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ULTRASTRUCTURE OF THE CORONARY MICROVESSELS OF THE CAMEL (Camelus dromedarius): MORPHOLOGICAL EVIDENCE FOR AN ENDOCRINE FUNCTION OF THE VENTRICULAR MYCOCARDIUM

(With 9 Figs.)

By

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(Received at 15/4/1990)

تركيب التفرعات الدقيقة للشريان التاجي
في الجمل
ظواهر بنشاط هرموني للجدار العضلي للبطين

عبدالحميد عثمان

اهتمت هذه الدراسة بالتركيب الدقيق للتفرعات متناهية الصغر للشريان التاجي في الجمل والتي تقل اقطارها عن عشرة ميكرونات وذلك باستعمال الميكروسكوب الاليكتروني. ولقد اظهرت الدراسة أن تلك الأوعية تحيط بالخلايا الانقباضية من كل جانب موازية لمحاورها الطولية وأن جدرانها مبطنة بطبقة واحدة من الخلايا المضغه والخاليه من الفجوات محاطة بغشاء تحتي. تتصل نهايات تلك الخلايا بواسطة دسموزومات كما توجد بين هذه الخلايا فجوات تسمح باتصال تجويف الوعاء الدموى بالفراغ المحيط بهذا الوعاء ولكنها لاتسمح بمرور الدم خلالها وذلك لدقة تلك الفجوات البين خلوية تميزت أسطح تلك الخلايا والتي تواجه التجويف الوعائي بوجود بروزات حليميه الشكل تمتد لمسافات متفاوته كما تميز الجدار الخلوى بوجود انبعاجات السيتوبلازم ينتج عنها ظهور حويصلات غير حقيقيه. ولقد أوضحت الدراسة ظهور حويصلات بغشاء رقيق وتحوى ماده هلاميه تتبعث من جدران الخلايا العضلية الانقباضية الى الفراغات التى تفصل تلك الخلايا ومايحيطها من الأوعية الدموية الدقيقة ولقد ظهرت حويصلات مماثلة في تجاويف الشعيرات الدموية وبدايات الأوردة مما يبعث على الاعتقاد أن هناك نشاط هرموني للخلايا العضلية الانقباضيه المكونة للبطينان.

SUMMARY

The geometry and architecture of ventricular intramural coronary microvessels less than 10 um in diameter, were studed using both scanning and transmission electron microscopy. These vessels were oriented parallel to the longitudinal axes of the adjoining working cardiomyocytes in the different myocardial layers. They were constructed of attenuated endotheliocytes invested by a basal membrane. The endotheliocytes were non-fenestrated and showed a variable number of simple and compound membranous vesicles

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which were either attached to the plasmalemma proper or appear free in the cytoplasm. The luminal endothelial surface showed many villous-like flexible outgrowths projecting into the lumen resulting in the appearance of luminal artificial vesicles. The ends of the contiguous endotheliocytes were sealed together by typical desmosomes.

We observed the presence of striking membrane-bounded vesicles of different sizes, containing electron-lucent flocculent material in the minute spaces between myocytes and capillaries. Some of these vesicles appeared to be emanating from the sarcolemmae, and others were detected in the lumina of the adjacent capillaries and post-capillary venules. The present investigation concluded that these vesicles constitute a morphological basis for endocrine activity between the working ventricular cardiomyocytes, and the surrounding capillaries.

INTRODUCTION

The physiological importance of the intramural coronary microvessels on supplying cardiomyocytes with O_2 and nutrients, and their role in many heart diseases have recently drawn the attention of many investigators to their structure/function relationships. Endothelial vesicles are one of the most characteristic features of continuous capillaries (WAGNER and ROBINSON, 1984) and have been the subject of considerable study. Some authors believe that these vesicles act as shuttles between the luminal and abliuminal fronts of the endotheliocyte for efficient transport between the blood and the pericapillary tissue (PALADE and BRUNS, 1968). This shuttling phenomenon appeared inconsistent with the findings of WAGNER and ROBINSON (1984), WAGNER and ANDREWS (1985), WOOD, et al. (1987) and BUCHANAN, et al. (1988) who have clarified that these vesicles differ greatly in number and size according to the tissue thickness and fixative type. They increased in number in glutaraldehyde fixed tissues and in sections less than 200 A in thickness.

