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Histological Examination of Tissue Isolated from Fascia with a View of Meridian System

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경락의 관점에서 본 근막 분리조직의 조직학적 연구

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ABSTRACT

The threadlike structures of fascia were examined by light and electron microscopy. In order to distinguish its tissue organization, we used staining methods including hematoxylin-eosin, Masson's trichrome, Van Gieson's collagen fiber stain and Kluver-Barrera's luxol fast blue for nerve stain. Under the light microscope, the threadlike structures were composed of many collagen fibers and nerve. In higher magnification, they looked like as the bundle of tubular structures. Many myoid cell-spindle nuclei were observed in the tissue, which were taken from the fascia. It was identical with Bonghan duct known as one of meridian network theory. In the early 1960's the North Korean Bong-Han Kim showed the anatomical structures of the acupuncture points, and explained the meridian system as the concrete duct network system. According to Bonghan theory the Bonghan ducts spread throughout the body. Because it is believed that the duct could have the role of signal pathway, the theory was reinvestigated in these days. All of the threadlike sturctures isolated from fascia shows the abundance of collagen fibers. The electron microscope examination (TEM) could

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confirm the well arranged collagen fiber and nerve. This investigation reveals that superficial Bohghan duct are nerve fiber parallel running with collagen fibers. We conjectured that the intermingled structure of collagen fiber, blood vessel and nerve fiber might have the role of meridian system. And the more, regardless of histological research, the study on collagen fiber as response transmitter in acupuncture treatment are in need.

Key words : Meridian system, Bonghan duct, Collagen, Fascia, Connective tissue

INTRODUCTION

Acupuncture originated in ancient China and systematically developed to manage various clinical disorders. Based on the anticipated effects and minimal side effects, acupuncture has been widely accepted as promising alternative treatment.

In recent years, acupuncture treatment appears to be effective for the cases of hypertension (Babichenko, 2000), stroke, nausea, drug detoxification (Ceniceros & Brown, 1998), induction of analgesia (Cao, 2002), and pain control (Lewit, 1979; Carlsson, 2002). During acupuncture treatments needles are inserted at specific locations of the body known as acupuncture points. In the aspect of oriental medicine, acupuncture points are lined a network of 'meridians system'. Acupuncture effects are based on the theory of meridians and energy flow. The reaction to acupuncture needling known as energy flow remains largely unknown, but widely accepted as essential to the therapeutic effect of acupuncture.

Several researchers tried to reveal the meridian system and acupoints as visual structures to show the connection between meridian system and anatomical structure. A general perception of acupuncture has been considered that the effects of acupuncture essentially take place through the nervous system. Studies have confirmed that most acupuncture points in rats correspond to high electrical conductance and low skin resistance of sympathetic nerve on the body surface along the meridians (Chen & Ma, 2005). Some of researches tried to find new channels as a meridian system. Ma et al. (2003) reported that the perivascular space might have role of meridian system. Using a infrared thermography after moxibustion, Schlebusch et al. (2005) confirmed light channels on the body, which appear to be identical to what are known as meridians. Despite considerable efforts to understand the meridians, the reality of the structures remains unknown.

Anatomical research on meridian system also reported that the acupuncture meridians are believed to form a network, connecting peripheral tissues to each other and internal organ (Langevin et al., 2001a; Langevin & Yandow, 2002). In recent, Langevin and his colleague reported about connective tissue as a role of meridian system (Langevin et al., 2004; Ahn et al., 2005). Interstitial conntective tissue constitutes a continuous network linking muscles tendons, and chest walls. This tissue network is also continuous with more specialized connective tissues such as perimysium. They suggested that interstitial connective tissue might have role of biologic signal transmitter or signal pathway.

Several decades ago, similar studies were executed in north Korea. In the 1960's Dr Bong-Han Kim reported the existence of a new circulatory system in animals. He showed the anatomical structures of the acupoints and explained the meridian system as the duct system. They exist inside blood vessel (intravascular Bonghan duct), outside blood vessel (extravascular Bonghan duct), along the surface of internal organ or fascia (intra-extravascular Bonghan duct).

Very recently Bonghan theory was reinvestigated in Korea (Lee et al., 2004; Shin et al., 2005). We reviewed Bonghan theory and isolated threadlike structures from rabbit fascia. Studies aimed at understanding meridian system from fascia with a view of histological features that might distinguish connective tissue components and nerve.

