ULTRASTRUCTURE OF HUMAN INTRAMUSCULAR BLOOD VESSELS IN DEVELOPMENT

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Muscles from human foetuses of nine weeks to nine months development were examined by electron microscopy. Capillaries, arteries and veins are frequent in the human foetal muscle at all stages of development. At nine weeks the vessels have the appearance of capillaries and basement membrane lies around the endothelial cells forming them. The capillaries are of continuous type which do not have apertures in their walls. Tight junctions are seen at some zones of adjacent endothelial cells of the capillaries. Sometimes, pericytes are also seen. At sixteen weeks vessels having the features of veins and of arteries can be identified between the muscle cells. Regarding the arteries, some of the endothelial cells are united to the smooth muscle cells and the intimal elastic lamina is interrupted where these cells approximate. The significance of this junction may be to anchor the intima to the media.

MATERIAL AND METHODS

The material used in this investigation is derived from 27 human foetuses ranging from nine weeks to nine months development. They have been studied in detail in longitudinal and transverse sections by electron microscopy; some 300 grids were examined. Foetuses of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 24, 28, 32, 36 and 40 weeks were studied.

The age of the minute foetuses was established by the obstetricians who supplied the specimens and was calculated according to the crown-rump length. The ages of foetuses of 5, 7, 8 and 9 months development were based on the menstrual age. They were newborn prematures who died within 24 hours after birth. Specimens were removed soon after death in these cases to avoid the occurrence of post mortem changes in the muscle.

The muscle was usually taken from the thigh, even that from the minute foetuses, and laid immediately on a piece of card and kept slightly stretched by means of

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pins applied to either end of the specimen. The specimens were then immersed in cold 3% glutaraldehyde in Sörensen's phosphate buffer at pH 7.4. After 2 hours of fixation the muscle held by the pins was released and cut in small pieces of about 1-2mm thick. These were washed in two changes of Sörensen's buffer at pH 7.4 for 15 minutos each.

Post fixation was carried out for 2 hours at room temperature in cold 1% osmium tetroxide in Michaelis' Veronal-acetate buffer at pH 7.4. After being washed in distilled water and dehydrated in ascending grades of alcohols, they were placed successively in propylene oxide and in a mixture of equal parts of propylene oxide and Epon 812.

Finally they were embedded in fresh Epon mixture. Sections, 1-2 μ thick were cut on an LKB Ultratome and stained with toluidine blue by the method of Trump, Smuckler and Benndítt (1961) and examined by light microscopy. Thin sections of appropriate regions were collected on copper grids, stained by uranyl acetate and lead citrate (Reynolds, 1963) and examined in a Siemens Elmiskop I.

RESULTS

Capillaries, veins and arteries can be readily identified in the muscle of the human foetus of nine weeks.

The capillaries are formed of closely applied endothelial cells which at some zones of contact exhibit specialised, electron dense tight junctions (Fig. 1). The endothelial cells proliferate by mitosis (Fig. 2) and the tight junctions persist between the adjacent cells even when the cells are in mitosis. The occurence of mitosis in the endothelial cells of the capillary wall does not interfere with the capacity of the vessel to transmit blood corpuscles. A layer of basement membrane lies on the outer aspect of the endothelial cells and within spaces in the basement membrane there occur pericytes. Pinocytotic vesicles are frequent in both the pericytes and the endothelial cells forming the capillaries of the muscle in foetuses of fifteen weeks development (Fig. 1).

Vessels which have the structural appearance of veins are seen in foetuses of sixteen weeks development (Fig. 3). The veins are lined by flattened endothelial cells and tight junctions occur at some adjacent parts of the adjacent membranes of the endothelial cells. The nucleus of the endothelial cells to be ovoid in shape and is often indented. It has a dense narrow rim of chromatin and the rest of the chromatin usually is granular. The cytoplasm of the lining cells is moderately electron dense. Around the endothelial cells lies a narrow space separating the endothelial cells from the plain muscle cells which form the medial coat. These muscle cells are loosely arranged and spaces occur between them and the endothelial cells which they surround. Many fibrils of collagen lie between the cells of the media and the loosely arranged cells which form the external coat of the vein. These cells have a relatively large nucleus which is dark and granular. They contain a large amount of cytoplasm which possesses rough endoplasmic reticulum, ribosomes, mitochondria and many fine filaments.

Arteries (Fig. 4) are also readily identified in the foetus of sixteen weeks development. They are lined by compactly arranged columnar shaped cells which enclose an irregular shaped lumen. Tight junctions are located at frequent zones of contact of all the cells forming the endothelial lining or intima of the artery. These columnar cells are of two distinct electron dense appearances (Fig. 4 and 5): the majority are fairly electron dense with a round or elongated nucleus which is granular with a very electron dense narrow margin. Their cytoplasm contains besides mitochondria considerable numbers of electron dense bodies of varying shape and also rough endoplasmic reticulum and ribosomes. Interspersed amongst these dark cells are cells which have fairly clear cytoplasm. These cells are much less frequent than the electron dense cells. A distinct space separates the dark endothelial cells from the layer of plain muscle cells forming the media of the artery, and in this space lies filamentous material corresponding to the internal elastic lamina. The light cells however make direct contact with the cells of the media and no space or internal elastic lamina intervenes between them (Fig. 4 and 5).

