature of the EC (Lewis, 1925, 1931; Wakui, 1988). In general, it is accepted that the lumen of the capillary sprout is formed between the adjacent endothelial processes (Figs. 12,

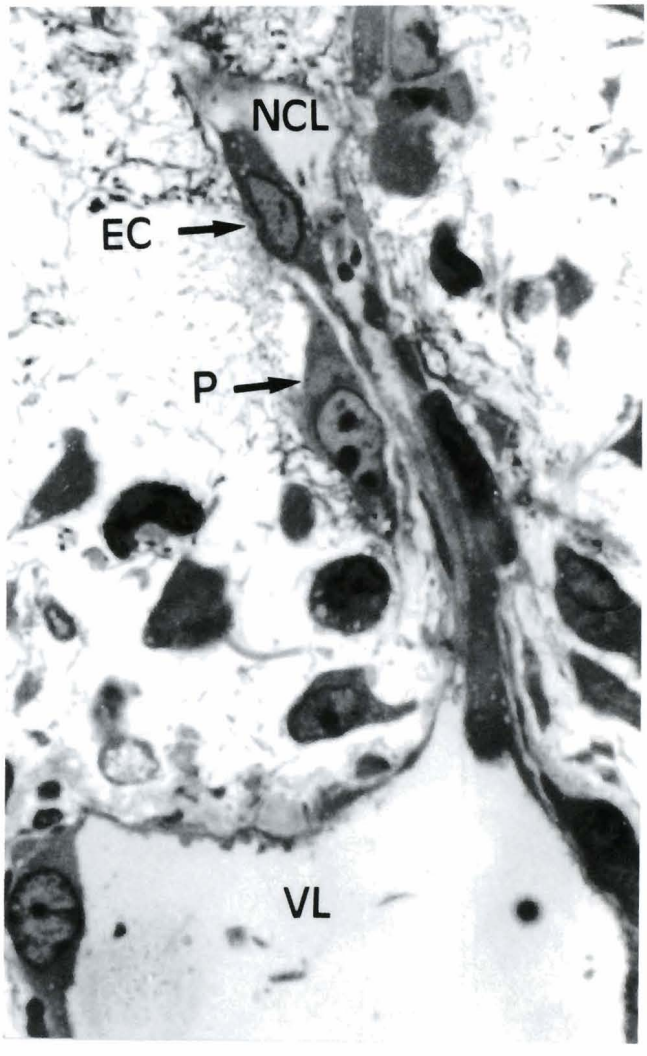

Fig. 12. Formation of the new capillary vessel lumen by intercellular canalization of adjacent endothelial cells. NCL: new capillary lumen; VL: lumen of the parent vessel; EC: endothelial cells; P: pericytes. Semithin section; toluidine blue. x 900

13), budding off the wall of the parent vessels and conserving their cellular polarity (Wakui, 1988). The exact moment in which the lumen of the new capillary connects with the parent vessel lumen has not been clarified. It may be from the beginning or in the early phases of the sprout formation (Yamagami, 1970; Bar and Wolff, 1972; Cavallo et al., 1973; Dvorak et al., 1988; Wakui, 1988; Paku and Paweletz, 1991). Some authors consider that the new lumen evolves while the sprouting EC are in the wall of the parent vessel (Wakui, 1988). In other words, the capillary sprout lumen may be an elongation of the parent vessel lumen (Wakui et al., 1988). Other authors are of the opinion that the new vessel lumen appears first in the sprout, merging later with the mother vessel (Ausprunk and Folkman, 1977; Folkman, 1984). So that the lumen is developed by transversal division of the EC (Nicosia et al., 1982).

Pericytes in angiogenesis:

In the same way that EC are recognised as the principal cellular component of neovascularization, there is a controversy concerning the involvement of pericytes before and during the different phases of capillary sprouting (Burger and Klintworth, 1981). Regarding this problem, four aspects should be taken into account: a) the behaviour of the preformed microvasculature pericytes; b) the involvement of pericytes in the different stages of angiogenesis, including their incorporation to the newly formed capillaries; c) the origin of pericytes in

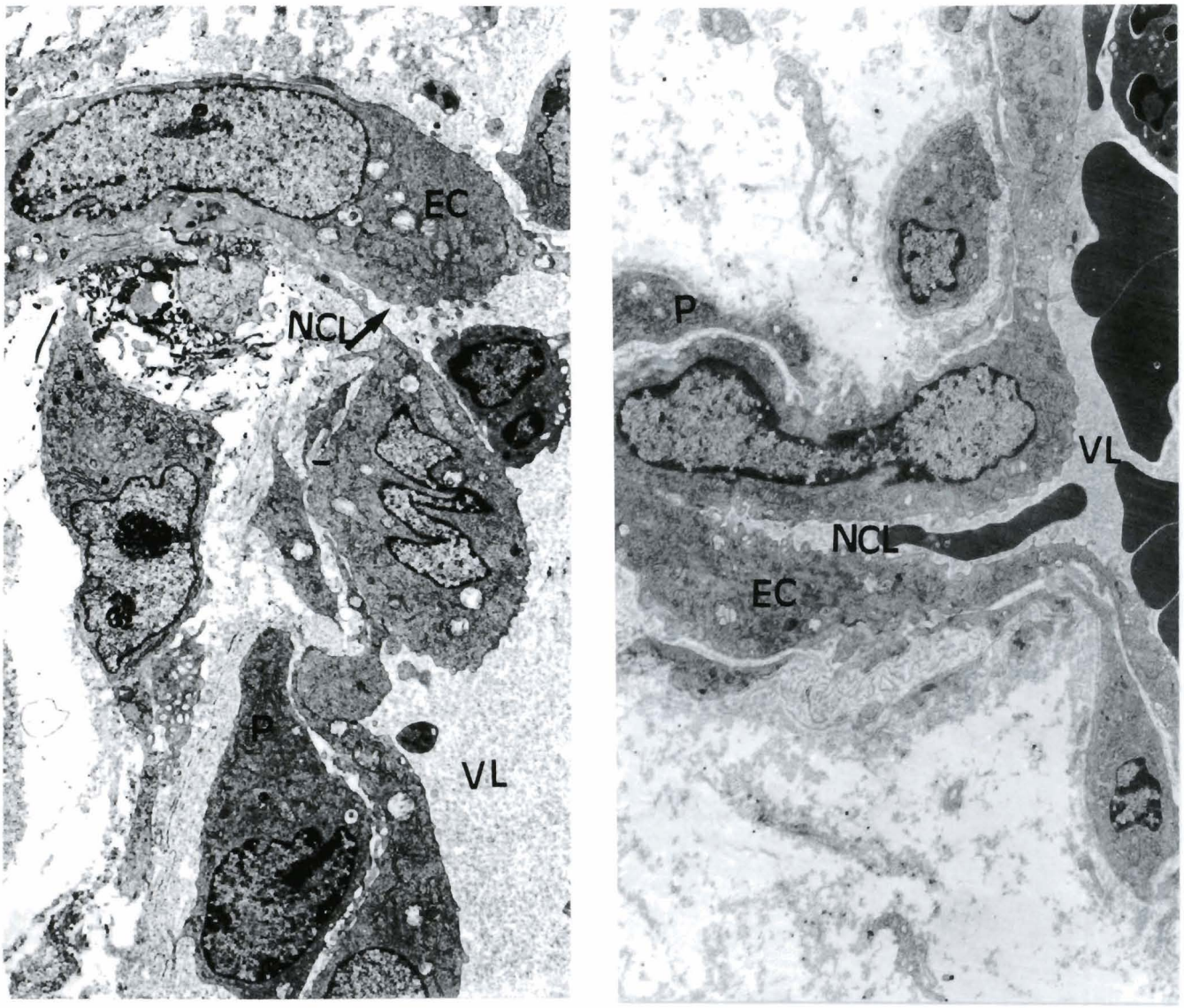

Fig. 13. Different stages of new capillary vessel lumen formation. NCL: new capillary lumen; VL: lumen of the parent vessel; EC: endothelial cells; P: pericytes. Ultrahin section; uranyl acetate and lead citrate. x 13,500

the newly formed vessels; and d) the role of pericytes in the regulation of EC proliferation during angiogenesis.

Information regarding modifications of pericytes at the level of the preformed (pre-existing) capillaries and postcapillary venules, from which the new blood vessels must develop, is scarce (Cavallo et al, 1973; McCraken et al., 1979; Burger and Klintworth, 1981; Diaz-Flores et al., 1992). The studies in preformed microvasculature pericytes reveal a sudden, brief and intense proliferation during the initial phase of angiogenesis (Diaz-Flores et al., 1992). Indeed, it has been pointed out that, after angiogenic stimulus, the pericytes undergo hypertrophy, with shortened processes, prominent nucleoli and dispersal of ribosomes into their free form (Fig. 7). Likewise, a decrease in contact surfaces between pericytes and endothelium, disruption and fragmentation of the pericyte basal lamina in some areas, and pericytic projections into the extravascular space have been observed (Fig. 6). In these conditions, many of the pericytes undergoing mitosis and autoradiographic studies show an increased DNA synthesis in pericytes and EC of the parent vessels (Fig. 10) (Schoefl, 1963; Cavallo et al., 1972, 1973; Sholley et al., 1977a,b; Burger and Klintworth, 1981; Diaz-Flores et al., 1992). In other words, the preformed microvasculature pericytes are substantially activated during post-natal angiogenesis, suggesting that they may contribute to the origin of new cells (Diaz-Flores and Dominguez, 1985; Diaz-Flores et al., 1988, 1989, 1990a,b, 1991a,b, 1992).

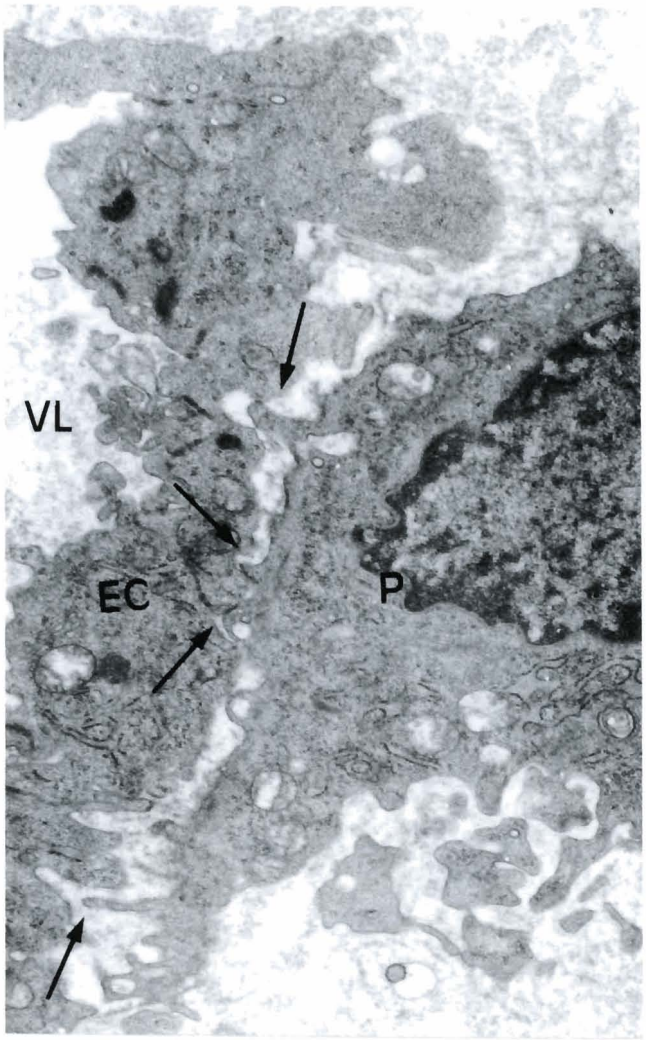

Fig. 14. The relationship between an endothelial cell and a pericyte in a growing capillary is shown. Cytoplasmic processes of the pericyte (P) and EC caving in on each other are observed (arrows). VL: vascular lumen. Ultrathin section; uranyl acetate and lead citrate. x 14,000

The second aspect is concerned with the question of pericyte involvement in the different stages of capillary sprouting. In the early stages of angiogenesis, the relationship between pericyte and endothelium remains unclear. Most of the authors are of the opinion that the involvement of capillaries with pericytes occurs at the end of the proliferative stage, following lumen formation (Folkman and Haudenschild, 1980; Folkman and Klagsbrun, 1987; D'Amore and Thompson, 1987; Paweletz and Knierim, 1989; Blood and Zetter, 1990). However, the number of pericytes increases when there is vascular proliferation (Schlingemann et al., 1990; Diaz-Flores et al. 1992) and EC may undergo mitosis when they are closely associated with pericytes (Cavallo et al., 1973; Sholley et al., 1977; Diaz-Flores et al., 1992a). The alternative possibility of an early recruitment of pericytes during angiogenesis has also been pointed out (Crocker et al., 1970; Inomata et al., 1971; Verhoeven and Buyssens, 1988; Schlingemann et al., 1991; Nehls et al., 1992). Even the authors that consider an early incorporation of pericytes to the vascular sprouts, do not agree on the moment of occurrence. Thus, some authors describe the presence of pericytes surrounding the buds when the endothelial cells extend and/or migrate to form the endothelial sprout (Fig. 9), or when slit-lumen appear in them (Yamagami, 1970; Crocker et al., 1970; Inomata et al., 1971; Wakui, 1988). Other researchers believe that slit-like lumens develop when no pericytes are visible, pointing out that the first appearance of pericytes around the newly formed vessels bears no relationship to the development and organization of the basal lamina (Paku and Paweletz, 1991). The type of relationship between endothelial sprouts and nascent pericytes is also not totally clear. The fusion of pericytes with the endothelium at the point of active angiogenesis (Crocker et al., 1970; Inomata et al., 1971), and the presence of cytoplasmic processes of pericytes and EC caving in on each other (Wakui, 1988; Furusato et al., 1990) have been observed in the early stages of neovascularization (Fig. 14). Recently, nascent pericytes showing cellular processes advancing at the tips of endothelial sprouts have been described during angiogenesis. In this way, the gaps between opposing endothelial sprouts are bridged by pericytic processes, suggesting that pericytes may serve as guiding structures for EC outgrowth (Nehls et al., 1992). Therefore, capillary sprouting could

include coordinated growth of both EC and pericytes (Nehls et al., 1992).

Another open question is concerned with the origin of pericytes in the newly formed vessels. The hypotheses of a primitive origin from mesenchymal cells derived from blood monocytes around new vessels (Crocker et al., 1970), or from the EC of pre-existing vessels (Nakayasu, 1988) have been abandoned. Several observations suggest that they may evolve from perivascular fibroblasts (Nakayasu, 1988; Rhodin and Fujita, 1989; Nehls et al., 1992). In the corneal stroma, for example, pericytes of newly invading vessels may develop from keratocytes (Nakayasu, 1988). Likewise, formation of capillary-like tubes by vascular EC cocultivated with keratocytes has been described (Nakayasu et al., 1992). The following steps have been hypothesized in the supposition that fibroblasts acquire pericytic characteristics (Nehls et al., 1992): a) establishment of contacts between endothelial sprouts and fibroblasts; b) the periendothelial cells may start expression of desmin; and c) the periendothelial cells, or pericytes acquire smooth muscle-like features and begin to express SM alpha actin (Nehls and Drenckhahn, 1991). It has also been indicated that preformed pericytes of nonmuscular pericytic microvasculature contribute to the origin of new pericytes or other related cells (Cliff, 1976; Diaz-Flores et al., 1991a,b). Finally, the vascular smooth muscle cells have been considered as a possible precursor of pericytes due to the phenotypic similarities between both types of cells (Diaz-Flores et al., 1994), including certain smooth-muscle type proteins (Joyce et al., 1984a,b, 1985; Herman and D'Amore, 1985; Fujimoto and Singer, 1987; Skalli et al., 1989), as well as the gradual transition existing between both types of cells (Sims, 1986; Diaz-Flores et al., 1991b).

Finally, among the functions attributed to pericytes is a role in angiogenesis regulation (Kuwabara and Cogan, 1963; Crocker et al., 1970; Ordlidge and D'Amore, 1987). The absence of pericytes at the tips of migrating EC is believed to stimulate EC mitosis, while their presence in the older regions of the growing capillaries may inhibit EC proliferation and migration (Ordlidge and D'Amore, 1987; Sato and Rifkin, 1989).

Changes in extracellular matrix. Formation of a new basal lamina:

Although the basal lamina degradation has already been described, it will be considered along with the changes in the extracellular matrix. Indeed, during angiogenesis, local proteolysis of the basement lamina of the parent vessels and subsequent degradation of interstitial matrix are observed (Rifkin et al., 1982; Kalebic et al., 1983; Madri et al., 1983). In quiescent microvasculature, laminin, fibronectin, entactin, heparan sulphate proteoglycan and collagen types IV and V are present in the basement lamina (Kanwar and Farquhar, 1979; Foidart et al., 1980; Bender et al., 1981; Pepper and Montesano, 1990). Initially, the active EC secrete matrix degrading enzymes, such as plasminogen activator and collagenase, causing fragmentation of the basement lamina (Folkman, 1982). Likewise, the components of the microvascular extracellular matrix, such as fibronectin, laminin and collagen Types I, III, IV and V, undergo dramatic changes (Nicosia and Madri, 1987), while EC undergo tube formation and extension (Folkman and Haudenschild, 1980; Folkman, 1986). In the initial stages of developing microvessels, fibronectin is the predominant component of the provisional matrix, making up a delicate fibrillary network. Fibrils of type V collagen, patchy amorphous deposits of laminin and Type IV collagen, and rare to absent fibrils of Type I and III collagen have also been described (Nicosia and Madri, 1987). Progressively, the deposits of fibronectin decrease, becoming discontinuous, while laminin and type IV collagen increase, accumulating and forming a continuous feltwork in the subendothelial space. In the late stages of angiogenesis, increased amounts of Types I and III collagen in the perivascular space are observed.

In some conditions, greatly thickened and/or multilayered basal lamina have been described in newly matured vessels (Szalay and Pappas 1970; Smelser and Ozanics, 1972). The possibility that this is due to repeated episodes of EC death and regrowth has been considered (Vracko and Benditt, 1970).

Capillary loop formation:

A short distance away from the parent vessel, the

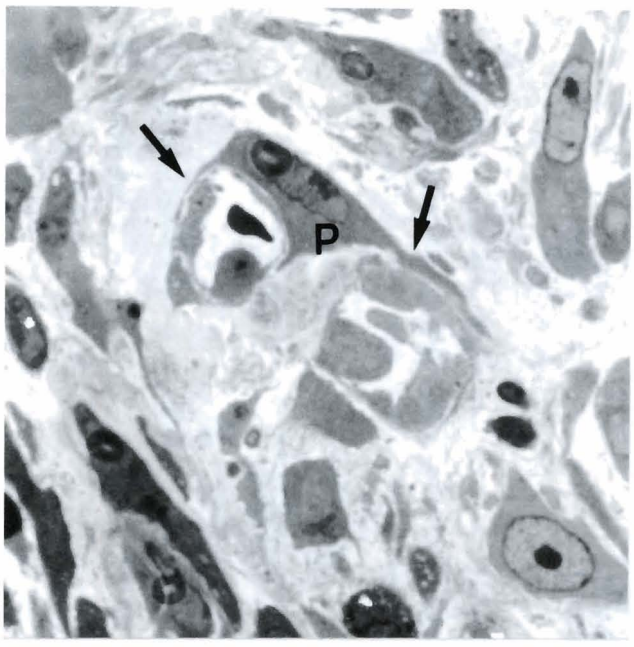

Fig. 15. The processes (arrows) of a pericyte (P) bridge between two capillary sprouts and they may serve as guiding structures for the fusion of sprouts into continuous capillary loops. Semithin section; toluidine blue. x 900

capillary sprouts, whose tips contain migrating EC, begin to branch and join other tips to form capillary loops. Other capillary sprouts then appear from these loops to form a plexus.

How capillary sprouts find each other to fuse into continuous capillary loops is an unsolved problem. It has been suggested that pericytic processes, which seem to bridge the gap between the leading edges of opposing endothelial sprouts, may serve as guiding structures for the outgrowth of EC (Nehls et al., 1992) (Fig. 15).

The capillary loop formation is well demonstrated during angiogenic reaction in the cornea, wherein secondary sprouts develop from the growing tips of initial sprouts, leading to a "brush border" morphology (Muthukkaruppan and Auerbach, 1979; Muthukkaruppan et al., 1982).

Early changes in the newly formed vessels. Persistence, involution and differentiation:

During angiogenesis, a substantial number of newly formed vessels regress (Meyer, 1852; Clark and Clark, 1939; Szalay and Pappas, 1970; Ausprunk et al., 1978; Latker and Kuwabara, 1981; Azmi and O'Shea, 1984; Latker et al., 1986; Spanel-Borowski and Mayerhofer, 1987). Indeed, immature vessels seem to require angiogenic stimuli to persist (Ausprunk et al., 1978), regressing when the stimuli are removed.