MATERIALS AND METHODS

Materials

Female New Zealand White rabits weighing 2.0~2.5 kg were used for the isolation of the fascia and connective tissue. Isolated connective tissue and fibers were directly observed without staining using upright light microscope (Olympus, Japan). The threadlike samples were divide into 3 part for direct observation without staining, staining for light microscopic observation and electron microscopic observation.

Light microscopy

Isolated samples were fixed in 10% neutral buffered formalin, dehydrated through a graded ethanol, and embedded in paraplast (Polyscience, USA). Then the paraffin blocks were sectioned at 8 μm by microtome (Reichert-Jung, USA) and stained with Harris hematoxylin-eosin method, Masson's trichrome method, van Gieson method and Kluver-Barrera's luxol fast blue (LFB) method. All of sections were deparaffinated in xylene and hydrated on the down grade of alcohol and tap water.

Harris's hematoxylin-esoin method was routinely stained with hematoxylin and eosin solutions for general morphological study. Masson' trichrome method and van Gieson method were used to differentiate collagen and nerve fiber. To identify myelin and nerve cell, the sections were stained with luxol fast blue and counterstained with 0.1% cresyl echt violet solution. The stained sections were mounted with canada balsam after dehydration in alcohol and examined in a light microscope (Olympus, Japan). For more specific observation of myelin sheath in nerve fiber with light microscope, we used simple modified staining method. The embedded tissues for observation was sectioned 0.6 μm by ultramicrotome and stained with 1% methylene blue for 20 seconds. The sections were washed in tap water, dried in the air and observed using light microscope.

Electron microscopy

Transmission electron mciroscopy

For transmission electron microscopic (TEM) observations, the specimens were fixed in 2.5% glutaraldehyde for 2 hr at 4°C, washed in 0.1 M phosphate buffer, pH 7.4 and then postficed in 1% osmium tetroxide in the same buffer for 90 min. Then the specimens were dehydrated through a graded series of ethanol, exchanged through propylene oxide, and embedded in a mixture of Epon. Subsequently, ultrathin sections were obtained by ultramicrotome (Richert-Jung, USA) with a diamond knife. Ultrathin sections were double stained with uranyl acetate and lead citrate, and examined in a trensmission electron microscope (JEOL-1200EX II, Japan) at 80 kV.

Scanning electron microscopy

For scanning electron microscopy (SEM) observations, the specimens were fixed in 2.5% glutaraldehyde for 2 hr. at 4°C, washed in 0.1 M phosphate buffer, pH 7.4 and then post fixed in 1% osmium tetroxide in the same buffer for 90 min at 4°C, dehydrated in a series of ethyl alcohol and critical point dried. The specimens were coated with gold-palladium and then examined in a scanning electron microscope (JEOL JSM-6300, Japan) at 30 kV.

RESULTS

The threadlike structure adhered to the fascia. Careful observation can find the threadlike structures on the superficial abdominal fascia. During the isolation of the structures it was observed that a line of blood vessel were running with the threadlike structures (Fig. 1). The structures can be traced through into the muscle (Fig. 2). The diameter of the structures were about 1 mm, respectively. Purified threadlike samples seems to consisted of fiber-like striae. After removing the blood vessel, the thread were observed to be clean, semi-transparent and white color (Fig. 3).

Light microscopy

The samples can be mounted on a slide in isotonic saline, and a coverslip added. The structure contain blood vessels filled with red blood cells, fiber bundles and surrounding fibers (Fig. 4). The isolated dense colored bundle looked like aggregated duct (Fig. 5). With the staining methods, two kinds of tissue fibers can usually be discerned illustrated in figures. With the hematoxylin and eosin stained collagen is eosinophilic (Figs. 6a, 7a). Hematoxylin and eosin staining of obtained structures was performed for discriminating nuclei and cytoplasm. Collagen appears as different colors in various selective staining methods such as bright blue in masson's trichrome and red in Van Gieson solution (Figs. 6b, 6c, 7b). Nerve fiber showed dark blue in Kluver's luxol fast blue stain (Fig. 6d). The stained nerve was marked in each section. The threadlike structures were mainly composed of collagen fibers and nerve fibers. For abundant collagen fiber, nerve bundle showed mixed blue colors when it stained with masson's trichrome (Fig. 8). Sometimes large rod shaped Schwann cells whose length is almost 50 μm could be observed inside the nerve fiber structures. Section of the threadlike structure shows well preserved nerve fibers. The typical appearance of small peripheral nerve branches was illustrated through various staining methods. Small nerves are enclose with abundant collagen fibers. At higher magnification, Schwann cell nuclei are seen to be elongated in the long axis of the nerve. The relatively sparse fibroblasts are distinguished by their more slender, condensed nuclei. Intact myelin sheath and perineurium were observed in modified 1% methylene stain (Figs. 9-10). A threadlike structure It showed several bundles of nerve fiber.

