Int. J. Dev. Biol. 38: 117-120 (1994)

Short Contribution

Reorganization of blood vessels after their partial section in the chick embryo chorioallantoic membrane

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ABSTRACT In this study the sequence of vascular reorganization of the vascular network after partial section of a branch of the left chorioallantoic artery is described in the chick embryo chorioallantoic membrane. After several hours (24-72), the defect of the artery is consistently by-passed by a collateral interconnecting of the stumps of the damaged vessel in such a way as to re-establish its continuity, according to a precise scheme which was similar in all cases that underwent the same type of ablation.

KEY WORDS: *morphogenesis, angiogenesis, chorioallantoic membrane*

Two processes may be involved in the formation of blood vessels during embryonic development, vasculogenesis, i.e. development of blood vessels from *in situ* differentiating endothelial cells, and angiogenesis, which is the process through which new blood vessels arise from preexisting ones (Risau and Lemmon, 1988; Risau *et al.*, 1988; Pardanaud *et al.*, 1989; Poole and Coffin, 1989).

The factors and molecular mechanisms involved in the formation of embryonic blood vessels are not completely clarified. The initial establishment of blood vessels seems to be genetically predetermined and, in addition, epigenetic factors, such as metabolic, mechanical or hemodynamic, play a significant role in the vessel formation (Hudlicka, 1991). Angiogenic factors have also been identified in developing organs, such as kidney and brain (Risau, 1986; Risau and Ekblom, 1986; Breier *et al.*, 1992). In areas of the vascular bed which were irreversibly damaged or excised, the restoration of circulation is accomplished by an ingrowth of new capillaries to reestablish a new vascular network (Schoefl, 1964). *In vitro,* a wounded confluent monolayer of endothelial cells has been used to study the mechanisms and the kinetics of endothelial cell regeneration (Sholley *et al.*, 1977; Ryan *et al.*, 1982).

The chorioallantoic membrane (CAM) of the chick is supplied by two primary chorioallantoic arteries drained by a single chorioallantoic vein and its vascular network is currently used as an assay to study *in vivo* the effects of angiogenic and anti-angiogenic substances (Auerbach *et al.*, 1991).

In this study the CAM has been utilized to analyze the morphological patterns of reorganization of blood vessels *in ovo* after the partial section of a branch of the left chorioallantoic artery.

The chosen vessel (Fig. 1A) was cut and a portion of it was removed causing serious hemorrhage (Fig. 1B). After a short time (1-3 min), a clot which corresponded to the proximal and distal vessel stumps was formed (Figs. 1C, 2B). At the level of the distal part of the lesion, the vessel was of a reduced calibre (Fig.1D, arrowhead) and its small collaterals appeared exsanguine (Figs. 1D, 2C, double arrowheads).

A reorganization of the vascular network took place 24-72 h later: the vascular interruption was by-passed and the continuity of the vessel was re-established. The frequency of occurrence of the described mechanism of revasculature was of 70% of the total number (50) of the performed experiments.

The vessel branches arising from the proximal and distal portions of the interrupted vessel modified their course and grew to the extent that they had been anastomosed (Figs. 1D-F, 2D), by-passing at times over a pre-existing vessel (Fig. 1F, arrowhead). Thus, a half formed vessel connected the two stumps of the lesioned vessel (Figs. 1F, 2F).

Sometimes, a small vessel appeared directly connecting the proximal stump to the distal one (Fig. 2E, arrowhead). Once the continuity between the two stumps was guaranteed by the process previously described, this smaller vessel regressed (Fig. 2F, the area marked by an asterisk).

One of the most debated problems regarding the dynamics of embryonic development, concerns the possibility of establishing the existence of morphogenetic fields which promote the growth and organization of biological systems, a vascular one for example, and how these fields can be modified by environmental influences (Thompson, 1917; Thom, 1980; Bard, 1990).

As far as the vascular system is concerned, the development of a new vascular geometry, i.e. a network, from one or more blood vessels, appears to be the consequence of a balance of physical

Abbreviations used in this paper: CAM, chorioallantoic membrane.