Endothelial secretory activity of the coronary microvessels of the bovine heart has been studied by GERRITSEN and PRINTZ (1981) who detected endothelial prostaglandin synthesis, and they concluded that it is released into the coronary circulation. Adenylate cyclase was also detected in the microvessels of the rabbit myocardium.

Fenestrated intramural capillaries have been described in connection with the conductive cardiomyocytes of various mammals (WEIHE and KALMBACH, 1978) and with the atrial myoendocrine cells of man and some other mammals (FORSSMANN, 1989).

This investigation aimed to study comprehensively, for the first time, the ultrastructure the coronary intramural capillaries of the camel's heart, emphasizing their relation to the ventricular cardiomyocytes.

MATERIAL and METHODS

The hearts of 5 adult camels were collected immediately after slaughter, in Zagazig slaugher-house. Specimens from the right and left ventricular myocardium were obtained and immediately prepared for scanning and transmission electron microscopic examination.

Scanning electron microscopy:

5 mm cubes of the myocardial tissue were immersed in 10% formol-saline for 2 weeks, washed in 0.1 M cacodylate buffer at pH 7.4 for 1 hour, then transfered to 1% solution of tannic acid for 2 hours at room temperature (CACECI and FRANKUM, 1987). The specimens were washed again in buffer, postifixed for 2 hours in 1% osmium tetroxide, also prepared in cacodylate buffer at pH 7.4. The postifixed material was firstly washed in the same buffer and then denydrated in graded series of ethanol and critical -point dried in CO2. They were then coated with gold/palladium for 3 minutes and examined with a JEOL JSM 35C scanning electron microscope at 25 KV.

Transmission electron microscopy:

3 mm cubes of the myocardial tissue were fixed in a cold solution of 5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 for 2 hours. Specimens were washed in 0.1 M cacodylate buffer, post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 hour, washed in buffer again, dehydrated in a series of ethanol and embedded in Epon. Thin sections were double stained with lead citrate and uranyl acetate and studied with a JEOL CX-II transmission electron microscope at 80 KV.

RESULTS

The SEM observations have clarified that the ventricular microvessels were almost exclusively disposed parallel to the longitudinal axes of the individual cardiomyocytes to which they were in close proximity. Some of these vessles were seen lodged into a channel on the surface of the myocyte and running parallel to the myofibrils inside them (Fig. 1).

The blood capillaries often exhibited circular cross sectional profiles which were always non-collapsed. Their diameter ranged between 5 and 7 um; however, capillaries as small as 2.5 um and as large as 9.5 um were also observed (Fig. 1). Individual capillaries showed caliber irregularity throughout their course which were correlated with the presence or absence of blood cells (Fig. 2).

The individual capillaries were invested with a network of fine fibrils and thicker branched fibers radiating from the fibrous investment of the surrounding myocytes (Fig. 2).

The TEM observations have also shown the close proximity of the individual capillaries to the surrounding myocytes (Fig. 3). Both structures were always in parallel orientation to each other in the different myocardial bundles.

The capillary wall was composed of attenuated endothelial cells surrounded by a prominent basal membrane (Fig. 4). The capillary circumference was surrounded by one to three endotheliocytes whose abutting edges were sealed by desmosomes (Fig. 4). The endotheliocyte included the perikaryon and the attenuated cytoplasmic extension. The perikaryon represented the broadest portion of the endotheliocyte and it was mostly occupied by an irregularly oval or flattened nucleus (Fig. 4, 5). The latter was characterized by a dense peripheral heterochromatin (Fig. 4, 5). The nuclear diameter ranged between 0.7-2 um. The perinuclear cytoplasm was characterized by paucity of organelles. Scarce mitochondria, electron-dense granules, and some vesicular bodies were observed paranuclearly (Fig. 4, 5).

Far-off from the nucleus, the rest of the endotheliocyte was an attenuated plate-like structure encircling the capillary lumen. A small amount of cytoplasm was usually sandwiche between the inner (luminal) and outer (abluminal) plasmalemmae. The thickness of such endothelial plates ranged between 50 to 370 nm. In some regions, both plasmalemmae were in close apposition with an inconspicuous bit of cytoplasm in between (Fig. 6). The thicknes in such regions was about 17 nm.