Electron microscopy

The ultrastructural features of peripheral nerves are seen in this example which contains both myelinated and non-myelinated fibers (Fig. 11). Each fascicle is surrounded by a condensed layer of collagenous connective tissue. Bundles of collagen fibers are seen in transverse and longitudinal section in the nerve fascicle. The endoneurium consists of aligned collagen fibers lying parallel to the nerve fibers. Nerve fibers are surrounded by Schwann cell cytoplasm (Fig. 12). Scanning microscopic observation of threadlike structures showed abundant collagen fibrils and fibers surrounding the structures (Fig. 13). A peeled off threadlike sample showed several columnar constitutions at inner part of the structures (Fig. 14).

DISCUSSION

For the meridian system being an important concept in oriental medicine, scientists have tried to find an anatomic network explainable acupuncture effect. Since the 1960's, a variety of phenomena along meridians have been reported, among which quite a few suggest that along meridians there is a fluid pathway but not blood vessels or lymphatics. Bong-Han Kim claimed to find a completely new circulating system that was composed of the acupuncture meridians in human body and animals such as rabbits and rats (Kim, 1963, 1965). Although his claim was partially confirmed by Fujiwara and Yu, their work has been considered unreasonable theory in the aspect of biology. Superficial Bonghan duct in the skin that correspond to the classical acupuncture meridians. Yet, no others have confirmed his theory despite intense search for Bonghan ducts in 1960's. The basic properties we observed are consistent with Bong-

Han Kim's report. This threadlike structure is semi-transparent, milky white and elastic, and about 20~200 μm in diameter and also has many myoid cell-like spindle nuclei. Our findings on the microscopic examinations of the threadlike structure agree with provious histological studies (Kim, 1963). Myoid cell that they examined correspondent to Schwann cell and Bong-Han Kim's diagram on superficial Bonghan duct have the similarity with fascicle of peripheral nerve. There is a clear correlation between our threadlike structures and Bonghan duct. As our method to obtain the samples from the fascia was done with a researcher who Bong-Han Kim's disciple at the time of 1960's in North Korea. Bonghan ducts from organ surface and intravascular space were isolated and most of the samples showed positive features suggesting nerve fiber. In this article we report the results of confirming the extra-vascular superficial threadlike structures which are part of the Bonghan's network. We present collagen fiber and nerve fiber which exhibit histological structure of superficial Bonghan ducts. The use of masson's trichrome and other staining methods that distinguish collagen from nerve were useful in our research. Under the light microscope, the threadlike structures were composed of many collagen fibers and nerve. The peripheral nerves shown in this research consist of fascicles surrounded by perineurium containing small blood vessels. A distinct feature of peripheral nerves is that the fibers follow a longitudinal zigzag course which permits stretching during movement. In higher magnification, they seemed like as the bundle of tubular structures. All of the threadlike structures isolated from fascia shows the abundance of collagen fibers. The transmission electron microscope (TEM) examination could confirm the well arranged numerous collagen fiber and nerve. Collagen fibers are parallel running with nerve.

Because it is believed that the Bonghan duct could have the role of signal pathway, the theory was reinvestigated. In recent, Soh and his colleagues reported on Bonghan duct from the surfaces of internal organs and intravascular space (Lee et al., 2004; Shin et al., 2005). The threadlike sturctures that they discovered were compared with lymphatic vessels. They showed the rod shaped nuclei distribute on the endothelial layers of the subducts. It was one of the hallmarks on Bonghan ducts. The features of the nuclei of the threadlike structure are in agreement with Bonghan Kim's description. We studied on the threadlike structures taken from the organ surfaces and intravascular space and sometimes it looked like nerve fiver bundles. The Bonghan ducts discovered from organ surface and intravsacular space are needs to be histological staining to discriminate their components.

