Occurrence of Cardiac Muscle in the Hepatic Portal Vein Wall of the Mouse and Rat

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Cardiac myocytes have been shown to occur in the tunica media ABSTRACTand adventitia at the region near the hepatic end of the portal vein of the mouse and rat, and have been studied by electron microscopy in the mouse portal vein. They measured 3–10 μm in breadth at their nuclear level, possessed centrally located nuclei, and were connected with each other by the intercalated disk. In these myocytes in the mouse portal vein, sarcoplasmic reticulum was represented by a rather simple and loose network of the anastomosing tubules. The membrane-bound granules, which closely resemble the atrial specific granules, were found in many of the mouse portal vein myocytes. Transverse tubules, 40-200 nm in diameter, were sometimes detectable at the Z line level. The nexus occupied about 3-5% of the whole junctional area between cardiac myocytes in the tunica media, whereas in the tunica adventitia the corresponding value was about 17%. Blood capillaries with fenestrated endothelium supplied the cardiac myocytes in the adventitia of mouse portal vein. The closest relationship between the adrenergic axon and portal vein cardiac myocytes was observed to be ca. 0.3 µm apart. The significance of these findings is discussed in relation to pulsations of the portal vein.

The hepatic portal vein of some mammals (mouse, rat, guinea pig, rabbit, and cat) is known to show rhythmical spontaneous contractions with a frequency of 3–30 per min, as well as a propagation of the contractile wave toward the liver (Attardi, 1955a,b; Booz, 1959; Funaki and Bohr, 1964; Holman et al., 1968; Johansson and Ljung, 1967a,b; Mislin, 1963, 1969). Histological studies of the vessel wall have revealed the occurrence therein of smooth muscle cells, leading to the general assumption that the latter are the only muscular element responsible for portal vein contraction (Hammersen and Jungst, 1968; Holman et al., 1968; Ts'ao et al., 1970; Schipp et al., 1971).

We have recently found cardiac myocytes in the hepatic portal vein wall of the mouse and rat, and distributions and fine structural features of these myocytes are described in this paper.

MATERIALS AND METHODS

Thirty-five ddy strain mice of 20–40 gm body weight and 8 Wistar strain rats of 200–400 gm body weight were used. These were all regarded as normal adult animals; individual body weight and sex were recorded only for about one-seventh of the animals and no care was taken to obtain their exact ages. The animals were sacrificed by decapitation or deep ether anesthesia, and perfused through the superior mesenteric vein with 10 ml (mouse) or 20 ml (rat) of the fixative 4% formaldehyde in M/15 phosphate buffer (pH 7.4) for light microscopy, or 1% glutaraldehyde and 4% paraformaldehyde in the same buffer for electron microscopy (Karnovsky, 1965). The hepatic portal vein with its main branches for the hepatic lobes was then dissected out together with the

adjacent hepatic and common bile ducts, the hepatic propria artery, and additionally, in the case of mice, the cystic duct.

For light microscopy, the tissue blocks excised from 1B mice and 4 rats were further immersed in the fixative overnight, and then were dehydrated, cleared, and embedded in paraffin. Sections 5 or 7 μ m thick were cut serially in a plane parallel to the long axis of the portal vein and were stained by Masson-Goldner's trichrome stain or with Heidenhain's iron hematoxylin. One of the Epon-embedded mouse tissue blocks prepared by the method for electron microscopy (see below) was also serially sectioned at 1 μ m and stained with toluidine blue for light microscopy.

For electron microscopy, the tissue blocks from 22 mice and 4 rats were further fixed by immersion in the ice-cold fixative for 1 hr, which was followed by osmication, dehydration in ethanol and dibutyl glycidil ether (QY-1), and embedding in Epon 812. Thin sections from the Epon blocks, stained with uranyl acetate and lead citrate, were viewed in an electron microscope.

Measurements of the length of junctional plasma membranes of the cardiac myocytes were carried out on electron micrographs magnified to 14,000–17,000 using an AM 01 Kontron Image Analyzer.

OBSERVATIONS

Light Microscopy

Striated muscle cells in the hepatic portal vein wall of the mouse and rat were distinguished from other cellular elements by virtue of their dark-stained cytoplasm

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with myofibrillar striations (Figs. 1-5). Intercalated disks were detectable under a high-power view of these striated muscle cells (insets in Figs. 2 and 5), thus making it possible to identify the latter as cardiac myocytes.

Cardiac myocytes occurred usually in small groups among the compactly arranged smooth muscle cells in the tunica media, within the dense connective tissue of the inner adventitia, or within the loose connective tissue of the outer adventitia of the vein. The distribution of cardiac myocytes along the course of the portal vein and its main branches, as revealed by studies of serial sections from five mice (two of which were females each with a 20-gm body weight) and 2 rats (one of which was male with a 400-gm body weight and another was a 250gm female), is shown in Figure 6. In the other 8 mice and 2 rats that were similarly processed for light microscopy serial section studies, no cardiac myocytes were detectable along the hepatic portal vein. A 20-gm female mouse and a 250-gm male rat were included in the group of 10 animals with the negative result.