The sequence of events in the vessel regression is variable (Ausprunk et al., 1978; Azmi and O'Shea, 1984; Spanel-Borowski and Mayerhofer, 1987). Two main types of vascular regression may be considered (Ausprunk et al., 1978; Azmi and O'Shea, 1984). In the first type (Ausprunk et al., 1978), aggregation of

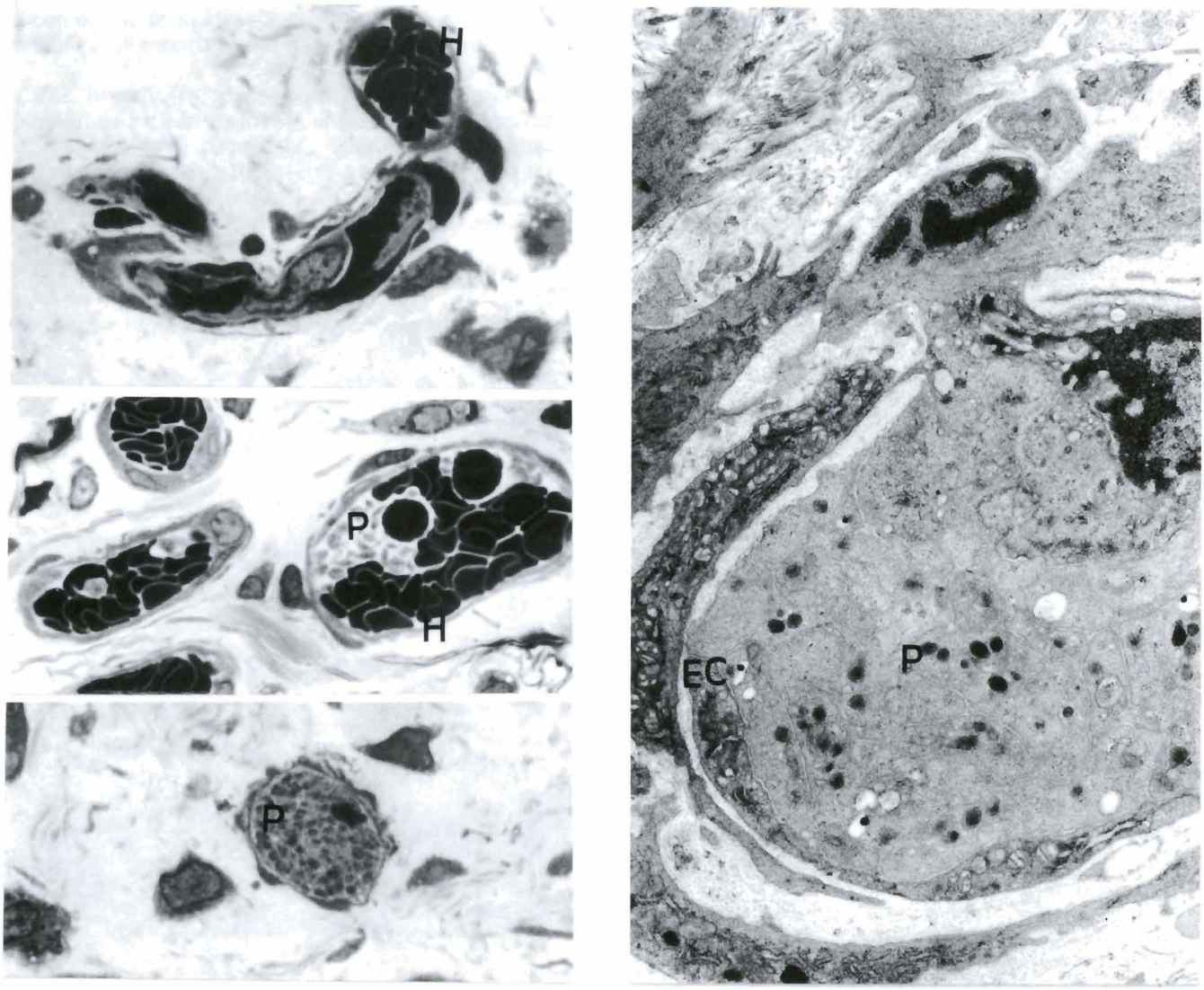

Fig. 16. Vascular regression during angiogenesis. Aggregation of platelets (P), stasis of blood (H) and degeneration of endothelial cells (EC) are observed. A, B and C. Semithin sections; toluidine blue x 900. D. Ultrathin section; uranyl acetate and lead citrate. x 13,500

platelets, stasis of blood, vessel occlusion and degeneration of vessel wall cells are the most important findings (Fig. 16). The platelets accumulate and adhere to the endothelium of the regressing vessels. Furthermore, fibrin polymerization, stasis of blood and vessel occlusion by erythrocytes occur. Some of the erythrocytes also leak out into the interstitium. Due to the above, the presence of immature and involutive capillary aggregates, containing accumulated but frequently degranulated platelets, may behave as a "paracrine organ". On occasions, the EC become very thin or fenestrate. At other times the EC at the distal tips of the capillaries show organelle swelling, vacuolization, plasma membrane disruption and cytolysis followed by granular material deposition into the interstitium. Finally, mononuclear cells remove vascular debris.

In the second type of vascular regression (Azmi and O'Shea, 1984), the endothelial delection is the greatest finding (Fig. 17). The following steps need to be considered: a) protrusion of some individual EC into capillary lumen; b) formation of adherence junctions between the protruded ECs and other EC; c) nuclear and cytoplasmic condensation; d) cellular and nuclear lobation and fragmentation; e) disruption of cell organelles and loss of plasma membrane integrity and cytoplasmic density; and f) engulfment of degenerate cell fragments by viable mural EC.

Once some of the new vessels reach the source of the stimulus, there is a flow decrease in the less advanced vessels which then regress (Auerbach et al., 1991). At the same time, the vascular regression seems to occur first in the smallest distal capillary branches, probably because blood flow within them is sluggish (Ausprunk et al., 1978).

The regressive behaviour of blood vessels depends on the tissue. For example, in the cornea of some animals the newly formed vessel may disappear when the angiogenic stimulus is removed or ceases (Zauberman et

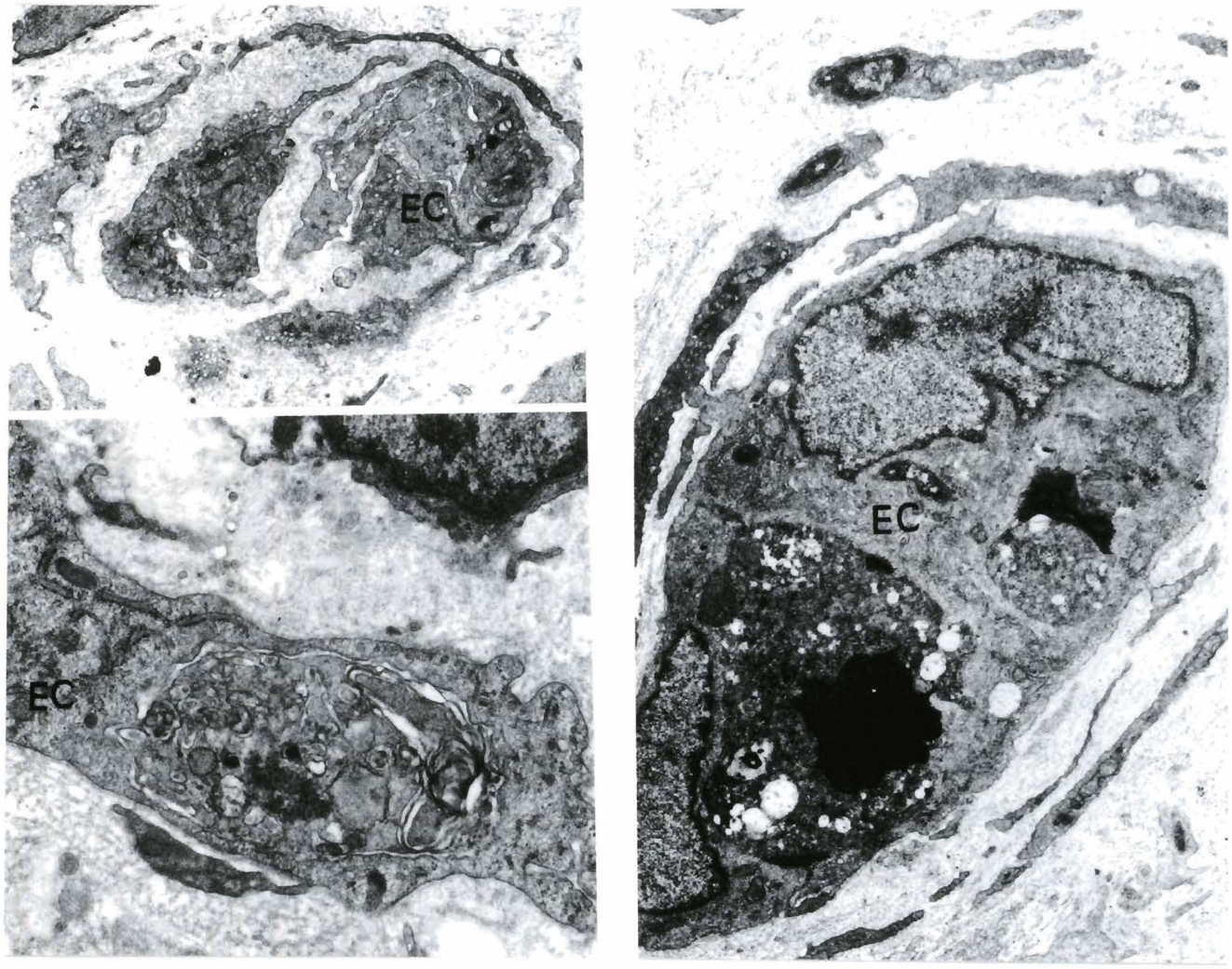

Fig. 17. Vascular regression during angiogenesis. Endothelial delection and engulfment of degenerate cell fragments by viable mural endothelial cells are observed. EC: endothelial cells. Ultrathin sections; uranyl acetate and lead citrate. x 13,500

al., 1969; Ausprunk et al., 1978). On the contrary, newly formed blood vessels can persist in human corneas (Cogan, 1949). Likewise, in normally vascular tissues, regenerating blood vessels do not all regress (Jennings and Florey, 1970).

With regard to differentiation, the newly formed microvasculature may be influenced by the tissues within which it develops. Thus, although neovascularization in metastasic tumours arises from the blood vessels of the receptor site, it can acquire the morphological characteristics of the primary organ vessels in which the neoplasm originates. In other words, the new vessels can differentiate according to tissue specificity.

Capillary network formation and organization into larger microvessels:

The mechanism by which a functional circulation is established during post-natal angiogenesis is difficult to explain (Phillips et al., 1991). This mechanism may be undertaken in two different ways, both of which are probably associated (Burger et al., 1983; Philips et al., 1991; Diaz-Flores et al., 1994): 1) by remodelling the newly formed capillaries (Fig. 18) and their parent vessels; and/or 2) by anastomoses of the new capillaries with pre-existing vessels of greater calibre in the venous and arterial sides of the circulation. The first possibility is based on the development of arteries and veins from capillary vessels, which would require a lateral EC proliferation and the presence of appropriate mural cells (pericytes and smooth muscle cells). Consistent with the second possibility for the venous side of the circulation, recent observations have demonstrated anastomoses of the microvessels originating from the small venules and capillaries with others arising from the veins (Diaz-Flores et al., 1994). As far as the arterial side of the circulation is concerned, a similar procedure does not seem likely, since new vessels have not been observed arising from arteries or arterioles. Nevertheless, an inverse process is possible, since ingrowing capillaries in the arterial wall have been described in several conditions (Diaz-Flores and Dominguez, 1985; Diaz-Flores et al., 1990a).

Peculiar forms of angiogenesis

Numerous forms of angiogenesis have been described. By way of examples, the vascularization of naturally avascular structures and tissue grafts, the

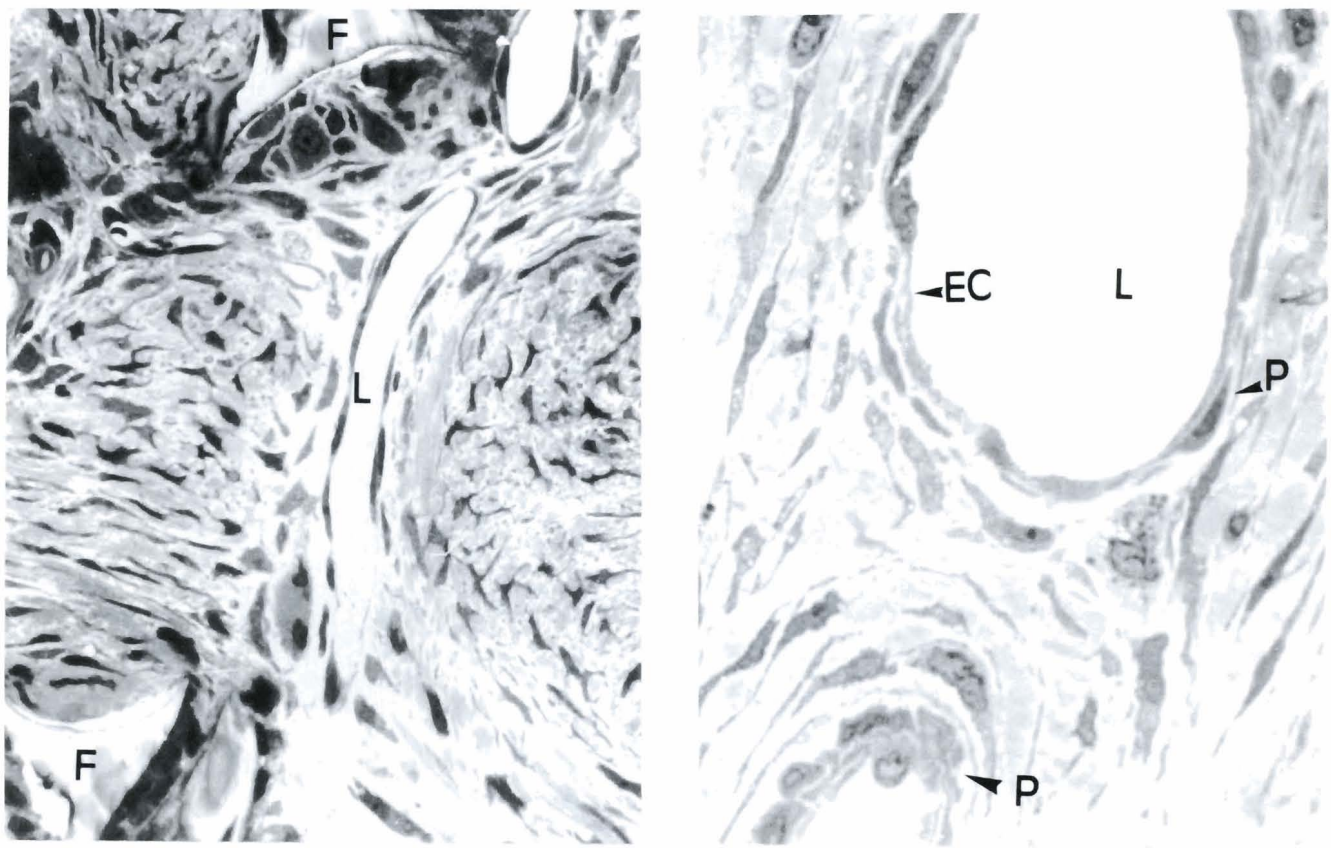

Fig. 18. Remodelling of the newly formed capillaries growing into a polyvinyl foam (F). L: vessel lumen; EC: endothelial cells; P: perivascular cells. Semithin section; toluidine blue. x 900

growth intussusceptive in the post-natal pulmonary microcirculation, the angiogenesis during the formation of neovascular collateral vessels, the intimal vascularization of arteries and the tumour angiogenesis will be considered.

Vascularization of naturally avascular structures:

An example is the invasion of the cornea by blood vessels from the pericorneal plexus, an event that occurs widely in a variety of pathological states (Cogan, 1949).

The resistance of certain tissues to vascular invasion has been studied by means of explanting them onto chick chorioallantoic membrane (Eisenstein et al., 1973). In these conditions, the tissues, which normally have a blood supply, are rapidly invaded by host vessels. The tissues devoid of blood vessels behave in two opposing ways. Thus, hyalin cartilage is impenetrable by neovascularization, unless it is calcified, while the stroma of the cornea is readily penetrable, although Descemet's membrane forms a barrier against invasion by host microvessels.

Neovascularization of tissue grafts:

Neovascularization of tumours using syngenic in vivo models and of normal tissue grafts has been investigated (Ausprunk and Folkman, 1977; Warren, 1979a; Paku and Paweletz, 1991). When re-vascularization of normal tissue grafts occurs, it is the result of the pre-existing graft vessels fusing with the host circulation (Folkman, 1976; Sasaki et al., 1991). Thus, newborn mouse cerebral cortex tissues transplanted into the third ventricle of rats show endothelial cells originating from both the host brain and the grafted mouse cerebral cortex, the latter expressing mouse-specific I ad antigen (Kohsaka et al., 1989). During neovascularization in the early stages of rat splenic autografts, it has been suggested that pre-existing sinus endothelial cells rearrange themselves after devascularization and reconstitute a characteristic complex structure that anastomoses with the invading capillaries from the connective tissue surrounding the graft (Sasaki et al., 1991).

In zones of focal necrosis or in related circumstances, such as grafts of autologous tissues, new EC sprouts from uninjured vessels can grow within the old basal lamina of necrotic vessels (Fig. 19), which provide a scaffold for the new vessels (Vracko and Benditt, 1970).

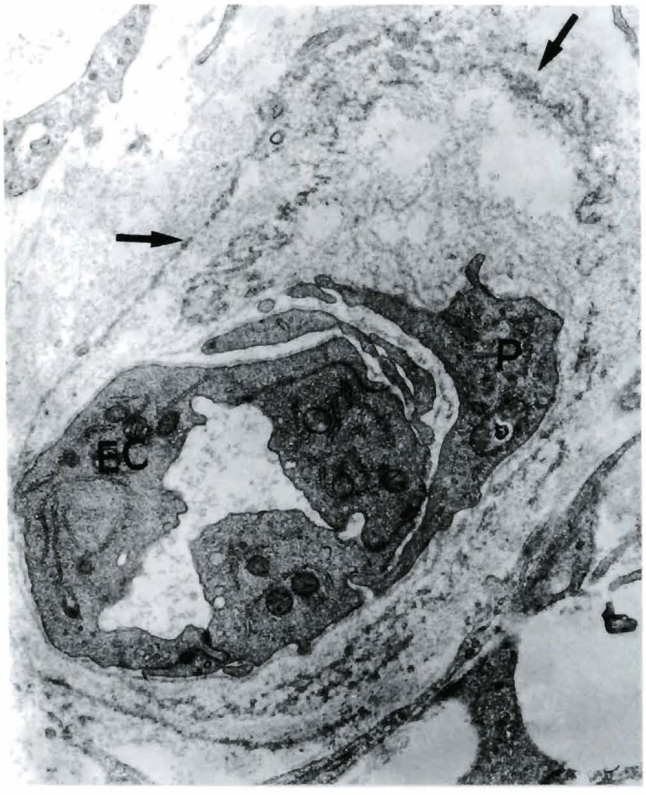

Fig. 19. A new capillary growing within the old basal lamina of a necrotic vessel (arrows) is observed. EC: endothelial cell; P: pericyte. Ultrathin section; uranyl acetate and lead citrate. x 12,000

Growth intussusceptive in the postnatal pulmonary microcirculation:

An example of another proposed mechanism of microvascular growth is that termed as growth intussusceptive in the post-natal pulmonary microcirculation. The authors who present this hypothesis point out that the capillary bed grows by forming slender intravascular tissue pillars (Caduff et al., 1986; Burri and Tarek, 1990), in which interendothelial bridges are the contacts between opposite capillary walls. Subsequently, the contact areas are sealed off by building up interendothelial junctions, with a central perforation in the capillary layer. Successive perforations by cytoplasmic extensions of pericytes, myofibroblasts and interstitial fibres will transform the pillars into normal capillary meshes.

Angiogenesis in the formation of neovascular collateral vessels:

Although angiogenesis normally does not occur in most adult organs, an alternate route of blood supply may arise from preformed or neovascular collateral vessels in response to ischemic stimuli, such as that of an ischemic heart (Schaper and Vandesteene, 1967; de Brabander et al., 1973; Roth et al., 1990; Sasayama and Fujita, 1992; Carroll et al., 1993). This process of collateralization has been experimentally studied in chronic myocardial ischemia using dogs (Bloor and White, 1972; White et al., 1978; Unger et al., 1991) and pigs (Roth et al., 1987; White et al., 1992). In dogs, with an enormous potential for collateral growth, the collateral development is rapid and extensive, while in pigs, with a collateral angiogenesis more comparable to that found in human hearts, it is rapid but limited.

During collateralization, the underlying mechanisms in the genesis of newly formed vessels are likely to be similar to those involved in neovascular growth elsewhere (D'Amore and Thompson, 1987). Normal and ischemic myocardium contain heparin-binding angiogenic growth factor (D'Amore and Thompson, 1987; Thompson et al., 1988. 1989; Eghbali, 1989; Kardami and Frandrich, 1989; Quinkler et al., 1989; Sasaki et al., 1989; Casscells et al., 1990; Schmidt et al., 1991) and the release of mitogens in human (Kumar et al., 1983) and experimental animal (Galloway et al., 1984) hearts has been demonstrated. Likewise, heparin treatment accelerates coronary collateral development in experimental coronary artery occlusion (Carroll et al., 1993), increasing collateralization by potentiating the action of an ischemic-derived angiogenic factor (Fujita et al., 1988, 1991).

Intimal vascularization of arteries:

The microvessel invasion of the artery wall from the adventitia has been demonstrated in human pathology (Koester, 1876; Paterson, 1936, 1938; Barger et al., 1984; Eisenstein, 1991; Zhang et al., 1993) and in experimental conditions (Fig. 20) (Diaz-Flores et al., 1990a). The neovascularization of the arterial wall may play several important roles in the pathogenesis of intimal thickening and atherosclerosis, such as: a) supplying plasma components (albumin, fibrinogen ...) and thus nourishing the thickening wall during plaque growth (Koester, 1876; Le Compte, 1967; Groszek and Grundy, 1980; Zhang et al., 1993); b) causing intimal hemorrhages by rupturing (Paterson, 1938; Barger and Beeuwkes, 1990); and c) augmenting the process of arterial intimal thickening by supplying more cells. Indeed, in occluded arterial segments, it has been suggested that pericytes and EC of the ingrowing vessels from the arterial microcirculation are sources of myointimal cells at the intimal thickening and of endothelium at the luminal surface, respectively (Diaz-Flores and Dominguez, 1985; Diaz-Flores et al., 1990a, 1991b).