Outside the internal elastic lamina and adhering to the clear cells of the intima lie plain muscle cells: between the internal elastic lamina and the smooth muscle cells is a layer of basement membrane which is interrupted by the clear intima cells which make contact with the cells of the media (Fig. 6). The cytoplasm of the

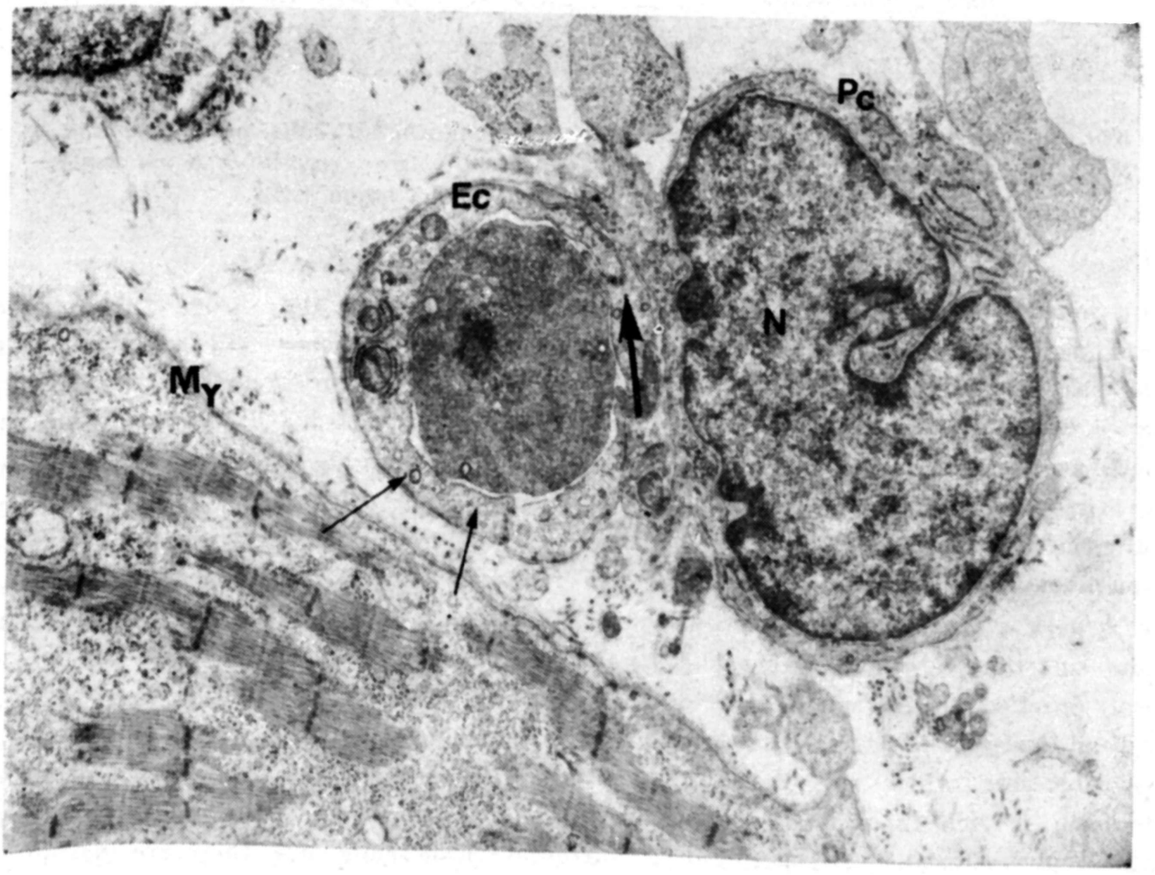

Fig. 1 — A small capillary which shows tight junctions (arrowed) between its endothelial cells which contain many pinocytotic vesicles (thin arrows) has a pericyte in its wall. The pericyte has a large indented nucleus X 10,000. Ec = endothelial cell, My = myotube, N = nucleus, Pc = pericyte.

