Long-term Histopathologic Changes in Canine Aneurysms Embolized with Guglielmi Detachable Coils

Michel E. Mawad, Janine K. Mawad, Joiner Cartwright, Jr, and Ziya Gokaslan

PURPOSE: To evaluate the long-term histologic changes, including those in the ultrastructure of the neoendothelium, occurring in experimental canine aneurysms obliterated with Guglielmi detachable coils. **METHODS:** Ten experimental aneurysms were surgically created in mongrel dogs using side-to-side jugular carotid fistulas that were subsequently ligated to form blind pouch venous aneurysms dependent on the carotid circulation. The aneurysms were obliterated with Guglielmi detachable coils, and the animals were kept in observation. Six months after the endovascular obliteration of the aneurysms, repeat carotid arteriography was performed to assess for potential recanalization of the aneurysms. The animals were then killed and submitted for autopsy. The carotid artery and the embolized aneurysm were resected and studied with light and electron microscopy. **RESULTS:** Both completely obliterated and recanalized aneurysms were excluded from the parent circulation by an endothelialized layer of connective tissue. The fundus of the aneurysm was completely obliterated by heavy reactive fibrous tissue surrounding the coils with very minimal, if any, inflammatory reaction. The neointima is composed of three well-identifiable layers, the most superficial of which is formed of new endothelial cells positioned next to each other in a cobblestone fashion over a basal membrane. **CONCLUSION:** In the absence of histologic data in human aneurysms obliterated with Guglielmi detachable coils, several observations made in our experimental study help in the understanding of the long-term results expected from this endovascular technique.

Index terms: Aneurysm, embolization; Interventional instrumentation, coils; Interventional neuroradiology, experimental; Animal studies

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The use of endovascular techniques in the treatment of intracranial aneurysms has evolved over the last few years. Whereas detachable balloons were once the treatment of choice, they have been abandoned because of the high percentage of recanalization and recurrence rate. Electrothrombosis of aneurysms using Guglielmi detachable coils (GDCs) is proving effective in the endovascular obliteration of aneurysms and their exclusion from the parent circulation (1, 2). Long-term histologic studies in human aneurysms treated with GDCs are lacking. We undertook an experiment in the dog to study the histopathologic changes induced by the GDC in the lumen and over the neck of the aneurysm, with special attention directed to the regrowth of the endothelium.

Materials and Methods

Ten experimental aneurysms were surgically created in mongrel dogs using side-to-side direct jugular carotid fistulas. The fistula was left to mature for approximately 10 to 15 days. Subsequently the cephalad and caudal ends of the draining vein were ligated, thus creating a blind pouch venous aneurysm dependent on the carotid circulation (Fig 1) (3–5). Carotid digital subtraction arteriograms were obtained after the ligation of the vein to document the creation of the aneurysm and patency of the carotid artery (Fig 2). The arteriograms were stored on magnetic tape for future comparison. The 10 aneurysms then were embo-

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From the Departments of Radiology (M.E.M.), Pathology (J.C.Jr), and Neurological Surgery (Z.G.), Baylor College of Medicine; and the Department of Pathology (J.K.M.), Park Plaza Hospital, Houston, Tex.

Address reprint requests to Michel E. Mawad, MD, Department of Radiology, The Methodist Hospital, MS# 033, 6565 Fannin, Houston, TX 77030.

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Fig 1. Surgical technique of creating a blind pouch venous aneurysm dependent on the carotid circulation.

lized under general anesthesia and systemic heparinization using electrothrombosis and GDCs. An effort was made to pack the aneurysms tightly with coils, although the degree of packing varied from one aneurysm to another. Postembolization arteriograms were obtained for all of the aneurysms treated with this technique. Six months after the endovascular treatment, repeat carotid arteriography was performed to verify the obliteration of the aneurysm and to assess for potential recanalization. The follow-up arteriogram was compared with the initial postembolization angiographic study. The animals were then killed and autopsies limited to the tissues of the neck were performed. The carotid artery, the embolized aneurysm, and the surrounding soft tissues were resected en bloc and submitted for pathologic examination. The carotid artery was opened along its longitudinal axis in a plane opposite the neck of the aneurysm. The gross appearance of the neck of the aneurysm was carefully examined and photographed. A Faxitron (Field Emission Corp MC, Minniville, Ore) radiograph of four of the specimens was obtained. The entire specimen then was fixed in formalin. The cross-sectioning of the specimen was severely hindered by the presence of the tightly packed coils. To cut the specimen, the coils had to be carefully and tediously removed. This was done from the fundus side of the aneurysm to avoid the tearing of the new intimal layer covering the neck. The cross-sectioned specimen was embedded in paraffin. Histologic sections were obtained and stained with hematoxylin and eosin. Additional elastic and trichrome stains also were obtained.

Four of the 10 specimens were submitted for electron microscopic studies of the intimal layer covering the neck of the aneurysm. To obtain optimal fixation of the endothelium, antemortem perfusion of the carotid artery with gluteraldehyde was performed through the angiography catheter. After the fixation, the intimal layer covering the neck of the aneurysm was carefully peeled off the underlying platinum coils, postfixed in 2% osmium tetroxide, and then submitted for scanning and transmission electron microscopy. For scanning electron microscopy, the specimen was critical point dried in liquid CO_2 and absolute alcohol and coated with gold in a sputter coater. For transmission electron microscopy, the specimen was dehydrated and embedded in epoxy resin. One-micron-thick sections were obtained as a first step. After orientation, thin sections (20 nm) were obtained and stained with uranyl acetate and lead citrate. Both electron microscopy studies were performed on a JEOL 100-C Temscan (Jeol, Tokyo) electron microscope.

Results

Angiographic Results

Nine of the 10 aneurysms were completely obliterated with coils at the time of initial embolization, although the degree of packing of the coils varied from one aneurysm to another. Six of the 9 completely obliterated aneurysms remained occluded on the follow-up arteriogram performed 6 months after the endovascular treatment (Fig 3). The remaining 3 aneurysms showed evidence of recanalization on the follow-up arteriogram. In all 3 recanalized aneurysms, the platinum coils were loosely posi-

Fig 2. Carotid arteriogram after the ligation of the draining veins documents the formation of the blind pouch venous aneurysm and the patency of parent carotid artery.

Fig 3. Long-term angiographic results in completely obliterated aneurysms. Follow-up carotid angiogram obtained 6 months after the embolization shows persistent and complete obliteration of the aneurysm.

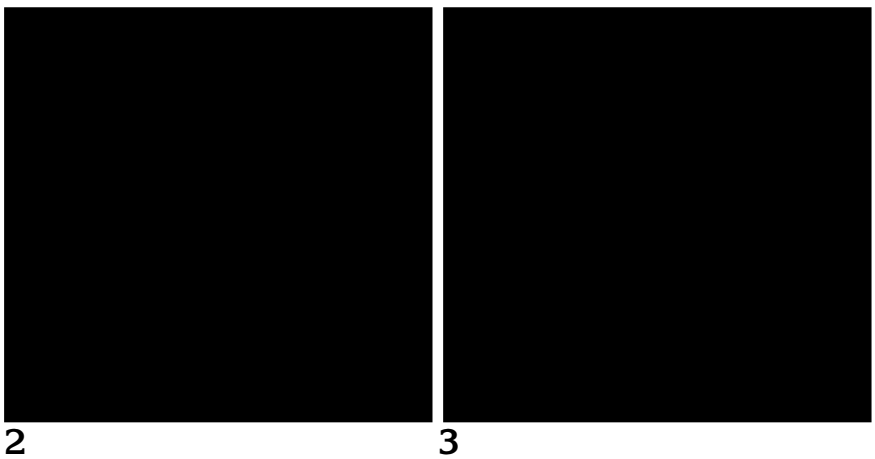

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Fig 4. A smooth, thin, and transparent continuous layer of neointima covers the neck of completely obliterated aneurysms. The convoluted coils can be seen through the transparent membrane.

Fig 5. A similar continuous thin layer of neointima covers the neck of incompletely obliterated or recanalized aneurysms.

tioned in the fundus and underwent reorientation and compression. One of the 10 aneurysms was incompletely treated at the time of the initial embolization and showed evidence of recanalization on the follow-up arteriogram.

