collagen fibers is one of the atherosclerosis markers. Taken together, our observations indicate that experimental HC induces pronounced atherosclerotic changes in pial arteries.

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Smooth Muscle Cell Heterogeneity in Intimal Thickenings of Various Genesis and in Organized Thrombi

B. V. Shekhonin, E. M. Tararak, and A. E. Zotikov

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A population of smooth muscle cells heterogeneous by the expression of myosin, h-caldesmon, and calponin is identified by indirect immunofluorescence in the intima of normal aorta and femoral artery, in the subendothelium in atherosclerosis and Takayasu's disease, and in organized thrombi. The similarity of the extracellular matrix in various forms of arteriosclerosis implies that its hyperproduction is a typical response of smooth muscle cells.

Key Words: *smooth muscle cells; intimal thickenings; thrombi*

Smooth muscle cells (SMC) are the major cell type responsible for the synthesis of the extracellular matrix (ECM) in the subendothelial layer. They contribute to the formation of diffuse intimal thickening (DIT) in elastic and muscle-elastic arteries which starts in childhood and propagates with age [9]. Increased synthesis of ECM has been observed in atherosclerosis and other types of arteriosclerosis and in organized mural thrombi. The attention of researchers has been focused on the behavior of SMC in atherosclerotic lesions. It has been hypothesized that in an atherosclerotic plaque SMC appear as a result of migration from the media into the subendothelial layer followed by their proliferation [12, 16]. However, this hypothesis was only indirectly confirmed by experiments with endothelial damage and by a wide variety of phenotypic modifications of SMC cultured *in vitro* [3,18]. So far, it remains unclear how SMC populate the subendothelial layer of the intima during the DIT development, how they appear in atherosclerotic plaques, and whether phenotypic modifications of SMC are specific for athe-

Institute of Experimental Cardiology, Cardiology Research Center, Russian Academy of Medical Sciences; A. V. Vishnevskii Institute of Surgery, Russian Academy of Medical Sciences, Moscow

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rosclerosis. Some progress in solving these problems can be achieved based on phenotypic features of subendothelial SMC. Previously, we used myosin, h-caldesmon, and calponin as SMC markers in normal and atherosclerotic human aorta [2,5]. In the present study we investigated the SMC phenotype not only in the aorta, but also at the distal anastomosis of the femoral artery after the aorta-femoral bypass in atherosclerosis and Takayasu's disease.