The cytoplasm of the non-nuclear portion was characterized by the presence of many plasmalemmal vesicles which were either attached to the plasmalemma proper or existed freely in the cytoplasm (Fig. 9). The vesicles were of different shape and size and both simple and compound forms were observed. The simple vesicles were usually spherical, smaller in size (about 100 nm in diameter) and showed electron-lucent cores; meanwhile, the compound form was larger in size (about 307 nm in diameter) occupying most of the endotheliocyte's thickness; and the vesicular core showed further membranous structures (Fig. 4, 6). Free and attached dense granules (25 nm in diameter), probably ribosomes, were observed beside the plasmalemmal vesicles (Fig. 6).

The luminal (and to lesser extent abluminal) endotheliocyte surfaces were thrown into a number of tubular extensions projecting into the capillary lumen and the periendothelial basal membrane respectively (Fig. 4, 6). The luminal projections were thinner, longer and more flexible, and therefore, some of them appear as luminal vesicles (Fig. 4, 6). The abluminal ones were shorter and tended to branch, but they were never extended beyond the basal membrane (Fig. 6). Discrete desmosomal junctions were observed joining the contiguous endotheliocytes together (Fig. 4, 6). Each desmosomal region showed a permanent 23-nm wide intercellular gap.

A prominent, continuous basal membrane was seen entirely surrounding the endotheliocyte. It was made up of an inner, homogeneous, moderately electrondense layer

appearing as a soft feltwork; and an outer interrupted, loose microfilamentous layer (Fig. 6). The latter layer was attached either to anchoring fibers inserting into the sarcolemma of the surrounding contractile myocytes (Fig. 4) or to the fibrous and amorphous components of the interstitium (Fig. 5, 6). The total thickness of the basal membrane ranged between 72 and 487 nm.

The capillary lumen was 1.2 to 7.5 um in diameter, and at any caliber it was suitable for the passage of the elliptical erythrocytes. Erythrocyte diameters at the mid-portions ranged between 0.6 to 1.3 um. The capillary lumen showed, in addition to the erythrocytes, sporadic membrane-bounded vesicles. Those were different from the plasmalemmal vesicular structures artificially formed as a result of the flexibility of the luminal plasmalemmal projections, in being surrounded by a very thin membrane (non-laminated); and their electron-lucent cores showed a finely granular or flocculent material (Fig. 5). The same vesicles were observed in the lumina of the post-capillary venules in which the artificial vesicles were missing (Fig. 7).

Similar membrane-bounded vesicles were always present in some regions of the interstitium between the sarcolemma of the contractile myocytes and the associated capillaries (Fig. 3, 5). The largest vesicles pressed on the capillary wall, causing its invagination with substantial luminal narrowing (Fig. 3, 8). Some of the largest interstitial vesicles showed localized areas of invaginations, the summits of which exhibited condensed osmiophilic accumulations.

Similar smaller vesicles were seen closely adjacent to the myocyte sarcolemmae (Fig. 4).

The perivascular interstitial tissue in between the muscular bundles showed a prominent increase in the connective tissue fibers, fibroblasts and mast cells (Fig. 9) but it was entirely devoid of the interstitial membrane-bounded vesicles.

DISCUSSION

The intramural coronary microvessels examined in the present investigation were identified primarely by the presence of the characteristic elliptical erythrocytes in their lumina, secondarely on the basis of their ultrastructural characteristics as previously described in other mammals (WEIHE and KALMBACH, 1978; WAGNER and ROBINSON, 1984; ONE, et al. 1986; BROWN and EGGINTON, 1988), and finally on their caliber which was determined to be less than 10 um.

The capillaries in different layers of the ventricular myocardium were always parallel to the longitudinal axes of the individual myocytes between which they were insinuated. Thus, the capillaries were crossing over each other according to the geometrical arrangement of the myocardial bundles resulting in the formation of an extensive

capillary net. This characteristic arrangement has been described in man and some animals (PETELENZ, 1965; ONO, et al. 1981; IRINO, et al. 1982). The existence of capillaries between the adjacent myocytes in all of the sectional profiles leads to the conclusion that each individual myocyte is cicumferentially surrounded by more than one capillary. This archeticture might be essential for the cardiomyocyte to tolerate its extensive demands for 0_2 and nutrients, for continuous contraction and relaxation. On the other hand, it also suggests that the stenosis or even occlusion of a particular capillary will not dramatically affect the integrity of the neighboring myocytes unless the arterial stem, from which these capillaries have emerged, is stenosed or occluded.