Gross Pathology

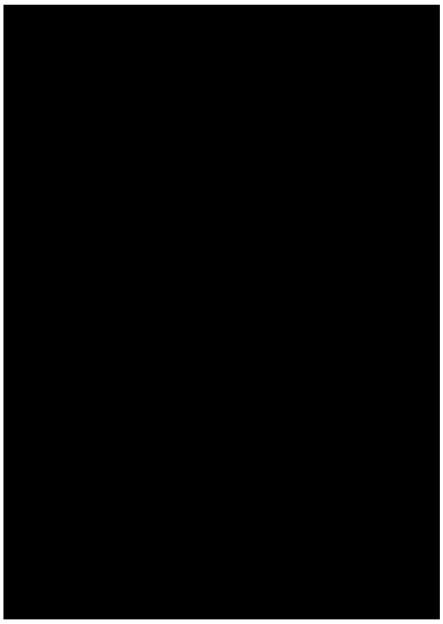
In the six aneurysms that remained obliterated, the gross examination showed the neck of the aneurysm to be completely covered by a smooth, thin, and glistening layer of membranous tissue. The same layer blended imperceptibly with the endothelial surface of the parent carotid vessel and bridged over the underlying coils, which could be seen through it (Fig 4). A similar layer also was found covering the neck of the incompletely embolized and recanalized aneurysms (Fig 5).

Light Microscopy

The aneurysm lumen was obliterated by richly vascularized fibrous tissue, the periphery of which was dense and collagenized. The fibrous tissue completely surrounded and bridged in between the casts of the platinum coils (Fig 6). There was only a mild foreign body reaction associated with the coils. It consisted of a few histiocytes and foreign body–type giant cells lined along the casts of the coils (Fig 7). In the large aneurysms, the center of the fibrous tissue was more cellular and less organized than at its periphery, suggesting a centripetal maturation process. It contained plump fibroblasts, hemosiderin laden histiocytes, and prominent endothelium of newly formed investing capillaries (Fig 8). There was no identifiable thrombus material. In most of the aneurysms, the boundary between the lumen obliterated with coils and the wall of the aneurysm was indistinct. This loss of boundary was most pronounced where the fibrous tissue replaced the muscular layer of the aneurysm wall. The neck of the aneurysm was covered by a new layer of intima (neointima) growing over and tightly adhering to the coils (Fig 9). This neointima was organized in three identifiable layers. The most superficial layer was made of endothelium continuous with that of the parent carotid artery. The middle layer of the neointima consisted of smooth muscle cells aligned parallel to the long axis of the carotid artery. In the deepest layer of the neointima, the smooth muscle cells were haphazardly arranged and separated by abundant ground substance. Multiple neocapillaries were present in the deep layer (Fig 10). The electron microscopy showed to better advantage the various layers of the neointima.

Scanning Electron Microscopy

This technique provided an "en face" view of the endothelial layer covering the neck of the aneurysm as seen from inside the carotid artery lumen. The neointima covered by endothelium adhered tightly to the underlying coils and invested itself within the crevasses present between them. The manipulation of the specimen often resulted in the tearing and stripping of the neointima from the underlying coils (Fig 11). At higher magnification and where it remained intact, the endothelial layer was formed of adjoining individual cells arranged in a typical cobblestone pattern (Fig 12). The scanning electron microscopy showed the geographic distribution



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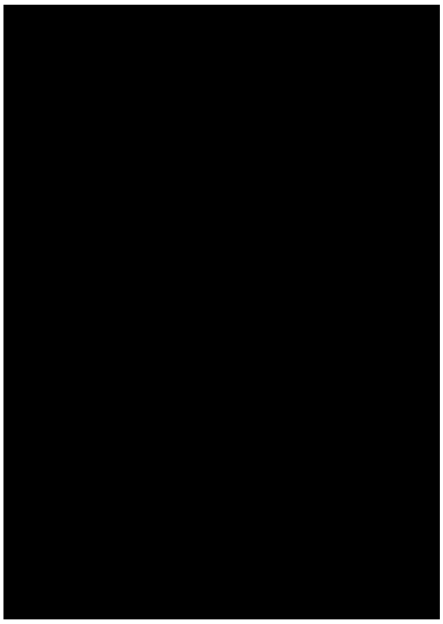

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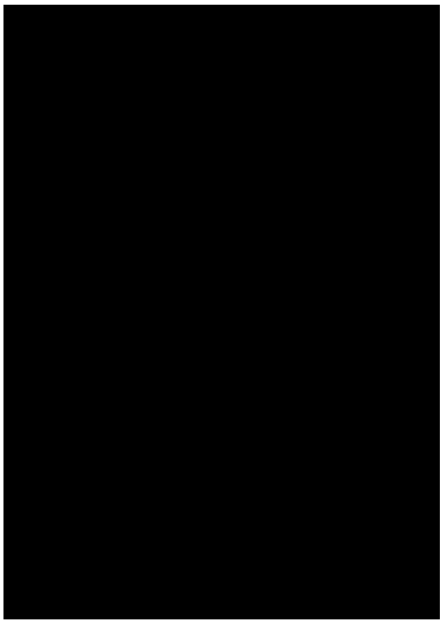

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Fig 6. The casts of the coils (*arrows*), which have been removed for cutting purposes, are seen embedded in dense fibrous tissue obliterating the aneurysm lumen (hematoxylin and eosin stain; magnification ×25).

Fig 7. A small number of multinucleated foreign body–type giant cells (*arrow*) are stretched along and abutting the periphery of the coil casts (*curved arrow*) (hematoxylin and eosin stain; magnification ×200).

Fig 8. In the center of the largest aneurysms, there is organizing granulation with newly formed capillaries and prominent fibroblasts (hematoxylin and eosin stain; magnification ×400).

of the continuous endothelial layer over the randomly arranged coils exquisitely (Fig 13).

Transmission Electron Microscopy

The transmission electron microscopy showed to better advantage the neointima layers (Fig 14) and confirmed the true endothelial nature of the continuous, most superficial cellular layer of the neointima. The elongated cells were supported by a continuous basal lamina and connected to each other by cell to cell junctions (Fig 15).

The cytoplasmic membrane showed numerous pinocytotic vesicles and a few short villous luminal projections (Fig 16). Rare Weibel-Palade bodies were found in the cytoplasm. In the midlayer, the individual smooth muscle cells were separated by collagen fibers and a granular matrix. Their cytoplasm was filled with thin filaments with dense bodies and attachment plates (Fig 17).

Discussion

There are a number of reports in the literature dealing with the experimental obliteration of intracranial aneurysms with tissue adhesive (6),

Fig 9. Elastic stain shows the transition between the parent vessel wall (*arrow*) and the neointima (*double arrow*) covering the casts of the coils (magnification ×100).

Fig 10. The neointima, which separates the lumen of the parent vessel from the coils, is organized in three identifiable layers: a neoendothelium (*arrow*), a midlayer of smooth muscles (*star*), and a deep collagenized layer (*open star*) (polychromatic stain; magnification ×400).

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Fig 11. Scanning electron microscopy shows the neointima investing itself over and between the coils. Note the artifactual tears of the neointima caused by handling and processing of the specimen (magnification ×100).

Fig 12. The lumenal side of the neointima consists of a continuous layer of endothelial cells, which are arranged in a uniform cobblestone pattern seen here "en face" on scanning electron microscopy (magnification ×1500).

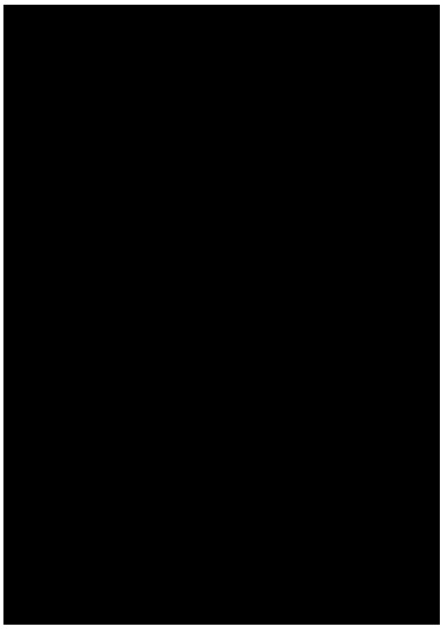
Fig 13. The neoendothelium covers the coils and insinuates itself in the shallow crevices formed between them (scanning electron microscopy; magnification ×1000).

ferromagnetic silicone, and iron-acrylic (7). Histologic data in human aneurysms obliterated with occlusive devices or material are, however, lacking. Guglielmi (1) and Ahuja (8) documented some of the histologic changes occurring in experimental aneurysms obliterated with GDCs. In the absence of long-term histologic data in human aneurysms treated with GDCs, several observations made in our experimental protocol can help in the understanding of the long-term results expected from this technique. Although the hemodynamics of our experimental animal model (9) do not resemble those found in human aneurysms, the histologic data remain reliable.

The follow-up arteriograms obtained 6 months after the endovascular treatment showed a direct correlation between the degree of packing of the coils and the potential recanalization of the aneurysms. Densely packed aneurysms have a significantly higher chance of remaining obliterated than those aneurysms in which the coils were loosely packed. Our experimental aneurysms had a relatively large neck, which is a complicating factor predisposing to recanalization particularly in those aneurysms that were insufficiently filled with coils.