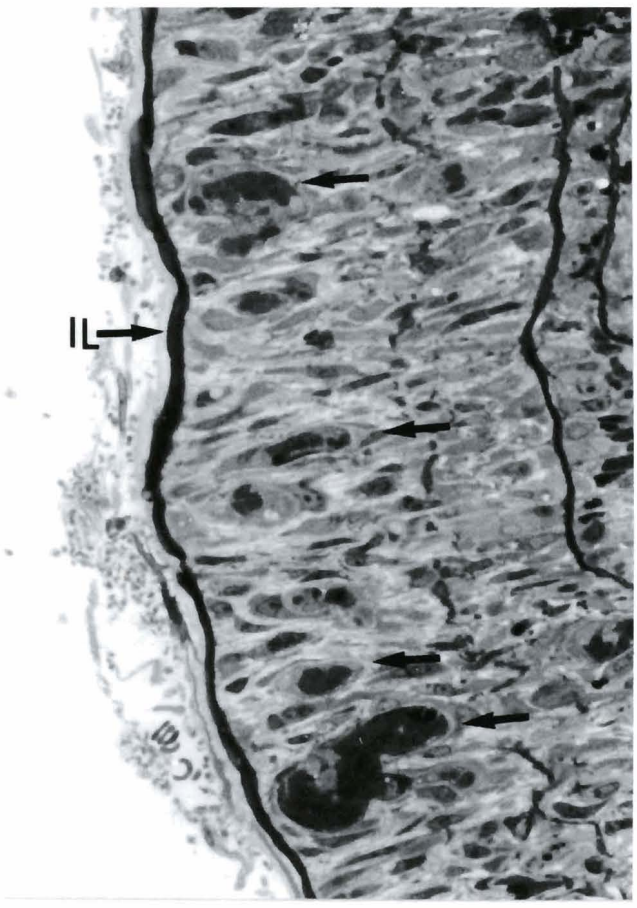

Fig. 20. Microvessel invasion of the artery wall. Several capillaries (arrows) are observed in the media of rat femoral artery after arterial wall injury. IL: Internal elastic lamina. Semithin section; toluidine blue. x 900

Tumour angiogenesis:

Tumour growth is accompanied by neovascularization (Ide et al., 1939; Warren and Shubik, 1966; Eddy and Cassarett, 1973; Yamaura and Sato, 1973; Tannock, 1968, 1970) (Fig. 21), since all solid tumours require stroma if they are to grow beyond 1 to 2 mm in size (about 10^6 cells) (Algire et al., 1945; Folkman et al., 1963; Folkman and Cotran, 1976; Reinhold and Van den Berg-Block, 1984; Folkman, 1985a,b,c, 1990). The tumour stroma is composed of new blood vessels, inflammatory cells and connective tissue (Dvorak, 1986). Thus, tumour growth beyond a few milimeters is dependent on angiogenesis (Folkman and Cotran, 1976; Folkman, 1971, 1972; Folkman and Haudenschild, 1980; Folkman and Klagsbrun, 1987), which is of great interest to cancer biology, metastasis, diagnosis and therapy. This newly formed microvasculature is induced by angiogenic substances (Algire et al., 1945; Folkman et al., 1971; Tuan et al., 1973; Klagsbrun and D'Amore, 1991) released by the tumour and by the inflammatory cells, predominantly macrophages (Polverini and Leibovich, 1984; Folkman and Klagsbrun, 1987). Furthermore, the decrease of angiogenesis inhibitors, such as thrombospondin, may occur when the cells undergo malignant transformation and become angiogenic (Rastinejad et al., 1989, Zajchowski et al., 1990).

The ingrowth of new blood vessels and other components of the stroma into the tumour cells closely resembles the granulation tissue of healing wounds (Dvorak et al., 1979a,b; Folkman, 1985a,b,c). Nevertheless, the organization of the microvasculature in the tumours is not so strict as in normal tissues. Tumours show phenotypic changes like dilated and irregular vessels (Warren, 1979a; Rofstad, 1984; Grunt et al., 1986), a high proliferation rate of the EC (Tannock, 1970; Cavallo et al., 1973; Denekamp, 1982) and increased permeability (Jain, 1985; Heuser and Miller,

1986).

Acquisition of an angiogenic phenotype is an important fact in the transition from hyperplasia to neoplasia (Folkman et al., 1989a,b). Indeed, two phases in the development of tumours can be considered: prevascular; and vascular (Folkman and Hochberg, 1973; Gimbrone et al., 1974; Chodak et al., 1980; Sillman et al., 1981; Jensen et al., 1982; Folkman et al., 1989a,b). The study of the latter may be of interest as a marker for preneoplastic and neoplastic processes of different organs, such as cutaneous melanoma and carcinoma of the breast and bladder (Brem et al., 1977, 1978; Chodak et al., 1980; Sillman et al., 1981; Jensen et al., 1982; Srivastava et al., 1986, 1988). Transgenic mice expressing an oncogene in the pancreatic islet beta cells develop a progression from normal to hyperplasia and to neoplasia. Using these mice, it has been demonstrated that angiogenic activity and consequent neovascularization both precede tumour formation (Folkman et al., 1989a,b).

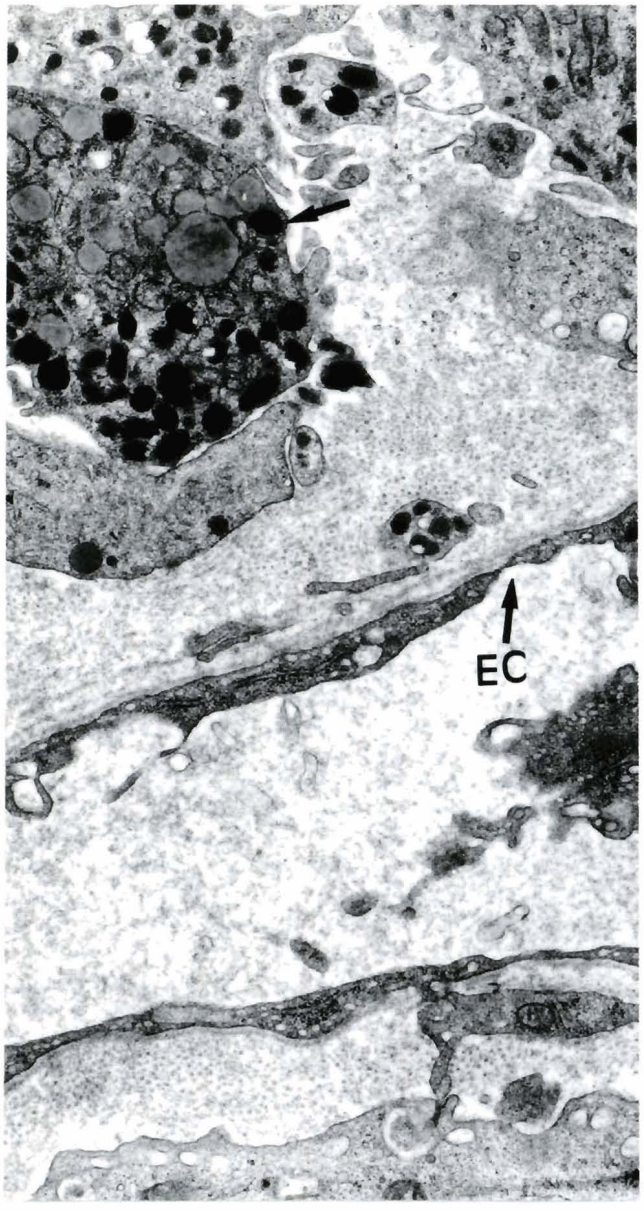

Fig. 21. The field shows a newly formed capillary in a melanoma composed of atypical cells containing melanosomas. (arrows). EC: fenestrated endothelial cell. Ultrathin section; uranyl acetate and lead citrate. x 14,500

The intensity of vascularization in a tumour can predict the probability of metastasis (Liotta et al., 1974; Srivastava et al., 1986, 1988; Herlyn et al., 1987; Weidner et al., 1991), since there is a correlation between the density of tumour microvessels and the occurrence of metastases (Weidner et al., 1991). The fragmented basal lamina of growing vessels as well as collagenases and plasminogen activator secreted by the EC, make them more penetrable than mature vessels. Therefore, they give the tumour cells a greater chance to enter into the circulation (Liotta et al., 1974; Weidner et al., 1991).

Previously, we pointed out that neovascularization in metastasic tumours can acquire the morphological characteristics of the primary organ vessels in which the neoplasm originates. Likewise, the type of metastasic angiogenesis might be determined by the vascular peculiarities of the receptor organ. For example, liver metastases may be of sinusoidal or portal type. The first is formed by large convoluted vessels, devoid of immunohistochemically detectable basal lamina, while the portal type is characterized by numerous small vessels with basal lamina (Paku and Lapis, 1993).

Systems to analyze angiogenesis

In vivo

From the classical studies on new blood vessel morphogenesis and permeability (Sandison, 1928; Clark and Clark, 1935, 1939; Abell, 1946; Cogan, 1949; Friedman and Byers, 1962; Schoefl, 1963; Sugiura and Matsuda, 1969; Szalay and Pappas, 1970; Yamagami, 1970; Haar and Ackerman, 1971; Warren et al., 1972; McKinney and Panner, 1972; Cavallo et al., 1973), a large number of "in vivo" and "in vitro" systems have been used to understand the phenomenon of angiogenesis (Auerbach et al., 1991; Passaniti et al., 1992). The development of inert, biocompatible, slow- release polymer pellets, which permit a sustained release of angiogenic or antiangiogenic factors (Folkman et al., 1971, Langer and Folkman, 1976; Rhine et al., 1980; Hsieh et al., 1981; Murray et al., 1983), has been of interest for some of the experimental techniques. The "in vivo" experimental models include procedures where the angiogenic response in specific areas can be assessed, such as:

a) rabbit (Gimbrone et al., 1974; Brem and Folkman, 1975), rat (Fournier et al., 1981), mouse (Muthukkaruppan and Auerbach, 1979), or guinea pig

corneas, where neovascularization is produced by lesions, tumours (Gimbrone et al., 1974), or slow release polymers containing angiogenic substances implanted in "pockets" created in their central regions. Through this procedure, a linear quantitation of growing capillaries from the limbus is possible (Proia et al., 1988; Haynes et al., 1989; Culton et al., 1990). The cornea is an appropriate place to demonstrate neovascularization, since it is normally completely avascular and the new capillaries can be distinguished from parent vessels of the limbus (Ausprunk and Folkman, 1977; Henkind, 1978). Therefore, this procedure provides an "in vivo" avascular and transparent substratum in which neovascularization can be continuously monitored (Polverini et al., 1977a; Greenburg and Hunt, 1978). Several hypotheses have been proposed to explain the neovascularization in the cornea (Klintworth, 1991), such as liberation of angiogenic factors (Maurice et al., 1966; Eliason and Elliot, 1987; Vlodavsky et al., 1987; Baudouin et al., 1990; Soubrane et al., 1990), destruction of anti-angiogenic substances (Kuettner et al., 1974), inflammation (Fromer and Klintworth, 1975a,b; Polverini et al., 1977b; Klintworth, 1977; Epstein and Stulting, 1987), hypoxia and corneal swelling (Cogan, 1949). Corneal neovascularization could be the result of a local imbalance between angiogenic and anti-angiogenic factors (Kaminska and Niederkorn, 1993) originating from inflammatory cells that invade the cornea or from the cornea itself, together with loosening of the stroma during the inflammatory process.

b) The chorioallantoic membrane (Alfthan 1956; Sorgente et al., 1975; Auerbach, 1981; Folkman 1982; Shing et al., 1985; Fett et al., 1985; Olivo et al., 1992), or the yolk sac (Taylor and Folkman, 1982; Shing et al., 1985; Rosenbruch, 1989; Takigawa et al., 1990a,b) of the early chick embryo, which lacks a mature immune system allowing the growth of xenogeneic graft. Furthermore, by means of this second "in vivo" procedure a more rapid assay of either angiogenesis inhibitors or angiogenic factors is possible before selecting a few to be tested in the cornea (Folkman, 1985a,b,c). The main problem is the possibility of false positives produced by almost any irritant or wound, which may be avoided by incubating the embryos in Petri dishes (Auerbach et al., 1974).

c) Other "immunologically privileged" sites, such as the hamster cheek pouch (Warren and Shubik, 1966; Schreiber et al., 1986) and the anterior eye chamber (Greene, 1943), which allow the growth of allogeneic or xenogeneic grafts with a minor cell-mediated immune response (Greene, 1943; Folkman et al., 1989a,b; Weidner et al., 1991).

d) The subcutaneous air "pouch", dorsal air sac or "blister" method (Selye, 1953), the "sandwich" observation chamber, and the rabbit ear chamber.

e) The mesentery (Norrby et al., 1986; Williams et al., 1989; Norrby et al., 1990a,b).

f) The subcutaneous implants of polyurethane foam cylinders (Bishop et al., 1989, 1990) and the disc angiogenesis assay. In the latter, discs of polyvinyl alcohol sponges, containing a slow-release polymer core and their flat sides sealed with millipore filters, are used (Fajardo et al., 1988). By means of this procedure the penetration of cells and the angiogenic response may be studied.

g) The implants of matrices, such as fibrin or gelatin, which act as angiogenesis initiators and enable the introduction of test substances (Dvorak et al., 1987).

h) Injection of cells or drugs entrapped in alginate (Plunkett and Hailey, 1990; Robertson et al., 1991).

In vitro systems

Angiogenesis or parts of this process have been investigated morphologically by different in vitro systems (Madri and Stenn, 1982; Furcht, 1986; Ingber et al., 1986; Dvorak et al., 1988; Heimark and Schwartz, 1988; Ingber and Folkman, 1988).

After the introduction of methods for EC culture (Jaffe et al., 1972; Gimbrone et al., 1973), EC isolated from both small and large vessels have been shown to be capable of forming random networks of capillary-like tubes "in vitro", when grown under appropriate culture conditions (Folkman and Haudenschild, 1980; Maciag et al., 1982; Kubota et al., 1982; Montesano and Orci, 1985; Pepper et al., 1990; Nguyen et al., 1992).

The "in vitro" systems with formation of capillary-like structures have been undertaken in cultured EC from various kinds of blood vessels (Jaffe et al., 1973; Folkman et al., 1979), such as human umbilical veins (Maciag et al., 1982), bovine aortas (Feder et al., 1983), bovine capillaries (Folkman and Haudenschild, 1980; Montesano et al., 1983) and rat capillaries (Madri et al., 1983; Sato et al., 1987). These procedures, along with the establishment of microvascular pericytes in culture enable the assessment of the following parameters (D'Amore and Thompson, 1987; Auerbach et al., 1991): 1) The recovery rate of a denuded surface in a confluent EC monolayer. In these conditions, the EC migration provides a quantitative assessment of the angiogenic response (Pepper et al., 1987, 1989, 1990). 2) EC locomotion and directionality (chemokinesis and chemotaxis, respectively) using different procedures (Auerbach et al., 1974, 1991; Obeso and Auerbach, 1984; Stokes et al., 1990; Taraboletti et al., 1990) such as phagokinetic track assay, in which individual cells move on a gold monolayer and phagocyte the colloidal particles, leaving migration tracks (Zetter, 1980, 1988; Rupnick et al., 1988; Weber et al., 1989). 3) The degree of EC proliferation in culture with test factors is determined by DNA synthesis, nuclear staining, DNA content, etc (Watt and Auerbach, 1986; Folkman and Ingber, 1987; Folkman and Klagsbrun, 1987; Ryan, 1988; Simionescu and Simionescu 1988, 1991). 4) Changes in EC function during angiogenesis, such as modulations in the production of cytokines (Shepro, 1988), protease release and fibrinolytic activity (Sueishi et al., 1989), and basal lamina synthesis. 5) Tube formation in relation to the

substrate (Maciag et al., 1982; Maciag, 1984; Madri and Pratt, 1986; Nicosia and Ottinetti, 1990). 6) The role of the pericytes in angiogenesis using coculture of both pericytes and EC (Orlidge and D'Amore, 1987, 1988). These possibilities for the study of EC function have been increased by utilizing 3-dimensional angiogenesis assays (Madri et al., 1988; Goto et al., 1993; Williams, 1993).

Angiogenesis control

Neovessel formation depends on several angiogenic stimuli to initiate and direct the proliferation and migration of ECs in the connective tissue (Folkman, 1982, 1985a,b,c; Furcht, 1986).

The most important factors in the control of angiogenesis seem to be the following: a) the angiogenic factors, capable of stimulating the EC migration and/or proliferation; b) the angiogenic inhibitors; c) the extracellular matrix modifications (role of the changes in extracellular matrix); and d) the intercellular interactions, for example, the "free edge effect" by absence of neighbouring EC (Schwartz et al., 1982). We shall consider the first three points.

Angiogenic factors

Angiogenesis is thought to be initiated by diffusible angiogenic factors, either after local activation of genes encoding them, or by release from their storages. In the past decade, several molecules have been shown to induce angiogenesis by acting in a direct or indirect way, including a variety of growth factors (Folkman and Klagsbrun, 1987; Klagsbrun and D'Amore, 1991; Folkman and Shing, 1992). An angiogenic factor is called "direct" when it is capable of inducing endothelial proliferation and/or migration "in vivo" and of stimulating endothelial cells "in vitro". When the "in vitro" action fails, or is inhibited, the angiogenic factor is considered to be "indirect" assuming that it mobilizes other direct factor(s) or cell(s) "in vivo". Likewise, the angiogenic factors may act mainly, or specifically on the EC, or on the contrary, may be pleiotropic, other cells also intervening, such as fibroblasts, smooth muscle cells, etc. Taking the above into account, the angiogenic factors may be chemotactic, mitogenic, chemotactic and mitogenic at the same time, or neither mitogenic nor chemotactic, but rather induce angiogenesis indirectly (Klagsbrun and Folkman, 1991). Among the direct angiogenic factors are the basic fibroblast growth factor (bFGF) (Folkman and Klagsbrun, 1987; Gospodarowicz et al., 1987; Klagsbrun and Vlodavsky, 1988), acidic fibroblast growth factor (aFGF) (Folkman and Haudenschild, 1980; Folkman and Klagsbrun, 1987; Gospodarowicz et al., 1987), vascular endothelial growth factor (VEGF) (Connolly et al., 1989; Keck et al., 1989; Leung et al., 1989) and platelet- derived endothelial growth factor (PD-EGF) (Thomas et al., 1985; Gospodarowicz et al., 1987; Miyazono et al., 1987; Ishikawa et al., 1989; Ferrara et al., 1989; Leung et al., 1989). The indirect angiogenic growth factors cover a great number of substances, such as, Transforming growth factor alpha (TGF-alpha), Epidermal growth factor (EGF) (Schreiber et al., 1986), Transforming growth factor beta (TGF-beta) (Roberts et al., 1986), Tumour Necrosis factor alpha (TNF-alpha) (Frater-Schröder et al., 1987), Platelet derived growth factor (PDGF) (Sato et al., 1993), E series prostaglandin (Ziche et al., 1982), angiogenin (Fett et al., 1985; Hallahan et al., 1991), monobutyrin (Dobson et al., 1990), nicotinamide (Kull et al., 1987), adenosine, Okadoic acid (Oikawa et al., 1992), hydroxyeicosatrienoic acid (Masferrer et al., 1991), some copper complexes (Ziche et al., 1982; Raju et al., 1984; Folkman and Klagsbrun, 1987; Brem et al., 1990), hyaluronic acid degradation products (West et al., 1985) and age-associated glycosylation end-products (Cozzolino et al., 1990).

Some angiogenic molecules are present in adult tissues where angiogenesis is absent (Gullino, 1981); the possibility that the angiogenic response depends on local activation, or inactivation of these molecules has been considered (Ziche et al., 1992). For example, corneal tissue under angiogenic stimulation becomes richer in sialic acid (Ziche et al., 1989) and copper ions (Ziche et al., 1982; Raju et al., 1982). Likewise, changes in the ratio of different substances in local tissue composition may modify the angiogenic response. Thus, corneal neovascularization induced by angiogenic factors is stimulated, or repressed in the cornea by reduction or enhancement of the GM3/GD3 ratio of tissue gangliosides (Ziche et al., 1992).

Some of the known angiogenic factors have been completely purified and characterized, while others are still being studied. We shall consider some of the most important angiogenic factors.

Fibroblastic growth factors:

bFGF and aFGF, with a strong affinity for anionic glycosamin-glycan heparin (Baird and Ling, 1987, see role of heparin on angiogenesis) are able to stimulate both angiogenesis in vivo and vascular endothelial cell growth in vitro (Thomas et al., 1985, Montesano et al., 1986; Gospodarowicz et al., 1987). Beta fibroblast growth factor, a multifunctional peptide of 146 aminoacids (Esch et al., 1985) is one of the more potent angiogenic factors and has the ability to stimulate the following features (Gospodarowicz et al., 1987): a) migration of EC and SMC which change their morphology becoming bipolar (Terranova et al., 1985; Gospodarowicz et al., 1985; Montesano et al., 1986; Moscatelli et al., 1986; Tsuboi et al., 1990); b) stimulation of cell proliferation with mitogenesis of vascular endothelial cells, SMC and a wide variety of cell types (Schweigerer et al., 1987; Winkles et al., 1987; Sato and Rifkin, 1988); c) EC production of a urokinase-type plasminogen activator, procollagenese (Gross et al., 1983; Moscatelli et al., 1986;

Presta et al., 1986; Montesano et al., 1986; Gospodarowicz et al., 1987; Banda et al 1987; Rifkin and Moscatelli, 1989), a membrane bound enzyme that degrades type IV collagen and prostomelysin; d) synthesis and deposition of extracellular matrix proteins, with effects on EC collagen, fibronectin and proteoglycan production (Tseung et al., 1982; Gospodarowicz, 1983; Gospodarowicz et al., 1987); for example, sprouting, or migrating EC switch their synthetic pattern to types I and III collagen (Madri et al., 1983); on the contrary, quiescent capillary EC synthesize type IV collagen; and e) differentiation of EC, influencing their phenotypic expression (Vlodavsky and Gospodarowicz, 1979; Vlodavsky et al., 1979; Greenberg et al., 1980).

bFGF is expressed during vascularization in the embryo (Risau et al., 1988) and in the adult, as that occuring in the ischemic heart (McNeil, 1980; Tomanek et al., 1989; Sasaki, 1989). Furthermore, in vivo administration of bFGF, by means of slow release polymers, induces intense angiogenesis (Baird and Bohlen, 1989). Growth of vasa-vasorum into the intima and media is observed in response to bFGF when infused onto the normal adventitia, or into the injured media of the rat carotid artery (Cuevas et al., 1991).

bFGF is mainly confined within the cells producing it (Folkman and Klagsbrun, 1987). Likewise, a high concentration of its inactive complex is stored within the extracellular matrix from which it can be released as a biologically active form by the actions of degradative enzymes (Folkman et al., 1988). When the EC are activated, they dissolve the extracellular matrix and bFGF is released. The latter bind with receptors on EC (Friesel et al., 1986) and vascular smooth muscle cells (Winkles et al., 1987) in which the binding of FGF to heparan sulphate is a prerequisite (Kiefer et al., 1990). Although bFGF seems to exert its effects on EC via a paracrine mode, it has been shown that endogenous bFGF produced by EC is important for EC migration and plasminogen activator production (Sato and Rifkin, 1988).