The interstitial tissue space separating the basal membrane of both a contractile myocyte and the adjoining microvessel was in many areas, very narrow so that it reached less than 27 nm in width (Fig. 7). This space was absent in some areas (Fig. 4) with corresponding continuity between the opposing myocyte and microvascular walls. Thus, the close proximity between the working myocyte and the adjacent capillaries form a morphological myocyte-blood barrier. This barrier was built up of: a) the endothelial wall, b) the minute interstitial tissue space and c) the myocyte sarcolemma; alternatively, it consisted of: a) the endothelial wall and b) the sarcolemma. This barrier might play an important physiological role concerning active transport between myocytes and capillaries. Another structure of special importance is the intercellular gaps between the contiguous endotheliocytes. These gaps construct a direct pathway between the capillary lumen and the pericapillary environment. Hence, we concluded that efficient fluid exchange between a working myocyte and the associated closely adjacent capillary could be possible although obvious capillary fenestrae are missing. Fenestrated capillaries were observed in the atrioventricular node and atrioventricular bundle of the mammalian hearts (WEIHE and KALMBACH, 1978; FORSSMANN, et al. 1983). Anchoring fibers crossing transversely between the opposing endothelial and myocyte walls were observed. Similar fibers had been described between cardiomyocytes and lymph capillaries (LEAK and BURKE, 1968; BOUCHER, et al. 1985).

The most conspicuous morphologic feature of the endotheliocyte was the presence of plasmalemmal vesicles which were either attached to the plasmalemma proper or appeared free in the cytoplasm. These vesicles seem to be invaginations from the endotheliocytic plasmalemma rather than true cytoplasmic membranous structures. This opinion coincides the findings of WAGNER and ANDREWS (1985) who have clarified that these endothelial vesicles increase in number in glutaraldehyde-fixed capillaries compared to fresh-frozen tissue. Moreover, the studies of BUCHANAN, et al. (1988) indicated that the vesicular number was significantly greater in the thinner sections. Formerly, some authors had believed that these vesicles are independent structures associated with transendothelial transport by shuttling between the luminal and abluminal endothelial fronts.

The interstitial spaces between the working myocytes and the associated capillaries exhibited unique membrane-bounded vesicles of different sizes. Some of these vesicles were observed in close proximity to the sarcolemma as if they are emanating from it. These vesicular structures were surrounded by a continuous or interrupted thin and non-laminant membrane and they contained electron-lucent flocculent material. The largest vesicles pressed on the endothelial wall causing its deformity and some of them showed villous-like invaginations whose summits were covered by a condensation of electron-dense osmiophilic material. It is strikingly observed that similar smaller vesicles had been detected in the capillary lumina and the post-capillary venules intermingling with the erythrocytes. From the aforementioned data it may be suggested that the working ventricular myocytes of the camel produce a membrane-bounded material budding from the sarcolemma and transmitted to the capillary lumina through the endothelial wall. This may also indicate that these myocytes play an endocrine function. In any event, further studies are necessary to clarify the nature of these vesicles, the biochemical composition of their contents and how far the pericapillary and intraluminal vesicles are structurally similar.

Recently, it has been established that some of the atrial working myocytes in man, cattle, pig, rat and mouse have an endocrine function, by producing a family of cardiac hormones regulating the cardiovascular and renal activities (FLYNN, et al. 1983; CURRIE, et al. 1984; CANTIN and GENEST, 1985; FORSSMANN, 1986 and 1989). None of the aforementioned authors described a morphological evidence of endocrine activity among the ventricular working cardiomyocytes, although FORSSMANN (1989) has described the transmission of peptide-containing vesicles (ANF; atrionatriutic factor) from the atrial myoendocrine cells of several mammalian species to the surrounding capillaries. Our investigation is the first study indicating a morphological basis for the possible endocrine activity of the ventricular working myocytes.

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LEGENDS

Fig. (1): Scanning electron micrograph of cross sectioned ventricular myocardiocytes (M), showing many uncollapased blood capillaries (C) in between and a capillary piercing a myocyte (arrowhead). Mag. x 1800.

Fig. (2): Scanning electron micrograph showing a blood capillary (C) parallel to the longitudinal axes of 2 adjacent myocytes (M). Note the caliber irregularity of the capillary alongside its course and its investment with thin and coarse fibers fixing it to the adjacent myocytes. Mag. x 3200.

