THE SPHINCTER MUSCLE PATTERN AS FOUND IN THE MICROCIRCULATION OF THE DOG'S LIVER[1]

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The existence of muscle sphincter components in capillaries was first introduced through the work of Rouget (1873) who described the Rouget cells along the capillaries of the nictitating membrane of the frog's eye. These cells have been the subject of some controversy, and it is strongly suspected that the Rouget cells of frogs are different from the sphincter muscles of mammals. Work is presently being carried on in this laboratory to clarify the identity of these cells. Although Rouget cells are reported to resemble smooth muscle cells which lack the birefrigent myofibrils characteristic of smooth muscle, they are thought to be responsible for the contraction of capillaries; however, Midsuno (1931), Clark (1936) and others reported that endothelial cells may contract independently of these primitive stellate cells. The findings in this laboratory tend to uphold the concept that only the true muscle sphincters are responsible for the contraction of capilliaries.

The importance of these true muscle sphincters is becoming increasingly apparent since various workers have effectively demonstrated that selected vasodilators and constrictors, in various concentrations, can initiate activity in these cells independent of the muscular components of larger blood vessels, thus indicating that capillaries play a far more important role in circulation than previously suspected.

The presence of these sphincters has been demonstrated in few mammalian organs and since their presence is apparently of some importance, work has been carried on in this laboratory to demonstrate their presence in several major mammalian tissues. In the course of these investigations, the capillary components (metarterioles, precapillaries and capillaries) have received considerable attention and certain morphological variations, from organ to organ, have been encountered. Such differences may prove to be of some interest to those interested in the physics of flow in circulation and vessel wall permeability.

The present study deals with the histology of the capillary components of the dog's liver and the demonstration of sphincter muscles associated with metarterioles and precapillaries.

Materials and Methods

Mongrel dogs were anesthetized by intravenous injection of diallyl-barbituric acid. An abdominal incision was made, the liver was exposed and blocks of tissue were taken from various areas of the lobes. The tissue was immediately placed in the fixative. The gradual fixation technique (Provenza and Scherlis, 1958) was employed involving frequent changes in varying concentrations of formalin. The tissue was finally washed in running tap water, dehydrated by the usual alcohol series, cleared in xylene and embedded. Cross and longitudinal sections were made at 5 microns in thickness from the various blocks and serially arranged. Alternate slides were stained with Gomori's trichrome (Gomori, 1950) and Bodian's (Bodian, 1936) while the others were stained with Haematoxylin-Eosin and Bodian's. The Gomori trichrome was used for connective tissue fibers, Bodian's for nerve fibers and Haematoxylin-Eosin for muscle fibers.

Results

In the dog's liver, the larger arteries anastomose freely in the connective tissue septa. The arteries of greater caliber are gradually reduced in diameter until the size of the terminal arteriole is reached. Generally, bifurcation of the larger vessels (arteries) occurs in the interlobular septa in conjunction with partitioning of the septal tissue. The branching, characteristic of arteries, is of a dichotomous type in which the two daughter arteries are approximately the same size. The arteries in the liver, like those found elsewhere, are composed of three coats, the intima, the media and the adventitia. Two interesting observations, however, were made relative to the arteries found in the dog's liver. The subendothelium and the elastic membrane of the tunica intima are generally present in the smaller arterioles and consistently present in the larger ones. The tunica adventitia consists of dense areolar connective tissue continuous with that found in the trabecular areas.

The terminal arterioles differ in size from the parent vessel. The intima consists of an endothelial lining which rests on a poorly developed subendothelium. The internal elastic membrane, when present, is poorly defined. The media consists of at least one layer of muscle cells which are contiguously arranged (fig. 1). The adventitia is composed of fine collagenic fibers which invade the spaces between the parenchyma cells of the lobules.