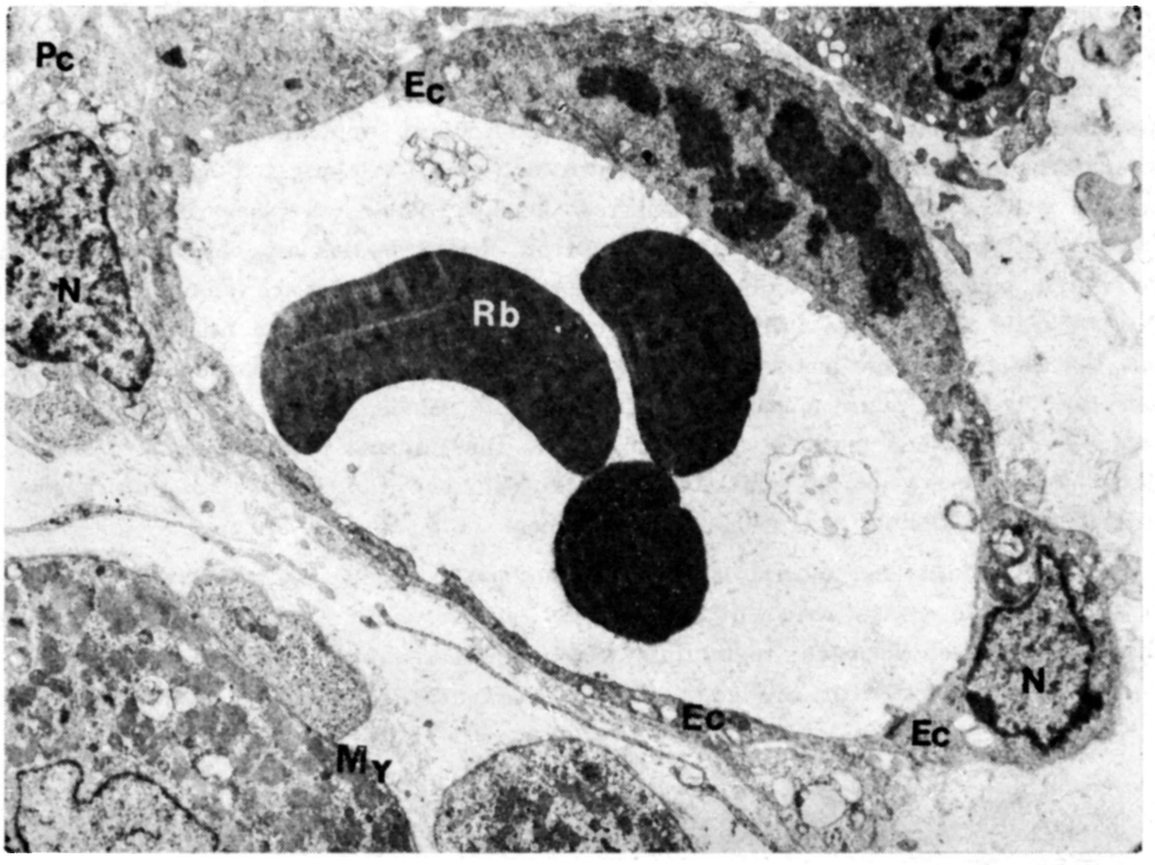

Fig. 2 — A blood capillary is lined by four endothelial cells one of which is in mitosis. X 3,000. Ec = endothelial cell, My = myotube, N = nucleus, Pc = pericyte, Rb = red blood cells.

plain muscle cells contains an abundance of thin filaments and throughout the cells there are numerous irregular shaped very electron dense zones which appear to be condensations of the filaments. In addition there are some rounded and irregular shaped dense bodies as well as collections of glycogen granules and some mitochondria. There are several layers of smooth muscle cells and thight junctions exist between these adjacent cells. The nuclei of the plain muscle cells are irregular in shape and rather granular with a large amount of small, very electron dense chromatin throughout their substance. Nucleoli are frequent and the margin of the nucleus has a dark rim of chromatin of variable thickness. Between the outer cells of the media and the adjacent external coat there occurs a layer of basement membrane closely applied to the plain muscle cells (Fig. 6). The cells of the external coat have an irregular shaped nucleus and a large amount of cytoplasm which forms long processes which are disposed concentrically around the medial coat. Their cytoplasm is rich in rough endoplasmic reticulum, ribosomes, mitochondria and fine filamentous material. Collagen fibrils surround these cells but they are devoid of basement membrane.

As maturation advances the number of endothelial cells increases. Fig. 7 shows a well developed blood capillary of the muscle of a human foetus of twenty weeks development.