Fig. (3): Transmission electron micrograph showing a blood capillary lodged in between two adjacent parallel myocytes (M). Note the close proximity of the capillary and myocytes. Membrane-bounded vesicles (V) with electron-lucent cores pressing the capillary wall with corresponding luminal narrowing. Mag. x 5200.

Fig. (4): Transmission electron micrograph of a capillary sectioned obliquely. The nucleus (N) of the endotheliocyte has a peripheral heterochromatin and a prominent nucleolus; the abutting ends of the endotheliocytes are attached with a desmosome (arrowhead); the luminal endotheliocytic surface exhibiting flexible projections (F); luminal artificial vesicles (A); plasmalemmal vesicles (V); anchoring fibers (arrow); membrane-bounded vesicles (B) adjacent to the sarcolemma. Mag. x 20000.

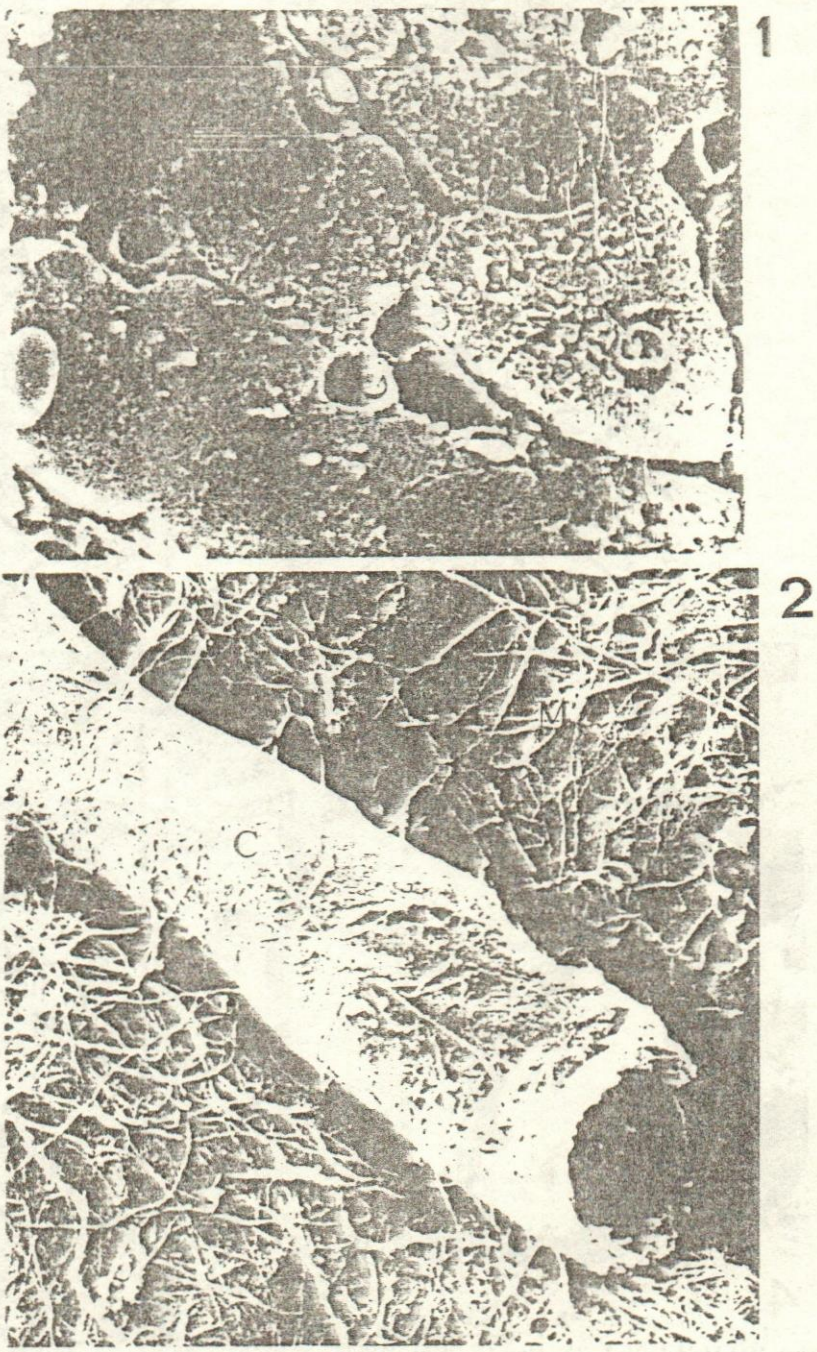
Fig. (5): Transmission electron micrograph showing circulating membrane-bounded vesicles (V) beside an erythrocyte (E); pericapillary fibrocyte (F); interstitial membrane-bounded vesicles (S); prominent basal membrane (arrowhead). Mag. x 10000.

