The Mammalian Lacteal: its Histological Structure in Relation to its Physiological Properties.

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(Plates 3–5.)

1. *Introduction.*

One of us (H. W. F.) (7, 8) has already noted that the mesenteric lacteals contract on appropriate stimulation in a number of animals. He also observed in the guinea-pig and the rat that the vessels were rhythmically contractile. In the latter species the nuclei of circular muscle fibres in the lacteal wall could be seen in the living condition. The difficulties, however, of making out the part played by smooth muscle cells and nuclei in such contractions are very great in the living preparation. It was, therefore, resolved to supplement these observations on the living mesentery by others on fixed and stained material. In some cases the excised lacteal had actually been observed to contract in the living mesentery.

The mesenteric lacteals of the cat have been taken as a type, and special attention has been paid to the distribution of smooth muscle fibres in the lymphatics in the various species studied.

2. *Methods.*

An apparatus enabling microscopic observation to be made on lacteals in the living animal has already been described by Florey elsewhere (7). For the preparation of fixed specimens, areas of mesentery containing lacteals were lightly stretched over a wide glass tube; a thread was tied round the tube; the mesentery outside the thread was cut away; the tube and the stretched portion of mesentery were then fixed in 2·5 per cent. or 5 per cent. formalin. Another method of obtaining uncurled pieces of mesentery was to place a piece of filter paper beneath the mesentery; a portion of the latter containing lymphatics was then cut out with the underlying filter paper, which was then dropped, mesentery downwards, into a Petri dish of the fixative. The great

majority of mesenteries so treated remained lightly adherent during fixation to the paper. They were easily detached prior to staining.

A number of stains were used, but the most suitable general stains for whole preparations were found to be the iron hæmatoxylins of Heidenhain and of Weigert. Both these methods, after careful differentiation beneath a staining microscope, give good pictures of lacteals.

Whole preparations made in the ways referred to above can be studied with powers up to a 3, and even a 2 mm., apochromatic oil-immersion objective. Sections were prepared by fixing pieces of mesentery in 5 per cent. formalin, cutting at 5μ, and staining by the usual methods.

3. *Histology of the Lacteals.*

A. *The lacteals of the cat.*—Sections of mesenteric lacteals show :—

(i) An endothelial layer. The cells comprising this are very similar to the endothelium of a vein, except that their arrangement and outlines are more irregular. Often the nuclei of the lacteal endothelium are rather obliquely set (*see* Plate 3, fig. 4). This, of course, is the result of the more irregular imbrication of the endothelial cells in lymphatics.

(ii) An adventitial layer. In a fairly large lacteal (see Plate 4, fig. 6), of 150 by 450 μ in diameter, the wall of the lacteal is made up of fine collagen fibres. Between these are small and rather darkly staining nuclei which we identify as connective tissue nuclei. The outermost fibres of the adventitia merge into those of the mesentery.

An elastic framework, mingled with the collagen fibres, is constant in lacteals other than lymphatic capillaries. These fibres are mainly longitudinal with frequent cross-anastomoses. The latter are often cut through obliquely in transverse sections of the vessel (see Plate 4, fig. 7).

The collagen fibres of a lacteal (Plate 4, fig. 6) are very thin as compared with those of a vein of approximately the same calibre (Plate 4, fig. 9). When the vessel contains lymph at the moment of fixation, the proteid is precipitated to form a very fine granular coagulum. Two small lacteals, closely applied against the larger vessel, are also shown in fig. 6 ; their walls are composed of endothelium and a few very fine collagen fibrils. Capillary blood-vessels are often found closely applied to the lacteal's wall.

The above description merely confirms that given in the well-known works on microscopic anatomy [*e.g.*, Schafer (14), Delamarre in Poirier (11), Prenant and Bouin (12) ; Bartels in Bardeleben (1)].

Smooth muscle fibres, when they occur, are intimately mingled with the collagen fibres and the connective-tissue cells of the lacteal wall. Some care is necessary to distinguish between smooth muscle and the other elements, and we have found the following criteria valuable :—

(i) The nucleus of the smooth muscle fibre is relatively larger than that of the connective tissue cell.