Our study demonstrated the regrowth of a new intima layer over the neck of all aneurysms filled with coils. Both completely obliterated and recanalized aneurysms were isolated from the parent circulation by this neointima, which was covered by endothelium. A similar endothelialized layer of connective tissue obliterating the mouth of experimental aneurysms was reported by other investigators (5, 10) after direct injection of Bucrylate in the aneurysm. In that particular model, early platelet adhesion and agregation over the injected Bucrylate was the initiating event (10). Our study, however, details the late endothelial changes at the neck of the aneurysm.

The possible significance of the neoendothelial layer remains unclear in terms of its role in the prevention of recanalization of aneurysms obliterated with coils, particularly those that were loosely packed with platinum. Nevertheless, the absolutely smooth and regular nature of the new endothelial layer is encouraging and suggests that it plays a major protective role against platelet aggregation and thrombus formation. We should, however, emphasize that all of our histologic studies were performed several months after the obliteration of the aneurysms and therefore do not reflect the early changes that may occur at the interface between the platinum coils and the lumen of the parent artery. Specifically, any potential thrombotic complication that can occur at the surface of the coils in the early hours or days after the obliteration of the aneurysms cannot be excluded nor inferred based on our long-term and somewhat late histologic studies. More so, we cannot predict from our findings when and how early the layer of new endothelium develops over the neck of the obliterated aneurysms. In clinical practice, therefore, low-dose anticoagulation and/or antiplatelet therapy may be a useful precautionary measure to prevent platelet aggregation and thrombus formation at the neck of



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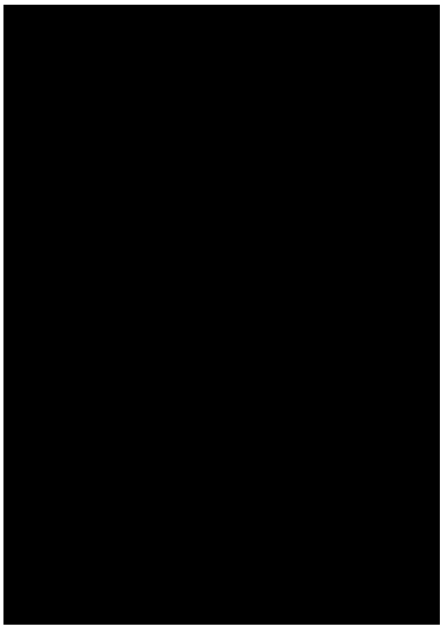

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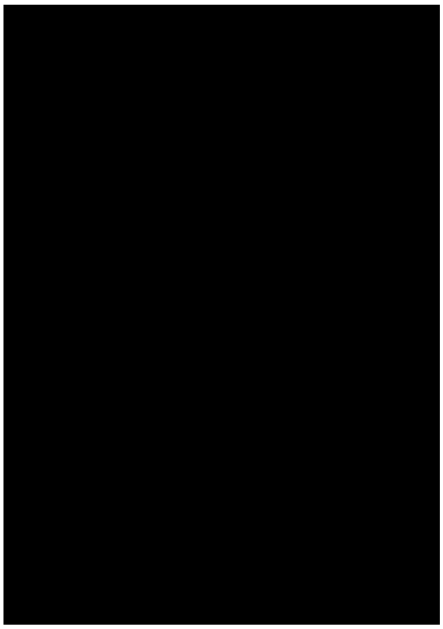

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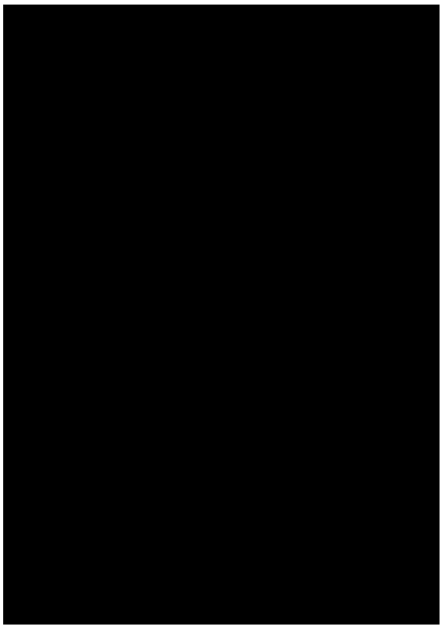
Fig 14. Transmission electron microscopy shows the organization of the neointima in three layers: a luminal endothelium (*arrow*), a midlayer of smooth muscle cells (*open arrows*) arranged parallel to the endothelial surface, and a deep layer of collagen separating haphazardly arranged smooth muscle cells (*arrowheads*) (magnification ×5000).

Fig 15. A continuous basal lamina (*arrows*) runs along the basal endothelial cytoplasmic membrane. Cell-to-cell junctions (*double arrow*) unite the interdigitating apposed endothelial cell borders (transmission electron microscopy; magnification ×48 000).

Fig 16. Numerous pinocytotic vesicles protrude from the endothelial cell cytoplasmic membrane (*arrows*). Note the underlying smooth muscle cell with filaments (*star*) (transmission electron microscopy; magnification ×64 000).

Fig 17. Smooth-muscle cells of the neointima are filled with thin filaments. Typical dense bodies (*arrows*) and attachement plates (*arrowheads*) are also noted (transmission electron microscopy; magnification ×25 400).



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the embolized aneurysm in the few hours or days after its endovascular obliteration.

Guglielmi et al (1) demonstrated a similar layer of new endothelium totally lining the free luminal surface of experimental swine aneurysms obliterated with the same platinum coils. As in our study, their results reflect rather long-term histologic changes occurring 2 to 6 months after the endovascular obliteration. Ahuja et al (8) showed earlier electron microscopy changes occurring over the platinum coils within a few hours of the endovascular occlusion. Their experimental work, however, dealt primarily with increasing the thrombogenicity of the coils with the addition of variety of polyurethanes.

Unlike other embolic agents such as fibered coils, derivatives of cyanoacrylate, or polyvinyl alcohol particles (11, 12), the GDCs were associated with a minimal foreign body and inflammatory reaction. In all 10 aneurysms the fundus was filled with variably organized fibrous tissue.

The dense fibrous tissue that tightly adhered to and surrounded the coils was mature and collagenized with no evidence of identifiable thrombus. It is not clear from our study whether the lack of thrombus is related merely to the relatively long time (6 months) between the endovascular treatment and the autopsy or to the fact that thrombus never formed. In prior experimental studies, Moringlane (13) found that organized reactive fibrous tissue had completely replaced the fibrin sealant used to obliterate the aneurysm within 3 weeks after injection. It is conceivable, although purely speculative, that a mechanism other than thrombosis is responsible for the fibrous reaction observed around the coils.

Conclusion

The use of endovascular techniques in the treatment of intracranial aneurysms has become an alternative to surgical clipping. The initial experience with electrothrombosis of aneurysms using GDCs in humans is showing encouraging results although long-term follow-up is still needed. Particularly, long-term histologic studies of human aneurysms treated with GDCs is lacking. Our experimental animal studies offer alternative results that can be extrapolated to the human aneurysms. They convincingly show a dense fibrous reaction that surrounds the coils and results in the obliteration of the aneurysm. The light and electron microscopic findings confirm the unquestionable growth of a thin layer of new endothelium over the surgical neck of the embolized experimental aneurysm excluding it from the parent circulation.