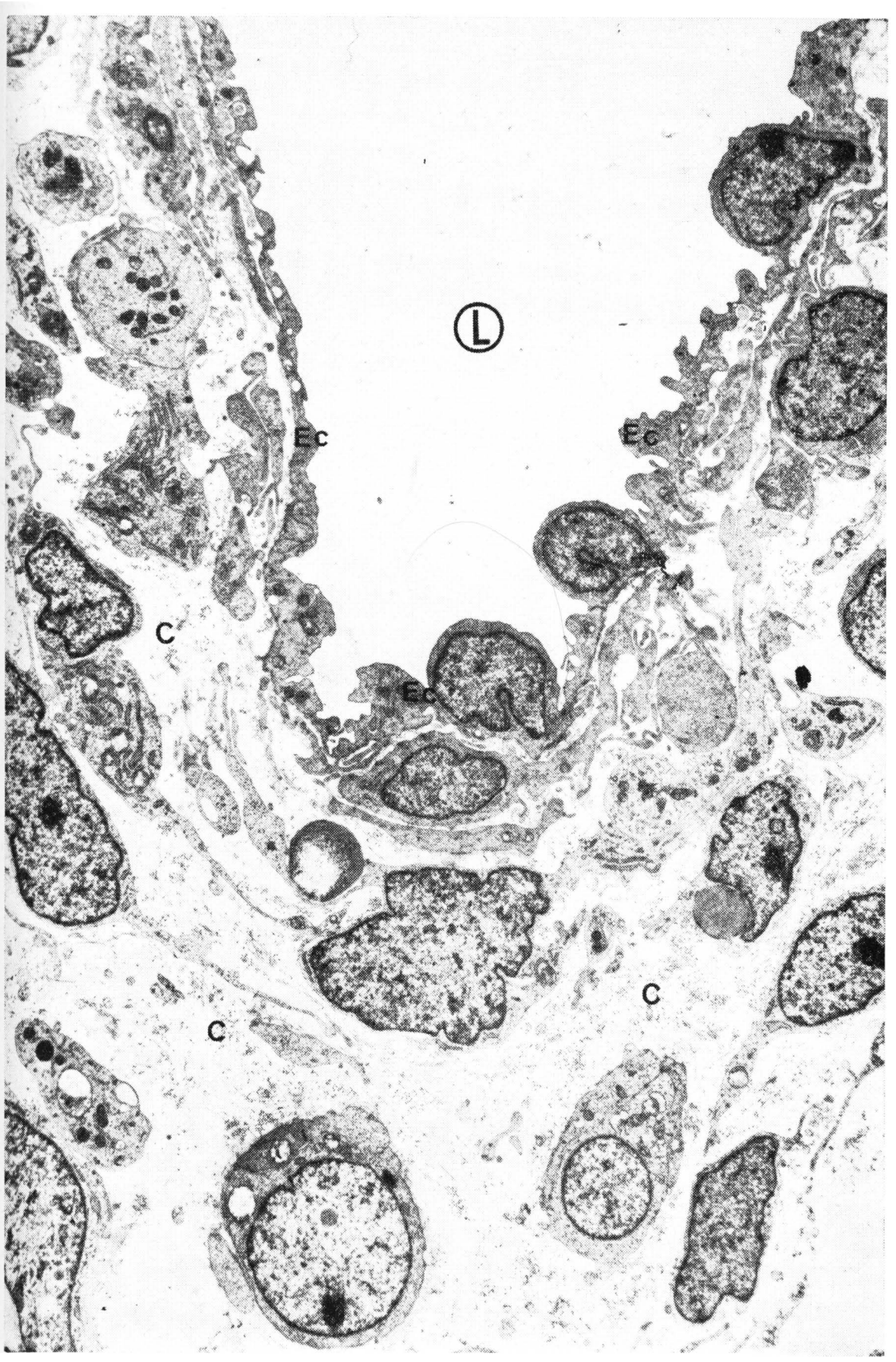

Fig. 3 — A blood vessel which has the appearance of a vein is lined by layers of endothelial cells which are fairly electron dense. This inner layer of cells is surrounded by layers of less compactly grouped cells which are less electron dense. Groups of collagen fibrils occur between these cells forming the outer layer of the vessel. X 2,700. C = collagen, Ec = endothelial cell, L = lumen.