The distal portion of the terminal arteriole is observed to communicate with a sphincter-type capillary, the metarteriole (fig. 2). Contrary to the descriptions given in most textbooks, the arteriole is sharply demarcated from the metarteriole inas-

much as the continuous muscle coat of the arteriole ends abrupt-
ly rather than tapering off gradually. The metarteriole consists
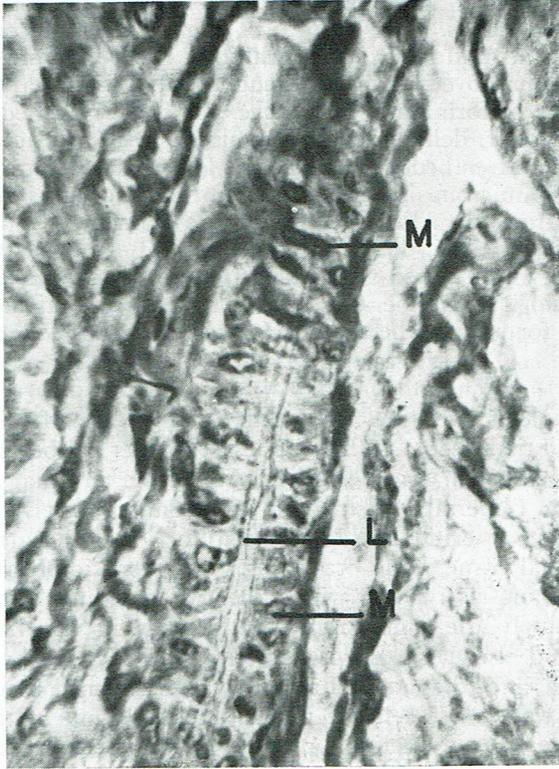

Figure 1.— Terminal arteriole in dog liver. Gradual Formalin fixation.
Haematoxylin-Eosin. X970.
Note: transverse muscle fibers abutting one another along lateral margins.
M: muscle fiber nucleus; L: lumen of arteriole.

of an endothelial lining, one cell in thickness, which is con-
tinuous with the lining of the arteriole. These endothelial cells
are not observed to rest on a subendothelium and an internal
elastic lamina is not seen. At varying intervals along the length
of the metarteriole are found muscle fibers which constitute a
sphincter. The number of muscle fibers comprising a sphincter
may vary from 1 to 3. Generally, the distance between the
sphincters is increased as the terminal of the metarteriole is
reached. Although the number of muscle fibers constituting a
sphincter is usually decreased towards the distal end of the

metarteriole, this condition need not prevail since sphincters consisting of 2 or 3 fibers commonly follow those consisting of only 1 fiber. These vessels are found interlobularly as well as
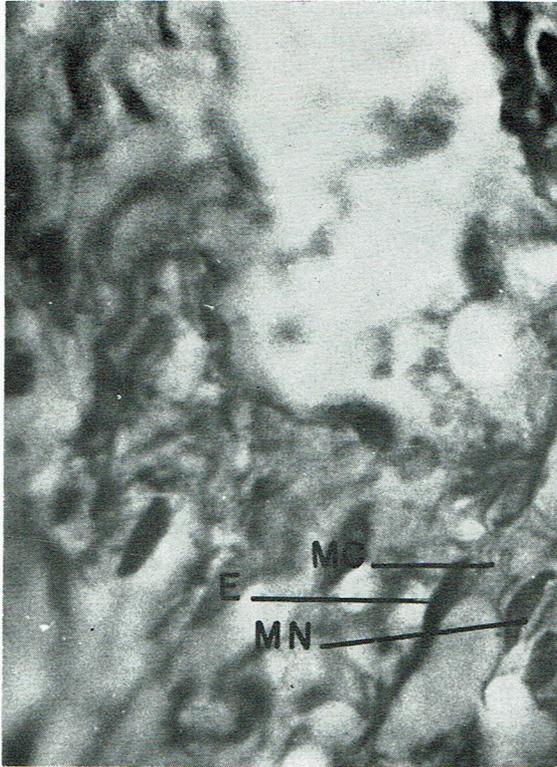

Figure 2.— Metarteriole in dog liver. Gradual formalin fixation. Haematoxylin-Eosin. X1450.
Note: muscle sphincter consisting of 1 muscle fiber circumferentiating endothelial lining of metarteriole. MN: muscle nucleus; MC: muscle cytoplasm; E: endothelial nucleus.

intralobularly and there does not appear to be any difference in their structure when seen intralobularly.

Another sphincter-type capillary found in the hepatic lobules and the septa, is the precapillary (fig. 3). In this type of vessel a sphincter, consisting of 1 to 3 muscle fibers, is located at the junction of its attachment to the parent arteriole or metarteriole. The entire region distal to the precapillary sphincter is indistinguishable from the true or classic capillary in that the sole compostion of the vessel is endothelial and devoid of muscle fibers (fig. 4).