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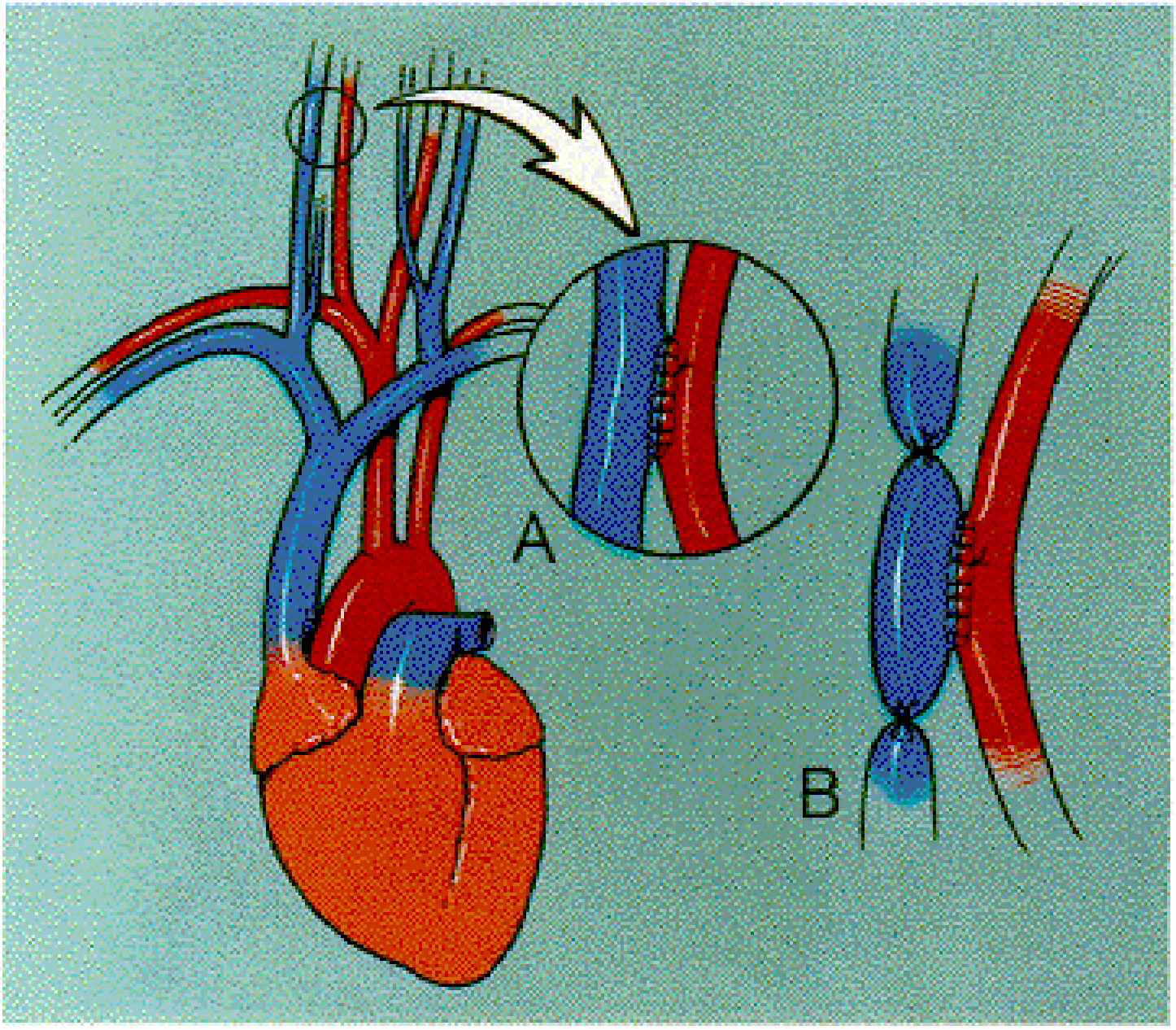
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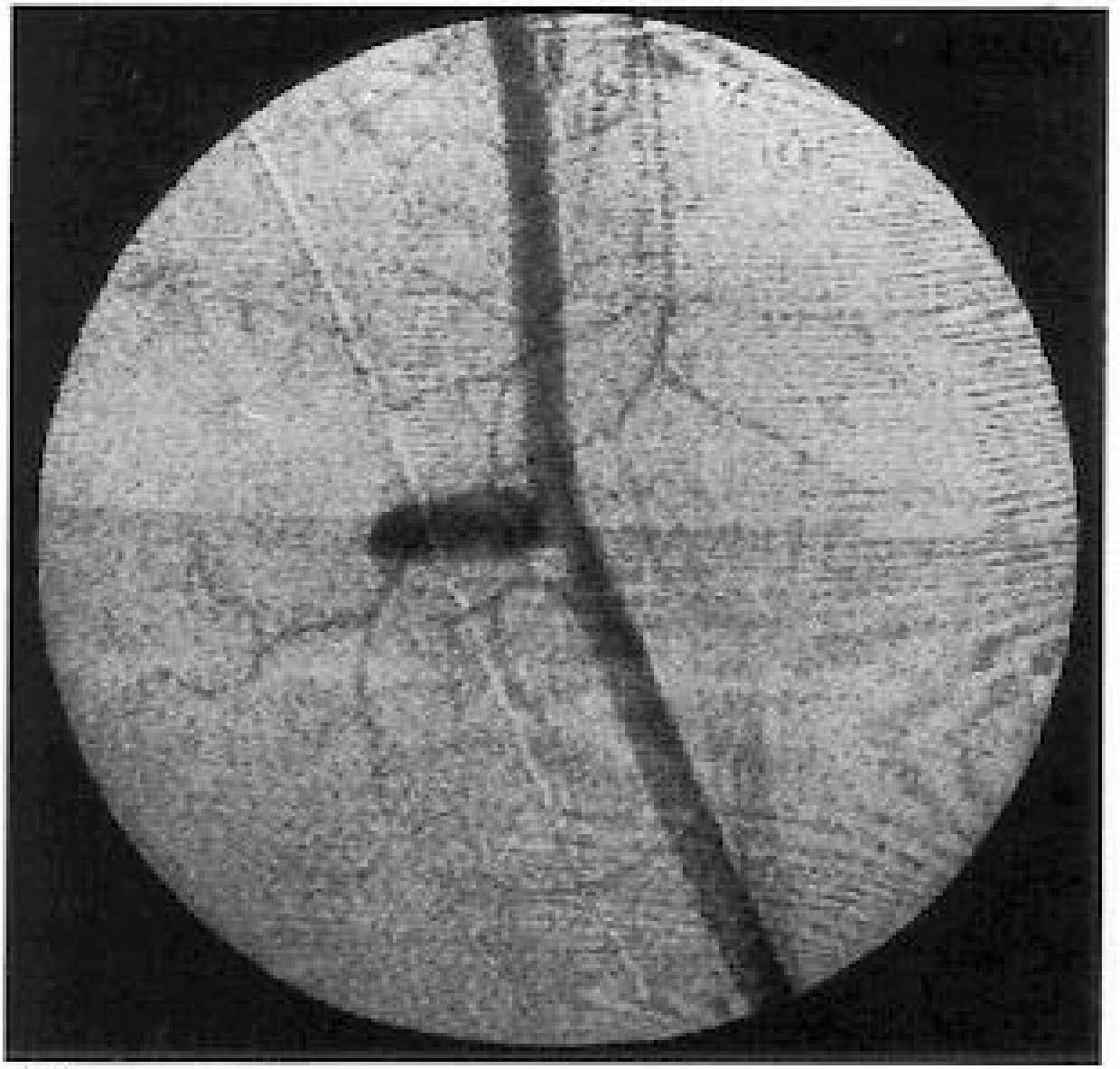

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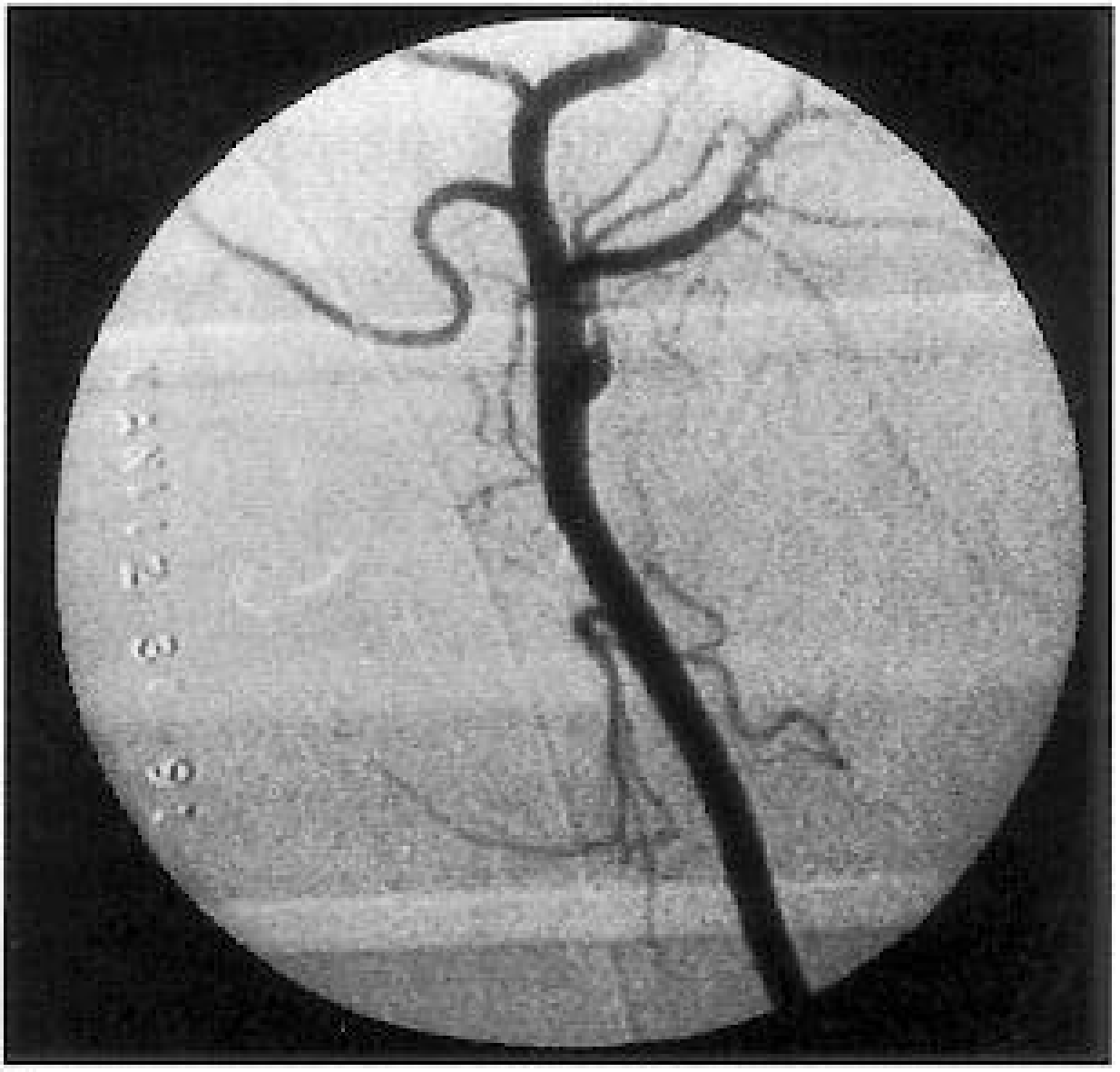