(ii) Its tendency to take up stains is generally less marked in one and the same preparation.

(iii) The smooth muscle nucleus is transversely or rather obliquely orientated with regard to the long axis of the lacteal. It is never parallel to the latter. This is in contrast with the endothelial nuclei.

(iv) The spiral nuclear fold of the smooth muscle nucleus is often a very useful criterion in fixed and stained specimens. With certain metallic impregnation methods, such as the Cajal method for the Golgi bodies, this spiral structure is deeply impregnated, as shown by Rio Hortega (13), and confirmed by one of us [H.M.C.; 2]. It then looks like a spiral band wrapped around the nucleus. In carefully differentiated sections stained in Heidenhain's iron hæmatoxylin, the spiral "band" is seen really to be a rather irregular fold or incision running spirally along the nuclear membrane of the smooth muscle fibre (Champy and Carleton) (3). It can also be observed in unstained preparations mounted in a medium of low refractive index.

We have made use of this structure to distinguish between the nuclei of smooth muscle, and, for example, those of connective tissue. We have never seen this spiral incision in cells other than smooth muscle cells; on the other hand, not all smooth muscle nuclei show it. So that, while a nucleus showing spiral folding can be branded as belonging to a smooth muscle fibre, a nucleus in which this structure is not present cannot, on this score alone, be eliminated from smooth muscle. It is necessary, in such cases, to assess the type of cell on points i, ii, and iii also.

Regarding the nature of the spiral nuclear fold, it is quite possibly an artefact, in the sense that it represents a contortion due to abnormal contraction of the muscle fibre when brought into contact with the fixative.

We must point out that the identification of an isolated smooth muscle fibre, lying between collagen fibres and connective-tissue nuclei, cannot be established by staining reactions only. For instance, although van Gieson's stain will selectively stain the collagen fibres red, an isolated smooth muscle fibre will

not be stained yellow. It will tend rather to stain like the surrounding (fuchsinophil) collagen elements.

Examination of many sections and whole preparations has led us to conclude as follows: The medium-sized lacteals (*i.e.*, of 100 to 200μ in diameter) contain typical smooth muscle nuclei. In transverse sections of lacteals they are difficult to find. This is because they are relatively few, as compared with the endothelial and connective-tissue nuclei. In longitudinal sections which have shaved the outer wall of the vessel smooth muscle nuclei are more easily found. In whole preparations they can be identified with facility when present (see Plate 3; figs. 2 and 3).

The spiral nuclear folds are shown in fig. 3; in fig. 2 the region of the lacteal depicted does not show these spiral incisions—a rare condition. But even here the orientation, size and position of these nuclei in the connective-tissue framework of the vessel wall enables one to identify them.

In the smaller lacteals smooth muscle nuclei cannot be seen. In Plate 3, fig. 1, is shown a segment of such a vessel some 30 μ in diameter. The endothelial nuclei on the upper and lower surfaces can be seen; a few connective-tissue cells are scantily grouped along the vessel. But there are no smooth muscle elements, nor can any be seen after careful examination of the entire length (4 mm.) of the vessel included in the fragment of mesentery. Yet lacteals of this size in the cat contract on strong stimulation by a faradic current.

B. *The lacteals of the squirrel.*—The vessels in this animal may easily be seen to contract on stimulation by a faradic current applied by means of a fine unipolar electrode. An annular contraction appears at the point stimulated, slightly spreading along the vessel. The diameter of the latter may be reduced by about one-third.

The general histology of the lacteals is much as in the cat. Extensive search for smooth muscle nuclei or fibres in small and medium-sized lacteals (*i.e.*, in vessels up to 300 μ in diameter) has convinced us that muscular elements are nearly—if not entirely—absent. We say "nearly absent" although we have not seen any smooth muscle nuclei in vessels up to the limit mentioned (300 μ diameter). But obviously the total length of lacteal examined beneath the microscope is far inferior to that in a whole mesentery. Still stronger evidence, however, that contraction in these vessels may be independent of muscle, is furnished by the excision and careful examination of segments of lacteal which had previously been seen to contract in the living. Examination of such segments, each over 4 mm. in length, has failed to reveal smooth muscle nuclei or fibres.