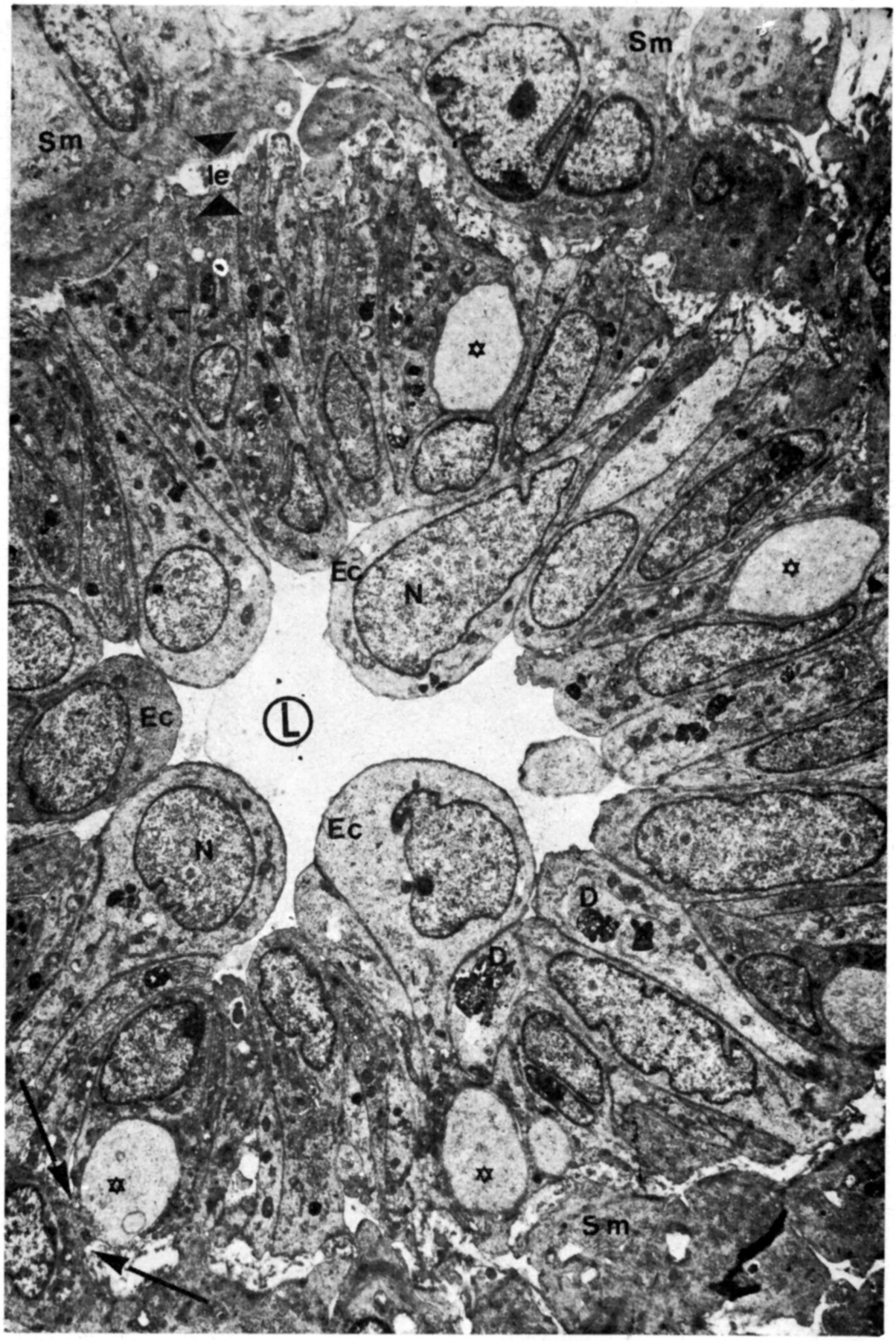

Fig. 4 — The developing arteries are lined by tall compactly arranged elongated cells lying around the lumen. Interspersed amongst these cells are less frequent cells (starred) which have a much less electron dense appearance and contain filamentous and granular material. The internal elastic lamina is sometimes interrupted by the clear cells which lie in close contact with the cells of the muscle coat (arrowed). X 2,600. D = dense bodies, Ec = endothelial cell, Ie = internal elastic lamina, L = lumen, N = nucleus, Sm = smooth muscle cell.