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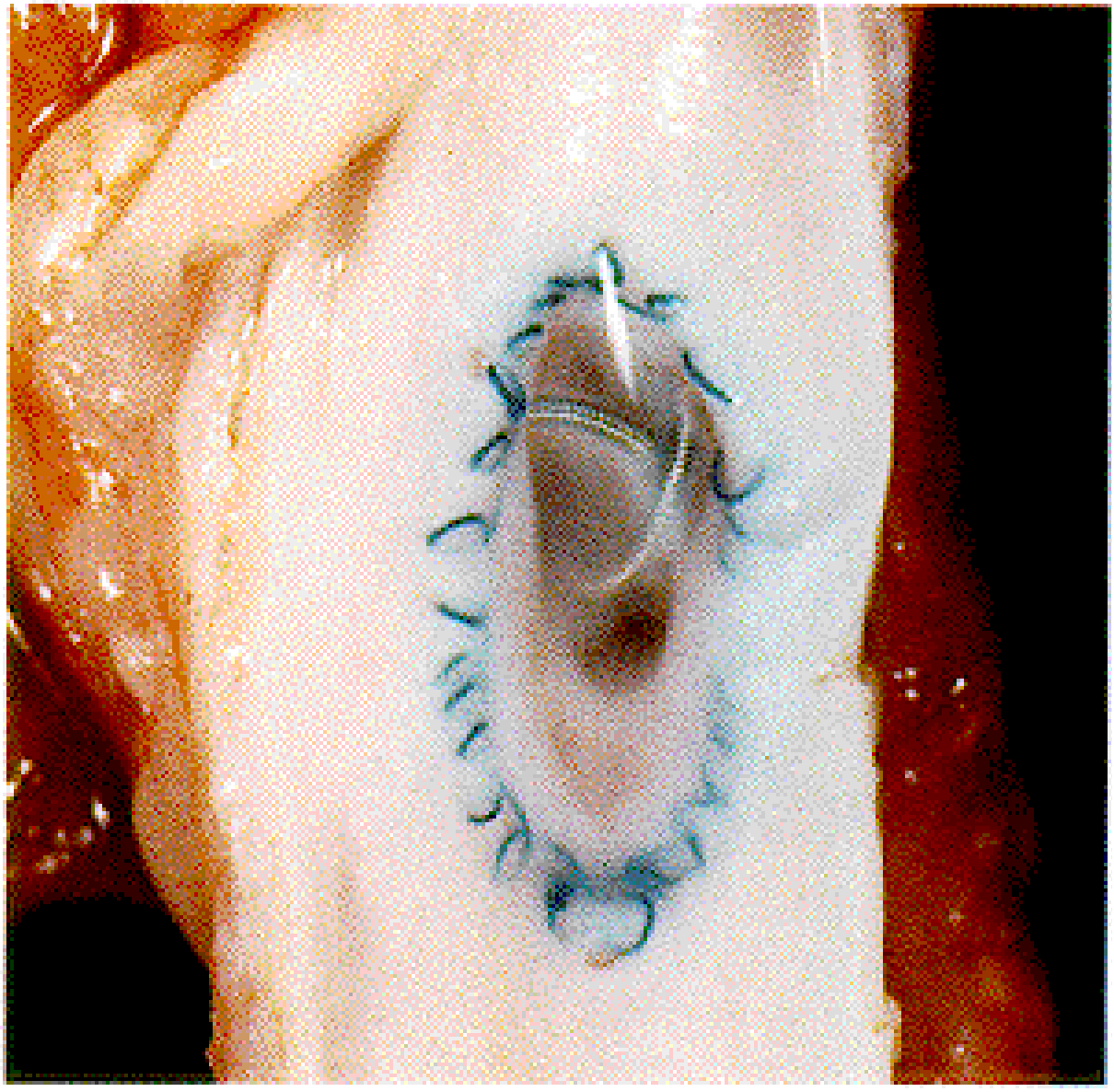

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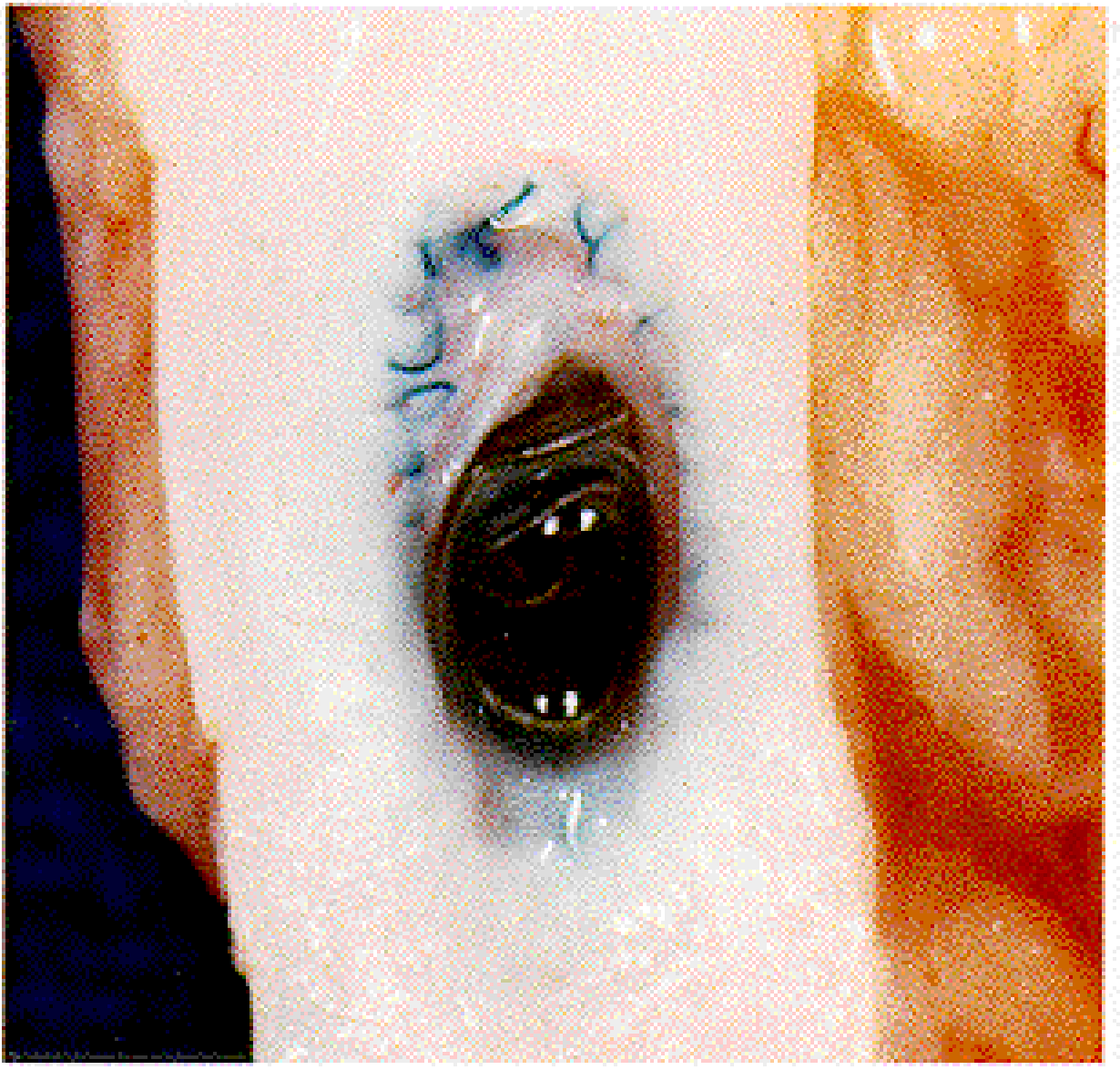