In Plate 3, fig. 4, one side of a lacteal from the squirrel's mesentery is shown. The diameter of this vessel, in a fixed and stained whole preparation, is 110 μ; its length, 11·25 mm. No smooth muscle nuclei or fibres can be found. The slightly oblique and pale nuclei (E.2) are seen, on focussing, to be obviously endothelial. The darkly stained and sometimes oblique nuclei (X in the figure) are easily identified on focussing, with the nuclei of the fat cells and their connective tissue framework lying about the lacteal.

C. *The distribution of smooth muscle nuclei and fibres in some other animals.*—*In the guinea-pig, the rat and the mouse*, the medium-sized lacteals contain abundant smooth muscle. In the rat smooth muscle nuclei and fibres are also found in fairly small vessels. Thus, in Plate 4, fig. 5, there are abundant smooth muscle nuclei, the majority of which show the spiral folding. The outline of a muscle fibre (rarely seen in whole preparations) is shown at M.F. in this figure. The diameter of this vessel was 50 μ.

In some specimens of lacteal of the *dog*, the *hedgehog*, and the *pig*, smooth muscle was plentiful in the larger vessels, particularly in the dog. Our scanty preparations of these species show no small lacteals, so we cannot say whether these contain muscle or not.

In the *rabbit* smooth muscle nuclei are easily identified.

(4) *The Nerves of the Lacteals.*

Observations were made on the nerves of these vessels by vital staining with methylene blue. The dye used was "methylen blau, rectif, nach Ehrlich of Grübler," and was administered in warm saline intraperitoneally.

In the guinea-pig the nerves of the lacteals were very clearly shown after injections of 0·5 per cent. and 0·25 per cent. solutions of the dye. The amount injected varied from 25 c.c. to 75 c.c. The animals were killed 1½ hours after the injection. Pieces of mesentery, stretched out on filter paper, were fixed in a saturated aqueous solution of ammonium picrate. They were mounted, examined and photographed also in this medium.

The nerves of the lacteal wall pursue a mainly longitudinal course and are finely varicose (Plate 5, fig. 10). They are amyelinated. No nerve cells are visible.

In the squirrel (Plate 5, figs. 11 and 12) the peri-lymphatic nervous network was demonstrated by an intraperitoneal injection of 0·5 per cent. methylene blue, and the animal was killed 1 hour afterwards.

In the large lacteals (*i.e.*, in those of 200 μ in diameter and above) the nerve fibres anastomose freely and form a network. Between the anastomoses nerve

cells can sometimes be seen. Fine twigs can also be observed to run towards, and to touch, the smooth muscle fibres which exist in the large lacteals in the squirrel. The relation of the nerves to the muscle fibres cannot be satisfactorily shown in a microphotograph on account of the thickness of the vessels and the refringency of the fat around them.

In the small lacteals, devoid of smooth muscle, no nerves can be made out. This would suggest that the nerve supply of the lacteal in the squirrel is motor, but we would not, at present, go further than suggesting this as a possibility. We have only examined the nerve supply of the squirrel's lacteals in one animal. The methylene blue method is capricious, and a negative result, with regard to the nerve supply to the endothelial portion, unless repeatedly confirmed, must be accepted with some reserve.

The results here recorded on the nerve supply of lacteals are in agreement with the observations of Dogiel (6) and Kytmanof (10) who, however, studied other lymphatics than those of the mesentery.

5. *Discussion.*

From observations recorded elsewhere (7 and 8), it was found that in two species—the rat and the guinea-pig—rhythmically contractile lacteals were present. Other species so far examined under precisely similar conditions have not given any evidence of rhythmicity, though found to be contractile to various stimuli. In searching for a possible cause for this the present histological investigation was made.

It would appear that there is no essential difference detectable in the lacteals of the guinea-pig and rat on the one hand and the cat on the other, except that the two former species have a rather more abundant supply of smooth muscle. When it is said that a lacteal of the guinea-pig is not nearly so rich in muscle as a mesenteric vein of corresponding size, some idea will be gained of the small complement of smooth muscle which is capable of imparting rhythmical propagated contractions to these vessels.