Being cognizant of the fact that Rouget cells have been reported to be associated with the true capillaries, sections of true capillaries were stained by the methylene blue technique (Vimtrup, 1922), and critically examined with both light and phase microscopes, but the only cellular constituents observed to be asso-
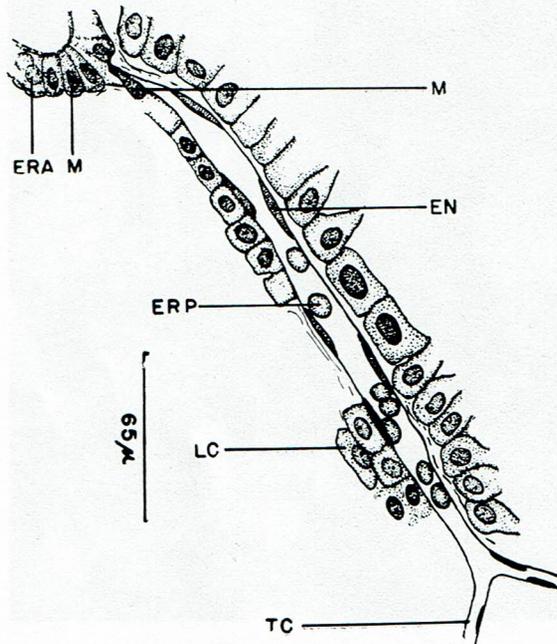
Figure 3.— India ink drawing of precapillary. Gradual formalin fixation. Haematoxylin.
Note: muscle sphincter at junction of arteriole and precapillary. ERA: erythrocyte in lumen of arteriole; M: muscle nucleus; EN: endothelial nucleus; ERP: erythrocyte in lumen of precapillary; LC: liver cell; TC: true capillary.

ciated with true capillaries, as the perithelium, were stellate mesenchymal cells, fibroblasts, mast cells and an occasional macrophage; no cells were seen which could be interpreted as the classic Rouget cell as described by Rouget (1873, 1879) and Vimtrup (1922, 1923).

Nerve bundles invade the liver via the porta, associated with the connective tissue. Upon entering the liver, the nerve bundles divide. Although the branching of nerve bundles is usually associated with the lobulation of the substance of the liver, fibers are frequently seen branching from the parent trunk. Daughter nerve fibers thus originated, usually travel to the larger accompanying artery and vein but they may undergo further
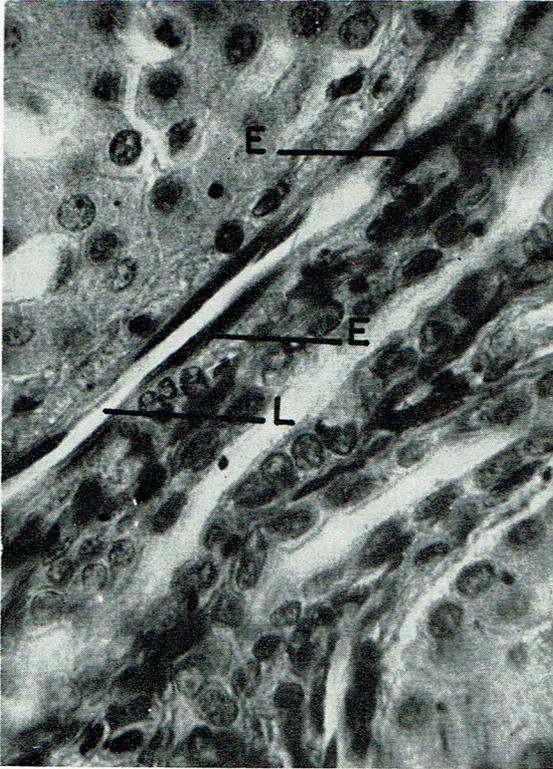

Figure 4.— True capillary in dog liver. Gradual formalin fixation. Haematoxylin-Eosin. X970.
Note: simple endothelial lining without muscle sphincter. E: endothelial nucleus; L: lumen of true capillary.