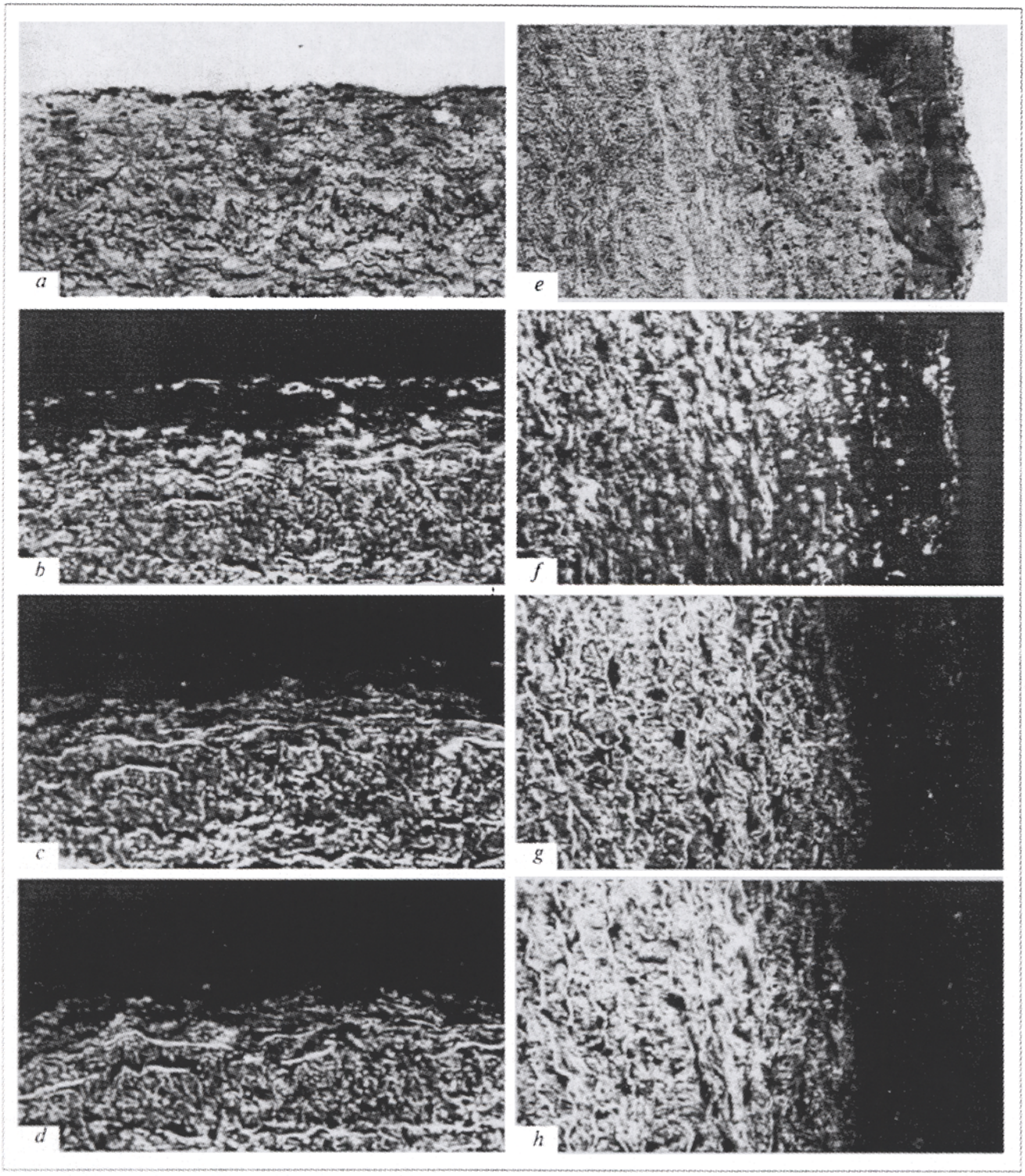

Fig. 1. SMC phenotypes in normal intima and lipid streak. *a-d*) thoracic aorta, 28-year-old man, ×200; *e-h*) lipid streak in the aorta, ×160. *a*, *e*) Oil Red O and hematoxylin staining; *b*, *f*) SMC myosin; *c*, *g*) h-caldesmon; *d*, *h*) calponin.

MATERIALS AND METHODS

Using the method of indirect immunofluorescence, we examined normal intima and atherosclerotic lesions in the thoracic aorta and femoral artery (18 autopsy specimens from 27-68-year-old patients), sections of the distal anastomosis of the femoral arteries removed during reparative surgery in 39-67-year-old patients with atherosclerosis (n=16) and Takayasu's disease (n=3), and mural thrombi from the anastomosis area. SMC were identified with monoclonal antibodies to the SMC myosin heavy chains, h-caldesmon, and calponin [5]. In addition, polyclonal rabbit antibodies to types I, III, IV, and V collagen, fibrinogen/fibrin, fibronectin (Inpharm, Moscow) were used to verify thrombotic masses and to study the organization processes in thrombi. Serial 5-μ sections were prepared in a cryostat and fixed in absolute acetone for 5 min at room temperature. Some sections from each series were fixed in formalin and stained with hematoxylin and eosin, Oil Red O, and by the method of Vergoff to visualize elastic fibers.

RESULTS

In normal intima of the thoracic aorta and femoral artery, a mixed population of SMC was identified by the expression of SMC myosin. Some SMC contained h-caldesmon and calponin, while many endothelial SMC were lacking both markers or one of them, h-caldesmon more frequently (Fig. 1, *a-d*). The phenotype of SMC in the intima remained unchanged with age during the development of DIT.