Fig. (6): Partial view of a capillary showing plasmalemmal vesicles (V) surrounded by electron-dense granules; desmosome with a discrete gap (arrow head); luminal (L) and abluminal (A) endothelial extensions; erythrocyte (E); basal membrane constructed of 2 layers. Mag. x 20000.

Fig. (7): Partial view of a post-capillary venule showing circulating membrane-bounded vesicles (V) beside the erythrocyte (E). Mag. x 7000.

Fig. (8): A circumscribed interstitial structure (V) made up of flocculent material existing between a myocyte (M) and a capillary (C); nucleus of pericyte (P) adjacent to an endotheliocytic perikaryon (K). Mag. x 8240.

Fig. (9): Transmission electron micrograph showing abundant connective tissue elements in between myocardial bundles. Connective tissue fibers (arrowhead); mast cell (M); fibroblast (F). Mag. x 8240.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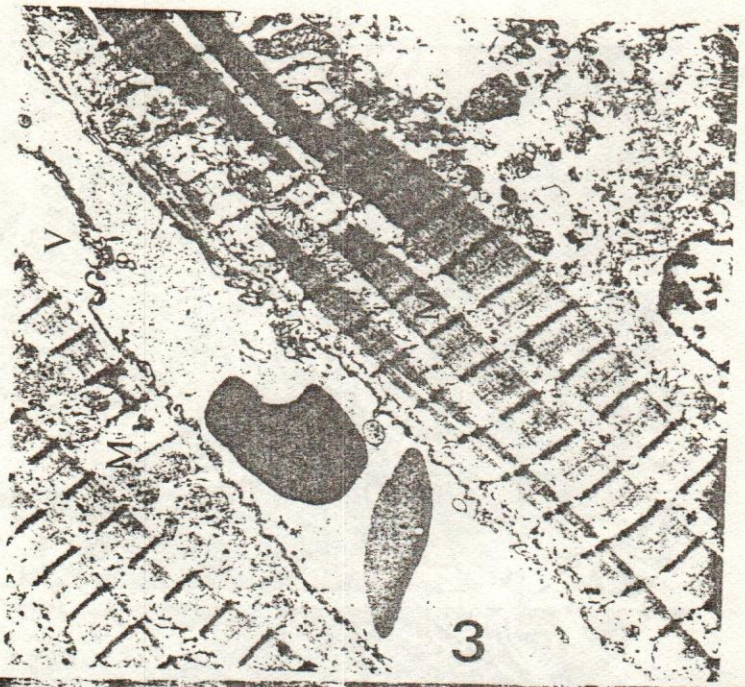
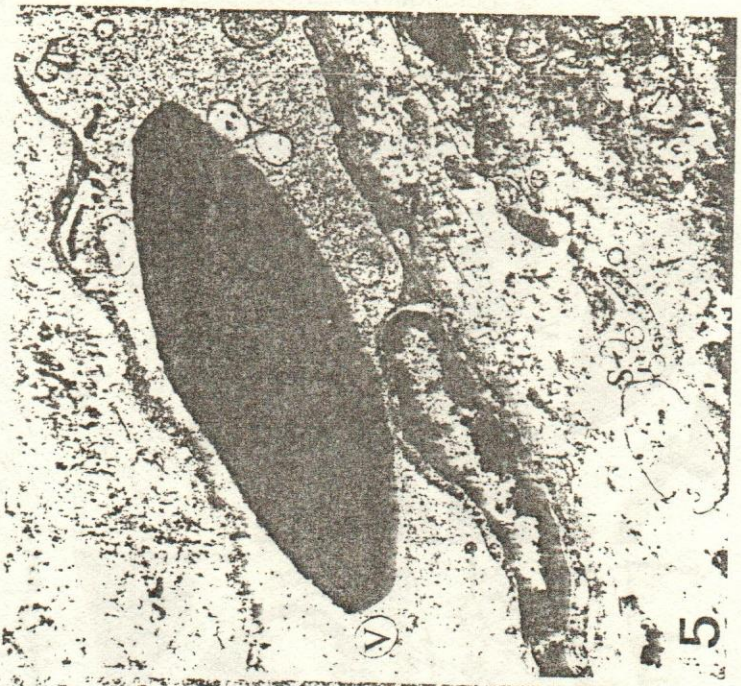
CORONARY MICROVESSELS, CAMEL