Histological examination of collagen and nerve in this research strongly suggest that collagen fiber might have the important role in superficial Bonghan system and meridian system. Ho and Knight (1998) reported the hypothesis on acupuncture system that collagen fibers provide proton conduction pathways for rapid intercommunication throughout the body, enabling the organism to function as a coherent whole. Langevin et al. (2001b, 2002) suggested that the mechanism of action of acupuncture also involves extraneural tissues. Their results supports the data that connective tissue winding is the mechanism responsible for the increase in pullout force induced by needle rotation. They have demonstrated that acupuncture needle rotation results in a measurable deformation of connective tissue. Pulling of collagen fibers and deformation of extracellular matrix during needle manipulation might have strong effects on local cells between extracellular matrix and intracellular cytoskeleton. Their new conception about connective tissue resulted in functional collagen fiber may explain acupuncture effect. Guimberteau et al. (2005) reported subcutaneous sliding system in humans. Beteween skin and muscle, there are only tissue connections, the vessels and muscles. They noted the existence of a type of system composed of cables structures that they term the multimicrovaculoar collagen dynamic absorption system (MCDAS). This notion of microvaculoes of collagen can explain the space filling ability and fast movement of the connective tissue in human body.

Collagen is not an electroconductor under physiological conditions, but rather a biological insulator (Tzukert et al., 1980). Collagens are centrally involved in the formation of fibrillar and microfibrillar networks of the extracellular matrix, basement membranes as well as other structures of the extracellular matrix. All microvessels are embedded in an extensive connective tissue matrix, referred to as the interstitium. Guilford and Gore demonstrate that there are mechanical interactions between arterioles and the interstitium that are mediated both through direct connections and through the movement of extracellular fluid through the connective tissue network. Connective tissues carry blood vessels and mediate the exchange of metabolites between tissues and the circulatory system. Besides the biomechanical aspects, collagens are also involved in additional functions. Specific receptors mediate the interaction with collagens, like integrin or specialized proteoglycan receptors (Levain & Nishiyama, 1996; Vogel, 2001). Signaling by these receptors defines adhesion, differentiation, growth, cellular reactivities as well as the survival of cells in multiple ways. Collagens contribute to the entrapment, local storage and delivery of growth factors and cytokines and therefore play important roles during organ development, wound healing and tissue repair (Hay, 1981; Yamaguchi et al., 1990).

Mechanical interactions with the interstitium may affect the length-tension characteristics of vessels, and thus affect their reactivity during contraction and relaxation The arterioles appeared to be connected to adjacent fibroblasts and fibrocytes by collagen fibrils (Guilford & Gore, 1995). Intermingled structure between collagen fiber and blood vessel enables the acupuncture increase blood flow in skin and muscle (Blom et al., 1993; Sandberg et al., 2003; Sandberg et al., 2004).

The fibrous components of connective tissue are of three main types: collagenous, reticular and elastic. These fibers are present in all connective tissues but occur in varying proportions. Elastin is a protein found in varying proportions in most connective tissues where in confers elastic properties which enable recovery of tissue shape following deformation or stretching. Collagen is the principal fiber type found in the matrix of all connective tissues. The regularity and arrangement of collagen fibers also varies according to function. Collagen fibers in connective tissue have the sufficient conditions of signal induction and transmission when acupuncture needle is inserted.

Until now, no anatomical or histological structures corresponding to the acupoint have been found. The present study showed histological examination of Bonghan network in fascia between skin and muscle and aimed at examine the relationship between Bonghan duct and meridian system described in oriental medicine. The histological examination of threadlike structures in fascia and Bonghan duct suggests reasonable explanations for meridian system. The physiological function of the threadlike structures has yet to be studied. We conjectured that the intermingled structure of collagen fiber, blood vessel and nerve fiber might have the role of meridian system. A histological understanding of the acupuncture points with biological concepts could establish an anatomical reality of meridian system. And the more, with histological research, the functional study on connective tissue in acupuncture treatment are in need.