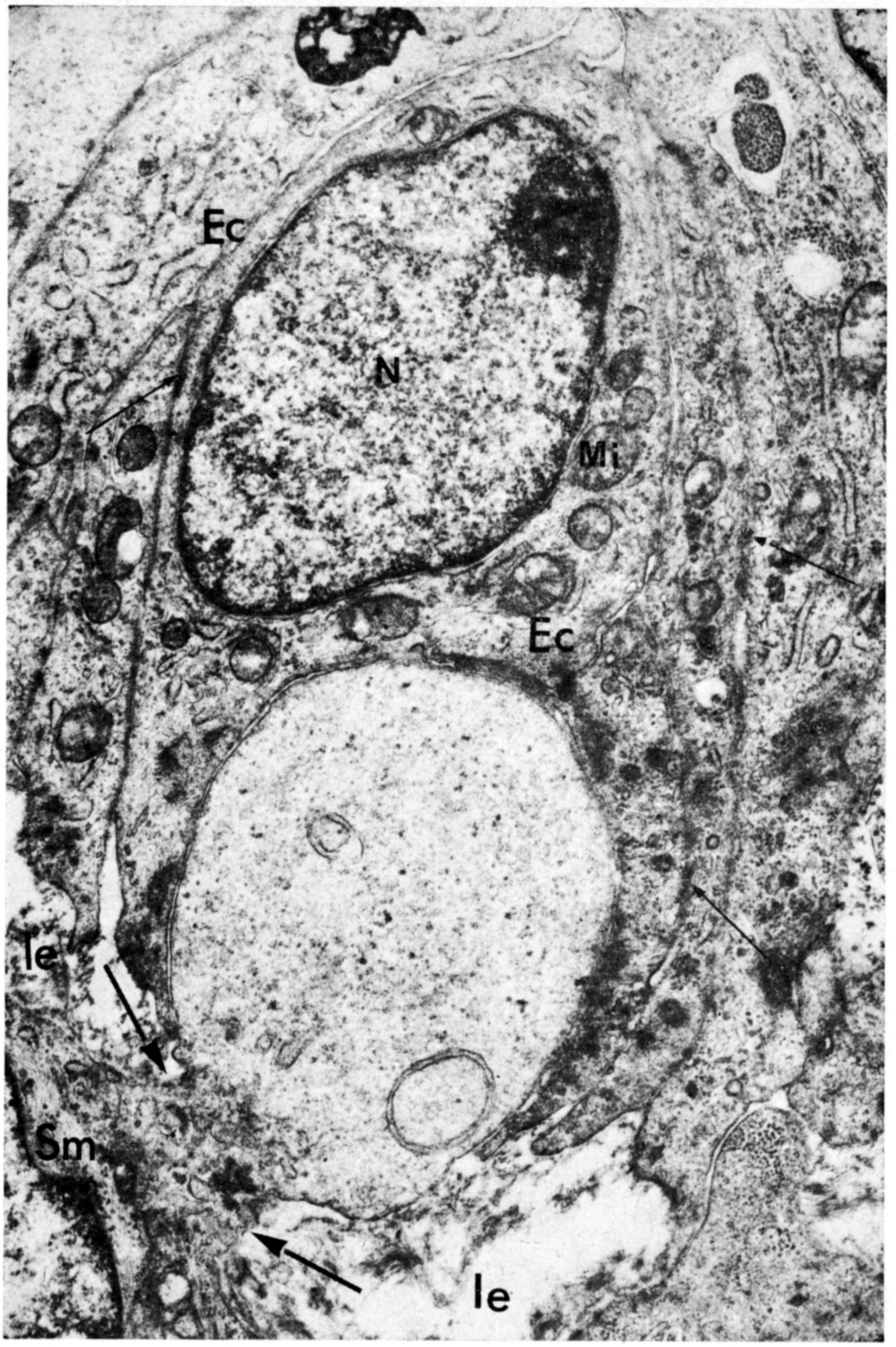

Fig. 5 — This picture shows the close relationship of the clear cells and the cells of the smooth muscle coat at greater magnification (arrowed in Fig. 4). The internal elastic lamina is interrupted at the zone of contact (arrowed). The clear cells are closely embraced by the dark cells. Tight junctions (thin arrows) occur between the dark cells. X 13,000. Ec = endothelial cell, Ie = internal elastic lamina, Mi = mitochondria, N = nucleus, Sm = smooth muscle cell.