The question arises as to the cause of this rhythmic action. Is it the result of nervous influences, or is the smooth muscle solely responsible? In preparations from guinea-pig mesentery no nerve cells have been rendered visible by the methods used, but, on the other hand, in the squirrel, definite nerve cells are present. It is therefore difficult to deny the possibility that the rhythmicity present in the guinea-pig's lacteals may owe its origin to the presence of nerve cells—though none have been seen in our preparations.

One species—the squirrel—has a very scanty supply of smooth muscle. It

is only in vessels above 400 μ that any can be detected. In the smaller lacteals of the cat also no smooth muscle can be found. These vessels have been seen to contract when stimulated by a strong faradic current or mechanically. It must be confessed that neither of these stimuli are physiological, and that the vessels without muscle nuclei do not contract to such drugs as adrenalin or pituitrin. Nevertheless, it would appear that one is dealing with vessels which are capable of contraction, though possessed only of an endothelial wall. There can be no question of the presence of such cells as the Rouget type to account for contraction. Smooth muscle, after the most careful search has not been found.

Tarchanoff (15), at the same time as he described the contraction of blood capillaries in the tadpole's tail, described similar though slower contractions in the lymphatic capillaries. The contention of Florey and Carleton (9) and E. R. and E. L. Clark (4, 5) that capillary contraction is quite independent of the presence of Rouget cells is strengthened by the above observations.

6. *Summary.*

1. The histological structure of the mesenteric lacteals of the guinea-pig, rat, cat, mouse, hedgehog, dog, pig and squirrel is described.

2. There is no histological evidence to show why the lacteals of the rat and guinea-pig are contractile and those of other species under the same conditions are not.

3. It is pointed out how small an amount of smooth muscle is responsible for the rhythmic contraction in the rat and guinea-pig.

4. The lacteals of the squirrel of less than 400 μ in diameter do not possess any smooth muscle. They are, however, contractile to electrical and mechanical stimuli. These phenomena are adduced as additional evidence that some endothelial structures are contractile.

5. The lacteals of the guinea-pig and squirrel have been shown to be innervated. The nerves are probably motor. Nerve cells have also been found on the vessels in the squirrel.

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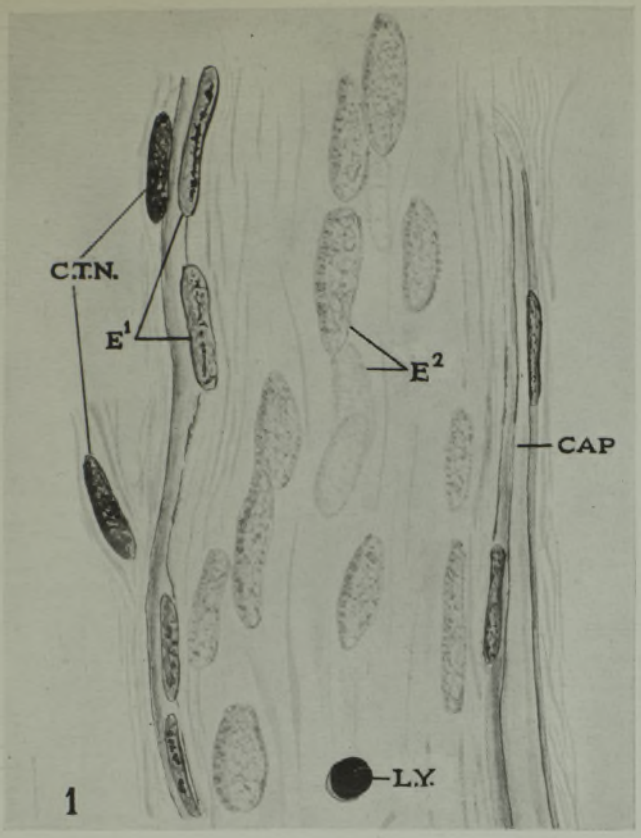