It has been pointed out that bFGF induces the production of prostaglandin E2 by microvascular EC and that PGE2 augments the production of cyclic AMP in these cells, stimulating their proliferation (Allison and Kowalski, 1989). Likewise, the angiogenic action of bFGF can be abolished by the systemic administration of drugs inhibiting prostaglandin synthesis (Fajardo et al., 1992).

Vascular endothelial growth factors (VEGF):

The VEGF (Ferrara and Henzel, 1989; Levy et al., 1989; Leung et al., 1989; Conn et al., 1990a,b; Ferrara et al., 1992), a secreted heparin-binding dimeric glycoprotein (Ferrara et al., 1991, 1992; Ferrara and Henzel, 1989), constitutes a family of angiogenic factors (Ferrara and Henzel, 1989; Leung et al., 1989; Conn et al., 1990a,b; Ferrara et al., 1992) also known as vascular permeability factor (Senger et al., 1983, 1986; Leung et al., 1989; Connolly et al., 1989; Keck et al., 1989; Ferrara and Henzel, 1989; Conn et al., 1990a,b; Tisher et al., 1991; Ferrara et al., 1992), or vasculotropin (Ploüet et al., 1989), in which their respective cDNAs have been cloned. At present, four different molecular species of VEGF are generated by alternative splicing mRNA (VEGF121, VEGF165, VEGF189, VEGF206) (Leung et al., 1989; Houck et al., 1991; Tisher et al., 1991; Ferrara et al., 1992), with 121, 165, 189 and 206 aminoacids. The VEGF mRNA is expressed in vascularized tissues (Ferrara et al., 1992; Berse et al., 1992) and during capillary proliferation (Phillips et al., 1990; Ravindranath et al., 1992). Likewise, VEGFs promote angiogenesis in vivo (Leung et al., 1989; Ploüet et al., 1989; Connoly, 1989) and they are EC-specific mitogens in vitro (Ferrara and Henzel, 1989; Ishikawa et al., 1989; Gospodarowicz et al., 1989; Conn et al., 1990a,b). VEGF binding sites have been identified in a majority of tissues and organs. The binding sites of VEGF in cultured EC have been described (Vaisman et al., 1990), where tyrosine kinase protein is its receptor. The above mentioned findings are consistent with the hypothesis that one of the physiological roles of VEGF is to promote neovascularization (Ferrara et al., 1992). In a study on spatial and temporal patterns of VEGF and VEGF receptor expression during natural angiogenic processes taking place within the female reproductive system, VEGF mRNA was found to be expressed in cells surrounding the expanding vasculature, while VEGF receptors were constitutively expressed in the endothelium, regardless of its proliferative status (Shweiki et al., 1993). Thus, VEGF may target the angiogenic response to specific areas. Furthermore, in this study, all cell types expressing VEGF were steroidogenic and/or steroid-responsive cells, which suggests that the VEGF expression is hormonally regulated (Shweiki et al., 1993).

It has been proposed that a tonic presence of VEGF may be required to maintain the differentiated state of microvessels and that suppressed expression of VEGF and/or its receptors may contribute to vessel regression (Ferrara et al., 1992).

Platelet-derived endothelial cell growth factor (PD-ECGF):

PD-ECGF, purified to homogeneity from human platelets (Miyazono et al., 1987), is a sequenced protein with a relative molecular mass of about 45,000 (Ishikawa et al., 1989), which stimulates EC growth and chemotaxis in vitro and angiogenesis in vivo (Ishikawa et al., 1989). Furthermore, PD-ECGF amplifies DNA synthesis activity of FGFs on EC (Folkman and Shing, 1992).

Tumour necrosis factor alpha (TNF):

The tumour necrosis factor alpha (TNF-alpha), a

cytokine mainly produced by macrophages and capable of inducing bFGF production in EC and of enhancing its secretion (Okamura et al., 1991), has a controversial role in angiogenesis (Frater-Schroder et al., 1987; Leibowich et al., 1987; Sato et al., 1987; Schweigerer et al., 1987; Ben-Ezra et al., 1990; Fajardo et al., 1992). TNF-alpha induces angiogenesis in vivo (Frater-Schröder et al., 1987, Leibowich et al., 1987), while it is a potent inhibitor of EC growth "in vitro" (Frater-Schroeder et al., 1987; Schweigerer et al., 1987) as well as of microvascular sprouts (Sato et al., 1987). Currently, it is known that this controversy is due to dose-dependent opposing effects of TNF, which determine a bimodal response (Fajardo et al., 1992). In vivo, low doses of m-TNF (0.01 - 1ng) induce angiogenesis (maximum at 0.1 ng), whereas high doses (1 and 5 μg) inhibit it (Fajardo et al., 1992). Owing to their pro-inflammatory actions, it has been suggested that production of prostaglandins by macrophages may mediate the angiogenic effect of TNF (Ben-Ezra et al., 1990). In vitro, TNF is chemotactic for microvascular EC, although not for large vessel EC. Likewise, TNF-alpha action depends upon the administration route. When TNF-alpha is injected intravascularly it may cause necrosis and when injected extravascularly, it is angiogenic (Folkman and Shing, 1992).

Platelet-derived growth factor:

EC themselves may synthetize PDGF (DiCorleto and Bowen-Pope, 1983; Starksen et al., 1987). Functional PDGF receptors have been demonstrated on microvascular EC of some tissues (Beitz et al., 1991; Heldin et al., 1991) which suggests that PDGF is an autocrine or paracrine modulator during angiogenesis. Recently, it has been pointed out that PDGF may accelerate capillary formation by activating connective tissue cells, such as myofibroblasts, in the vicinity of EC (Sato et al., 1993).

Prostaglandins:

Among the prostanoid lipids with angiogenic activity are the prostaglandins of the E series (PGE1 and PGE2) (Ben-Ezra 1978a,b; Ziche et al., 1982; Form and Auerbach, 1983; Dobson et al., 1985; Ziche et al., 1985, 1989). They have been used as strong angiogenesis triggers in the rabbit cornea and in the chick embryo chorioallantoic membrane, and could play an important part in the cascade of events underlying the neovascularization process (Ziche et al., 1989). Furthermore, extravascular PGE1 and PGE2, associated with triacetine, are capable of inducing capillary sprouting from veins (Diaz-Flores et al., 1994). Although it is not clear how PGE1 and PGE2 induce capillary growth, they have been considered as acting in vivo by some indirect pathway (Folkman and Klagsbrun, 1987). Indeed, prostaglandin levels are elevated in wounds, inflammatory exudates, tumours and activated macrophages (Form and Auerbach, 1983). In these conditions, the secretion of growth factors could be the result of PGE1 and PGE2 mobilizing and activating macrophages, or by some other unknown mechanisms (Folkman and Klagsbrun, 1987). Certain members of the prostanoid family, which are different from currently known prostaglandins, seem to be the factors of major angiogenic activity secreted by 3T3 adipocytes (Castellot et al., 1982; Dobson et al., 1985).

Angiogenin:

Angiogenin, isolated from human colon adenocarcinoma cell-conditioned medium (Fett et al., 1985), is a 14kD protein present in plasma at a relatively high concentration. In vivo, it induces intense angiogenesis (Fett et al., 1985) in the chicken chorioallantoic membrane (Fett et al., 1985) and in the rabbit cornea. Moreover, angiogenin has the following actions: a) it is a potent inhibitor of cell-free protein synthesis by specific ribonucleolytic inactivation of ribosomes (St. Clair et al., 1987); b) it activates EC phospholipase and phosphatase A2 (Bicknell and Vallee, 1988); and c) it stimulates EC prostacyclin secretion by activation of phopholipamase A2 (Bicknell and Vallee, 1989).

Role of heparin on angiogenesis:

Heparin facilitates neovascularization (Ribatti et al., 1987; Ehrlich et al., 1988) by the following mechanisms: a) release of basic FGF from the extracellular matrix (Bashkin et al., 1989); b) protection of both a- and bFGF from inactivation (Gospodarowicz and Cheng, 1986; Herbert et al., 1988); c) increased aFGF activity (Herbert et al., 1988); and d) facilitation of the interaction between bFGF and EC (Bashkin et al., 1989). High-affinity receptors on the cell surface require heparin-like molecules to bind with FGF (Yayon et al., 1991) and VEGF (Gitay-Goren et al., 1992). This is probably accomplished by inducing a conformational change in the growth factors to allow them to interact with their receptors (Yayon et al., 1991; Gitay-Goren et al., 1992).

Heparin stimulates the secretion and activation of plasminogen activator (Lijnen and Collen, 1986; Falcone, 1989). Furthermore, its binding to plasminogen activator inhibitor-1 (Ehrlich et al., 1991), potentiates its neutralization with thrombin.

Angiogenesis antagonists:

Although the term "angiogenesis inhibitor" was not introduced until 1975 (Brem and Folkman, 1975), numerous factors were identified as inhibiting vessel formation. The study of these factors is of great interest since it may facilitate the development of antagonists for treatment of angiogenic diseases. For example, the process of angiogenesis during tumour growth is a potential target for tumour therapy (Folkman and Cotran, 1976; Denekamp, 1982; Schor and Schor, 1983). The angiogenic inhibitors include the cartilage-derived

inhibitor identified as tissue inhibitor of metalloproteinases (Carmichael et al., 1986, Moses et al., 1990), thrombospondin (Rastinejad et al., 1989; Good et al., 1990, Iruela-Arispe et al., 1991), protamine (Taylor and Folkman, 1982), platelet factor-4 (Hiti-Harper et al., 1978; Taylor and Folkman, 1982; Maione et al., 1990), interferon (Tsuruoka et al., 1988; White et al., 1989), angiostatic antibiotics (Ingber et al., 1990) deoxymannojirimycin (Nguyen et al., 1992), steroids (Crum et al., 1985) and angiogenesis inhibitors effective as a combination (Folkman et al., 1989a,b), synthetic peptides containing the amino acid sequence Arg-Gly-Asp, minocycline (Tamargo et al., 1991), difluoromethyl ornithine (Takigawa et al., 1990a,b), sulphatin chitin derivates (Murata et al., 1991), DS-4152 sulphated polysaccharide from Arthrobacter, and bovine vitreous extract (Lutty et al., 1983).

Several studies have been undertaken to demonstrate that cartilage extracts can inhibit angiogenesis (Eisenstein et al., 1973, 1975; Brem and Folkman, 1975; Langer et al., 1976, 1980; Lee and Langer, 1983). The purification and characterization of neovascularization inhibitor from cartilage and from the conditioned media of chondrocytes have been recently reported (Moses et al., 1990). This cartilage derived protein negatively modulates the proliferation and migration of capillary EC. It is a powerful inhibitor of neovascularization "in vitro" and of embryonic and tumour-induced angiogenesis "in vivo". Furthermore, it is a collagenease and metalloproteinase inhibitor.

Thrombospondin, a high molecular weight multifunctional glycoprotein (Silverstein et al., 1986), stimulates different EC functions and modulates the activity of angiogenic factors (Taraboletti et al., 1990). Recently, it has been demonstrated that angiogenic macrophages produce not only positive but also negative angiogenesis regulators such as thrombospondin 1 (DiPietro and Polverini, 1993).

Protamine, an arginine-rich basic protein of 4,300 molecular weight, found in sperm with an affinity for heparin, is an angiogenesis inhibitor (Taylor and Folkman, 1982), although it is not used for the control of neovascularization because of its high toxicity (Folkman, 1985a,b,c).

Platelet factor-4, a platelet alpha-granule protein with high affinity for heparin, has an angiostatic effect which is probably due to specific inhibition of growth factor-stimulated EC proliferation. This angiostatic activity may be modulated through sulphated polysaccharides (Maione et al., 1990).

Interferons, regulatory proteins with potent biological activities, may be effective in inhibiting the proliferation of EC (Friesel et al., 1987; Sidky and Borden, 1987; Feldman et al., 1988). Interferons have been used as treatment for pathogenic neovascularization diseases such as Kaposi's sarcoma (Groopman et al., 1984; Rios et al., 1985; Real et al., 1986) and pulmonary hemangiomatosis (White et al., 1989).

A new class of angiostatic antibiotics, termed angioinhibins, have been identified among the fumagillin analogues to suppress the growth of a wide variety of tumours (Ingber et al., 1990). Among the angioinhibins is the O-(Chlroacetylcarbamoyl) fumagillol or AGM-1470 which is 50 times more active than the fumagillin parent and has relatively few side-effects (Ingber et al., 1990).

Angiogenesis inhibitors, effective as a combination, include heparin with hydrocortisone (Folkman et al., 1989a,b), as well as beta-cyclodextrintetradecasulphate with hydrocortisone (Folkman et al., 1989a,b).

Deoxymannojirimycin, which prevents synthesis of hybrid and complex-type oligosaccharides, inhibits capillary tube formation in vitro (Nguyen et al., 1992).

Role of the changes in extracellular matrix

The different structural organization and composition of the extracellular matrix during vascular sprouting (see changes in extracellular matrix) is principally due to the fact that the EC secrete enzymes, which digest the pre-existing basement membrane (Kalebic et al., 1983; Montesano and Vasalli, 1985), and synthesize glycoproteins, proteoglycans and collagen (Ausprunk, 1982), which make up the extracellular matrix of new microvessels. Thus, the EC play an important role in the remodelling of the extracellular matrix which surrounds them during capillary development.

At the same time, changes in the composition of the extracellular matrix surrounding the EC may have important regulatory effects on the various stages of microvascular morphogenesis, modifying the organization, morphological features, function and behaviour of EC, such as cellular phenotype, migration and proliferation (Folkman and Haudenschild, 1980; Delvos et al., 1982; Madri et al., 1983; Tseng et al., 1983; Montessano et al., 1983; Schor et al., 1983; Madri and Pratt, 1986; Nicosia and Madri, 1987). Thus, extracellular matrix proteins isolated from basal lamina facilitate differentiation of EC into tube-like structures, whereas interstitial collagens stimulate EC migration and proliferation (Madri and Williams, 1983; Montesano et al., 1983). These phenomena are associated with modifications in intracellular cytoskeletal components (Kocher and Madri, 1989). For example, EC plated onto basal lamina form tubelike structures after a short period in culture, but they neither proliferate nor migrate. EC plated onto a gel containing the extracellular matrix proteins, form capillary tubes within 24 h, but within 2-3 weeks when EC are plated onto a two-dimensional matrix, or onto plastic (Kubota et al., 1988). Likewise, EC embedded in a three- dimensional collagen matrix organize into a network of capillary-like tubes and acquire correct cellular polarization (Montesano et al., 1983). Therefore, the extracellular matrix influences the proliferation and migration of the vascular EC, and also their capacity to differentiate into capillary-like structures and to determine the correct cellular polarization (Montesano et al., 1983; Form et al., 1986;

Madri and Pratt, 1986; Ingber et al., 1987; Ingber and Folkman, 1989). Finally, the rates of EC recovery after injury depend on the matrix components (Young and Herman, 1985).

During angiogenesis, different basal lamina components distribute around newly formed vessels (Form et al., 1986; Nicosia and Madri, 1987; Murray and Leblond, 1988), and these components can induce lumen formation as tube-like structures (Kubota et al., 1982; Maciag et al., 1982; Ingber and Folkman, 1988).

When the synthesis and the degradation of the components of the basal lamina is disturbed, neovascularization is inhibited (Ingber and Folkman, 1988). This supports the hypothesis that formation of a new basal lamina plays an important role in the development of active vessels.

The integrins, cell surface molecules which mediate adhesion to either neighbouring cells or to the extracellular matrix, are likely to play a key role in angiogenesis. Thus, anti-integrin antibodies directed to the major integrin receptors for the tube-permissive matrices of collagen and fibrin, enhance capillary tube formation "in vitro". This fact suggests that restriction of specific cell-matrix interactions can enhance capillary formation, converting EC from a proliferative phenotype towards differentiation.

Acknowledgements: This work was in part supported by Dirección General de Universidades e Investigación. Gobierno de Canarias. Grant 92/006

References

Abell R.G. (1946). The permeability of blood capillary sprouts and newly formed blood capillaries as compared to that of older blood capillaries. Am. J. Physiol. 147, 237-241.

Alfthan O.S. (1956). A comparative study of the growth of skin and human skin tumors on the chorioallantoic membrane of the embryonated chicken eggs. Ann. Med. Exp. Biol. Fenn. 9 (Suppl. 34), 1-78.

Algire G.H., Chalkley H.W., Legallais F.Y. and Park H.D. (1945). Vascular reactions of normal and malignant tumors in vivo: I. Vascular reactions of mice to wounds and to normal and neoplastic transplants. J. Natl. Cancer Inst. 6, 46-73.

Allison A.C. and Kowalski J. (1989). Prostaglandins as transducers of proliferation signals in microvascular endothelial cells and the pharmacological control of angiogenesis. Vascular endothelium, receptors and transduction mechanisms. Catravas J.D., Gillis C.N., and Ryan U.S. (eds). Plenum Press. New York. pp 99-110.

Altschul R. (1954). Endothelium. Its Development, morphology, function and pathology. MacMillan Co. New York. pp 28-31.

Auerbach R. (1981). Angiogenesis-inducing factors: a review. In: Lymphokines. Vol IV. Pick E. (ed) Academic Press. New York. pp 69-88.

Auerbach R., Kubai L., Knighton D. and Folkman J. (1974). A simple procedure for the long-term cultivation of chicken embryos. Dev. Biol. 41, 391-394.

Auerbach R., Auerbach W. and Polakowski I. (1991). Assays for angiogenesis; a review. Pharmacol. Ther. 51, 1-11.

Ausprunk D.H. (1982). Synthesis of glycoproteins by endothelial cells in embryonic blood vessels. Dev. Biol. 90, 79-90.

Ausprunk D.H. and Folkman J. (1977). Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during tumor angiogenesis. Microvasc. Res. 14, 53-65.

Ausprunk D.H., Falterman K. and Folkman J. (1978). The sequence of events in the regression of corneal capillaries. Lab. Invest. 38, 284-294.

Azmi T.I. and O'Shea J.D. (1984). Mechanism of deletion of endothelial cells during regression of the corpus luteum. Lab. Invest. 51, 206-217.

Baird A. and Ling N. (1987). Fibroblast growth factors are present in the extracellular matrix produced by endothelial cells in vitro: implications for a role of heparinase-like enzymes in the neovascular response. Biochem. Biophys. Res. Commun. 142, 428-435.

Banda M.J., Herron G.S., Murphy G. and Werb Z. (1987). Regulation of metalloproteinase activity by microvascular endothelial cells. In: Angiogenesis. Mechanisms and pathophysiology. Rifkin D.B. and Klagsbrun M. (eds) Cold Spring Harbor Laboratory. New York. pp 101-109.

Bar Th. and Wollf J.R. (1972). The formation of capillary basement membranes during internal vascularization of the rat's cerebral cortex. Z. Zellforsch. 133, 231-248.

Barger A.C. and Beeuwkes R. (1990). Rupture of coronary vasa vasorum as trigger of acute myocardial infarction. Am. J. Cardiol. 66, 41G-43G.

Barger A.C., Beeuwkes R., Lainey L.L. and Silverman K.J. (1984). Hypothesis: vasa vasorum and neovascularization of human coronary arteries: A possible role in the pathophysiology of atherosclerosis. N. Engl. J. Med. 310, 175-177.

Bashkin P., Doctrow S., Klagsbrun M., Svahn C.M., Folkman J. and Vlodavsky I. (1989). Basic fibroblast growth factor binds to subendothelial extracellular matrix and is released by heparitinase and heparin-like molecules. Biochemistry 28, 1737-1743.

Baudouin C., Fredj-Reygrobellet D., Caruelle J-P., Barritault D., Gastaud P. and Lapalus P. (1990). Acidic fibroblast growth factor distribution in normal human eye and possible implications in ocular pathogenesis. Ophthalmic Res. 22, 73-81.

Bavisotto L.M., Schwartz S.M. and Heimark R.L. (1990). Modulation of Ca-dependent intercellular adhesion in bovine aortic and human umbilical vein endothelial cells by heparin-binding growth factors. J. Cell. Physiol. 143, 39-51.

Bender B.L., Jaffe R., Carlin B. and Chung A.E. (1981). Immunolocalization of entactin, a sulfated basement membrane component, in rodent tissues, and comparison with GP-2 (laminin). Am. J. Pathol. 103, 419-426.

Ben-Ezra D. (1978a). Neovasculogenic ability of prostaglandins, growth factors and synthetic chemoattractants. Am. J. Ophtalmol. 86, 455-461.

Ben-Ezra D. (1978b). Neovasculogenesis. Triggering factors and possible mechanisms. Surg. Ophthalmol. 24, 167-176.

Ben-Ezra D., Hemo I. and Maftzir G. (1990). In vivo angiogenesis activity of interleukins. Arch. Ophthalmol. 108, 573-576.

Beitz J.G., Kim I., Calabresi P. and Frackelton A.R. Jr. (1991). Human microvascular endothelial cells express receptors for platelet derived growth factor. Proc. Natl. Acad. Sci. USA 88, 2021-2025.

Berse B., Brown L.F., Van De Vater L., Dvorak H.F. and Senger D.R. (1992). Vascular permeability factor (vascular endothelial growth

factor) gene is differentially expressed in normal tissues, macrophages and tumors. Mol. Biol. Cell 3, 211-220.

Bicknell R. and Vallee B.L. (1988). Angiogenin activates endothelial cell phospholipase C. Proc. Natl. Acad. Sci. USA 85, 5961-5965.

Bicknell R. and Vallee B.L. (1989). Angiogenin stimulates endothelial cell prostacyclin secretion by activation of phosphorilase A2. Proc. Natl. Acad. Sci. USA 86, 1573-1577.

Birdwell C.R., Gospodarowicz D. and Nicholson G.L. (1978). Identification, localization, and role of fibronectin in cultured bovine endothelial cells. Proc. Natl. Acad. Sci. USA 75, 3273-3277.