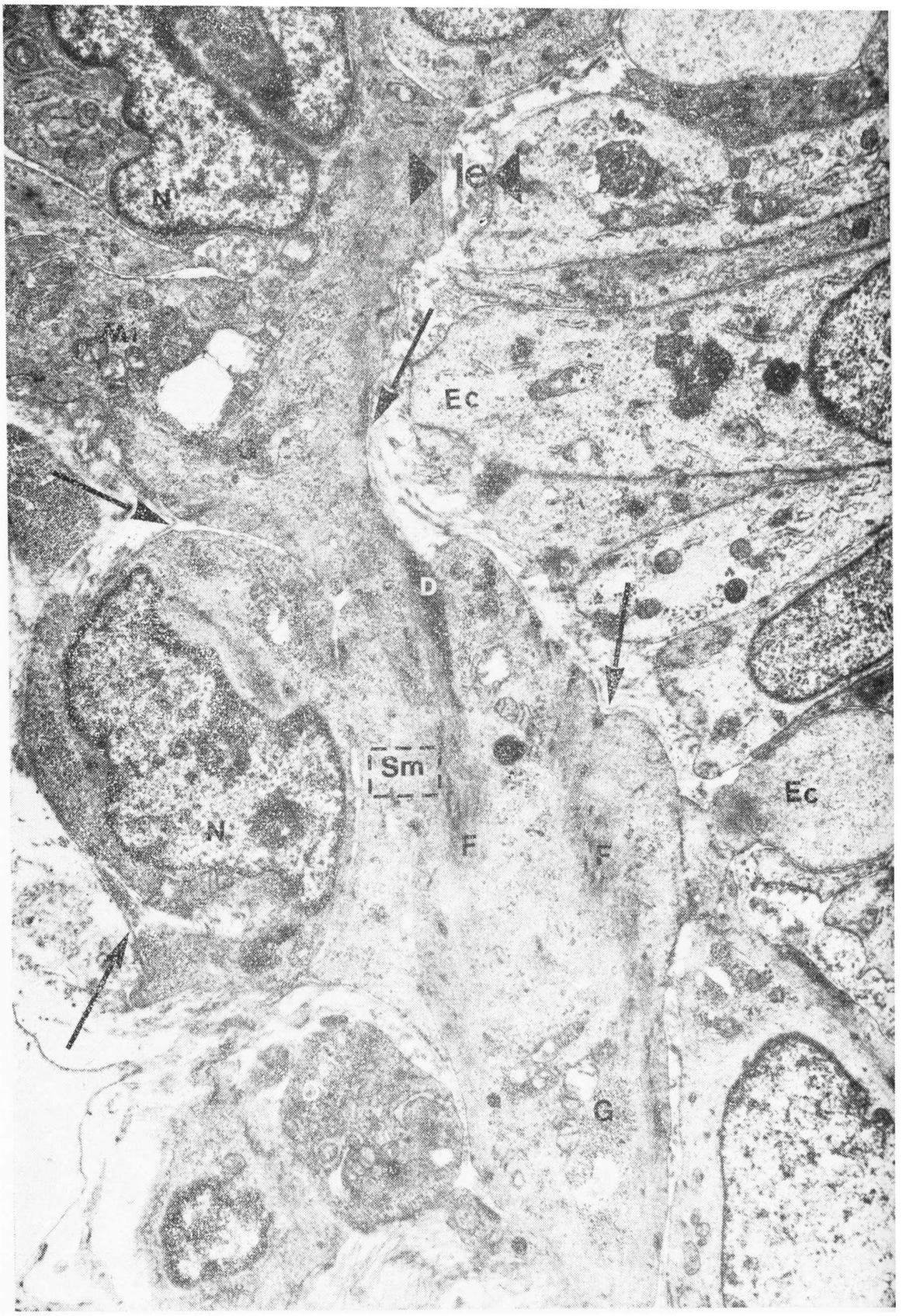

Fig. 6 — The tall endothelial cells lining the artery lie in contact with the filamentous material of the internal elastic lamina which is separated from the subjacent smooth muscle coat by a layer of basement membrane (arrowed). A layer of basement membrane appears also intimately apposed to the external aspect of the muscle cells (arrowed) which contain numerous thin filaments and dense bodies in their cytoplasm. X 5,500. D = dense bodies, Ec = endothelial cell. F = filaments, G = glycogen, Ie = internal elastic lamina, Mi = mitochondria, N = nucleus, Sm = smooth muscle cell.