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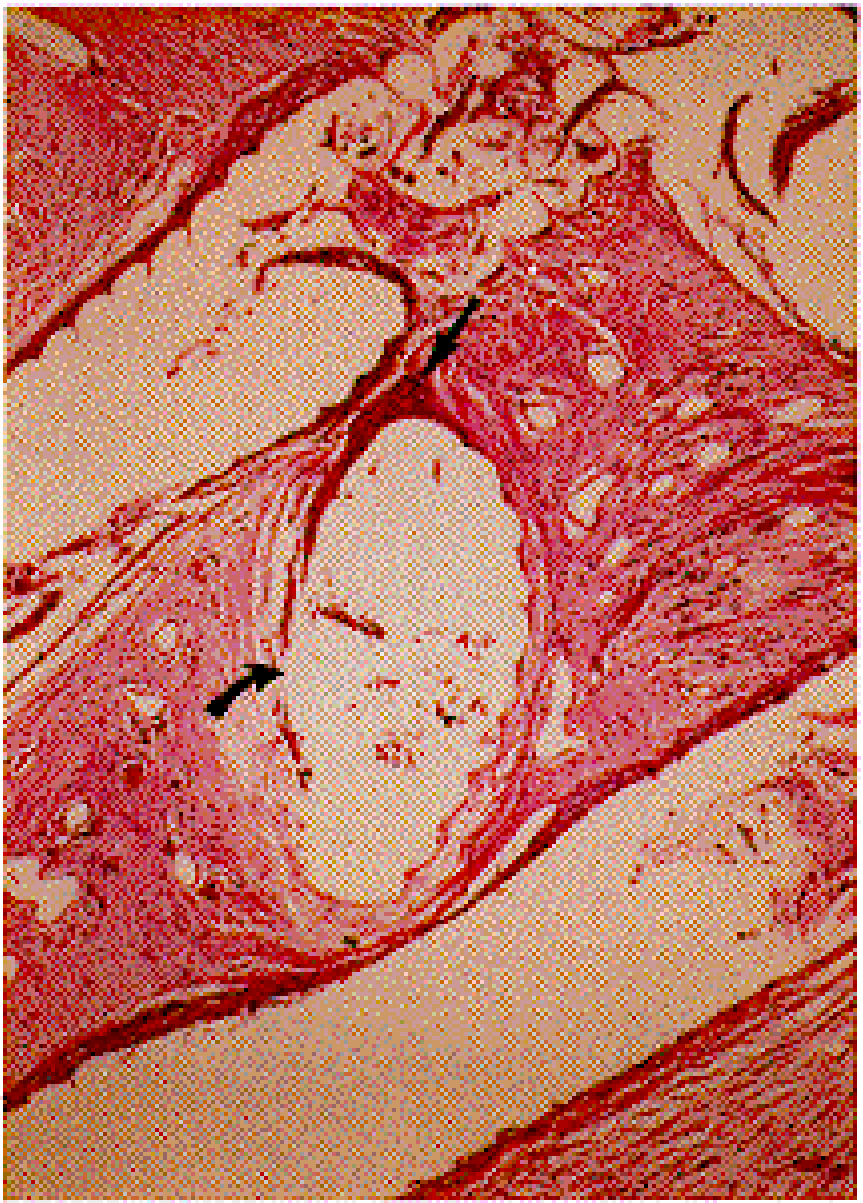

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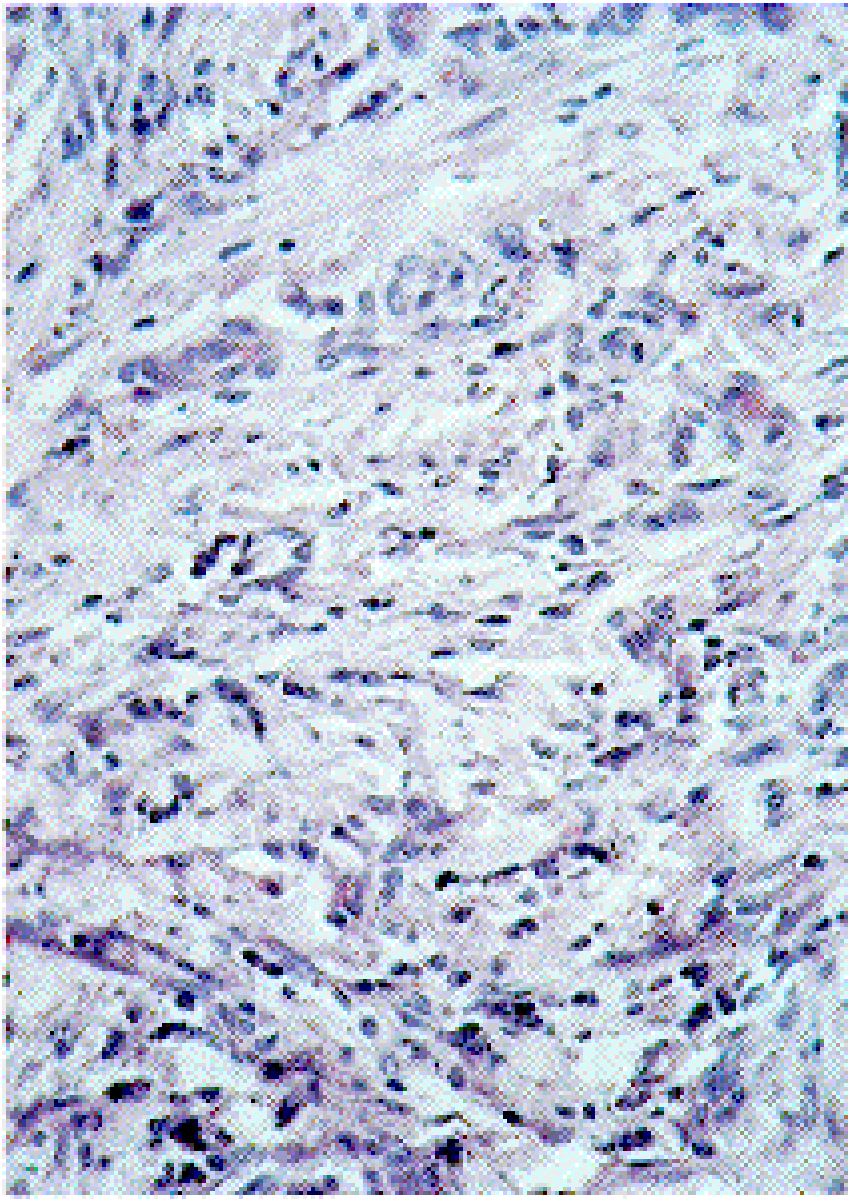