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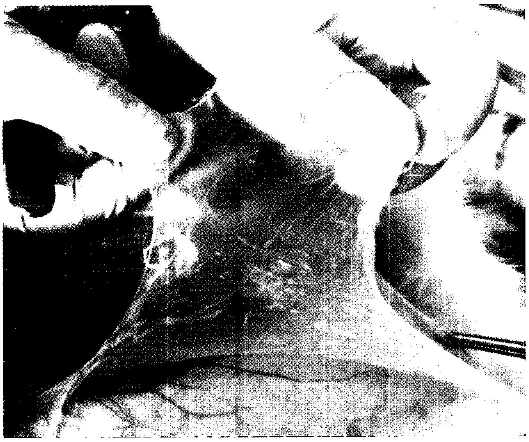
<국문초록>

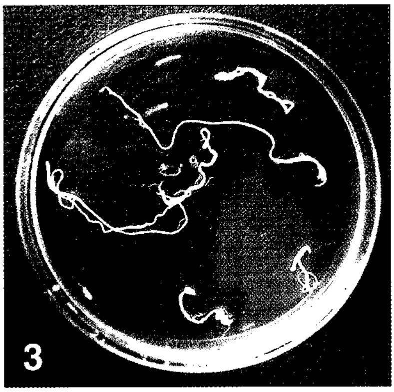
동양의학 이론의 주요 관심사인 경락의 연구에 있어 생체 내 신호전달 체계와 해부학적 실체에 대한 단서를 확인하기 위하여 본 실험을 수행하였다. 1960년대 이루어졌던 봉한학설의 이론과 문헌을 근거로 하여 토끼의 복막에 존재하는 봉한관으로 생각되는 반투명의 회백색의 탄성이 있는 섬유상 구조물을 분리하여 조직학적으로 관찰하였다. 각 조직 샘플은 봉한관 확인을 위하여 hematoxylin eosin과 Masson's trichrome 염색, 교원섬유는 Van Gieson's 염색, 신경은 Kluver Barrera's luxol fast blul 염색을 하여 비교함으로써 구성성분에 대한 분석을 실시하였다. 광학현미경상에서 관찰된 조직들은 교원섬유와 신경으로 구성된 집합체였으며 다발의 형태를 이루고 있었고, 여러 개의 myoid spindle 형태의 세포들이 종으로 배열되어 있었다. 이러한 세포들은 신경의 슈반세포이며 봉한관의 myoid 형태의 세포들과 같은 소견이었다. 같은 부위를 전자현미경으로 관찰한 결과 잘 배열된 교원섬유와 신경을 관찰할 수 있었으며 신경다발 사이로 교원섬유가 둘러싸고 있었다.

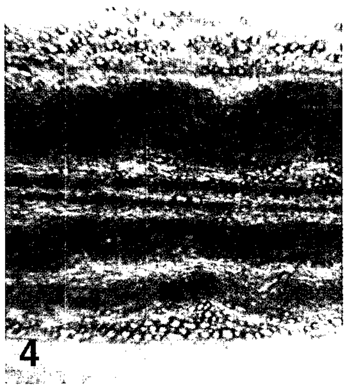
이상의 결과를 종합해 보면 복막에 존재하는 봉한관은 교원섬유와 어우러진 신경임을 확인할 수 있었고 신경과 어우러져 존재하는 교원섬유의 절연성과, 침의 치료에 있어 교원섬유가 주위의 신경과의 신호전달 체계에 영향을 미치는 점을 고려하여 볼 때 교원섬유와 신경 그리고 혈관의 유기적인 관계 속에서 교원섬유의 새로운 역할 규명에 대한 연구가 필요할 것으로 사료된다.

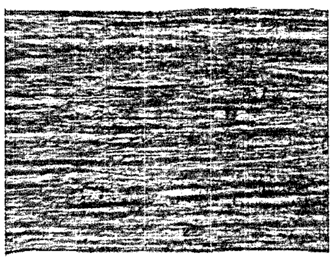
FIGURE LEGENDS

Fig. 1. Fascia just before taking the thread like structure.
Fig. 2. The isolated structures (arrow) from fascia connected through into the muscle.
Fig. 3. The isolated thread like structures. They all showed unique long threadlike feature.
Fig. 4. A light microscopic image of unstained threadlike structure showing blood vessel, dense fiber bundles and surrounding tissuse. The thread like structure isolated from the fascia showed hundreds of linear tissue materials (× 40).
Fig. 5. A part of the threadlike structure shown dense color image (× 100).
Fig. 6. The longitudinal section of thread like structure (× 100). Abundance of collagen fiber (arrow) and peripheral nerve fiber (dart) were stained. a: Hematoxylin-eosin stain, b: Masson's trichrome stain, c: van Gieson stain, d: Kluver luxol fast blue stain.
Fig. 7. Photomicrograph of a threadlike structure in cross section (× 40). Arrow: Collagen fiber, Dart: Nerve fiber, a: Hematoxylin-eosin stain, b: Masson trichrome stain.
Fig. 8. Photograph of a nerve fiber bundle stained with masson trichrome, showing intermingled blue color for collgen fiber (× 400).
Fig. 9. A photograph of threadlike structure showing myelin sheath stained with 1% methylene blue (× 100).
Fig. 10. A photograph showing conserved perineurium with 1% methylene blue (× 400).
Fig. 11. Cross section of a nerve contains myelinated and non-myelinated fibers. isolated from threadlike structure (scale bar=1 μm).
Fig. 12. Longitudinal section of nerve showing aligned collagen fibers (scale bar=1 μm).
Fig. 13. Scanning electron micrograph of threadlike structures (scale bar=1 μm).
Fig. 14. Scanning electron micrograph of a peeled off threadlike sample (scale bar=10 μm).