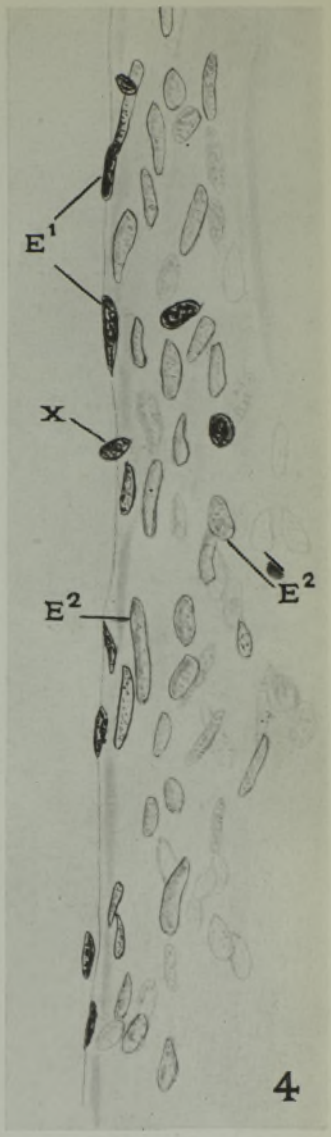

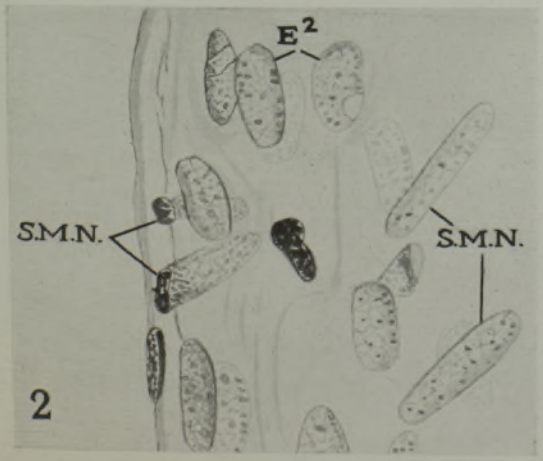

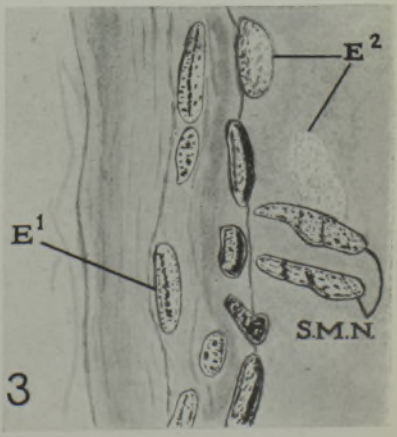

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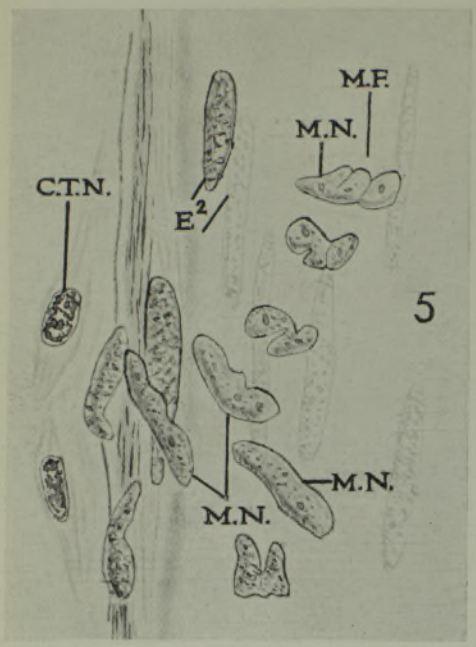