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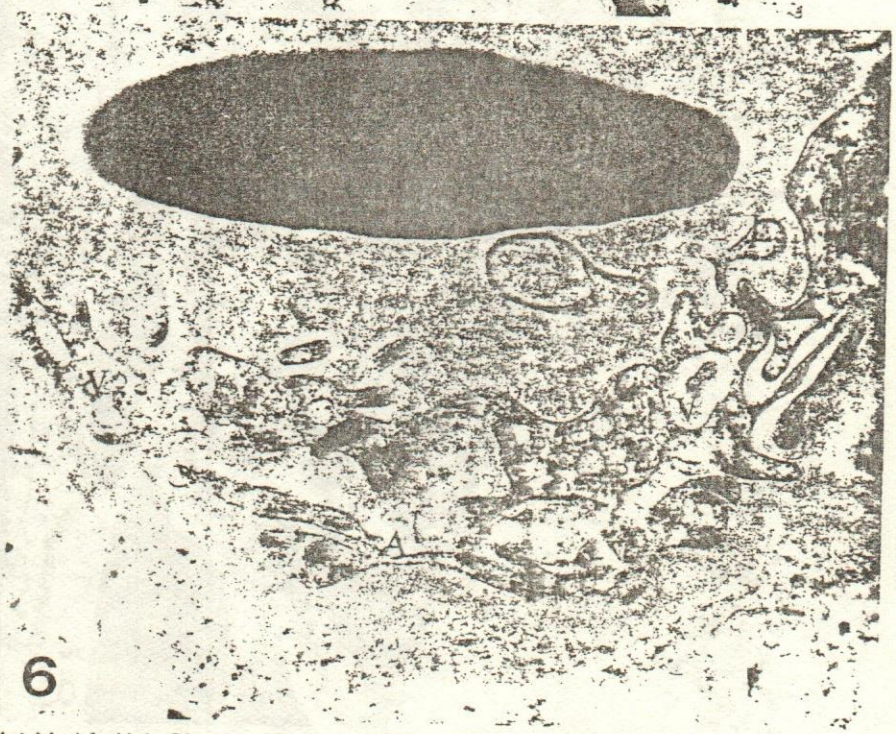
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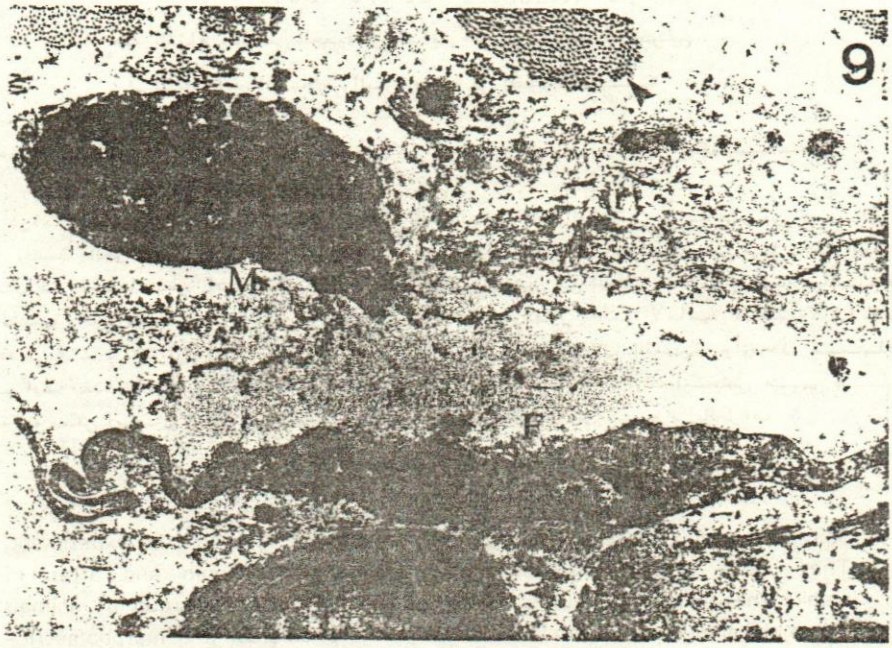
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