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0214-6282/94/$03.00

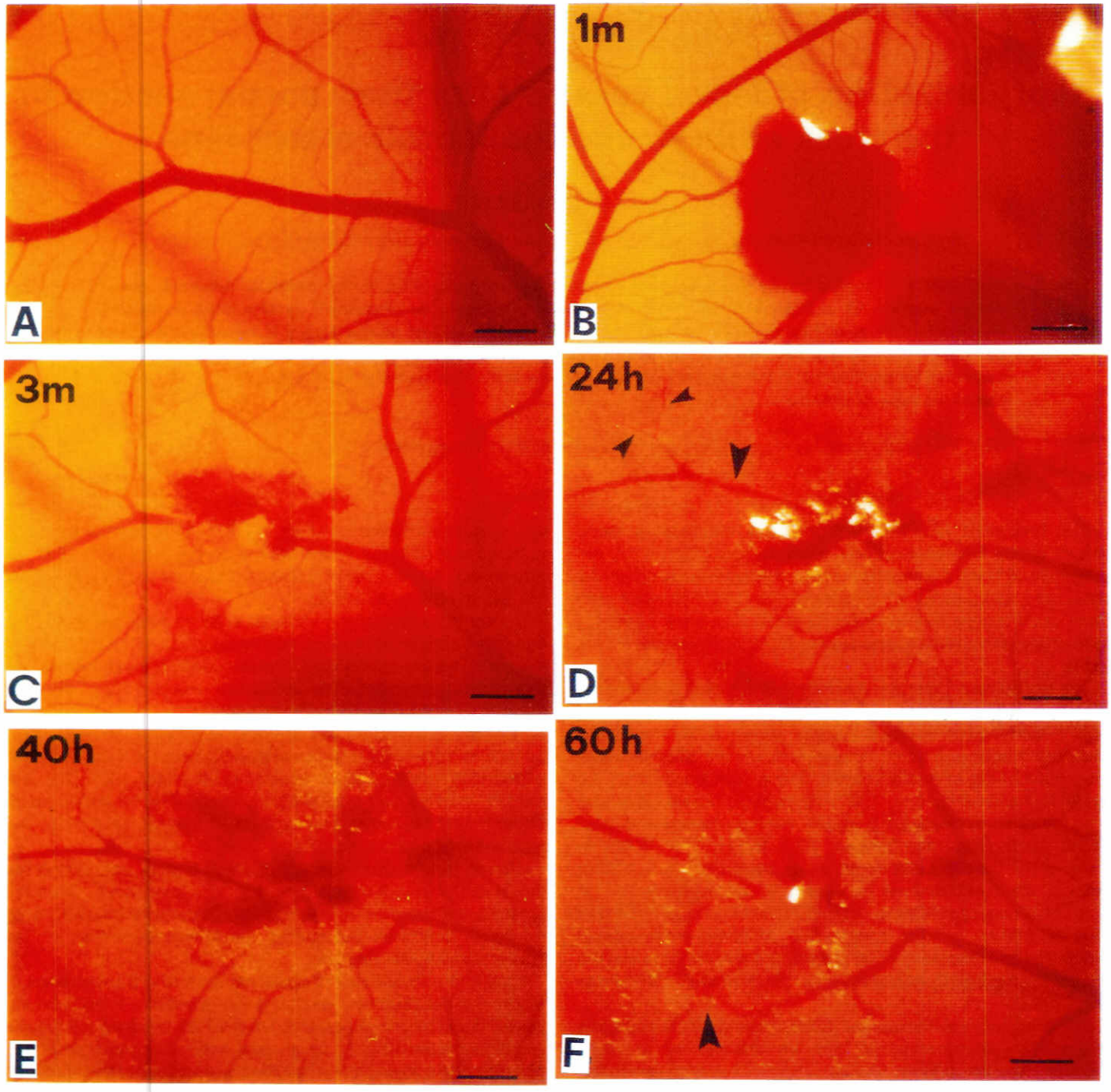

Fig. 1. Photographic time-lapse sequences of the reorganization processes in two chick embryo CAM's vascular networks after a partial section of a branch of the left chorioallantoic artery. *Scale bar, A,C, 500 μm; B, 1.25 mm; D,E, 310 μm; F, 200 μm.*

forces and the geometry of a living tissue (Wakman, 1981; Gaehltgens, 1992).

In our experimental model, the effect of ablation on a branch of an artery vessel leads to the reorganization of the vascular network according to a scheme which is different from the original one and functional for the hemodynamic situation, allowing the reconnection of the proximal stump to the distal one through a different route in the shortest time possible. Furthermore, this scheme is identical in all cases undergoing the same type of ablation, depending on the type of balance that has been altered and must be rebuilt.

These events take place in a very short time, between 24 and 72 h, suggesting the formation of collaterals by direct growth of new blood vessels. It is possible that the release of growth factors, specific to the blood vessels and highly concentrated in the chick