Bishop D.K., Jutila M.A., Sedmak D.D., Beattie M.S. and Orosz C.G. (1989). Lymphocyte entry into inflammatory tissues in vivo. Qualitative differences of high endothelial venule-like vessels in sponge matrix allografts vs isografts. J. Immunol. 142, 4219-4224.

Bishop D.K., Sedmak D.D., Leppink D.M. and Orosz C.G. (1990). Vascular endothelial differentiation in sponge matrix allografts. Hum. Immunol. 28, 128-133.

Blood C.H. and Zetter B.R. (1990) Tumor interactions with the vasculature: angiogenesis and tumor metastasis. Biochem. Biophys. Acta 1032, 89-118.

Bloor C.M. and White F.C. (1972). Functional development of the coronary collateral circulation during coronary artery occlusion in the conscious dog. Am. J. Physiol. 67, 483-500.

Brambell F.W.R. (1956). Ovarian changes. In: Marshall's Physiology of reproduction. 3nd ed. Vol. I. Parkes A.S. (ed). Lonsmans Green. London. pp 397-542.

Brem H. and Folkman J.H. (1975). Inhibition of tumor angiogenesis mediated by cartilage. J. Exp. Med. 141, 427-438.

Brem S.S., Gullino P.M. and Medina D. (1977). Angiogenesis: a marker for neoplastic transformation of mammary papillary hyperplasia. Science. 195, 880-882.

Brem S.S., Jensen H. and Gullino P.M. (1978). Angiogenesis as a marker of preneoplastic lesions of the human breast. Cancer. 41, 239-244.

Brem S.S., Zagzag D., Tsanaclis A.-M.C., Gately S., Elkouby, M.P. and Brien S.E. (1990). Inhibition of angiogenesis and tumor growth in the brain. Suppression of endothelial cell turnover by Penicillamine and the depletion of copper, an angiogenic cofactor. Am. J. Pathol. 137, 1121-1142.

Brenk H.A.S.V. (1955). Studies in restorative growth processes in mammalian wound healing. Br. J. Surg. 43, 525-550.

Bowersox J.C. and Sorgente N. (1982). Chemotaxis of aortic endothelial cells in response to fibronectin. Cancer Res. 42, 2547-2551.

Burger P.C. and Klintworth G.K. (1981). Autoradiographic study of corneal neovascularization induced by chemical cautery. Lab. Invest. 45, 328-335.

Burger P.C., Chandler D.B. and Klintworth G.K. (1983). Corneal neovascularization as studied by scanning electron microscopy of vascular casts. Lab. Invest. 48, 169-180.

Burri P.H. and Tarek M.R. (1990). A novel mechanism of capillary growth in the rat pulmonary microcirculation. Anat. Rec. 228, 35-45.

Caduff J.H., Fischer L.C. and Burri P.H. (1986). Scanning electron microscopic study of the developing microvasculature in the postnatal rat lung. Anat. Rec. 216, 154-164.

Carmichael D.F., Sommer A., Thompson R.C., Anderson D.C., Smith C.G., Welgus H.G. and Stricklin G.P. (1986). Primary structure and cDNA cloning of human fibroblast collagenase inhibitor. Proc. Natl. Acad. Sci. USA 83, 2407-2411.

Carroll S.M., White F.C., Roth D.M. and Bloor C.M. (1993). Heparin accelerates coronary collateral development in a porcine model of coronary artery occlusion. Circulation 88, 198-207.

Casscells W., Speir W., Sasse J., Klagsbrun M., Allen P., Lee M., Calvo B., Chiba M., Haggroth L, Folkman J. and Epstein S.E. (1990). Isolation, characterization and localization of heparin-binding growth factors in the heart. J. Clin. Invest. 85, 433-441.

Castellot J.J. Jr., Karnovsky M.J. and Spiegelman B.M. (1982). Differentiation-dependent stimulation of neovascularization and endothelial cell chemotaxis by 3T3 adipocytes. Proc. Natl. Acad. Sci. USA 79, 5597-5601.

Cavallo T., Sade R., Folkman J. and Cotran R.S. (1972). Tumor angiogenesis. Rapid induction of endothelial mitoses demonstrated by autoradiography. J. Cell Biol. 54, 408-420.

Cavallo T., Sade R., Folkman J. and Cotran R.S. (1973). Ultrastructural autoradiographic studies of the early vasoproliferative response in tumor angiogenesis. Am. J. Pathol. 70, 345-362.

Christianens G.C.M.L., Sixma J.J. and Haspels A.A. (1982) Hemostasis in menstrual endometrium: a review. Obstet. Gynecol Surv. 37, 281-303.

Chodak G.W., Haudenschild C., Gittes R.F. and Folkman J. (1980). Angiogenic activity as a marker of neoplastic and of preneoplastic lesions of the human bladder. Ann. Surg. 192, 762-771.

Clark R.A.F. (1985). Cutaneous tissue repair: Basic biologic considerations. I. J. Am. Acad. Dermatol. 13, 701-725.

Clark E.R. and Clark E.L. (1935). Observations on changes in blood vascular endothelium in the living animal. Am. J. Anat. 57, 385-438.

Clark E.R. and Clark E.L. (1939). Microscopic observations on the growth of blood capillaries in the living mammal. Am. J. Anat. 64, 251-301.

Clark R.A.F., Lanigan J.M., Dellepella P., Manseau E., Dvorak H.F. and Colvin R.B. (1982a). Fibronectin and fibrin provide a provisional matrix for epidermal cell migration during wound reepithelialization. J. Invest. Dermatol. 70, 264-269.

Clark R.A.F., Quinn J.H., Winn H.J., Lanigan J.H., Dellepella P. and Colvin R.B. (1982b). Fibronectin is produced by blood vessels in response to injury. J. Exp. Med. 156, 646-651.

Cliff W.J. (1963). Observations on healing tissue: A combined light and electron microscopic investigation. Philos. Trans. R. Soc. Lond. B. (Biol. Sci.) 246, 305-325.

Cliff W.J. (1965). Kinetics of wound healing in rabbit ear chambers: a time lapse cinemicroscopic study. Q. J. Exp. Physiol. 50, 79-89.

Cliff W.J. (1976). The extra-endothelial cells of blood vessel wall. In: Blood vessels, biological structure and function. Vol. 6. Harrison R.J., McMinn H.W. and Treherne K. (eds) Cambridge University Press. Cambridge. pp 68-72.

Coffin J.D. and Poole T.J. (1988). Embryonic vascular development: immunohistochemical identification of the origin and subsequent morphogenesis of the major vessel primordia in quail embryos. Development 102, 735-748.

Cogan D.G. (1949). Vascularization of the cornea: its experimental induction by small lesions and a new theory of its pathogenesis. Arch. Ophthalmol. 41, 406-416.

Conn G., Soderman D.D., Schaffer M.-T., Wile M., Hatcher V. and Thomas K. (1990a). Purification of a glycoprotein vascular endothelial cell mitogen from a rat glioma-derived cell line. Proc. Natl. Acad. Sci. USA 87, 1323-1327.

Conn G., Bayne M., Soderman L., Kwok P.W., Sullivan K.A., Palisi T.M., Hope D.A. and Thomas K.A. (1990b). Amino acid and cDNA sequence of a vascular endothelial cell mitogen homologous to

platelet-derived growth factor. Proc. Natl. Acad. Sci. USA 87, 2628-2632.

Connolly D.T., Heuvelman D.M., Nelson R., Olander J.V., Eppley B., Delfino J.J., Siegel R.N., Leingruber R.M. and Feder J. (1989). Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J. Clin. Invest. 84, 1470-1478.

Cozzolino F., Torcia G., Ziche M., Ogawa S., Brett J., Koga S., Vlassara H., Nawroth P. and Stern D. (1990). Advanced glycosylation endproducts (AGEs) stimulate endothelial cell (EC) growth in vitro and in vivo. Circulation. 82, III36.

Crocker D.J., Murad T.M. and Geer J.C. (1970). Role of the pericyte in wound healing. An ultrastructural study. Exp. Mol. Pathol. 13, 51-65.

Crum R., Szabo S. and Folkman J. (1985). A new class of steroids inhibits angiogenesis in the presence of heparin or a heparin fragment. Science 230, 1375-1378.

Cuevas P., Gonzalez A.N., Carcellar F. and Baird A. (1991). Vascular response to basic fibroblast growth factor when infused onto the normal adventitia or into the injured media of the rat carotid artery. Circ. Res. 69, 360-369.

Culton M., Chandler D.B., Proia A.D., Hickingbotham D. and Klintworth G.K. (1990). The effect of oxygen on corneal neovascularization. Invest. Ophthalmol. Vis. Sci. 31, 1277-1281.

Davis M.D. (1988). Diabetic retinopathy: a clinical overview. Diabetes/ Metabol. Rev. 4, 291-322.

D'Amore P.A. and Thompson R.W. (1987). Mechanisms of angiogenesis. Annu. Rev. Phisiol. 49, 453-464.

de Brabander M., Scharper W. and Verheyen F. (1973). Regenerative changes in the porcine heart after gradual and chronic coronary artery occlusion. Beitr. Pathol. Bd. 149, 170-185.

Delvos U., Gajdusek C., Sage H., Harker L.A. and Schwartz S.M. (1982). Interaction of vascular wall cells with collagen gels. Lab. Invest. 46, 61-72.

Demir R., Kaufmann P., Castellucci M., Erbengi T. and Kotowski A. (1989). Fetal vasculogenesis and angiogenesis in human placental villi. Acta Anat. 136, 190-203.

Denekamp J. (1982). Endothelial cell proliferation as a novel approach to targeting tumour therapy. Br. J. Cancer 45, 136-139.

Denekamp J. and Hobson B. (1982). Endothelial cell proliferation in experimental tumours. Br. J. Cancer 46, 711-720.

Diaz-Flores L. and Dominguez C. (1985). Relation between arterial intimal thickening and the vasa-vasorum. Virchows Arch. (A) 406, 165-177.

Diaz-Flores L., Rodriguez E., Gayoso M.J. and Gutierrez R. (1988). Growth of two types of cartilage after implantation of free autogeneic perichondrial grafts. Clin. Orthop. 234, 267-279.

Diaz-Flores L., Martin A.I., Garcia R. and Gutierrez R. (1989). Proliferative fasciitis: ultrastructure and histogenesis. J. Cutan. Pathol. 16, 85-92.

Diaz-Flores L., Valladares F., Gutierrez R. and Varela H. (1990a). The role of the pericytes of the adventitial microcirculation in the arterial intimal thickening. Histol. Histopath. 5, 145-153.

Diaz-Flores L., Martin A.I., Garcia Montelongo R. and Gutierrez R. (1990b). Role of pericytes and endothelial cells in tissue repair and related pathological processes. J. Cutan. Pathol. 17, 191-192.

Diaz-Flores L., Gutierrez R., Gonzalez P., and Varela H. (1991a). Inducible perivascular cells contribute to the neochondrogenesis in grafted perichondrium. Anat. Rec. 229, 1-8.

Diaz-Flores L., Gutierrez R., Varela H., Rancel N. and Valladares F. (1991b). Microvascular pericytes: a review of their morphological and functional characteristics. Histol. Histopath. 6, 269-286.

Diaz-Flores L., Gutierrez R., Lopez Alonso A., Gonzalez R. and Varela H. (1992). Pericytes as a supplementary source of osteoblasts in periostal osteogenesis. Clin. Orthop. 275, 280-286.

Diaz-Flores L., Gutierrez R., Valladares F., Varela H. and Perez M. (1994). Intense vascular sprouting from rat femoral vein induced by prostaglandins E1 and E2. Anat. Rec. 238, 68-76.

DiCorleto P.E. and Bowen-Pope D.F. (1983). Cultured endothelial cells produce a platelet-derived growth factor-like protein. Proc. Natl. Acad. Sci. USA 80, 1919-1923.

DiPietro L.A. and Polverini P.J. (1993). Angiogenic macrophages produce the angiogenic inhibitor thrombospondin 1. Am. J. Pathol. 143, 678-684.

Dobson D.E., Castellot J.J. Jr., Spiegelman B.M. (1985). Angiogenesis stimulated by 3T3-Adipocytes is mediated by prostanoid lipids. J. Cell Biol. 101, 109a.

Dobson D.E., Kambe A., Block E., Dion T., Lu H., Castellot J.J.Jr and Speigelman B.M. (1990). 1-Butyrylglycerol: a novel angiogenesis factor secreted by differentiating adipocytes. Cell. 61, 223-230.

Dvorak H.F. (1986). Tumors: Wounds that do not heal. Similarities between tumor stroma generation and wound healing. New Engl. J. Med. 315, 1650-1659.

Dvorak H.F., Orenstein N.S. and Carvalho A.C. (1979a). Induction of a fibrin-gel investment: an early event in line 10 hepatocarcinoma growth mediated by tumor-secreted products. J. Immunol. 122, 166-174.

Dvorak H.F., Dvorak A.M., Manseau E.J., Wiberg L. and Churchill W.H. (1979b). Fibrin gel investment associated with line 1 and line 10 solid tumor growth, angiogenesis, and fibroplasia in guinea pigs: role of cellular immunity, myofibroblasts, microvascular damage, and infarction in line 1 tumor regression. JNCI 62, 1459-1472.

Dvorak H.F., Harvey V.S., Estrella P., Brown L.F., McDonach J. and Dvorak A.M. (1987). Fibrin containing gels induce angiogenesis. Implications for tumor stroma generation and wound healing. Lab. Invest. 57, 673-686.

Dvorak H.F., Nagy J.A., Dvorak J.T. and Dvorak A.M. (1988). Identification and characterization of the blood vessels of solid tumors that are leaky to circulating macromolecules. Am. J. Pathol. 133, 95-109.

Eddy H.A. and Cassarett G.W. (1973). Development of the vascular system in the hamster malignant neurilemmoma. Microvasc. res. 6, 63-82.

Edwards R.G. (1980) Conception in the human female. Academic Press. London.

Eghbali M. (1989). Cellular origin and distribution of transforming growth factor-beta 1 in the normal rat myocardium. Cell Tissue Res. 256, 553-558.

Ehrlich H.P., Jung W.K., Costa D.E. and Rajaratnam J.B. (1988). Effects of heparin on vascularization of artificial skin grafting. Exp. Mol. Pathol. 48, 244-251.

Ehrlich H.J., Keijer J., Preissner K.T., Gebbink R.K. and Pannekoek H. (1991). Functional interaction of plasminogen activator inhibitor type 1 (PAI-1) and heparin. Biochemistry 30, 1021-1028.

Eisenstein R. (1991). Angiogenesis in arteries: Review. Pharmacol. Ther. 49, 1-19.

Eisenstein R., Sorgente N., Soble L.W., Miller A. and Kuettner K.E. (1973). The resistance of certain tissues to invasion: penetrability of explanted tissues by vascularized mesenchyme. Am. J. Pathol. 73, 765-774.

Eisenstein R., Kuettner K.E., Neopolitan C., Soble L.W. and Sorgente N. (1975). The resistance of certain tissues to invasion. III. Cartilage extracts inhibit the growth of fibroblasts and endothelial cells in culture. Am. J. Pathol. 81, 337-348.

Ekblom P., Sariola H., Karkinen M. and Saxen L. (1982). The origin of the glomerular endothelium. Cell Diff. 11, 35-39.

Eliason J.A. and Elliott J.P. (1987). Proliferation of vascular endothelial cells stimulated in vitro by corneal epithelium. Invest Ophthalmol. Vis. Sci. 28, 1963-1969.

Epstein R.J. and Stulting R.D. (1987). Corneal neovascularization induced by stimulated lymphocytes in inbred mice. Invest. Ophthalmol. Vis. Sci. 28, 1505-1513.

Esch F., Baird A., Ling N., Ueno N., Hill F., Denoroy L., Kleppe R., Gospodarowicz D., Bohlen P. and Guillemin R. (1985). Primary structure of bovine pituitary basic fibroblast growth factor (FGF) and comparison with the amino-terminal sequence of bovine acidic FGF. Proc. Natl. Acad. Sci. USA 82, 6507-6511.

Fajardo L.F., Kowalski J., Kwan H.H., Prionas S.D. and Allison A.C. (1988). The disc angiogenesis system. Lab. Invest. 58, 718-724

Fajardo L.F., Kwan H.H., Kowalski J., Prionas S.D. and Allison A.C. (1992). Dual role of tumor necrosis factor alpha in angiogenesis. Am. J. Pathol. 140, 539-544.

Falcone D.J. (1989). Heparin stimulation of plasminogen activator secretion by macrophage-like cell line RAW264.7: role of the scavenger receptor. J. Cell. Physiol. 140, 219-226.

Feder J., Marasa J.C. and Olander J.V. (1983). The formation of capillary-like tubes by calf aortic endothelial cells grown in vitro. J. Cell. Physiol. 116, 1-6.

Feinberg R.N., Sherer, G.K. and Auerbach R. (1991). The development of the vascular system. S. Karger. Basel.

Feldman D., Goldstein A.L., Cox D.C. and Grimley P.M. (1988). Cultured human endothelial cells treated with recombinant leukocyte A interferon: tubuloreticular inclusion formation, antiproliferative effect, and 2',5'oligoadenylate synthetase induction. Lab. Invest. 58, 584-589.

Ferrara N. and Henzel W.J. (1989). Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem. Biophys. Res. Commun. 161, 851-859.

Ferrara N., Leung D.W. and Phillips H.S. (1991). Molecular characterization and distribution of vascular endothelial growth factor. In: Neuroendocrine perspectives. Muller E.E. and McLeod R.B. (eds). Springer Verlag. New York. 9, 127-161.

Ferrara N., Houck K., Jakeman L. and Leung D.W. (1992). Molecular and biological properties of the vascular endothelial growth factor family of proteins. Endoc. Rev. 13, 18-32.

Fett J.W., Strydom D.J., Lobb R.R., Alderman E.M., Bethune J.L., Riordan J.F. and Vallee B.L. (1985) Isolation and characterization of angiogenin, an angiogenic protein from human carcinoma cells. Biochemistry 24, 5480-5486.

Findlay J.K. (1986) Angiogenesis in reproductive tissues. J. Endocrinol. 111, 357-366.

Foidart J.M., Bere E.W. Jr., Yaar M., Rennard S.I., Gullino M., Martin G.R. and Katz S.I. (1980). Distribution and immunoelectron microscopic localization of laminin, a noncollagenous basement membrane glycoprotein. Lab. Invest. 42, 336-342.

Foley M.E., Griffin B.D., Zuzel M., Aparicio S.R., Bradbury K., Bird C.C., Clayton J.K., Jenkins D.M., Scott J.S., Rajah C.M. and McNicol G.P. (1978). Heparin-like activity in uterine fluid. Br. Med. J. ii, 322-324.

Folkman J. (1971). Tumor angiogenesis. Therapeutic Implications. New Engl. J. Med. 285, 1182-1186.

Folkman J. (1972). Anti-angiogenesis: new concept for therapy of solid tumors. Ann. Surg. 175, 409-416.

Folkman J. (1975). Tumor angiogenesis. Adv. Cancer Res. 43, 175-203.

Folkman J. (1976). The vascularization of tumors. Sci. Am. 234, 58-73.

Folkman J. (1982). Angiogenesis: initiation and control. Ann. N.Y. Acad. Sci. 401, 212-227.

Folkman J. (1984). What is the role of endothelial cells in angiogenesis. Lab. Invest. 51, 601-602.

Folkman J. (1985a). Toward an understanding of angiogenesis search and discovery. Pers. Biol. Med. 29, 10- 36.

Folkman J. (1985b). Tumor angiogenesis. Adv. Cancer Res. 43, 175-203.

Folkman J. (1985c). Angiogenesis and its inhibitors. In: Important Advances in Oncology. De Vita V.T. Jr, Hellman S.A. and Rosenberg S.A. (eds) Lippincott. Philadelphia. pp 42-62.

Folkman J. (1986). How is blood vessel growth regulated in normal and neoplastic tissue. Cancer Res. 46, 467-473.

Folkman J. (1989). Sucessful treatment of an angiogenic disease. N. Engl. J. Med. 320, 1211-1212.

Folkman J. (1990). What is the evidence that tumors are angiogenesis dependent? J. Natl. Cancer Inst. 82, 4-6.

Folkman J. and Cotran R. (1976). Relation of vascular proliferation to tumor growth. Int. Rev. Exp. Pathol. 16, 207-248.

Folkman J. and Haudenschild C.C. (1980). Angiogenesis in vitro. Nature 288, 551-556.

Folkman J. and Hochberg M. (1973). Self-regulation of growth in three dimensions. J. Exp. Med. 138, 745-753.

Folkman J. and Ingber D.E. (1987). Angiostatic steroids. Method of discovery and mechanism of action. Ann. Surg. 206, 374-383.

Folkman J. and Klagsbrun M. (1987). Angiogenic factors. Science 235, 442-447.

Folkman J. and Shing Y. (1992). Angiogenesis. J. Biol. Chem. 267, 10931-10934.

Folkman J., Long D.M. and Becker F.F. (1963). Growth and metastasis tumor in organ culture. Cancer 16, 453-467.

Folkman J., Merler E., Abernathy C. and Williams G. (1971). Isolation of a tumor factor responsible for angiogenesis. J. Exp. Med. 133, 275-288.

Folkman J., Haudenschild C.C. and Zetter B.R. (1979). Long-term culture of capillary endothelial cells. Proc. Natl. Acad. Sci. USA 76, 5217-5221.

Folkman J., Klagsbrun M., Sasse J., Wadzinski M.G., Ingber D. and Vlodavsky I. (1988). A heparin-binding angiogenic protein-basic fibroblast growth factor is stored within basement membrane. Am. J. Pathol. 130, 393-400.

Folkman J., Watson K., Ingber D. and Hanahan D. (1989a). Induction of angiogenesis during the transition from hyperplasia to neoplasia. Nature 339, 58-62.

Folkman J., Weisz P.B., Joullie M.M., Li W.M. and Ewing W.R. (1989b). Control of angiogenesis with synthetic heparin substitutes. Science 243, 1490-1493.

Form D.M. and Auerbach R. (1983). PGE2 and angiogenesis. Proc. Soc. Exp. Biol. Med. 172, 214-218.

Form D.M., Pratt B.M. and Madri J.A. (1986). Endothelial cell proliferation during angiogenesis. In vitro modulation by basement membrane components. Lab. Invest. 55, 521-530.