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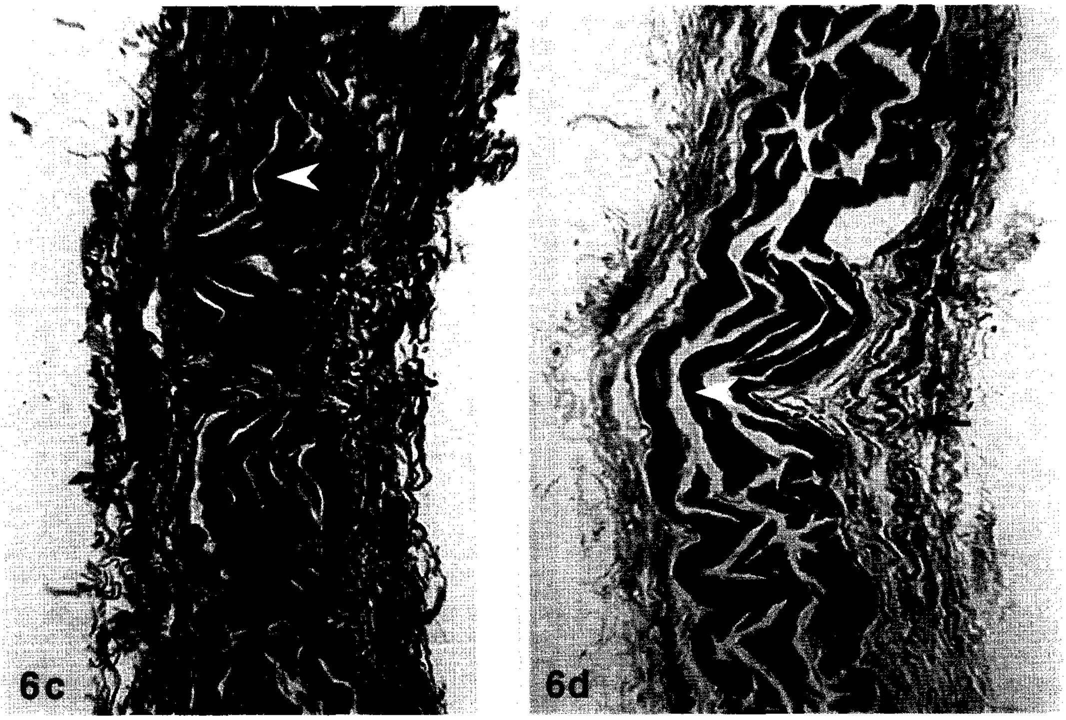
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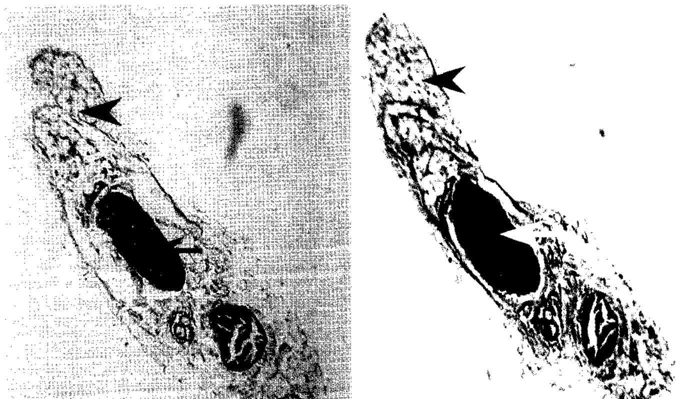
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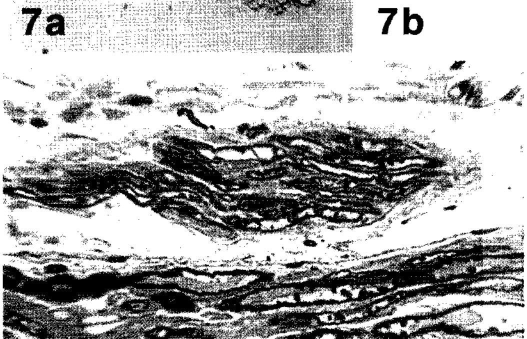