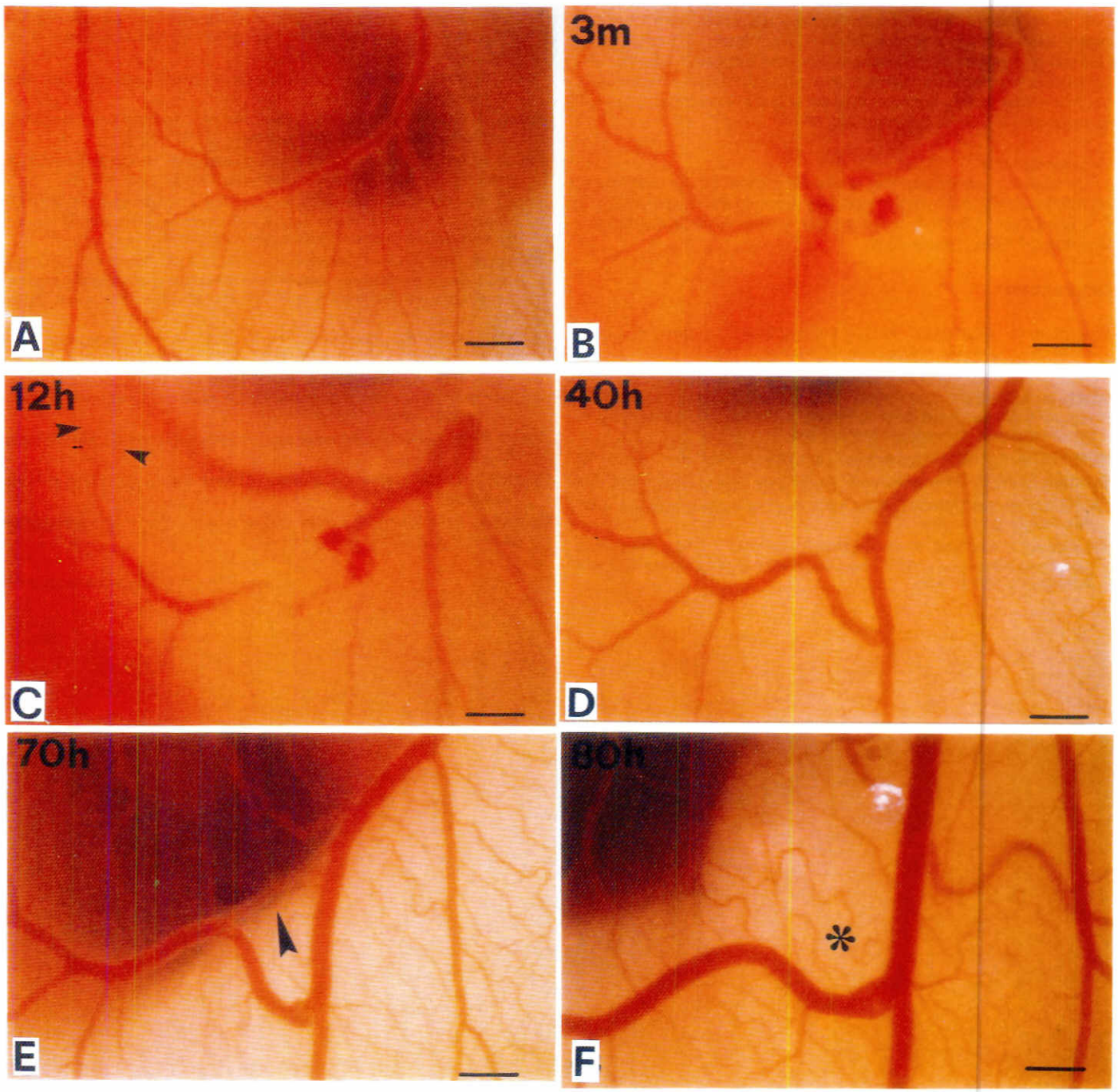

Fig. 2. Photographic time-lapse sequences of the reorganization processes in two chick embryo CAM's vascular networks after a partial section of a branch of the left chorioallantoic artery. *Scale bar, A, 830 μm; B,C,D, 500 μm; E,F, 310 μm.*

embryonic tissues (Risau, 1986; Risau and Ekblom, 1986), occurs besides the hemodynamic and mechanical factors. These angiogenetic factors are responsible for the stimulation of the growth of the pre-existing vessels and the neoformation of others. It is also quite likely that angiogenetic factors, deposited in normal conditions in inactive form in the extracellular matrix (Klagsbrun and D'Amore, 1991), are released after the vessel ablation.

Experimental procedures

The experimental procedures for this study followed the published *Guiding Principles in the Care and Use of Animals* approved by the Council of the American Physiological Society. Experiments were performed on 100 fertilized chicken eggs incubated at 37°C and 60% relative humidity. A square window was opened in the egg shell at the 3rd incubation day (i.d.) after removal of 2-3 ml of albumen to detach the developing CAM from the

shell. At the 6th i.d., the chorioallantoic vascular areas were examined under a Zeiss Stereomicroscope SR and a small tract (1-2 mm length) of a branch of the left chorioallantoic artery was surgically removed. The sequence of the process of reorganization of the CAM vessel network was documented by photographs taken under a Zeiss Stereomicroscope SR equipped with the Camera System MC 63.

Acknowledgments

The author is grateful to Prof. Luisa Roncali for critical reading and helpful comments on the manuscript. This research was supported in part by a grant from "Ministero dell'Università e della Ricerca Scientifica e Tecnologica" (60%), Rome, Italy.

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Accepted for publication: February 1994