Forsman A.D. and McCormack J.T. (1992). Microcorrosion casts of hamster luteal and follicular vasculature throughout the estrous

cycle. Anat. Rec. 233, 515-520.

Fournier G.A., lutty G.A., Watt S., Fenselau A. and Patz A. (1981). A corneal micropocket assay for angiogenesis in the rat eye. Invest. Ophthalmol. Vis. Sci. 21, 351-354.

Frater-Schroder M., Risau W., Hallmann, R., Gautschi P. and Bohlen P. (1987). Tumor necrosis factor type-alpha, a potent inhibitor of endothelial cell growth in vitro, is angiogenic in vivo. Proc. Natl. Acad. Sci. USA 84, 5277-5281.

Frederick J.L., Shimanuki T. and di Zerega G.S. (1984) Initiation of angiogenesis by human follicular fluid. Science 224, 389-390.

Freemont A.J. and Ford W. (1985). Functional and morphological changes in post-capillary venules in relation to lymphocytic infiltration into BCG-induced granulomata in rat skin. J. Pathol. 147, 1-12.

Friedman M. and Byers S.O. (1962). Excess lipid leakage: a property of very young vascular endothelium. Br. J. Exp. Pathol. 43, 363-372.

Friesel R., Burgess W.H., Mehlman T. and Maeiog T. (1986). The characterization of the receptor for endothelial cell growth factor by covalent ligand attachment. J. Biol. Chem. 261, 7581-7584.

Friesel R., Komoriya A. and Maciag T. (1987). Inhibition of endothelial cell proliferation by gamma-interferon. J. Cell Biol. 104, 689-696.

Fromer C.H. and Klintworth G.K. (1975a). An evaluation of the role of leukocytes in the pathogenesis of experimentally induced corneal vascularization. I. Comparison of experimental models of corneal vascularization. Am. J. Pathol. 79, 537-554.

Fromer C.H. and Klintworth G.K. (1975b). An evaluation of the role of leukocytes in the pathogenesis of experimentally induced corneal vascularization. II. Studies on the effect of leukocyte elimination on corneal vascularization. Am. J. Pathol. 81, 531-544.

Fromer C.H. and Klintworth G.K. (1976). An evaluation of the role of leukocytes in the pathogenesis of experimentally induced corneal vascularization. III. Studies related to the vasoproliferative capability of polymorphonuclear leukocytes and lymphocytes. Am. J. Pathol. 82, 157-170.

Fujimoto T. and Singer S.J. (1987). Immunocytochemical studies of desmin and vimentin in pericapillary cells of chicken. J. Histochem. Cytochem. 35, 1105-1115.

Fujita M., Sasayama S. Asanoi H., Nakajima H., Sakai O. and Ohno A. (1988). Improvement of treadmill capacity and collateral circulation as a result of exercise with heparin pretreatment in patients with effort angina. Circulation 77, 1022-1029.

Fujita M., Yamanishi K., Hirai T., Ohno A., Miwa K. and Sasayama S. (1991). Comparative effect of heparin treatment with and without strenuous exercise on treadmil capacity in patients with stable effort angina. Am. Heart J. 122, 453-457.

Furcht L.T. (1986). Critical factors controlling angiogenesis: Cell products, cell matrix and growth factors. Lab. Invest. 55, 505-509.

Furusato M., Fukunaga M, Kikuchi Y., Yokota S., Joh K., Aizawa S. and Ishikawa E. (1984). Two- and Three dimensional ultrastructural observation of angiogenesis in juvenile hemangioma. Virchows Arch. (Cell Pathol.). 46, 229-237.

Furusato M., Shimoda T., Yokota K., Joh K., Miyazaki H., Inomata I., Takaki K., Aizawa S. and Ishikawa E. (1985). Angiogenesis of juvenile hemangioma. In: Microcirculation annual 1985. Tsuchiya M., Asano M. and Mishima Y. (eds). Elsevier. Amsterdam. pp 101-107.

Furusato M., Wakui S., Suzuki M., Takagi K., Hori M., Asari M., Kano Y. and Ushigome S. (1990). Three dimensional ultrastructural distribution of cytoplasmic interdigitation between endothelium and pericyte of capillary in human granulation tissue by serial section reconstruction method. J. Electron. Microsc. Tokyo 39, 86-91.

Galloway A.C., Pelletier R. and D'Amore P.A. (1984). Do ischemic hearts stimulate endothelial cell growth?. Surgery 96, 435-438.

Garbett P.K. and Gibbins J.R. (1987). Experimental neovascularization in vivo: the early changes in a stable adult vasculature responding to angiogenic stimulation by a syngeneic neoplasm. Br. J. Exp. Pathol. 68, 625-635.

Gimbrone M.A. Jr., Cotran R.S. and Folkman J. (1973). Endothelial regeneration: studies with human endothelial cells in culture. Ser. Haematol. 6, 453-455.

Gimbrone M.A. Jr., Cotran R.S., Leapman S.B. and Folkman J. (1974). Tumor growth and neovascularization: An experimental model using the rabbit cornea. J. Natl. Cancer Inst. 52, 413-427.

Gitay-Goren H., Soker S., Vlodavsky I. and Neufeld G. (1992). The binding of vascular endothelial growth factor to its receptors is dependent on cell surface associated heparin-like molecules. J. Biol. Chem. 267, 6093-6098.

Gonzalez-Crussi F. (1971). Vasculogenesis in the chick embryo. An ultrasound study. Am. J. Anat. 130, 441-460.

Good D.J., Polverini P.J., Rastinejad F., Le Beau M.M., Lemons R.S., Frazier W.A. and Bouck N.P. (1990). A tumor suppressor-dependent inhibitor of angiogenesis is immunologically and functionally indistinguishble from a fragment of thrombospondin. Proc. Natl. Acad. Sci. USA 87, 6624-6628.

Goodman A.L. and Rone J.D. (1985) Detection of angiotropic (chemoattractant) activity released by rabbit luteal cells cultured in serum-free or serum-enriched media. Biol. Reprod. 32 (Suppl. 1),. 296 (Abstract).

Gospodarowicz D. (1983). Growth factors and their action in vivo and in vitro. J. Pathol. 141, 201-233.

Gospodarowicz D. and Cheng J. (1986). Heparin protects basic and acidic FGF from inactivation. J. Cell. Physiol. 128, 475-484.

Gospodarowicz D., Cheng J., Liu G.M., Fujii D.K., Baird A. and Bohlen P. (1985) Fibroblast growth factor in human placenta. Biochem. Biophys. Res. Commun. 128, 554-562.

Gospodarowicz D., Ferrara N., Schweigerer L. and Neufeld G. (1987). Structural characterization and biological functions of fibroblast growth factor. Endocr. Rev. 8: 95-114.

Gospodarowicz D., Abraham J.A. and Schilling J. (1989). Isolation and characterization of vascular endothelial mitogen produced by pituitary-derived folliculo stellate cells. Proc. Natl. Acad. Sci. USA 86, 7311-7315.

Goto F., Goto K., Weindel K. and Folkman J. (1993). Synergystic effects of vascular endothelial growth factor and basic growth factor on the proliferation and cord formation of bovine capillary endothelial cells within collagen gel. Lab. Invest. 69, 491-493.

Greenberg G. and Hunt T.K. (1978). The proliferative response in vitro of vascular endothelial and smooth muscle cells exposed to wound fluids and macrophages. J. Cell. Physiol. 97, 353-360.

Greenberg G., Vlodavsky I., Foidart J.M. and Gospodarowicz D. (1980). Conditioned medium from endothelial cell cultures can restore the normal phenotypic expression of vascular endothelium maintained in vitro in the absence of fibroblast growth factor. J. Cell. Physiol. 103, 333-341.

Greene H.S.N. (1943). The heterologous transplantation of embryonic mammalian tissues. Cancer Res. 3; 809-822.

Grillo H.C. (1963). Origin of fibroblasts in wound healing: an auto-radiographic study of inhibition of cellular proliferation by local X-irradiation. Ann. Surg. 157, 453-467.

Groopman J.E., Gottlieb M.S. and Goodman J. (1984). Recombinant alpha-2 interferon therapy for Kaposi's sarcoma associated with the acquired immunodeficiency syndrome. Ann. Intern. Med. 100, 671-676.

Gross J.L., Moscatelli D. and Rifkin D.B. (1983). Increased capillary endothelial cell protease activity in response to angiogenic stimuli in vitro. Proc. Natl. Acad. Sci. USA 80, 2623-2627.

Groszek E. and Grundy S.M. (1980). The possible role of the arterial microcirculation in the pathogenesis of atherosclerosis. J. Chronic Dis. 33, 679-684.

Grunt T.W., Lametschwandter A., Karrer K. and Staindl O. (1986). The angioarchitecture of the Lewis lung carcinoma in laboratory mice. Scanning Electron. Microsc. II, 557-573.

Gullino P.M. (1981). Angiogenesis factor(s). In: Handbook of experimental pharmacology. Baserga R. (ed). Springer-Verlag. New York. p 427.

Haar J.L. and Ackerman G.A. (1971). A phase and electron microscopic study of vasculogenesis and erythropoiesis in the yolk sac of the mouse. Anat. Rec. 170, 199-224.

Hadfield G. (1951). Granulation tissue. Ann. Roy. Coll. Surg. Eng. 9, 397-407.

Hallahan T.W., Shapiro R. and Vallee B.L. (1991). Dual site model for the organogenic activity of angiogenin. Proc. Natl. Acad. Sci. USA 15, 2222-2226.

Harris M.K. and Eakin R.M (1949) Survival of transplanted ovaries in rats. J. Exp. Zool. 112, 131-163.

Haynes W.L., Proia A.D. and Klintworth G.K. (1989). Effect of inhibitors of arachidonic acid metabolism on corneal neovascularization in the rat. Invest. Ophthalmol. Vis. Sci. 30, 1588-1593.

Heder G., Jakob W., Halle W., Mauersberger B., Kambach G., Jentzsch K.D. and Oehme P. (1979). Influence of porcine corpus luteum extracts on DNA synthesis and proliferation of cultivated fibroblasts and endothelial cells. Exp. Pathol. 17, 493-497.

Heimark R.L. and Schwartz S.M. (1988). The role of cell-cell interaction in the regulation of endothelial cell growth. In: The Molecular and cellular biology and wound repair. Clark R.A.F. and Henson P.H. (eds). Plenum. New York. p 359.

Heldin P., Pertoft H., Nordlinder H., Heldin C-H. and Laurent T.C. (1991). Differential expression of platelet-derived growth factor alpha and beta receptors on fat-storing cells and endothelial cells of rat liver. Exp. Cell Res. 193, 364-369.

Henkind P. (1978). Ocular neovascularization. The Krill memorial lecture. Am. J. Ophthalmol. 85, 287-301.

Herbert J.M., Laplace M.C. and Maffrand J.P. (1988). Effect of heparin on the angiogenic potency of basic and acidic fibroblast growth factors in the rabbit cornea assay. Int. J. Tissue Reac. X, 133-139.

Herlyn M., Clark W.H., Rodeck U., Macianti M.L., Jambrosic J. and Koprowski H. (1987). Biology of tumor progression in human melanocytes. Lab. Invest. 56, 461-474.

Herman I.M. and D'Amore P.A. (1985). Microvascular pericytes contain muscle and non-muscle actins. J. Cell Biol. 101, 43-52.

Heuser L.S. and Miller F.N. (1986). Differential macromolecular leakage from the vasculature of tumors. Cancer 57, 461-464.

His W. (1868). Untersuchungen uber die erste Anlage des Wirbelthierleibes. F.C.W. Vogel. Leipzig.

Hiti-Harper J., Wohl H. and Harper E. (1978). Platelet factor 4: An inhibitor of collagenase. Science 199, 991-992.

Houck K.A., Ferrara N., Winer J., Cachianes G., Li B. and Leung D.W. (1991). The vascular endothelial growth factor family: identification of a fourth molecular species and characterization of alternative splicing of RNA. Mol. Endocrinol. 5, 1806-1814.

Hsieh D.S.T., Langer R. and Folkman J. (1981). Magnetic modulation of release of macromolecules from polymers. Proc. Natl. Acad. Sci. USA 78, 1863-1867.

Ide A.G., Baker N.H. and Warren S.L. (1939). Vascularization of the Pearce rabbit epithelioma transplant as seen in the transparent ear chamber. Am. J. Roentgenol. 42, 891-899.

Ingber D.E. and Folkman J. (1988). Inhibition of angiogenesis through modulation of collagen metabolism. Lab. Invest. 59, 44-51.

Ingber D.E. and Folkman J. (1989). Mechanochemical switching between growth and differentiation during fibroblast growth factor-stimulated angiogenesis in vitro: Role of extracellular matrix. J. Cell Biol. 109, 317-330.

Ingber D.E., Madri J.A. and Folkman J. (1986). A possible mechanism for inhibition of angiogenesis by angiostatic steroids: Induction of capillary basement membrane dissolution. Endocrinology 119, 1768-1775.

Ingber D.E., Madri J.A. and Folkman J. (1987). Endothelial growth factors and extracellular matrix regulate DNA synthesis through modulation of cell and nuclear expansion. In Vitro Cell Dev. Biol. 23, 387-394.

Ingber D., Fujita T., Kishimoto S., Sudo K., Kanamaru T., Brem H. and Folkman J. (1990). Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth. Nature 348, 555-557.

Inomata H., Smelser G.K. and Polack F.M. (1971). Corneal vascularization in experimental uveitis and graft rejection-an electron microscopic study. Invest. Ophthalmol. 10, 840-850.

Iruela-Arispe M.L., Bornstein P. and Sage H. (1991). Thrombospondin exerts an antiangiogenic effect on cord formation by endothelial cells in vitro. Proc. Natl. Acad. Sci. USA 88, 5026-5030.

Ishikawa F., Miyazono K., Hellman U., Drexler H., Wernstedt C., Hagiwara K., Usuki K., Takaku F., Risau W. and Heldin C-H. (1989). Identification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor. Nature 338, 557-562.

Jaffe E. and Mosher D.F. (1978). Synthesis of fibronectin by cultured human endothelial cells. J. Exp. Med. 147, 1779-1791.

Jaffe E.A., Nachman R.L., Becker C.G. and Minick C.R. (1972). Culture of human endothelial cells derived from umbilical veins: identification by morphologic and immunologic criteria. J. Clin. Invest. 51, 46a.

Jaffe E., Nachman R.L., Becker C.G. and Minick C.R. (1973). Culture of human endothelial cells derived from umbilical veins: identification by morphologic and immunologic criteria. J. Clin. Invest. 52, 2745-2756.

Jain R.K. (1985). Determinants of tumor blood flow. A review. Cancer Res. 48, 2641-2658.

Jakob W., Jentzsch K.D., Mauersberger B. and Oehme P. (1977) Demonstration of angiogenesis activity in the corpus luteum of cattle. Exp. Pathol. 13, 231-236.

Jennings M.A. and Florey H.W. (1970). Healing. In: General pathology. 4th ed. Florey H.W (ed). Lloyd-Luke (Medical Books) Ltd., W.B. Saunders Co. Philadelphia. pp 480-548.

Jensen H.M., Chen I., DeVault M.R. and Lewis A.R. (1982). Angiogenesis induced by "normal' human breast tissue: a probable marker for precancer. Science 218, 293-295.

Joyce N.C., De Camilli P. and Boyles J. (1984a). Pericytes, like vascular smooth muscle cells, are immunocytochemical evidence for the

presence of two isomyosins in graded concentrations. J. Cell Biol. 100, 1387-1395.

Joyce N.C., De Camilli P. and Boyles J. (1984b). Pericytes, like vascular smooth muscle cells, are immunocytochemically positive for cyclic GMP-dependent protein kinase. Microvasc. Res. 28, 206-219.

Joyce N.C., Haire M.F. and Palade G.E. (1985). Contractile proteins in pericytes. II. Immunocytochemical evidence for the presence of two isomyosins in graded concentrations. J. Cell Biol. 100, 1387-1395.

Kalebic T., Garbisa S., Glaser B. and Liotta L.A. (1983). Basement membrane collagen: degradation by migrating endothelial cells. Science 221, 281-283.

Kaminska G.M. and Niederkorn J.Y. (1993). Spontaneous corneal neovascularization in nude mice. Local imbalance between angiogenic and anti-angiogenic factors. Invest. Ophthalmol. Vis. Sci. 34, 222-230.

Kaminski M., Majewski S., Jablonska S., and Pawinska, M. (1984). Lowered angiogenic capability of peripheral blood lymphocytes in progressive systemic sclerosis (scleroderma). J. Invest. Derm. 82, 239-243.

Kanwar Y.S. and Farquhar M.G. (1979). Presence of heparan sulfate in the glomerular basement membrane. Proc. Natl. Acad. Sci. USA 76, 1303-1307.

Kanzani H., Okamura H., Takemori K. and Mori T. (1985) Ultrastructural changes of corpus luteum capillaries through its life span in the rabbit. Biol. Reprod. 32 (Suppl. 1), 295 (Abstract).

Kardami K. and Frandrich R. (1989). Basic fibroblast growth factor in atria and ventricles of the vertebrate heart. J. Cell Biol. 109, 1865-1875.

Keck P.J., Hauser S.D., Krivi G., Sanzo K., Warren T., Feder J. and Connolly D.T. (1989). Vascular permeability factor, an endothelial cell mitogen related to PDGF. Science 246, 1309-1312.

Kiefer M., Stefhens J.C., Crawford K., Okino K. and Barr P.J. (1990). Ligand-affinity cloning and structure of a cell surface heparan sulfate proteoglycan that binds basic fibroblast growth factor. Proc. Natl. Acad. Sci. USA. 87, 6985-6989.

Klagsbrun M. and D'Amore P.A. (1991). Regulators of angiogenesis. Annu. Rev. Physiol. 53, 217-239.

Klagsbrun M. and Folkman J. (1991). Angiogenesis. Peptide growth factors and their receptors II. Sporn M.B. and Roberts A.B. (eds). Springer- Verlag. New York. pp. 549-586.

Klagsbrun M. and Vlodavsky I. (1988). Biosynthesis and storage of basic fibroblast growth factor (bFGF) by endothelial cells: Implication for the mechanism of action of angiogenesis. In: Growth factors and other aspects of woud healing: Biological and clinical implications. Riss A.R. Liss (ed). New York. pp 55-61.

Klintworth G.K. (1977). The contribution of morphology to our understanding of the pathogenesis of experimentally produced corneal vascularization. Invest. Ophthalmol. Vis. Sci. 16, 281-285.

Klintworth G.K. (1991). Hypotheses about the pathogenesis of corneal vascularization. In: Corneal angiogenesis. A comprehensive critical reviews. Klintworth G.K. (ed.) Springer-Verlag. Berlin. pp 23-29.

Kocher O. and Madri J.A. (1989). Modulation of actin mRNAs in cultured vascular cells by matrix components and TGF-beta 1. In Vitro Cell Dev. Biol. 25, 424-434.

Koester K. (1876). Endarteritis and arteriitis. Klin. Wochenschr. 13, 454-455.

Kohsaka S., Shinozaki T., Nakano Y., Takei K., Toya S. and Tsukada Y. (1989). Expression of Ia antigen on vascular endothelial cells in mouse cerebral tissue grafted into the third ventricle of rat brain. Brain Res. 484, 340-347.

Koos R.D. (1986) Stimulation of endothelial cell proliferation by granulosa cell-conditioned medium. Endocrinology. 119, 481-489.

Koos R. and LeMaire W. (1983). Evidence for an angiogenic factor from rat follicles. In: Factors regulating ovarian function. Greenwald G.S. and Terranova P.F. (eds). Raven Press. New York. pp 191-195.

Kubota Y., Kleinman H.K. Martin G.R. and Lawley T.J. (1988). Role of basement membrane in the morphological differentiation of human endothelial cells into capillary-like structures. J. Cell Biol. 107, 1589-1598.

Kuettner K.E., Croxen R.L., Eisenstein R. and Sorgente N. (1974). Proteinase inhibitor activity in connective tissue. Experientia 30, 595-597.

Kuwabara T. and Cogan D.G. (1963). Retinal vascular patterns: IV. Mural cells of the retinal capillaries. Arch. Ophthalmol. 69, 492- 502.

Kull F.C. Jr., Brent D.A., Parikh I. and Cuatrecasas P. (1987). Chemical identification of a tumor-derived angiogenic factor. Science 236, 843-845.

Kumar S., West D., Shahabaddin S., Arnold F., Haboubi N., Reid H. and Carr H. (1983). Angiogenesis factors from human myocardial infarcts. Lancet 2, 364-368.

Langer R. and Folkman J. (1976). Polymers for the sustained release of proteins and other macromolecules. Nature 263, 797-800.

Langer R., Brem H., Falterman K., Klein M. and Folkman J.H. (1976). Isolation of a cartilage factor that inhibits tumor neovascularization. Science 193, 70-71.

Langer R.S., Conn H., Vacanti J., Haudenschild C. and Folkman J. (1980). Control of tumor growth in animals by infusion of an angiogenesis inhibitors. Proc. Natl. Acad. Sci. USA. 77, 4331-4335.

Latker C.H. and Kuwabara T. (1981). Regression of the tunica vasculosa lentis in the postnatal rat. Invest. Ophthalmol. Vis. Sci. 21, 689-699.

Latker C.H., Feinberg R.N. and Beebe D.C. (1986). Localized vascular regression during limb morphogenesis in the chicken embryo: II. Morphological changes in the vasculature. Anat. Rec. 214, 410-417.

LeCompte P.M. (1967). Reactions of the vasa vasorum in vascular disease. In: Cowdry's arteriosclerosis, a survey of the problem. 2nd ed. Blumenthal H.T. (ed) Thomas C.C.Springfield. Illinois. pp 212-224.

Lee A. and Langer R. (1983). Shark cartilage contains inhibitors of tumor angiogenesis. Science 221, 1185-1187.

Leibovich S.J., Polverini P.J., Shepard H.M., Wiseman D.M., Shively V. and Nuseir N. (1987). Macrophage-induced angiogenesis is mediated by tumor necrosis factor alpha. Nature 329, 630-632.

Leung D.W., Cachianes G., Kuang W-J., Goeddel D.V. and Ferrara N. (1989). Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 246, 1306-1309.