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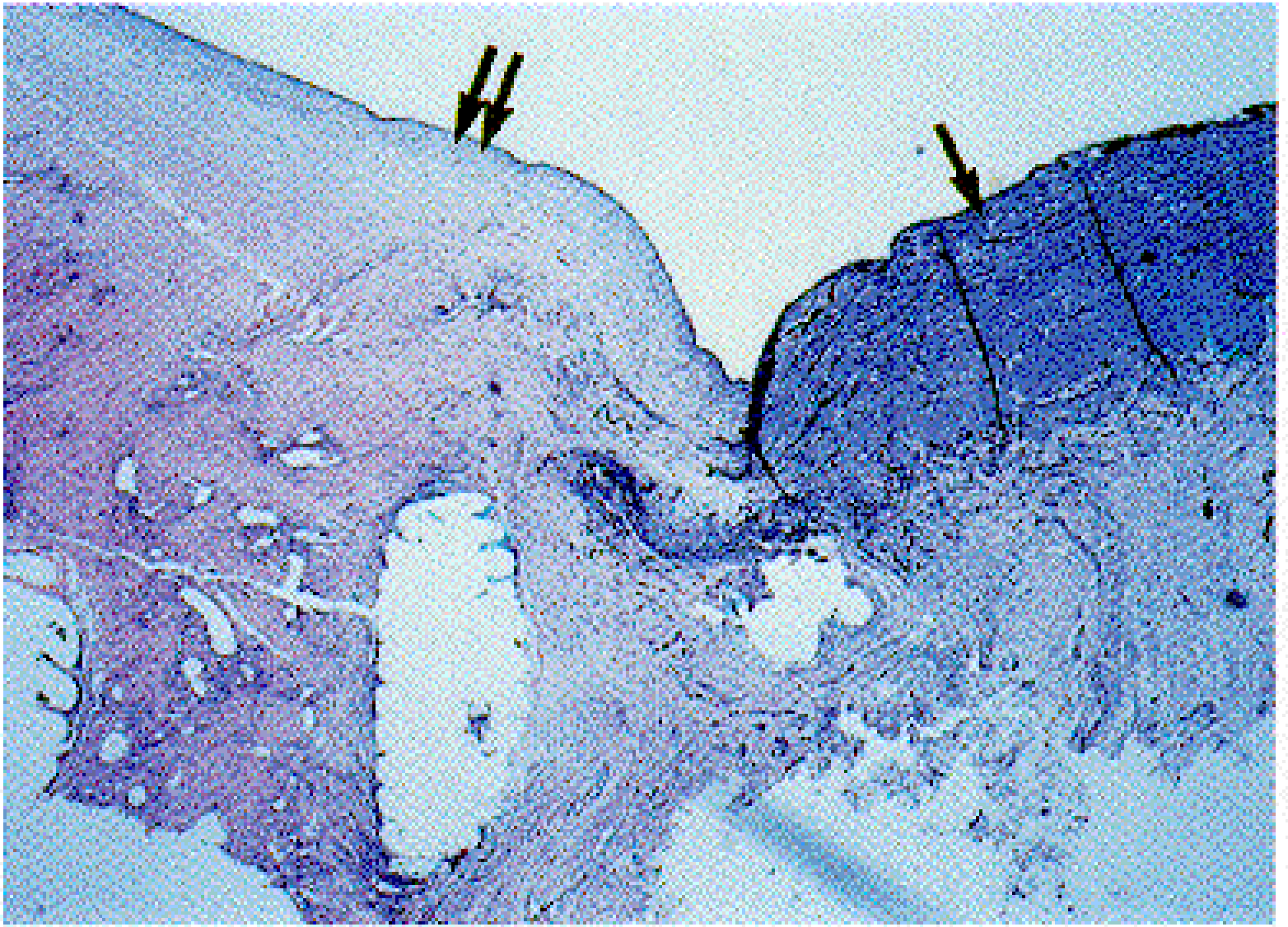

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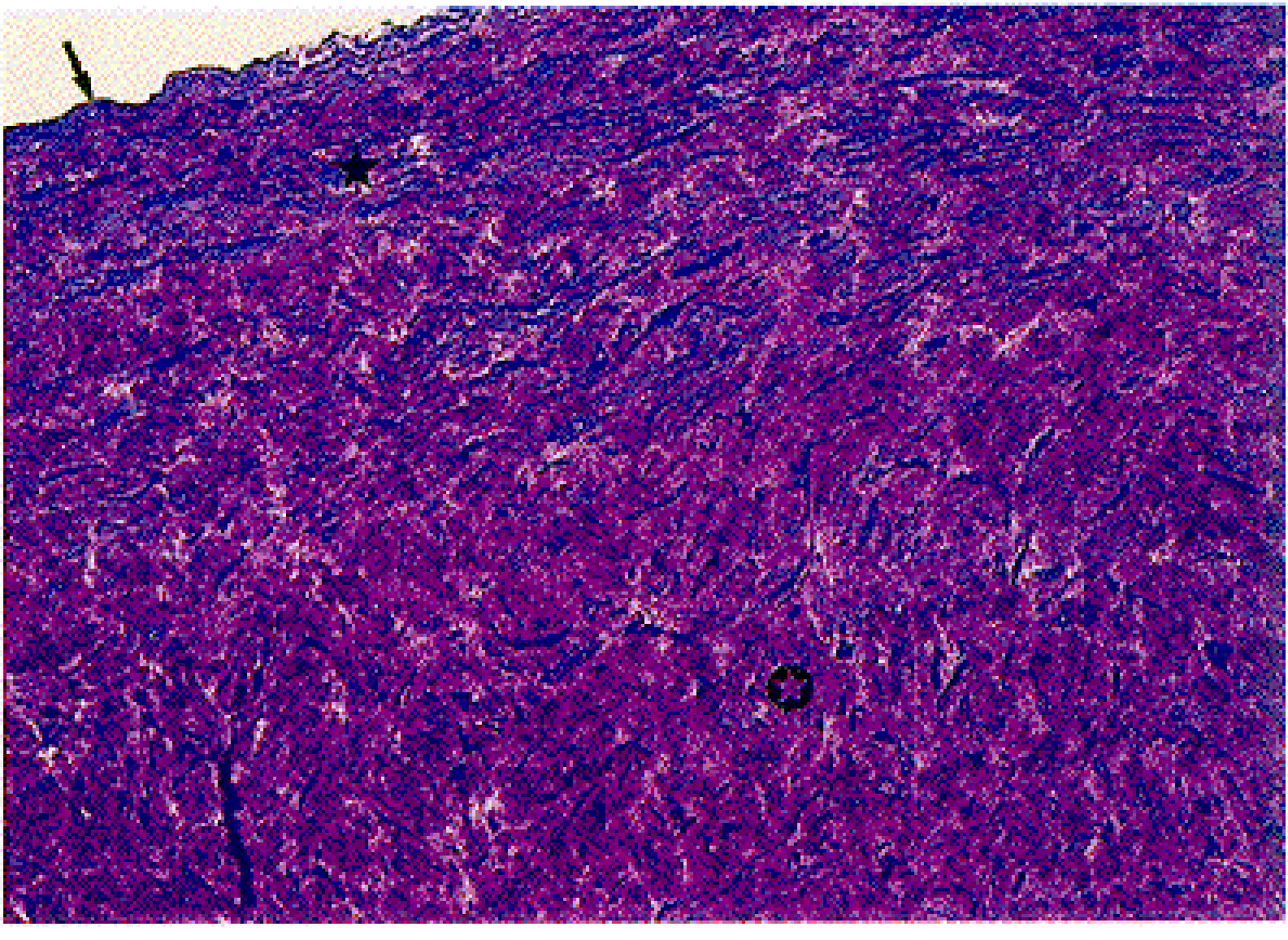

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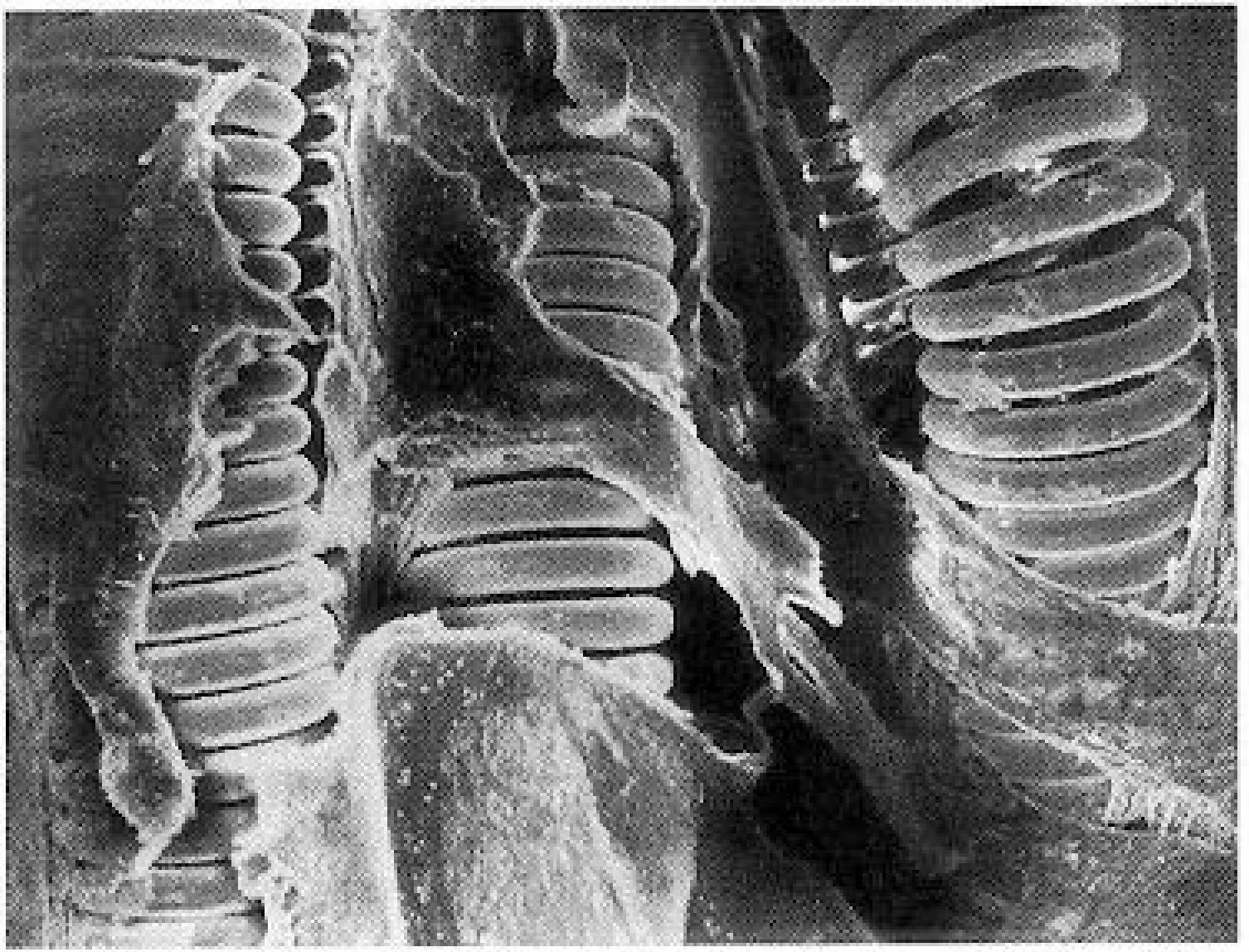