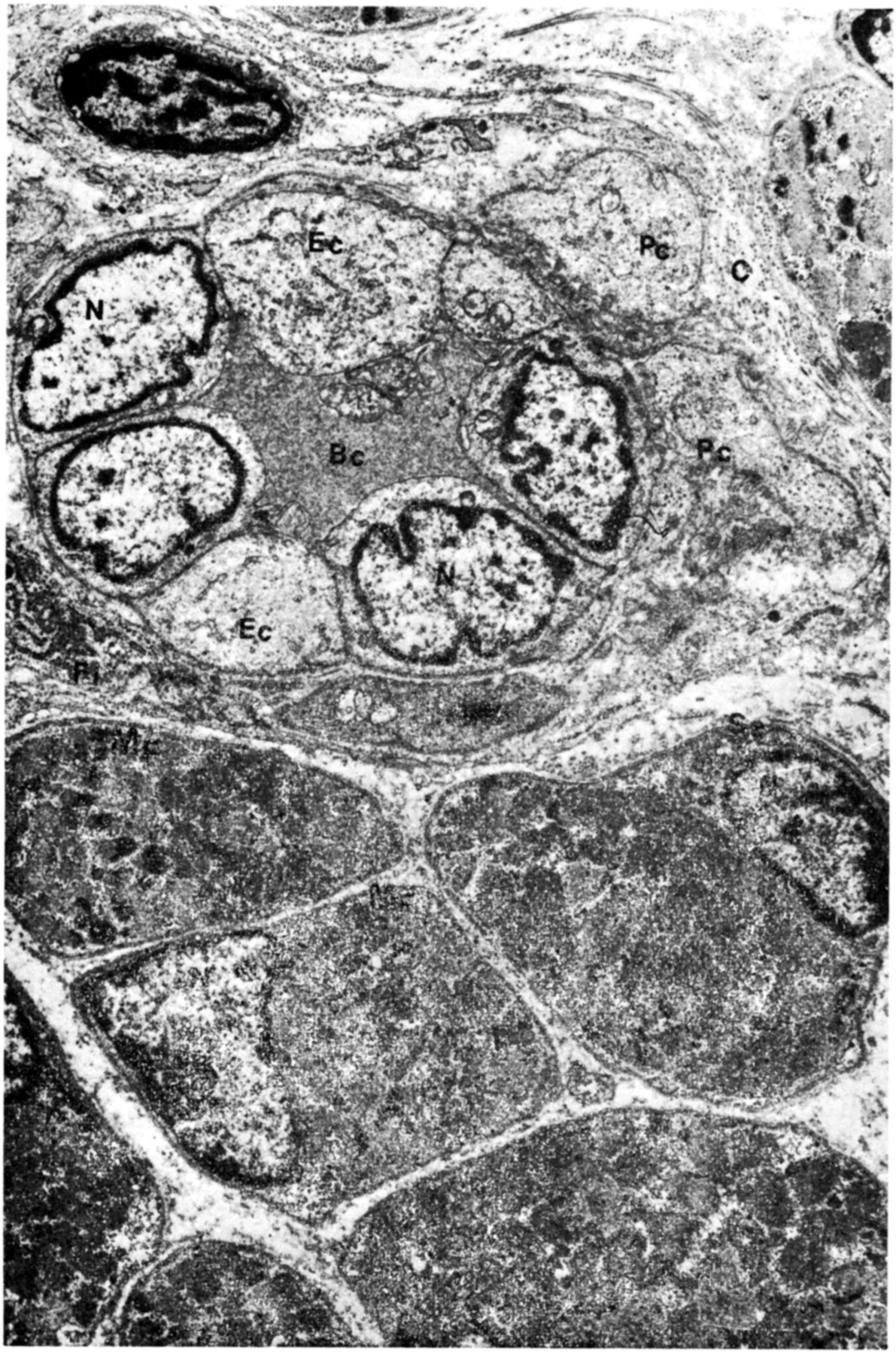

Fig. 7 — A blood capillary lined by seven endothelial cells lies between muscle fibres. Basement membrane surrounds the endothelial cells and pericytes occur in clefts in the basement membrane. X 2,700. C = collagen, Ec = endothelial cell. Fi = fibroblast. Mf = muscle fibre, N = nucleus, Pc = pericyte, Sa = satellite cell.

COMMENTS

Blood vessels are distributed widely amongst the foetal muscle cells at all stages of development examined in this investigation.

Regarding the capillaries they are lined by flattended endothelial cells on the outer surface of which is a covering of basement membrane: they are the so-called continuous type (Bloom and Fawcett, 1975) in which the endothelial cells are closely applied throughout their extent. This is in contrast to the capillaries of the fenestrated type which present openings in the capillary wall as in the visceral capillaries wich are concerned with the production and absorption of fluid. The adjacent endothelial cells of the capillaries of the muscle at nine weeks development present electron dense tight junctions at their surfaces. These junctional complexes are of the macula occludens type. Many endothelial cells in the capillar wall were undergoing mitosis and even in these cells tight junctions between them and their neighbouring endothelial cells are observed. In the endothelial cell cytoplasm of the capillaries in foetuses of fifteen weeks onwards occur many pinocytotic vesicles (Bailey et al., 1971).

Developing veins also are frequent in the muscle. Three coats can be distinguished in their walls, the intima, media and adventitia. The intima is formed of a continuous layer of flattended endothelial cells and tight junctions occur at zones of contact of adjacent cells. The medial coat however is ill defined even at sixteen weeks development and many gaps occur between the smooth muscle cells which form it. An abundance of collagen fibrils lie external to the media amongst the loosely arranged non-continuous cells of the adventitial coat.

Arteries can be identified in the foetus of sixteen weeks. They are formed of intimal, medial and adventitial coats with an interrupted layer of fibrils representing the internal elastic lamina separating the intima from the media. The intimal cells contrast markedly with those of the veins and capillaries in that they are columnar in shape and very numerous. They are of two types, one being much more electron dense than the other. However there are no reports in the literature which would indicate the functional difference between the two type of intimal cells. Tight juctions occur between the various cells of the intima. In addition, the internal elastic lamina is interrupted fairly frequently by foot processes of the clear cells making contact with the smooth muscle cells of the media. At these zones of contact the membranes of the contacting cells are ill defined. Here the basement membrane layer which covers the inner surface of the medial coat is also interrupted. A layer of basement membrane is closely related to the outer surface of the smooth muscle cells of the medial coat. The adventitial coat is formed of several layers of concentric elongated cells with collagen fibrils between them.

RESUMO

Ultraestrutura de vasos sanguineos intramusculares humanos em desenvolvimento.

Foram examinados por ultramicroscopia vasos sanguíneos provenientes da biópsia muscular de 27 fetos humanos de 9 semanas a 9 meses de desenvolvimento intra-uterino. Na nona semana de vida embrionária os vasos sanguíneos intramusculares têm a aparência de capilares cujas células endoteliais são envoltas por membrana basal e abaixo desta muitas vezes são identificados pericitos. Tais capilares são do tipo contínuo. Nas áreas de contato entre as células endoteliais ocorrem desmossomas.

Veias e artérias bem formadas são vistas a partir da décima sexta semana Nas artérias são observadas amplas zonas de contato entre a camada endotelial e a camada seguinte formada pelas células musculares lisas. Tais zonas de contato interrompem periodicamente a lâmina elástica interna e são originárias, na verdade, de células claras que situadas entre as células endoteliais mantêm com estas uma íntima relação através de numerosos desmossomas. Não foi possível identificar os núcleos de tais células cuja função parece ser a de fixar a íntima na camada média.

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