Leavesley D.I., Schwartz M.A., Rosenfeld M. and Cheresh D.A. (1993). Integrin beta1 and beta3-mediated endothelial cell migration is triggered through distinct signaling mechanisms. J. Cell Biol. 121, 163-170.

Levy A., Tamargo R., Brem H. and Nathans D. (1989). An endothelial cell growth factor from the mouse neuroblastoma cell line NB41. Growth Factors 2, 9-19.

Lewis W.H. (1925). The transformation of mononuclear blood cells into macrophages, epithelioid cells, and giant cells. Harvy Lect. 21, 77-112.

Lewis W.H. (1931). The outgrowth of endothelium and capillaries in tissue culture. Bull. Johns Hopk. Hosp. 48, 242-253.

Liaw L. and Schwartz S.M. (1993). Microtubule disruption stimulates DNA synthesis in bovine endothelial cells and potentiates cellular response to basic fibroblast growth factor. Am. J. Pathol. 143, 937-948.

Lijnen H.R. and Collen D. (1986). Stimulation by heparin with plasmin-mediated conversion of single-chain to two-chain urokinase-type plasminogen activator. Thromb. Res. 43, 687-690.

Liotta L. and Kleinerman J. and Saidel G. (1974). Quantitative relationships of intravascular tumor cells, tumor vessels, and pulmonary metastases following tumor implantation. Cancer Res. 34, 997-1004.

Lutty G.A., Thompson D.C., Gallup J.Y., Mello R.J., Patz A. and Fenselau A. (1983). Vitreous: an inhibitor of retinal extract-induced neovascularization. Invest. Ophthalmol. Vis. Sci. 24, 52-56.

Maciag T. (1984). Angiogenesis. Prog. Haemostat. Thromb. 7, 167-182.

Maciag T., Kadish J. Wilkins L., Stemerman M.B. and Weinstein R. (1982). Organizational behavior of human umbilical vein endothelial cells. J. Cell Biol. 94, 511-520.

Madri J.A. and Pratt B.M. (1986). Endothelial cell-matrix interactions: In vitro models of angiogenesis. J. Histochem Cytochem 34, 85-91.

Madri J.A. and Pratt B.M. (1988). Angiogenesis. In: The Molecular and Cellular Biology of Wound Repair. Clark R.A.F. and Henson P.M. (eds.). Plenum. New York. p 337.

Madri J.A. and Stenn K.S. (1982). Aortic endothelial cell migration. I. Matrix requirements and composition. Am. J. Pathol. 106, 180-186.

Madri J.A. and Williams S.K. (1983). Capillary endothelial cell cultures: matrix requirements and composition. 97, 153-156.

Madri J.A., Williams S.K., Wyatt T., and Mezzio C. (1983). Capillary endothelial cell cultures: Phenotypic modulation by matrix components. J. Cell Biol. 97, 153-165.

Madri J.A., Pratt B. and Tucker A. (1988). Phenotypic modulation of endothelial cells by transforming growth factor beta depends upon the composition and organization of the extracellular matrix. J. Cell Biol. 106, 1375-1384.

Maione T.E., Gray G.S., Petro J., Hunt A.J., Donner A.L., Bauer S.I., Carson H.F. and Sharpe R.J. (1990). Inhibition of angiogenesis by recombinat human platelet factor-4 and related peptides. Science. 247, 77-79.

Majewski S., Tigalonowa M., Jablonska S., Polakowski I. and Janczura E. (1987). Serum samples from patients with active psoriasis enhance lymphocyte-induced angiogenesis and modulate endothelial cell proliferation. Arch. Dermatol. 123, 221-225.

Makris A., Ryan K.J., Yasumizu T., Hill C.L. and Zetter B.R. (1984). The non luteal porcine ovary as a source of angiogenic activity. Endocrinology 115, 1672-1677.

Markee J.E. (1940) Menstruation in intraocular endometrial transplants in the rhesus monkey. Cont. Embryol. 77, 221-308.

Masferrer J.L., Rimarachin J.A., Gerrifsen M.E., Falck J.R., Yadagiri P., Dunn M.W. and Laniado S.M. (1991). 12(R)-Hydroxyeicosatrienoic acid, a potent chemotactic and angiogenic factor produced by the cornea. Exp. Eye Res. 52, 417-424.

Matsuhashi K. (1961). Electron microscopic observation of the corneal vascularization. (Report I). J. Clin. Ophthalmol. 14, 121-127.

Matsuhashi K. (1962). Experimental study on the corneal vascularization. II. Electron microscope observation on the corneal vascularization. Acta Soc. Ophthalmol. Jpn. 66, 939-952.

Maurice D.M., Zauberman H. and Michaelson I.C. (1966). The stimulus to neovascularization in the cornea. Exp. Eye Res. 5, 168-184.

Mc Cracken J.S., Burger P.C. and Klintworth G.K. (1979). Morphologic observations on experimental corneal vascularization in the rat. Lab. Invest. 41, 519-530.

Mc Donald R.A. (1959). Origin of fibroblasts in experimental healing wounds: autoradiographic studies using tritiated thymidine. Surgery. 46, 376-382.

McKinney R.V. and Panner B.J. (1972). Regenerating capillary basement membrane in skeletal muscle wounds. Lab. Invest. 26, 100- 113.

Meyer J. (1852). Über die Neubildung von Blutgefassen in plastischen Exsudaten seroser Membranen und in Hautwunden. Ann. Charite (Berlin). 4, 41-140.

Miyazono K., Okabe T., Urabe A., Takaku F. and Heldin C-H. (1987). Purification and properties of an endothelial cell mitogen from human platelets. J. Biol. Chem. 262, 4098-4103.

Montesano R. and Orci L. (1985). Tumor-promoting phorbol esters induce angiogenesis "in vitro". Cell 42, 469-477.

Montesano R., Orci L. and Vassalli P. (1983). In vitro rapid organization of endothelial cells into capillary-like networks is promoted by collagen matrices. J. Cell Biol. 97, 1648-1652.

Montesano R., Vassalli J-D., Baird A., Guillemin R. and Orci L. (1986). Basic fibroblast growth factor induces angiogenesis in vitro. Proc. Natl. Acad. Sci. USA 83, 7297-7301.

Moscatelli D. and Rifkin D.B. (1988). Membrane and matrix localization of proteases: A common theme in tumor invasion and angiogenesis. Biochim. Biophys. Acta 948, 67-85.

Moscatelli D., Gross J.L. and Rifkin D.B. (1981). Angiogenic factors stimulate plasminogen activator and collagenase production by capillary endothelial cells. J. Cell Biol. 91, 201a (Abstract).

Moscatelli D., Presta M. and Rifkin D.B. (1986). Purification of a factor from human placenta that stimulates capillary endothelial cell protease production, DNA synthesis, and migration. Proc. Natl. Acad. Sci. USA 83, 2091-2095.

Moses M.A., Sudhalter J. and Langer R. (1990). Identification of an inhibitor of neovascularization from cartilage. Science 248, 1408-1410.

Murata J., Saiki I., Makabe T., Tsuta Y., Tokura S. and Azuma I. (1991). Inhibition of tumor-induced angiogenesis by sulfated chitin derivatives. Cancer Res. 51, 22-26.

Murray I.C. and Leblond Ch.P. (1988). Immunoelectron microscopy of endothelial cells in rat incisor suggest that most basement membrane components are produced by young cells, whereas heparan sulfate proteoglycan is produced by both young and old cells. J. Histochem. Cytochem. 36, 763-773.

Murray J.B., Brown L., Langer R. and Klagsbrun M. (1983). A microsustained release system for epidermal growth factor. In Vitro 19, 743-748.

Muthukkaruppan V. and Auerbach R. (1979). Angiogenesis in the mouse cornea. Science 205, 1416-1418.

Muthukkaruppan V.R., Kubai L. and Auerbach R. (1982). Tumor-induced neovascularization in the mouse eye. J. Natl. Cancer Inst. 69, 699-708.

Nagy J.A., Brown L.F., Senger D.R., Lanir N., Van de Water L., Dvorak A.M. and Dvorak H.F. (1988). Pathogenesis of tumor stroma generation; critical role for leaky blood vessels and fibrin deposition. Biochim. Biophys Acta. 948, 305-326.

Nakayasu K. (1988). Origin of pericytes in neovascularization of rat cornea. Jpn. J. Ophthalmol. 32, 105-112.

Nakayasu K., Hayashi N., Okisaka S. and Saro N. (1992). Formation of

capillary-like tubes by vascular endothelial cells cocultivated with keratocytes. Invest. Ophthalmol. Vis. Sci. 33, 3050-3057.

Nehls V. and Drenckhahn D. (1991). Heterogeneity of microvascular pericytes for smooth muscle type alpha-actin. J. Cell Biol. 113, 147-154.

Nehls V., Denzer K. and Drenckhahn D. (1992). Pericyte involvement in capillary sprouting during angiogenesis in situ. Cell Tissue Res. 270, 469-474.

Nguyen M., Folkman J. Bischoff J. (1992). 1-Deoxymannojirimycin inhibits capillary tube formation in vitro. Analysis of N-linked oligosaccharides in bovine capillary endothelial cells. J. Biol. Chem. 267, 26157-26165.

Nicosia R.F. and Madri J.A. (1987). The microvascular extracellular matrix. Developmental changes during angiogenesis in the aortic ring-plasma clot model. Am. J. Pathol. 128, 78-90.

Nicosia R.F. and Ottinetti A. (1990). Growth of microvessels in serum-free matrix culture of rat aorta. A quantitative assay of angiogenesis in vitro. Lab. invest. 63, 115-122.

Nicosia R.F., Tchao R. and Leighton J. (1982). Histiotypic angiogenesis in vitro. Light microscopic, ultrastructural and autoradiographic studies. In Vitro 18, 538-549.

Norrby K., Jakobsson A. and Sorbo J. (1986). Mast-cell-mediated angiogenesis: a novel experimental model using the rat mesentery. Virchows Arch. (B). 52, 195-206.

Norrby K., Jakobsson A. and Sorbo J. (1990a). Quantitative angiogenesis in spreads of intact rat mesenteric windows. Microvasc. Res. 39, 341-348.

Norrby K., Jakobsson A. and Sorbo J. (1990b) On mast-cell mediated angiogenesis in the rat mesenteric window assay. Agents Actions 30, 231-233.

Obeso J.L. and Auerbach R. (1984). A new microtechnique for quantitating cell movement in vitro using polystyrene bead monolayers. J. Immunol. Meth. 70, 141-152.

Oikawa T., Suganuma M., Ashino-Fuse H. and Shimamura M. (1992). Okadaic acid is a potent angiogenesis inductor. Jpn. J. Cancer Res. 83, 6-9.

Okamura K., Sato Y., Matsuda T., Hamanaka R., Ono M., Kohno K. and Kuwano M. (1991). Endogenous basic fibroblast growth factor-dependent induction of collagenase and interleukin-6 in tumor necrosis factor-treated human microvascular endothelial cells. J. Biol. Chem. 266, 19162-19165.

Olivo M., Bhardwaj R., Schulze-Osthoff K., Sorg C., Jacob H.J. and Flamme I. (1992) A comparative study on the effects of tumor necrosis factor alpha (TNF-alpha), human angiogenic factor (h-AF) and basic fibroblast growth factor (bFGF) on the chorioallantoic membrane of the chick embryo. Anat. Rec. 234, 105-115.

Orlidge A. and D'Amore P.A. (1987). Inhibition of capillary endothelial cell growth by pericytes and smooth muscle cells. J. Cell Biol. 105, 1455-1462.

Ordlidge A. and D'Amore P.A. (1988). Endothelial cell-pericyte co-cultures produce activated TGF-beta inhibits endothelial growth. ARVO Abstracts. Invest. Ophthalmol. Vis. Sci. 29 (Suppl.), 109-119.

Paku S. and Lapis K. (1993). Morphological aspects of angiogenesis in experimental liver metastases. Am. J. Pathol. 143, 926-936.

Paku S. and Paweletz N. (1991). First steps of tumor-related angiogenesis. Lab. Invest. 65, 334-346.

Pardanaud L.C., Altmann C., Kitos P., Dieterlenlievre F. and Buck C.A. (1987). Vasculogenesis in the early quail as studied with a monoclonal antibody recognizing endothelial cells. Development 100, 339-349.

Passaniti A.P., Taylor A.M., Pili R., Guo Y., Long P.V., Haney J.A., Pauly R.R., Grant D.S. and Martin G.R. (1992). A simple, quantitative method for assesing angiogenesis and antiangiogenic agents using reconstituted basement membrane, heparin, and fibroblast growth factor. Lab. Invest. 67, 519-528.

Paterson J.C. (1936). Vascularization and hemorrhage of the intima of arteriosclerotic coronary arteries. Arch. Pathol. 22, 313-324.

Paterson J.C. (1938). Capillary rupture with intimal hemorrhage as a causative factor in coronary thrombosis. Arch. Pathol. 25, 474-487.

Paweletz N. and Knierim M. (1989). Tumor-related angiogenesis. Crit. Rev. Oncol/Hematol. 9, 197-242.

Peacock E.E. (1984). Wound repair. W.B. Saunders Co., Philadelphia. p 127.

Pepper M.S. and Montesano R. (1990). Proteolytic balance and capillary morphogenesis. Cell Diff. Dev. 32, 319-328.

Pepper M.S., Vassalli J.D., Montesano R. and Orci L. (1987). Urokinase-type plasminogen activator is induced in migrating capillary endothelial cells. J. Cell Biol. 105, 2535-2541.

Pepper M.S., Spray D.C., Chanson M., Montesano R., Orci L. and Meda P. (1989). Junctional communication is induced in migrating capillary endothelial cells. J. Cell Biol. 109, 3027-3038.

Pepper M.S., Belin D., Montesano R., Orci L. and Vassalli J-D. (1990). Transforming growth factor-beta 1 modulates basic fibroblast growth factor-induced proteolytic and angiogenic properties of endothelial cells in vitro. J. Cell Biol. 111, 743-755.

Phillips H.S., Hains J., Leung W. and Ferrara N. (1990). Vascular endothelial growth factor is expressed in rat corpus luteum. Endocrinology 131, 254-260.

Phillips G.D., Whitehead R.A. and Knighton D.R. (1991). Initiation and pattern of angiogenesis in wound healing in the rat. Am. J. Anat. 192, 257-262.

Ploüet J., Schilling J., Gospodarowicz D. (1989). Isolation and characterization of a newly identified endothelial cell mitogen produced by AtT-20 cells. EMBO J. 8, 3801-3806.

Plunkett M.L. and Hailey J.A. (1990). An in vivo quantitative angiogenesis model using tumor cells entrapped in alginate. Lab. Invest. 62, 510-517.

Polverini P.J. and Leibovich S.J. (1984). Induction of neovascularization in vivo and endothelial proliferation in vitro by tumor-associated macrophages. Lab. Invest. 51, 635-642.

Polverini P.J., Cotran R.S., Gimbrone M.A. Jr. and Unanue E.R. (1977a). Activated macrophages induce vascular proliferation. Nature 269, 804-806.

Polverini P.J., Cotran R.S. and Sholley M.M. (1977b). Endothelial proliferation in the delayed hypersensitivity reaction: an autoradiographic study. J. Immunol. 118, 529-537.

Presta M., Moscatelli D., Joseph-Silverstein J. and Rifkin D.B. (1986). Purification from a human hepatoma cell line of a basic fibroblast growth factor-like molecule that stimulates capillary endothelial cell plasminogen activator production, DNA synthesis, and migration. Mol. Cell Biol. 6, 4060-4066.

Proia A.D., Chandler D.B., Haynes W.L., Smith C.F., Suvarnamani C., Erkel F.H. and Klintworth G.K. (1988). Quantitation of corneal neovascularization using computerized image analysis. Lab. Invest. 58, 473-479.

Quinkler W., Maasberg M., Bernotat-Danielowski S., Luthe N., Sharma H. and Scharper W. (1989). Isolation of heparin-building growth factors from bovine, porcine and canine hearts. Eur. J. Biochem.

181, 67-73.

Raju K.S., Alessandri G., Ziche M. and Gullino P.M. (1982). Ceruloplasmin copper ions and angiogenesis. J. Natl. Cancer Inst. 69, 1183-1188.

Raju K.S., Alessandri G. and Gullino P.M. (1984). Characterization of a chemoattractant for endothelium induced by angiogenesis effectors. Cancer Res. 44, 1579-1584.

Rastinejad F., Polverini P.J. and Bouck N.P. (1989). Regulation of the activity of a new inhibitor of angiogenesis by a cancer suppresor gene. Cell 56, 345-355.

Ravindranath N., Little-Ihrig L., Phillips H.S., Ferrara N. and Zeleznick A.J. (1992). Vascular endothelial growth factor mRNA expression in the primate ovary. Endocrinology 131, 254-260.

Real F.X., Oettgen H.F. and Krown S.E. (1986). Kaposi's sarcoma and the acquired immunodeficiency syndrome: treatment with high and low doses of recombinant leukocyte A interferon. J. Clin. Oncol. 4, 544- 551.

Reinhold H.S. and van den Berg-Blok A. (1984). Factors influencing the neovascularization of experimental tumours. Biorheology 21, 493-501.

Rhine W., Hsieh D.S.T. and Langer R. (1980). Polymers for sustained macromolecule release: procedures to fabricate reproducible delivery systems and control release kinetics. J. Pharm. Sci. 69, 265-270.

Rhodin J.A.G. and Fujita H. (1989). Capillary growth in the mesentery of normal young rats. Intravital video and electron microscope analyses. J. Submicrosc. Cytol. Pathol. 21, 1-34.

Ribatti D., Roncali L., Nico B. and Bertossi M. (1987). Effects of exogenous heparin on the vasculogenesis of the chorioallantoic membrane. Acta Anat. 130, 257-263.

Richards J.S. (1980). Maturation of ovarian follicles: actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. Physiol. Rev. 60, 51-89.

Rifkin D.B. and Moscatelli D. (1989). Recent developments in the cell biology of basic fibroblast growth factor. J. Cell Biol. 109, 1-6.

Rifkin D.B., Gross J.L., Moscatelli D. and Jaffe E. (1982). Proteases and angiogenesis: Production of plasminogen activation and collagenase by endothelial cells. In: Pathobiology of the endothelial cell. Nossel H.L. and Vogel H.J. (eds). Academic Press Inc. New York. pp 191-197.

Rios A., Mansell P.W., Newell G.R., Reuben J.M., Hersh E.M. and Gutterman J.U. (1985). Treatment of acquired immunodeficiency syndrome-related Kaposi's sarcoma with lymphoblastoid interferon. J. Clin. Oncol. 3, 506-512.

Risau W. (1986). Developing brain produces an angiogenesis factor. Proc. Natl. Acad. Sci. USA. 83, 3855-3859.

Risau W. and Ekblom P. (1986). Production of a heparin-binding angiogenesis factor by the embryonic kidney. J. Cell Biol. 103, 110-1107.

Risau W. and Lemmon V. (1988). Changes in the vascular extracellular matrix during embryonic vasculogenesis and angiogenesis. Dev. Biol. 125, 441-450.

Risau W., Sariola H., Zerwes H.G., Sasse J., Ekblom P., Kemler R. and Doetschman T. (1988). Vasculogenesis and angiogenesis in embryonic-stem-cell derived embryoid bodies. Development. 102, 471- 478.

Roberts A.B., Sporn M.B., Assoian R.K., Smith J.M., Roche N.J., Wakefield L.M., Heine U.I., Liotta I.A., Falanga V., Kehrl J.H. and Fauci A.S. (1986). Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proc. Natl. Acad. Sci. USA 83, 4167-4171.

Robertson N.E., Discapani C.M., Downs E.C., Hailey J.A., Sarre O., Runkle R.L. Jr. Popper T.L. and Plunkett M.L. (1991). A quantitative in vivo mouse model used to assay inhibitors of tumor-induced angiogenesis. Cancer Res. 51, 1339-1344.

Rofstad E.K. (1984). Growth and vascular structure of human melanoma xenografts. Cell Tissue Kinet. 17, 91-101.

Rone J.D. and Goodman A.L. (1985). Detection of angiotropic activity from intact rabbit follicles cultured in serum-free medium. Biol. Reprod. 32 (Suppl. 1), 294 (Abstract).

Rosenbruch M. (1989). Granulation tissue in the chick embryo yolk sac blood vessel system. J. Comp. Pathol. 101, 363-373.

Ross R., Everett N.B. and Tyler R. (1970). Wound healing and collagen formation. VI. The origin of wound fibroblast studied in parabiosis. J. Cell Biol. 44, 645-654.

Roth D.M., Maruoka Y., Rogers J., White F.C., Longhurst J.C. and Bloor C.M. (1987). Development of coronary collateral circulation in the left circumflex ameroid occluded swine myocardium. Am. J. Physiol. 253, H1279-H1288.

Roth D.M., White F.C., Nichols M.L., Dobbs S.L., Longhurst J.C. and Bloor C.M. (1990). Effect of long-term exercise on regional myocardial function and coronary collateral development after gradual coronary artery occlusion in pigs. Circulation 82, 1778-1789.

Rupnick M.A., Stokes C.L., Williams S.K. and Lauffenburger D.A. (1988). Quantitative analysis of random motility of human microvessel endothelial cells using a liner under-agarose assay. Lab. Invest. 59, 363-372.

Ryan U.S. (1988). Endothelial cells. CRC Press. Boca Raton. Los Angeles.

Ryan G.B. and Spector W.G. (1970). Macrophages turnover in inflamed connective tissue. Proc. R. Soc. Lond. (Biol.) 174, 269-292.

Sabin F.R. (1920). Studies on the origin of the blood vessels and of red blood corpuscles as seen in the living blastoderm of chick during the second day of incubation. Contr. Embryol. 9, 215-262.

Sandison J.C. (1928). Observations on the growth of blood vessels as seen in the transparent chamber introduced in the rabbit's ear. Am. J. Anat. 41, 475-496.

Sasaki H., Hoshi H., Hong Y-M., Suzuki T., Kato T., Saito M., Youki H., Karube K., Konno S., Onoderag M., Saito T. and Aoyagi S. (1989). Purification of acidic fibroblast growth factor from bovine heart and its localization in the cardiac myocytes. J. Biol. Chem. 264, 17606-17611.

Sasaki K., Kiuchi Y., Sato Y. and Yamamori S. (1991). Morphological analysis of neovascularization at early stages of rat splenic autografts in comparison with tumor angiogenesis. Cell Tissue Res. 265, 503-510.