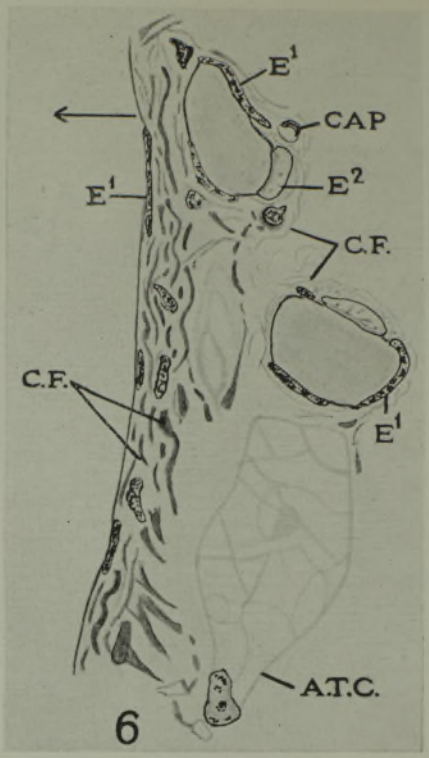

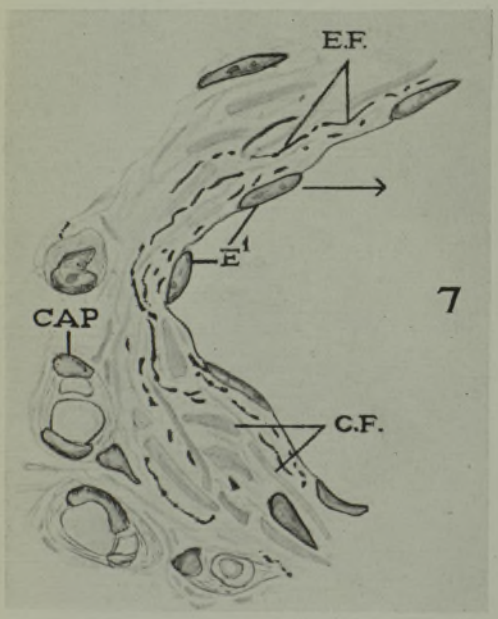

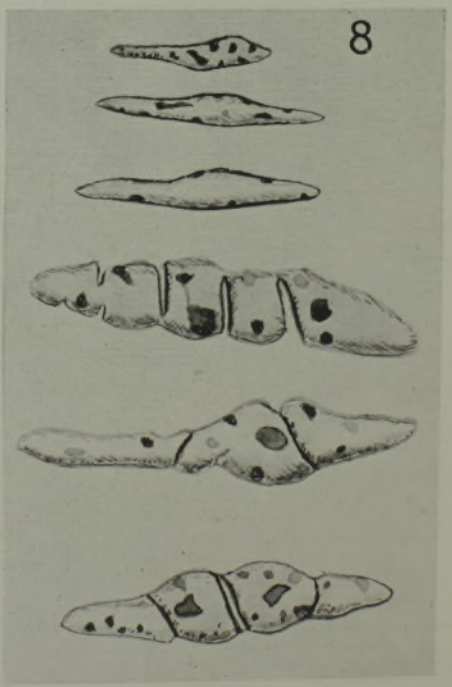

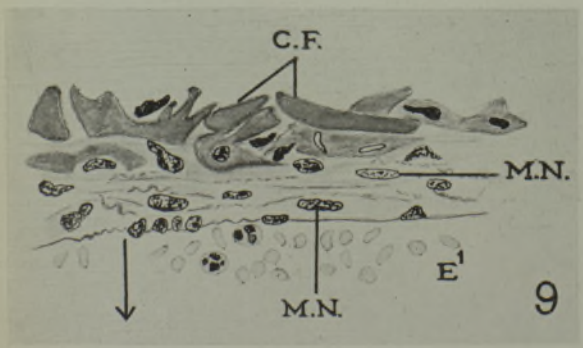

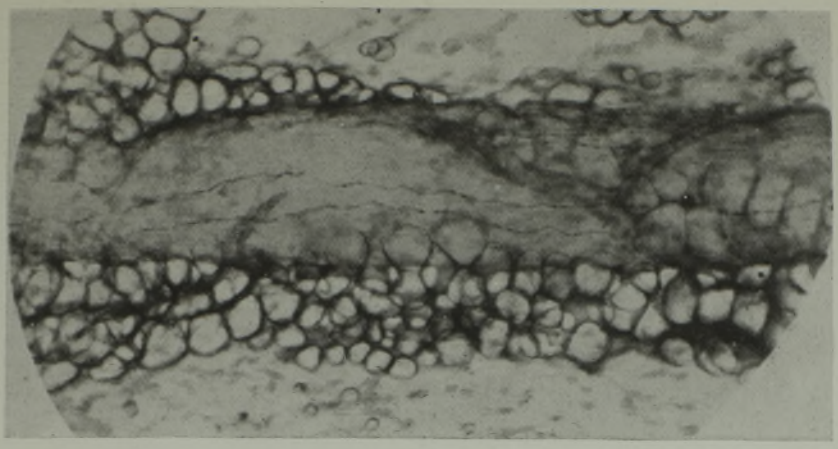

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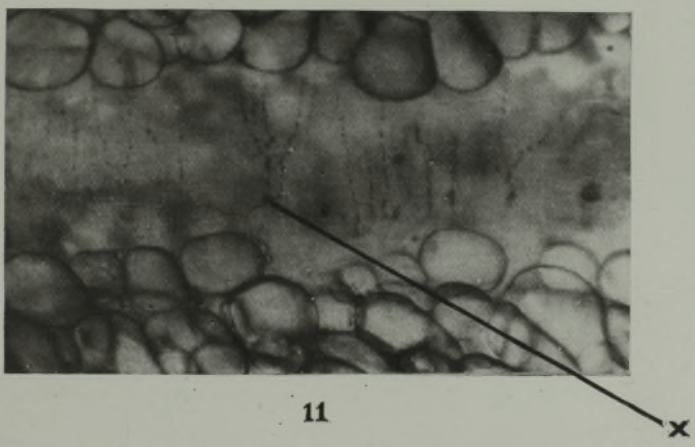

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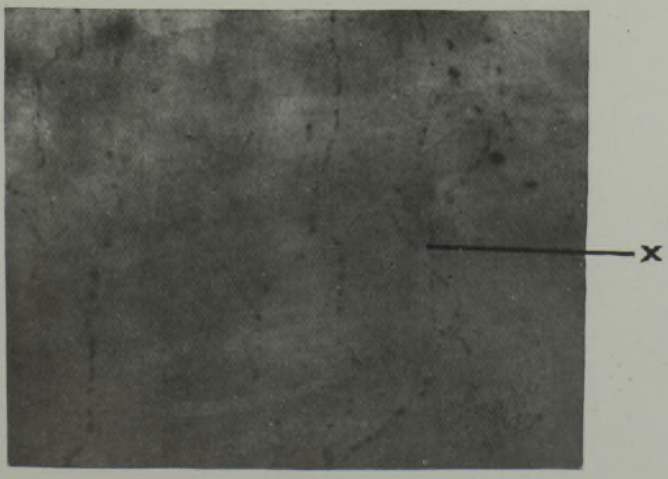

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(8) EXPLANATION OF PLATES.

Plate 3.

All the figures are drawn with the Abbe camera lucida. Nos. 1 to 5 are from "whole preparations" of mesentery; in these the uppermost nuclei or cells are depicted darker than the underlying elements. Both endothelial surfaces (*i.e.*, upper and lower with regard to the objective) are conventionally demarcated in this manner, except in fig. 5, in which only the upper wall of the vessel is shown. Figs. 6 to 9 are all from sections of 5 μ in thickness.

The arrows point towards the lumen of the lacteal.

Lettering.—A.T.C. = adipose tissue cell; CAP = capillary blood-vessel; C.F. = collagen fibres; C.T.N. = connective-tissue nucleus; E^1 = endothelial nucleus seen in profile; E^2 = endothelial nucleus seen "en face"; E.F. = elastic fibres; LY = lymphocyte; M.F. = outline of smooth muscle fibre; S.M.N. = smooth muscle nucleus; ×, see the explanation of the figure.

Fig. 1 (× 1050).—A small lacteal in a whole preparation of mesentery of cat. Transverse diameter circa 30 μ. Formol; Weigert's iron hæmatoxylin—van Gieson.

Endothelial (E^1 and E^2) and connective-tissue nuclei (C.T.N.) can be seen, but no smooth muscle nuclei. The length of this vessel is 3 mm.; no smooth muscle nuclei or fibres can be seen anywhere along it.