division and these tertiary branches travel to the smaller vessels in the area. On reaching the vessel, the nerve fibers enter the media of the vessel and terminate as beads or knots in the vicinity of the nuclei of the muscle fibers. Other diminutive nerve trunks, resulting from further ramifications and branchings, are associated with the still smaller vessels (arterioles and

venules). Such smaller nerve fibers, which are specifically asso-
ciated with arterioles, run parallel to the vessels and at intervals
give rise to minute fibers which innervate other nearby small
blood vessels. A similar condition is found in the nerve fibers
associated with metarterioles and precapillaries; however, the
nerve fibers which are parallel to sphinctered capillaries give
off even smaller fibers and these arise from the parent trunk
only in the vicinity of the sphincters. The terminals of those
nerve fibers which invade the sphincters are in the form of
knotted fibrillar processes which lie in the vicinity of the nuclei
of the muscle cells (fig. 5). It is interesting to note that nerve-
end apparati were not observed to terminate in the endothelial
cells in either the sphinctered or non-sphinctered regions of the
capillary variants.

DISCUSSION

Dichotomous branching of the parent artery to give rise to
two daughter vessels of subequal size appears to be a consistent
anatomic feature in the liver. This condition is of some interest
since in the pulp of human teeth (Provenza, 1958), in the
ventricular myocardium of the dog (Provenza and Scherlis, 1959)
two types of branching are found. The more frequent type, asso-
ciated with the larger vessels, is one where the daughter vessels are
distinctly unequal in diameter. The diameters of the larger
daughter vessels is gradually reduced while traveling through the
tissue; the diameter of the smaller daughter vessel is rapidly
reduced and soon terminates in a capillary network. It is not
inconceivable that the function of the larger vessel is primarily
one of transportation of blood to the extremities while that of
the smaller vessel is to vascularize the tissues in the immediate
area. The condition, as found in the dog's liver, is interpreted
to result from a difference in degree of metabolic activity since
in the connective tissue septa of the liver there is a reduction
of cellular elements.

The anatomic structure of the arterioles of the liver appears
to be compatible with the histologic features of the surrounding
tissue. This is borne out by the presence of a subendothelium
and an internal elastic membrane in the hepatic arterioles. A
difference was reported in the architecture of the superficial and
deeper arterioles in the ventricular myocardium of the dog
(Provenza and Scherlis, 1959). In the myocardium, the intima of
the more superficially located arterioles consists of a subendothe-
lium with a distinct internal elastic membrane, while in the
deeper arterioles the subendothelium and internal elastic mem-
brane are wanting. It is postulated that the absence of these
structures in the arterioles found in deeper tissue may be directly
correlated with the high degree of external support from the
surrounding tissue since the more superficial vessels of the heart,
which lack a great degree of support from the muscle cells, are

compensated by a more highly developed intima. It is quite possible that the degree of development of the tunica intima of the hepatic arterioles is similarly increased to compensate for the lack of support on the part of the surrounding parenchyma.

Much has been written, pro and con, relative to the intrinsic ability of endothelial cells *per se* to contract. Some investigators (Midsuno, 1931; Clark, 1936; Sanders et al, 1940) reported the inward bulging of the capillarial endothelium to the extent
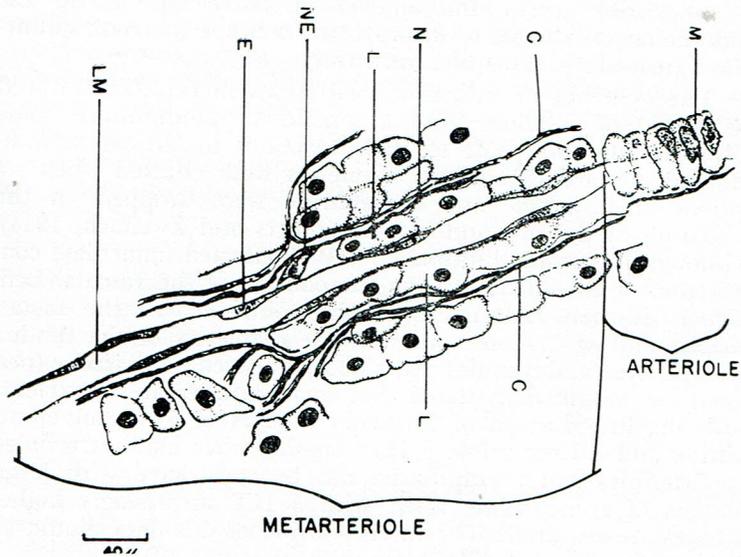