Sasayama S. and Fujita M. (1992). Recent insights into coronary collateral circulation. Circulation 85, 1197-1204.

Sato E., Ishibashi J. and Koide S.S. (1982). Inducement of blood vessel formation by ovarian extracts from mice injected with gonadotrophins. Experientia 38, 1248-1249.

Sato N., Sawasaki Y., Senoo A., Fuse Y., Hirano Y. and Goto T. (1987). Development of capillary networks from rat microvascular fragments in vitro: The role of myofibroblastics cells. Microvasc. Res. 33, 194-210.

Sato N., Fukuda K., Nariuchi H. and Sagara N. (1987). Tumor necrosis factor inhibiting angiogenesis in vitro. J. Natl. Cancer Inst. 79, 1383-1391.

Sato N., Beitz J.G., Kato J., Yamamoto M., Clark J.W., Calabresi P. and Frackelton A.R. Jr. (1993). Platelet-derived growth factor indirectly stimulates angiogenesis in vitro. Am. J. Pathol. 142, 1119-1130.

Sato Y. and Rifkin D.B. (1988). Autocrine activities of basic fibroblast growth factor: regulation of endothelial cell movement, plasminogen activator synthesis, and DNA synthesis. J. Cell Biol. 107, 1199-1205.

Sato Y. and Rifkin D.B. (1989). Inhibition of endothelial cell movement by pericytes and smooth muscle cells: Activation of a latent transforming growth factor-b1-like molecule by plasmin during co-culture. J. Cell Biol. 109, 309-315.

Schaper W. and Vandesteene R. (1967). The rate of growth of inter-arterial anastomoses in chronic coronary artery occlusion. Life Sci. 6, 1673-1680.

Schlingemann R.O., Rietveld F.J.R., de Waal R.M.W., Ferrone S. and Ruiter D.J. (1990). Expression of the high molecular weight melanoma-associated antigen by pericytes during angiogenesis in tumors and in healing wounds. Am. J. Pathol. 136, 1393-1405.

Schlingemann R.O., Rietveld F.J.R., Kwaspen F., van de Kerkhof P.C.M., de Waal R.M.W. and Ruiter D.J. (1991). Differential expresion of markers for endothelial cells, pericytes, and basal lamina in the microvasculature of tumors and granulation tissue. Am. J. Pathol. 138, 1335-1347.

Schmidt M., Sharma H., Schott R.J. and Scharper W. (1991). Amplification and sequencing of mRNA encoding acidic fibroblast growth factor (aFGF) from porcine heart. Biochem. Biophys. Res. Commun. 180, 853-859.

Schoefl G.I. (1963). Studies on inflammation. III. Growing capillaries: Their structure and permeability. Virchows Arch. Pathol. Anat. 337, 97-141.

Schor A.M. and Schor S.L. (1983). Tumour angiogenesis. Br. J. Pathol. 141, 385-413.

Schor A.M., Schor S.L. and Allen T.D.(1983). Effects of culture conditions on the proliferation morphology and migration of bovine aortic endothelial cells. J. Cell Sci. 62, 267-285.

Schreiber A.B., Winkler M.E. and Derynck R. (1986). Transforming growth factor-alpha: a more potent angiogenic factor than epidermal growth factor. Science 232, 1250-1253.

Schwartz S.M., Gajdusek C.M. and Owens G.K. (1982). Vessel wall growth control. In: Pathobiology of the endothelial cells. Nossel H.L. and Vogel H.J. (eds). Academic Press Inc. New York. pp 63-78.

Schweigerer L., Malerstein B. and Gospodarowicz D. (1987). Tumor necrosis factor inhibits the proliferation of cultured capillary endothelial cells. Biophys. Res. Commun. 143, 997-1004.

Selye H. (1953). On the mechanism through which hydrocortisone affects the resistance of tissues to injury. J. Am. Med. Ass. 152, 1207-1213.

Senger D., Galli S.J., Dvorak A.M., Peruzzi C.A., Harvey V.S. and Dvorak H.F. (1983). Tumor cells secrete a vascular permeability factor which promotes ascites fluid accumulation. Science 219, 983-987.

Senger D., Peruzzi C.A., Feder J. and Dvorak H.F. (1986). A highly conserved vascular permeability factor secreted by a variety of human and rodent cell lines. Cancer Res. 46, 5629-5632.

Shepro D. (1988). Endothelial cells, inflammatory edema, and the microvascular barrier: Comments by a "free radical". Microvasc. Res. 35, 247-264.

Shing Y., Folkman J., Haudenschild C., Lund D., Crum R. and Klagsbrun M. (1985). Angiogenesis is stimulated by a tumor-derived endothelial cell growth factor. J. Cell Biochem. 29, 275-287.

Sholley M.M., Gimbrone M.A. and Cotran R.S. (1977a). Cellular migration and replication in endothelial regeneration: a study using irradiated endothelial cultures. Lab. Invest. 36, 18-25.

Sholley M.M., Cavallo T. and Cotran R.S. (1977b). Endothelial cell proliferation in inflammation. I. Autoradiographic studies following thermal injured to the skin of normal rats. Am. J. Pathol. 89, 277-296.

Sholley M.M., Gimbrone M.A. and Cotran R.S. (1978). The effects of leukocyte depletion on corneal neovascularization. Lab. Invest. 38, 32-40.

Sholley M.M., Ferguson G.P., Seibel H.R., Montuor J.L. and Wilson J.D. (1984). Mechanisms of neovascularization. Vascular sprouting can occur without proliferation of endothelial cells. Lab. Invest. 51, 624-634.

Shweiki D., Itin A., Neufeld G., Gitay-Goren H. and Keshet E. (1993). Patterns of expression of vascular endothelial growth factor (VEGF) and VEGF receptors in mice suggest a role in hormonally regulated angiogenesis. J. Clin. Invest. 91, 2235-2243.

Sidky Y.A. and Borden E.C. (1987). Inhibition of angiogenesis by interferons: Effects on tumor- and lymphocyte-induced vascular responses. Cancer Res. 47, 5155-5161.

Sillman F., Boyce J. and Fruchter R. (1981). The significance of atypical vessels and neovascularization in cervical neoplasia. Am. J. Obstet. Gynecol. 139, 154-159.

Silverstein R.L., Leung L.K. and Nachman R.L. (1986). Thrombospondin: a versatile multifunctional glycoprotein. Arteriosclerosis 6, 245-253.

Simionescu N. and Simionescu M. (1988). Endothelial cell biology in health and disease. Plenum Press. New York.

Simionescu N. and Simionescu M. (1991). Endothelial cell dysfunction. Plenum Press. New York.

Sims D.E. (1986). The pericyte. A review. Tissue Cell 18, 153-174.

Skalli O., Pelte M-F., Peclet M-C., Gabbiani G., Gugliotta P., Bussolati G., Ravazzola M. and Orci L. (1989). Alpha-smooth muscle actin, a differentiation marker of smooth muscle cells, is present in micro-filamentous bundles of pericytes. J. Histochem. Cytochem. 37, 315-321.

Smelser G.K. and Ozanics V. (1972). Modern views of corneal anatomy. In: Symposium of the cornea. Castroviejo R. (ed). C.V. Mosby Co. St. Louis. pp 127-143.

Sorgente N., Kuettner K.E., Soble L.W. and Eisenstein R. (1975). The resistance of certain tissues to invasion. II. Evidence for extractable factors in cartilage which inhibit invasion by vascularized mesenchyme. Lab. Invest. 32, 217-222.

Soubrane G., Jerdan J., Karpouzas I., Fayein N.A., Glaser B., Coscas G., Courtois Y. and Jeanny J.C. (1990). Binding of basic fibroblast growth factor to normal and neovascularized rabbit cornea. Invest. Ophthalmol. Vis. Sci. 31, 323-333.

Spanel-Borowski K. and Mayerhofer A. (1987). Formation and regression of capillary sprouts in corpora lutea of immature superstimulated golden hamsters. Acta Anat. 128, 227-235.

Sparagen S.C., Bond V.P. and Dahl L.K. (1962). Role of hyperplasia in vascular lesions of cholesterol-fed rabbits studied with thymidine -H3 autoradiography. Circ. Res. 11, 329-336.

Srivastava A., Laidler P., Hughes L.E., Woodcock J. and Shedden E.J. (1986). Neovascularization in human cutaneous melanoma: a quantitative morphological and Doppler ultrasound study. Eur. J. Cancer Clin. Oncol. 22, 1205-1209.

Srivastava A., Laidler P. Davies R.P., Horgan K. and Hughes L.E. (1988). The prognostic significance of tumor vascularity in intermediate-thickness (0.76-4.0 mm thick) skin melanoma: a quantitative histologic study. Am. J. Pathol. 133, 419-423.

St. Clair D.K., Rybak S.M., Riordan J.F. and Vallee L. (1987). Angiogenin abolishes cell-free protein synthesis by specific ribonucleolytic inactivation of ribosomes. Proc. Natl. Acad. Sci. USA 84, 8330-8334.

Starksen N.F., Harsh G.R. IV., Gibbs V.C. and Williams L.T. (1987). Regulated expression of the platelet-derived growth factor. A chain in microvascular endothelial cells. J. Biol. Chem. 262, 14381-14384.

Stewart P.A. and Wiley M.J. (1981). Developing nervous tissues induced formation of blood-brain barrier characteristics in invading endothelial cells: A study using quail-chick transplantation chimeras. Dev. Biol. 84, 183-192.

Stokes C.L., Rupnick M.A., Williams S.K., and Laffenburger D.A. (1990). Chemotaxis of human microvessel endothelial cells in response to acidic fibroblast growth factor. Lab. Invest. 63, 657-668.

Sueishi K., Yasunaga C., Nakashima Y., Tsutsui H. and Ishii Y. (1989). Endothelium-fibrinolysis system interaction. Nippon Ketsueki Gakkai Zasshi 52, 1350-1358.

Sugiura S. and Matsuda H. (1969). Electron microspic studies on the corneal neo-vascularization. Acta. Soc. Ophthalmol. Jpn. 73, 1208-1221.

Sunderkötter C., Goebeler M., Schulze-Osthoff K., Bhardwaj R. and Sorg C. (1991). Macrophage derived angiogenesis factors. Pharmacol. Ther. 51, 195-216.

Szalay J. and Pappas G.D. (1970). Fine structure of rat corneal vessels in advanced stages of wound healing. Invest. Ophthalmol. 9, 354-365.

Takigawa M., Enomoto M., Nishida Y., Pan H-O., Kinoshita A. and Suzuki F. (1990a). Tumor angiogenesis and polyamines: a- d-ifluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase, inhibits B16 melanoma-induced angiogenesis in ovo and the proliferation of vascular endothelial cells in vitro. Cancer Res. 50, 4134-4138.

Takigawa M., Nishida Y., Suzuki F., Kishi J., Yamashita K. and Hayakawa T. (1990b). Induction of angiogenesis in chick yolk-sac membrane by polyamines and its inhibition by tissue inhibitors of metalloproteinases (TIMP and TIMP-2). Biochem. Biophys. Res. Commun. 171, 1264-1271.

Tamargo R.J., Bok R.A. and Brem H. (1991). Angiogenesis inhibition by minocycline. Cancer Res. 51, 672-675.

Tano Y., Chandler D.B. and Machener R. (1981). Vascular casts of experimental retinal neovascularization. Am. J. Ophthalmol. 92, 110-118.

Tannock I.F. (1968). The relation between cell proliferation and the vascular system in a transplanted mouse mammary tumour. Br. J. Cancer 22, 258-276.

Tannock I.F. (1970). Population kinetics of carcinoma cells, capillary endothelial cells, and fibroblasts in a transplanted mouse mammary tumor. Cancer Res. 30, 2470-2476.

Tannock I.F. and Hayashi S. (1972). The proliferation of capillary endothelial cells. Cancer Res. 32, 77-82.

Taraboletti G., Roberts D., Liotta L.A. and Giavazzi R. (1990). Platelet thrombospondin modulates endothelial cell adhesion, motility, and growth: A potential angiogenesis regulatory factor. J. Cell Biol. 111, 765-772.

Taylor S. and Folkman J. (1982). Protamine is an inhibitor of angiogenesis. Nature 297, 307-312.

Terranova V.P., DiFlorio R., Lyall R.M., Hic S., Friesel R. and Maciag T. (1985). Human endothelial cells are chemotactic to endothelial cell growth factor and heparin. J. Cell Biol. 101, 2330-2334.

Thomas K., Rios Candelore M., Gimenez-Gallego G., Di Salvo J., Bennet C., Rodkey J. and Fiuzpatrick S. (1985). Pure brain-derived acidic fibroblast growth factor is a potent vascular endothelial cell mitogen with sequence homology to interleukin 1. Proc. Natl. Acad. Sci. USA 82, 6409-6413.

Thompson N., Bazoberry F., Speir E., Casscells W., Ferrans V., Flanders K., Kondaiah P., Geiser A. and Sporn M.B. (1988). Transforming growth factor beta-1 in acute myocardial infarction in rats. Growth Factors 1, 91-99.

Thompson N.L., Flanders K.C., Smith J.M., Ellingsworth L.R., Roberts A.B. and Sporn M.B. (1989). Expression of transforming growth factor-beta 1 in specific cells and tissues of adult and neonatal mice. J. Cell Biol. 108, 661-669.

Tisher E., Mitchell R., Hartmann T., Silva M., Gospodarowicz D., Fiddes J. and Abraham J. (1991). The human gene for vascular endothelial growth factor. J. Biol. Chem. 266, 11947-11954.

Tomanek R.J., Schalk K.A., Marcus M.L. and Harrison D.G. (1989). Coronary angiogenesis during long-term hypertension and left ventricular hypertrophy in dogs. Circ. Res. 65, 352-359.

Tseng S.C.G., Savion N., Gospodarowicz D. and Stern R. (1983). Modulation of collagen synthesis by a growth factor and by the extracellular matrix: Comparison of cellular response to two different stimuli. J. Cell Biol. 97, 803-809.

Tseung S., Savion N., Stern R. and Gospodarowicz D. (1982). Fibroblast growth factor modulates synthesis of collagen in cultured vascular endothelial cells. Eur. J. Biochem. 122, 355-362.

Tsuboi R., Sato Y. and Rifkin D.B. (1990). Correlation of cell migration, cell invasion, receptor number, proteinase production, and basic fibroblast growth factor levels in endothelial cells. J. Cell Biol. 110, 511-517.

Tsuruoka N., Sugiyama M., Tawaragi Y., Tsujimoto M., Nishihara T., Goto T. and Sato N. (1988). Inhibition of in vitro angiogenesis by lymphotoxin and interferon-gamma. Biochem. Biophys. Res. Commun. 155, 429-435.

Tuan D., Smith S., Folkman, J. and Merler E. (1973). Isolation of the nonhistone proteins of rat Walker carcinoma 256: their association with tumor angiogenesis. Biochemistry 12, 3159-3165.

Unger E.F., Sheffield C.D. and Epstein S.E. (1991). Heparin promotes the formation of extracardiac to coronary anastomoses in a canine model. Am. J. Physiol. 260, H1625-H1634.

Vaisman N., Gospodarowicz D. and Neufeld G. (1990). Characterization of the receptors for vascular endothelial growth factor. J. Biol. Chem. 265, 19461-19466.

Verhoeven D. and Buyssens N. (1988). Desmin-positive stellate cells associated with angiogenesis in a tumour and non-tumour system. Virchows Arch. (B). 54, 263-272.

Vlodavsky I. and Gospodarowicz D. (1979). Structural and functional alterations in the surface of vascular endothelial cells associated with the formation of a confluent cell monolayer and with the withdrawal of fibroblast growth factor. J. Supramol. Struct. 12 (Suppl. 1) 73-114.

Vlodavsky I., Johnson L.K., Greenburg G. and Gospodarowicz D. (1979). Vascular endothelial cells maintained in the absence of fibroblast growth factor undergo structural and functional alterations that are incompatible with their in vivo differentiated properties. J.

Cell Biol. 83, 468-486.

Vlodavsky I., Folkman J., Sullivan R., Fridman R. Ishai-Michaeli R., Sasse J. and Klagsbrun M. (1987). Endothelial cell-derived basic fibroblast growth factor: Synthesis and deposition into sub-endothelial extracellular matrix. Proc. Natl. Acad. Sci. USA. 84, 2292-2296.

Vracko R. and Benditt E.P. (1970). Capillary basal lamina thickening: its relationship to endothelial cell death and replacement. J. Cell Biol. 47, 281-285.

Wagner R.C. (1980). Endothelial cell embriology and growth. Adv. Microcirc. 9, 45-75.

Wall R.T., Harker L.A. and Striker G.E. (1978). Human endothelial cell migration and stimulation by a released platelet factor. Lab. Invest. 39, 523-529.

Wakui S. (1988). Two and three dimensional ultrastructural observation of two cell type angiogenesis in human granulation tissue. Virchows Arch. (B) 56, 127-139.

Warren B.A. (1979a). The vascular morphology of tumors. In: Tumor blood circulation. Peterson H.I. (ed) CRC Press. Boca Raton. pp 1-47.

Warren B.A. (1979b). Tumor angiogenesis. In: Angiogenesis, vascular morphology and blood flow of experimental and human tumors. Peterson H.I. (ed) CRC Press. Boca Raton. pp 33-74.

Warren B.A. and Shubik P. (1966). The growth of blood supply to melanoma transplants in the hamster check pouch. Lab. Invest. 15, 464-478.

Warren B.A., Greenblatt M. and Kommineni V.R.C. (1972). Tumor angiogenesis: ultrastructure of endothelial cells in mitosis. Br. J. Exp. Pathol. 53, 216-224.

Watt S.L. and Auerbach R. (1986). A mitogenic factor for endothelial cells obtained from mouse secondary mixed leukocyte cultures. J. Immunol. 136, 197-202.

Weber J., Meyer K.C., Banda P., Calhoun W.J. and Auerbach R. (1989). Studies of bronchoalveolar lavage cells and fluids in pulmonary sarcoidosis. II. Enhanced capacity of bronchoalveolar lavage fluids from patients with pulmonary sarcoidosis to induce cell movement in vitro. Am. Res. Resp. Dis. 140, 1450-1454.

Weidner N., Semple J.P., Welch W.R. and Folkman J. (1991). Tumor angiogenesis and metastasis - Correlation in invasive breast carcinoma. New Engl. J. Med. 324, 1-8.

West D.C., Hampson I.N., Arnold F. and Kumar S. (1985). Angiogenesis induced by degradation products of hyaluronic acid. Science 228, 1324-1326.

White F.C., Sanders T.M. and Bloor C.M. (1978). Regional redistribution of myocardial blood flow after coronary occlusion and reperfusion in the conscious dog. Am. J. Cardiol. 43, 234-243.

White C.W., Sondheimer H.M., Crouch E.C., Wilson H. and Fan L.L. (1989). Treatment of pulmonary hemangiomatosis with recombinant interferon alfa-2a. New Engl. J. Med. 320, 1197-1200.

White F.C., Carroll S.M., Magnet A. and Bloor C.M. (1992). Coronary collateral development in the swine after coronary artery occlusion. Circ. Res. 17, 1490-1500.

Williams R.J., Robertson D. and Davies A.J. (1989). Identification of vascular endothelial cells in murine omentum using the lectin, Dolichos biflorus agglutinin: possible applications in the study of angiogenesis. Histochem. J. 21, 271-278.

Williams S. (1993). Angiogenesis in three-dimensional cultures. Lab. Invest. 69, 491-493.

Winkles J.A., Friesel R., Burgess W.H., Howk R., Mehlman T., Weinstein R. and Moeiag T. (1987). Human vascular smooth muscle cells both express and respond to heparin-binding growth factor 1 (endothelial cell growth factor). Proc. Natl. Acad. Sci. USA 84, 7124-7128.

Yamagami I. (1970). Electronmicroscopic study on the cornea. I. The mechanism of experimental new vessel formation. Jpn. J. Ophthalmol. 14, 41-58.

Yamaura H. and Sato H. (1973). Experimental studies on angiogenesis in AH109A ascites tumor tissue transplanted to a transparent chamber in rats. In: Chemoterapy of cancer dissemination and metastasis. Garattini S. and Franchi G. (eds) Raven. New York. pp 149-175.

Yayon A., Klagsbrun M., Esko J.D., Leder P. and Ornitz D.M. (1991). Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. Cell 64, 841-848.

Young W.C. and Herman I.M. (1985). Extracellular matrix modulation of endothelial cell shape and motility following injury in vitro. J. Cell Sci. 73, 19-32.

Zajchowski D.A., Band V., Trask D.K., Kling D., Connolly J.L. and Sager R. (1990). Suppression of tumor forming ability and related traits in MCF-6 human breast cancer cells by fusion with immortal mammary epithelial cells. Proc. Natl. Acad. Sci. USA 2314-2318.

Zauberman H., Michaelson I.C., Bergmann F. and Maurice D.M. (1969). Stimulation of neovascularization of the cornea by biogenic amines. Exp. Eye Res. 8, 77-83.

Zetter B.R. (1980). Migration of capillary endothelial cells is stimulated by tumor derived factors. Nature 285, 41-43.

Zetter B.R. (1988). Angiogenesis. State of the art. Chest 93, 159S-166S.

Zhang Y., Cliff W.J., Schoefl G.I. and Higgins G. (1993). Immunohistochemical study of intimal microvessels in coronary atherosclerosis. Am. J. Pathol. 143, 164-172.

Ziche M., Jones J. and Gullino P.M. (1982). Role of prostaglandin E1 and copper in angiogenesis. J. Natl. Cancer Inst. 69, 475-482.

Ziche M., Ruggiero M., Pasquali F. and Chiarugi V.P. (1985). Effects of cortisone with and without heparin on angiogenesis induced by prostaglandin E1 and by S180 cells, and on growth of murine transplantable tumours. Int. J. Cancer 35, 549-552.

Ziche M., Alessandri G. and Gullino P.M. (1989). Gangliosides promote the angiogenic response. Lab. Invest. 61, 629-634.

Ziche M., Morbidelli L., Alessandri G. and Gullino P.M. (1992). Angiogenesis can be stimulated or repressed in vivo by a change in GM3:GD3 ganglioside ratio. Lab. Invest. 67, 711-715.