Fig. 2 (× 1050).—A portion of the wall of a fair-sized lacteal in the same specimen. Diameter circa 125 μ. Smooth muscle nuclei (S.M.N.) are here present, in addition to those of the endothelium and connective tissue of the lacteal. The smooth muscle elements are only slightly, if at all, contracted. Hence the spiral nuclear incisions, so often shown in fixed and stained specimens of smooth muscle (see fig. 8) are not in evidence.

Fig. 3 (× 1050).—Another portion of the wall of a large lacteal of the cat. Diameter circa 220 μ. Formol; Weigert's iron hæmatoxylin. Two characteristic smooth muscle nuclei (S.M.N.) are seen lying outside the endothelium transversely to the long axis of the vessel. Spiral nuclear incisions are shown in these.

Fig. 4 (× 525).—Portion of the wall of a fair-sized lacteal of the squirrel. Whole preparation; formol; Weigert's iron hæmatoxylin. Diameter of vessel circa 110 μ.

Endothelial nuclei (E^1 and E^2) can be seen; so can dark nuclei (X), which, on focussing, are seen to form the pavement epithelium of the mesentery. Muscle nuclei or fibres cannot be made out in this or in other lacteals below and up to this size in the squirrel.

PLATE 4.

FIG. 5 (× 1050).—Part of the wall of a whole preparation of the lacteals of the rat. Formol; Weigert's iron hæmatoxylin. Abundant smooth muscle nuclei (S.M.N.) are present; their orientation is transverse or oblique to the long axis of the vessel; some of them show typical spiral nuclear incisions. The outlines of some of the muscle fibres can be faintly seen at M.F.

FIG. 6 (× 525).—Transverse sections of part of the wall of one large and two small lacteals of the cat. Formol; Weigert's iron hæmatoxylin—van Gieson. The endothelial lining (E^1) and the thin wall of delicate collagen fibres (C.F.) are shown in the larger vessel. Nuclei are scattered amongst the collagen fibres; the former are connective tissue rather than muscle nuclei.

The two small lacteals show only endothelium (E^1 and E^2), and a very delicate investment of collagen fibrils (C.F.).

FIG. 7 (× 1050).—Slightly oblique section of the wall of a lacteal in the cat. Formol; Weigert's elastin stain—lithium carmine.

The presence of elastic fibres (E.F.) as well as collagen ones (C.F.) is shown. So also are the capillary blood-vessels (CAP), often noted in close proximity of lacteals.

The arrangement of the elastic and collagen fibres, when studied in longitudinal sections or in whole preparations of the lacteals, is mainly parallel to the long axis of the vessel. But cross anastomoses are very frequent in the elastic meshwork.

FIG. 8 (× 2100).—Illustrates the differences between the nuclei of smooth muscle fibres and those of the connective tissue in the wall of a vein in a section of the cat's mesentery. Formol; Weigert's iron hæmatoxylin—van Gieson.

The vein being contracted, the characteristic spiral nuclear incisions of the smooth muscle nuclei are clearly shown. Three connective-tissue nuclei above, three smooth muscle nuclei beneath.

FIG. 9 (× 525).—Transverse sections of a medium-sized vein in the cat's mesentery. Formol; Weigert's iron hæmatoxylin—van Gieson.

Obvious smooth muscle nuclei (S.M.N.) and fibres can be seen in the tunica media. The collagen fibres (C.F.) of the tunica adventitia are far stouter than in the lacteals.

PLATE 5.

FIG. 10 (× 81).—Lacteal of guinea-pig, 200 μ in diameter. Nerve fibres longitudinal to the main axis of the vessel. The very refringent perilymphatic fat is well shown.

FIG. 11 (× 81).—Lacteal of squirrel, 220 μ in diameter. The nerve fibres here are mainly transverse. At × is a nerve cell.

FIG. 12 (× 354).—The same preparation at a higher magnification. Illustrating the fine anastomoses between the fibres. At × is the nucleus of a smooth muscle fibre.

All the above microphotographs were taken by Mr. F. Haynes with a Leitz "Micca" camera. They were subsequently enlarged by Mr. Wm. Chesterman. The present approximate magnifications are given for each figure. The microphotographs are untouched; they all represent nerves in the lacteal wall stained by vital intra-peritoneal injections of methylene blue and fixed by the ammonium picrate method.