Figure 5.— India ink drawing of arteriole-metarteriole junction showing nerve ending in muscle sphincter of metarteriole. Gradual Formalin fixation. Bodian stain.
M: muscle cell; C: collagenic fibers; N: nerve fiber; L: liver cell; NE: nerve ending in muscle sphincter. E: endothelial nucleus; LM: lumen of metarteriole.

that the lumina of these vessels were occluded. Subsequent investigators (Chambers and Zweifach, 1944; Kahn and Pollak, 1931), however, are of the opinion that the inward bulging of the endothelial cells is a passive reaction resulting from the contraction of the muscle fibers of the metarteriole and pre-capillaries. The previous investigations of capillary circulation in human tissues (Provenza and Biddington, 1958; Provenza and Scherlis, 1959) and in the dog's myocardium (Provenza and Scherlis, 1959) together with this paper, tend to corroborate the interpretations of Chambers and Zweifach and Kahn and Pollak in that contraction of the endothelial cells of the true capillaries

were not seen. Where endothelial cells demonstrated inward bulging of the nuclei, these were seen in areas adjacent to the sphinctered regions of the metarterioles and the precapillaries, and they were seen only when the muscles were in a state of contraction. Furthermore, although nerve fibers were found to be associated with the true capillaries and the non-sphinctered areas of the precapillaries and metarterioles, nerve-end apparati were not found associated with the endothelial cells. Since the contraction of blood vessels is generally believed to be stimulated by associated nerves, the absence of nerve endings in the endothelial cells tends to disprove the concept that non-sphinctered areas are capable of contracting.

The existence of anatomical variations in capillaries would be devoid of meaning from a functional standpoint if some difference did not exist in their reactions to various stimuli. Marked alteration of lumen diameters were effected when solutions of histamine and epinephrine were dropped on the omentum of the dog and rat (Chambers and Zweifach, 1944). Although histamine dilutions of 1:1000 effected immediate construction of all muscle bearing components of the vascular bed, higher dilutions affected only the sphincters of the metarterioles and of the precapillaries; the diameters of the lumina of arterioles and venules remained unaltered. Zweifach (personal communication) stated that small arteries are constricted with the introduction of threshold dosages of 5 mg/ml epinephrine and 1.0 mg/ml of 5 HT; on the other hand, arterioles, metarterioles and precapillaries can be constricted with lesser dosages of epinephrine, while with 5 HT surprisingly higher dosages are required. The interpretation of this data should be forthcoming from Zweifach.

It has been shown by Cannon and Rosenblueth (1933, 1937) that epinephrine does not act directly on the nervous system since sympathectomy does not impair its pharmacologic effects. Turner (1949) summarized such experiments to mean that epineprine actually affects directly the motor endings of myo-neural junctions interpolated between fibers and the smooth muscle cells. These interpretations are basically upheld by the authors' observations despite the fact that in capillary sphincters the myo-neural junction appears to be within the muscle fiber, near the nucleus.

SUMMARY

The terminal arteriole consists of two definite coats, the tunica intima and the tunica media. The media is composed of a layer of contiguously arranged muscle fibers which surrounds the intima. The intima consists of an endothelial lining which rests upon a poorly developed sub-endothelium with or without an internal elastic membrane. The tunica adventitia, when visible, consists of fine collagonic fibers.

In the liver, there are three capillary-types which arise from the terminal arterioles: metarteriole, precapillary and the true or classic capillary. The true capillary is a simple endothelial tube. Intermediate in composition between the terminal arteriole and the true capillary are the metarteriole and the precapillary. The metarteriole is a capillary-type whose intima consist of a simple endothelial lining. At varying intervals along the length of this vessel is found a single fiber or a group of muscle fibers constituting a sphincter. The areas between sphincters are indistinguishable from the true capillary for its composition is strictly endothelial. The precapillary is a capillary-type which consists of a single sphincter (1-3 muscle cells abutting one another) and this is limited in its location to the segmental area of attachment with the parent vessel (arteriole or metarteriole). The region distal to the precapillary sphincter is likewise indistinguishable from the true capillary since its sole composition is endothelial.

ACKNOWLEDGMENT

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