Lipid streaks were found in the aorta but not in the femoral artery. Staining with Oil Red O revealed extracellular lipids and foam cells in the streaks. SMC were found under the endothelium and among non-muscular foam cells. Most of these SMC did not express h-caldesmon and calponin or one of these antigens (Fig. 1, *e-h*). Uncomplicated fibrous atherosclerotic plaques in thoracic aorta contained SMC with various phenotypes. Some of these cells expressed neither h-caldesmon nor calponin, as it was observed in the subendothelial layer in DIT and in lipid streaks (Fig. 2, *a-d*). Large clusters in which SMC expressed myosin and calponin but not h-caldesmon were also identified. The third SMC phenotype in atherosclerotic plaques was represented by cells that similarly to medial SMC contained not only SMC myosin but also h-caldesmon and calponin. At the distal anastomosis of the femoral artery, complicated atherosclerotic plaques with atheronecrosis, calcification, mural and intramural thrombi were observed in most cases. However, atherosclerotic plaques in thoracic aorta and at distal anastomosis of the femoral artery did not differ in the phenotypical set of SMC.

In the femoral arteries removed during repeated reparative surgery in Takayasu's disease, pronounced sclerosis of the adventitia, atrophy and fibrosis of the media, and marked thickening of the intima were observed. Numerous SMC were identified in the subendothelium by the expression of SMC myosin. Many of these cells lacked both h-caldesmon and calponin or one of these markers (Fig. 2, *e-h*).

In the area of the distal anastomosis of the femoral artery, we usually observed either complete or partial organization of mural thrombi. At the early stages of thrombus organization, macrophages and neutrophils predominated. Later, cell composition changed, and SMC were the main cell type in fully organized thrombi. These SMC were identified by the presence of SMC myosin. Studies of ECM demonstrated its evolution during the connective tissue development. The early stages were characterized by the predominance of type III collagen and pronounced vascularization, while in fully organized thrombi occasional collapsed vessels were seen, and types I and V collagen predominated. In some organized mural thrombi, the content of type IV collagen was increased, collagen being diffusely scattered in ECM. At any stage of thrombus organization the thrombi were rich in firbonectin, while the content of fibrinogen/fibrin varied (Fig. 3, *a-e*). Many SMC in organized thrombi did not contain h-caldesmon and/or calponin (Fig. 3, *f-h*).

Normal intima of the aorta and femoral artery as well as atherosclerotic plaques contain SMC which similarly to embryonal SMC express cytokeratin-8 and ICAM-1 [8,10,15]. Previously, we showed that SMC lacking h-caldesmon and/or calponin are phenotypically similar to embryonal SMC and are present in the intima of adult human aorta and in uncomplicated atherosclerotic plaques [2,5]. This study demonstrates that such SMC are present in lipid streaks, complicated atherosclerotic plaques, intimal thickenings in Takayasu's disease, and organized mural thrombi. It should be noted that SMC were phenotypically identical in various forms of arteriosclerosis. Our findings show that the structure of ECM in organized thrombi is principally the same as that reported previously for atherosclerotic plaques and intimal thickenings in Takayasu's disease [1,19]. Presumably, the activation of ECM synthesis in subendothelial SMC is a typical reaction, although the influence of specific initiating factors cannot be ruled out in each particular case.

Mitotic activity of SMC in lipid streaks and early atherosclerotic plaques is increased [13,14]. In our investigations, large clusters of phenotypically identical SMC, which most probably stem from one pre-

cursor cell, were often observed in different sites of an atherosclerotic plaque.

We think that progression of an atherosclerotic plaque may be periodically attended by enhanced SMC proliferation. Presumably, proliferation of SMC in the subendothelium occurs in Takayasu's disease and during thrombus organization. The question arises: what type of precursor cells (populating the