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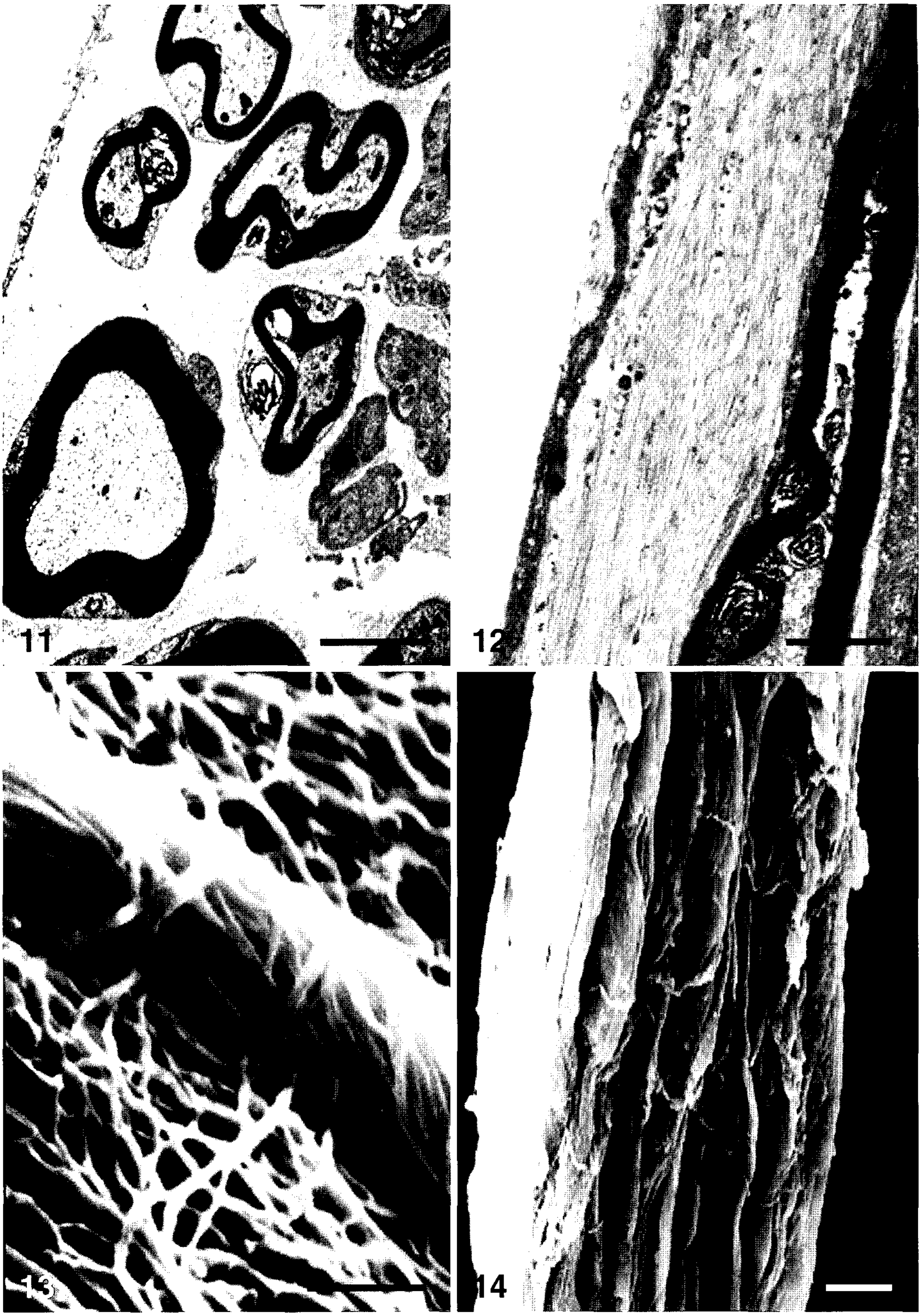
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