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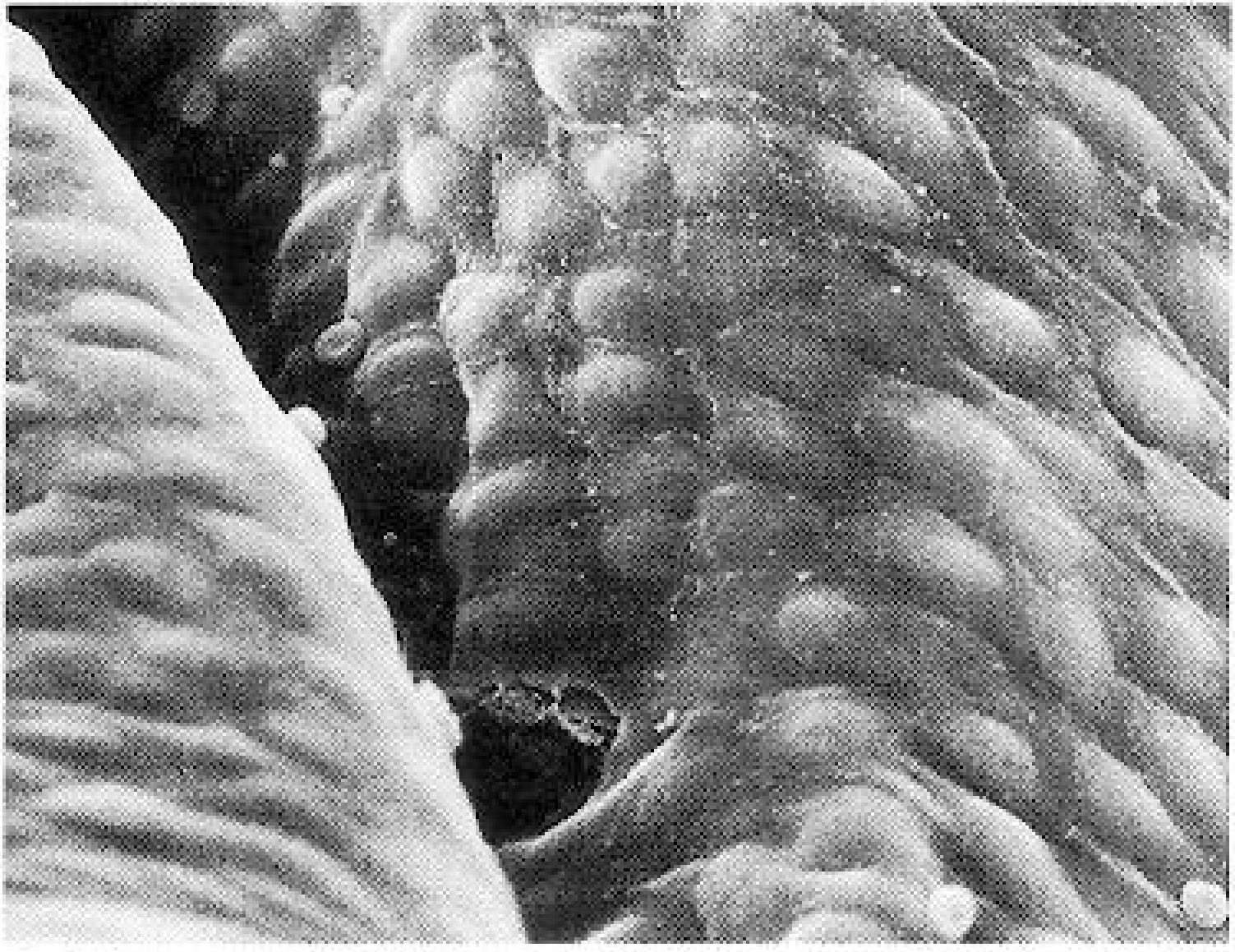

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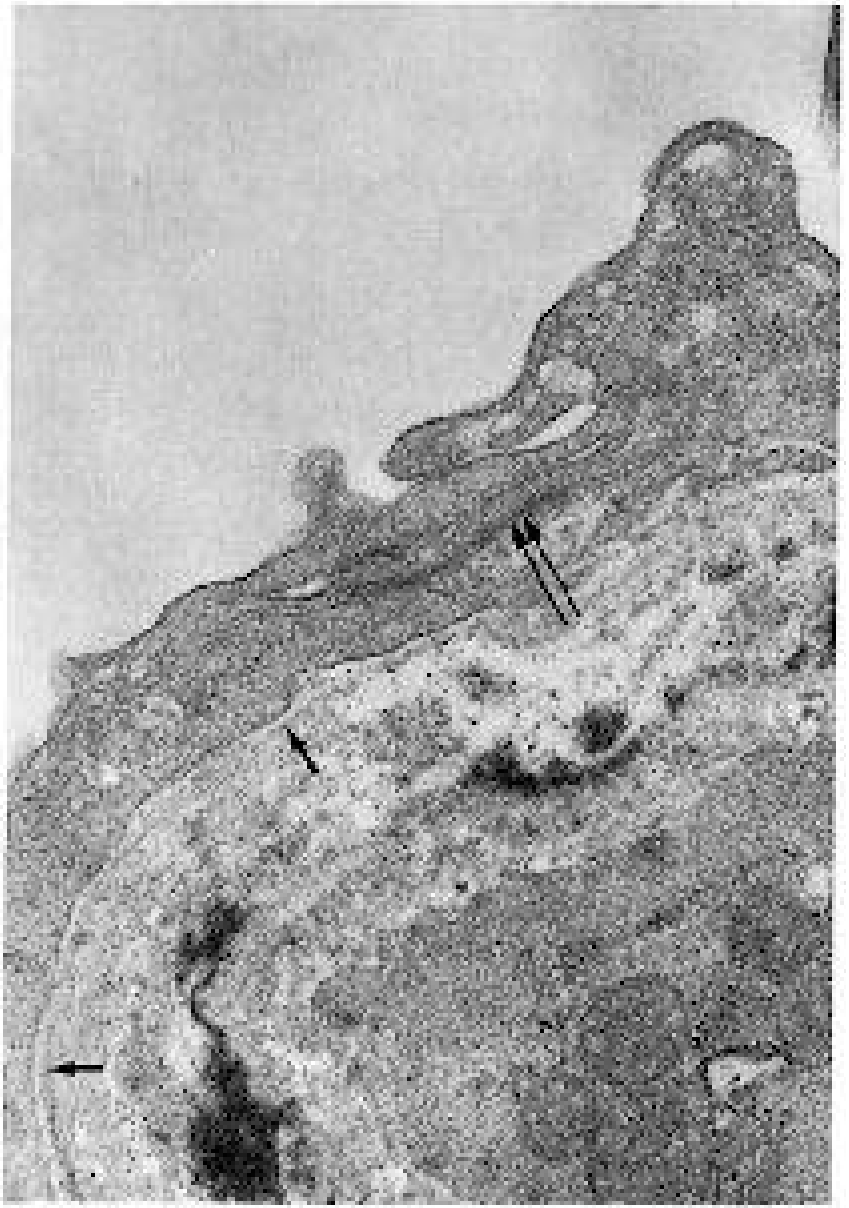

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