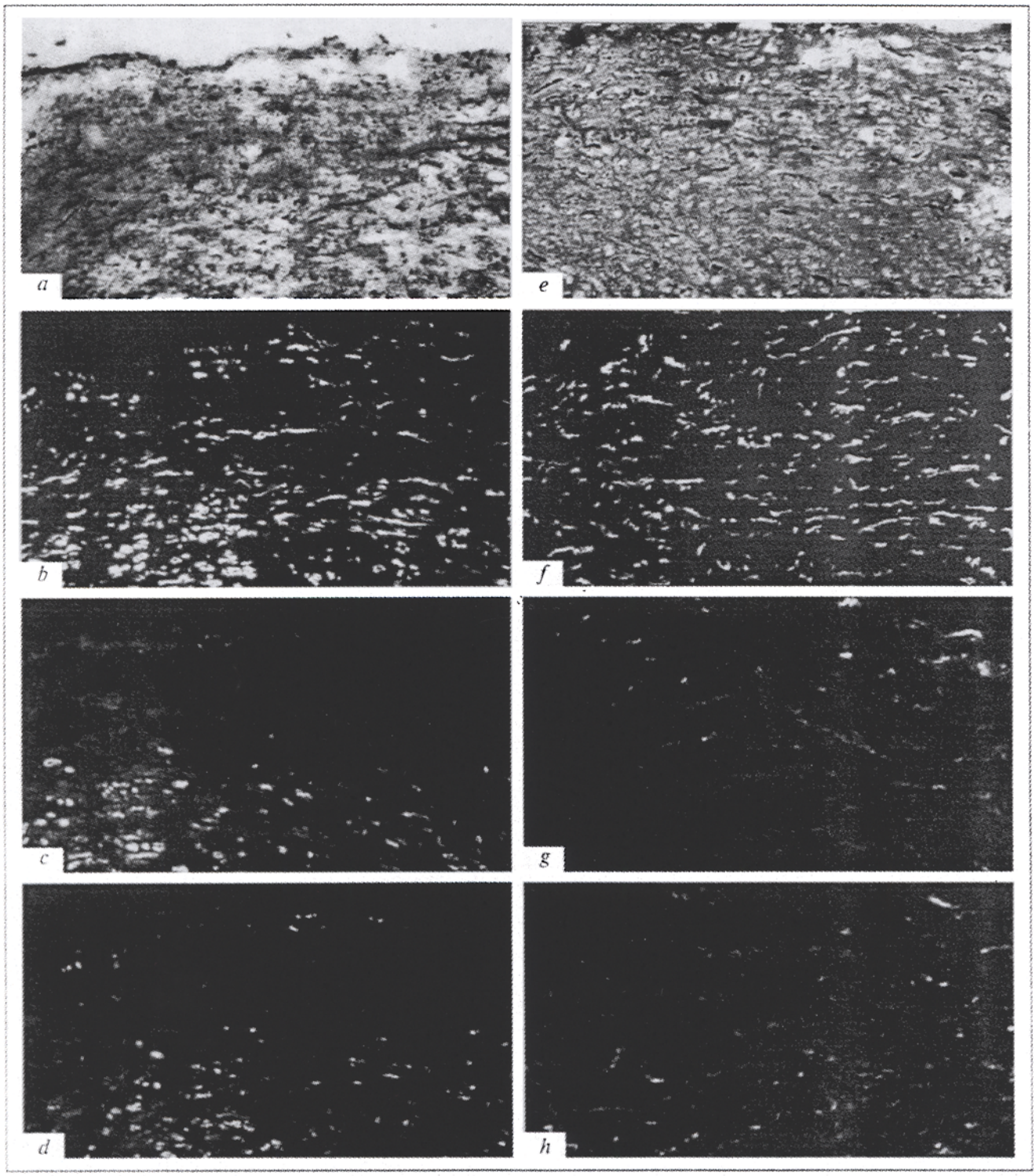

Fig. 2. SMC phenotypes in an atherosclerotic plaque and intimal thickening in Takayasu's disease, ×160. *a-d*) uncomplicated fibrous atherosclerotic plaque in the thoracic aorta; *e-h*) intimal thickening of the femoral artery. *a*, *e*) hematoxylin and eosin staining; *b*, *f*) SMC myosin; *c*, *g*) h-caldesmon; *d*, *h*) calponin.

intima or migrating from the media) is involved in proliferation? In adults, arterial lesions always arise on the basis of developed DIT. Our findings and previous studies of normal arterial intima and atherosclerotic plaques demonstrate an absolute identity of the SMC phenotypes in the intima in DIT and various forms of arteriosclerosis. However, ulrastructurally and phenotypically subendothelial SMC differ dramatically from medial SMC [6-8,10,11]. These observations support the hypothesis that in

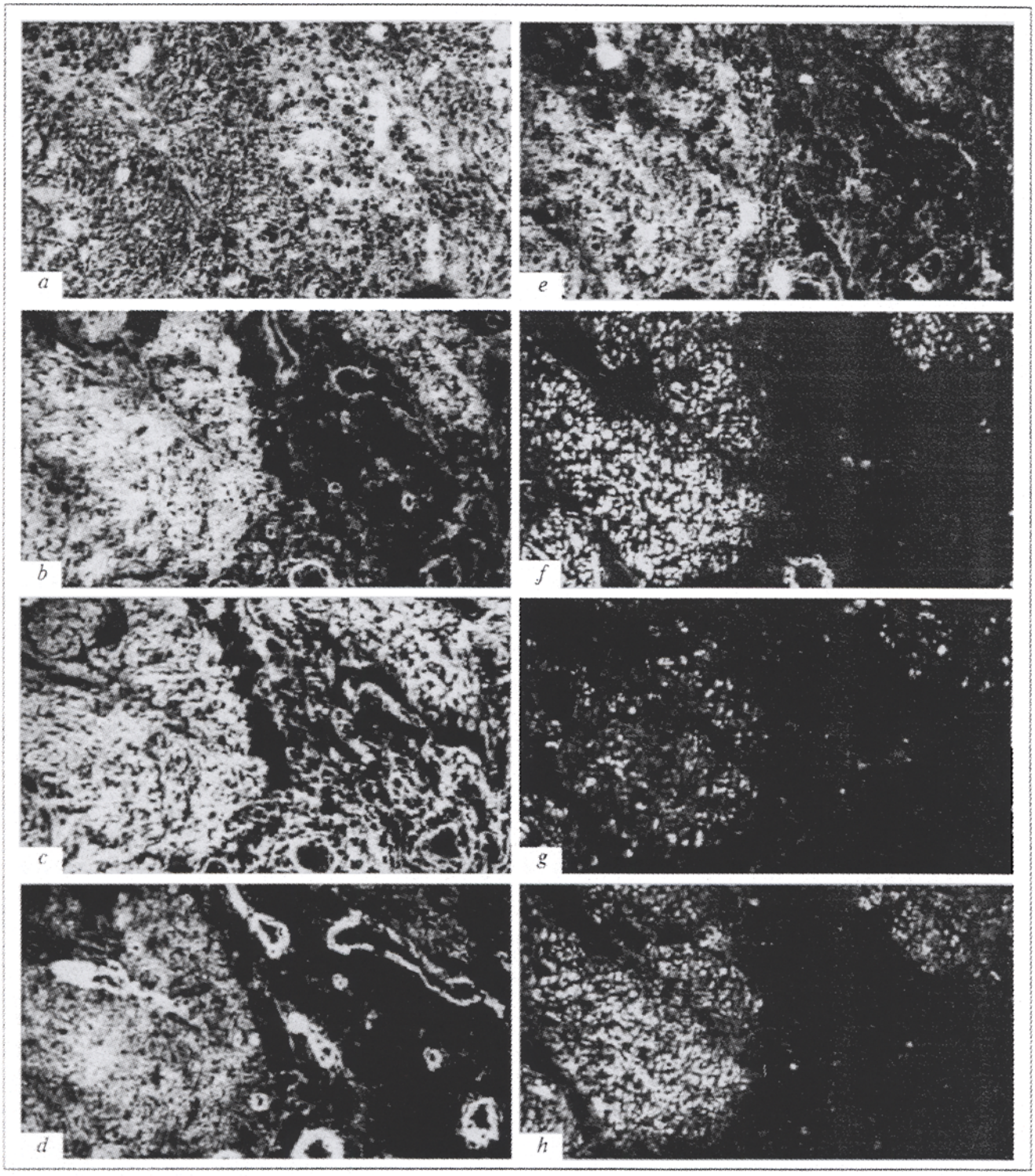

Fig. 3. Different stages of organization of a mural thrombus in the femoral artery, ×125. *a*) hematoxylin and eosin staining; *b*) type I collagen; *c*) type III collagen; *d*) type IV collagen; *e*) fibrinogen/fibrin; *f*) SMC myosin; *g*) h-caldesmon; *h*) calponin.

intimal thickenings of various genesis intimal SMC derive from preexistent subendothelial precursor cells. However, alternative origins of SMC cannot be excluded. For example, SMC in intimal thickenings in Takayasu's disease are presumed to originate from pericytes accompanying the vessels ingrowing from the adventitia [17]. Bearing in mind the active vascularization of organizing thrombi, pericytes can also play a role of SMC precursors. Hematogenous drift of SMC precursor cells into the intima and organizing thrombi was also considered [4,20].

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