In Mouse 1 and Mouse 2 in Figure 6, cardiac myocytes were present to a rather limited extent, whereas in Mouse 4 and Mouse 5 they showed a wider distribution in the hepatic end of portal vein trunk. In Mouse 2, a few myocytes were found in the adventitia of an intrahepatic portal vein branch. In Mouse 4, some cardiac myocytes were located intimately underneath the endothelium of a lymphatic vessel in the portal vein adventitia (Fig. 4).

In the two cases of the rat illustrated in Figure 6, cardiac myocytes were present only in the form of a single large mass in the adventitia of the portal vein trunk near its hepatic end (Fig. 5).

Electron Microscopy

The cardiac myocytes observed in the present study include those encountered in the tunica media (defined as the layer of compactly arranged smooth muscle cells) and inner fibrous adventitia (Group 1 cardiac myocytes), and in the outer, loose adventitia (Group 2 cardiac myocytes) of the extrahepatic main branch of the portal vein in one mouse, as well as those encountered in the wall of the small intrahepatic branch of the portal vein (Group 3 cardiac myocytes) in another mouse. In general, they had a large, centrally located nucleus, which contained the peripheral chromatin and one or two nucleoli in a sectional plane (Fig. 7). The cross-striation pattern of myofibrils consists of Z, H, and M lines and I and A bands. Mitochondria were rather evenly distributed throughout the sarcoplasm; their shape and size varied greatly (Fig. 7). Sarcoplasmic reticulum (SR) was arranged in a simple and loose network of branched tubules among the myofibrils. The flattened agranular sacs of SR formed peripheral couplings (Sommer and Johnson, 1970) with external sarcolemma. A transverse tubular system 40-200 nm in diameter and coupling of the T-tubules and SR accompanying the electron-dense material between the two structures were occasionally encountered (Fig. 8). Intercalated disks between the myocytes contained an intermediate junction, desmosome, and nexus.

Group 1 cardiac myocytes often intermingled with smooth muscle cells. However, no direct contact was found between a cardiac myocyte and a smooth muscle cell; the two kinds of cells were always separated by a

connective tissue space no less than 70 nm wide. Group 1 cardiac myocytes measured 5–10 μ m in breadth at the nuclear level and possessed well-organized myofibrils. Supercontraction of the myofibrils was seen in some of these myocytes. A unique feature of the Group 1 cardiac myocytes was their rich content of membrane-bound granules (Fig. 9), which resembled atrial specific granules (Jamieson and Palade, 1964). Cardiac myocytes with more granules from the portal vein did not have more extensive T-tubules as reported in the atrium by Forssmann and Girardier (1970). At the intercellular junctions of Group 1 cardiac myocytes the nexus was observed only occasionally. The length of the nexus was 2.9% of the whole length studied, 92.8 µm, of the junctional sarcolemma. No capillaries were found around the cardiac myocytes in the inner layer of the tunica media of the portal vein branch, whereas those in the outer layer of the tunica media were supplied by a few capillaries of a nonfenestrated type. Unmyelinated nerve fibers occurred around the Group 1 cardiac myocytes, but neuromuscular contacts were not seen.

Group 2 cardiac myocytes occurred in large clusters (Fig. 7), which were separated from each other by a relatively wide connective tissue space. These myocytes, 4-8 μm in breadth at the nuclear level, only rarely contained those cytoplasmic granules that resemble atrial specific granules (compare Figs. 7 and 10, which illustrate perinuclear regions of several Group 2 cardiac myocytes, with Fig. 9, illustrating the perinuclear region of a Group 1 cardiac myocyte). Myofibrils were arranged in uniform directions within some of the myocytes, but not so in others, so as to show their longitudinal, transverse, and oblique profiles in a sectional plane through a myocyte (Fig. 10). Even a disordered appearance of myofilaments can be seen. T-tubules were detectable (Fig. 8). At intercalated disks, the intermediate junction and desmosome were more extensive than the nexus, but the latter was estimated to occupy 4.6% of the whole junctional length (a total of 90.5 μ m was studied) of the sarcolemma. Group 2 cardiac myocytes were supplied by blood capillaries with a fenestrated endothelium (Figs. 7, 11); however, thin processes of fibroblasts usually intervened between the endothelium and myocytes. Profiles of unmyelinated nerve fibers consisting of a few axons were seen in the interstitial space (Fig. 7). Axon varicosities may contain small granular vesicles typical of adrenergic axons (Fig. 12). Naked axons with synaptic vesicles were abundant near the surface of Group 2 cardiac myocytes, and the closest neuromuscular distance was observed to be $0.3 \mu m$.

Group 3 cardiac myocytes measured 3–5 μ m in breadth at the nuclear level and contained no cytoplasmic granules at all (Figs. 13, 14). In many sectional levels their myofibrils were observed to be well organized in general, although the myofibrils in the small part were rather randomly oriented within a myocyte. The T-tubules, being 0.1 μ m in diameter, were detectable in many myocytes. At the intercalated disk the development of the nexus was most striking: It extended as far as 3-4 μ m in a plane of section. The nexus was estimated to occupy 17.0% of the whole length studied (56.5 μ m) of junctions between Group 3 cardiac myocytes. Blood capillaries were rarely found around these myocytes. Unmyelinated axons containing small granular vesicles were distributed near the surface of the cardiac my-

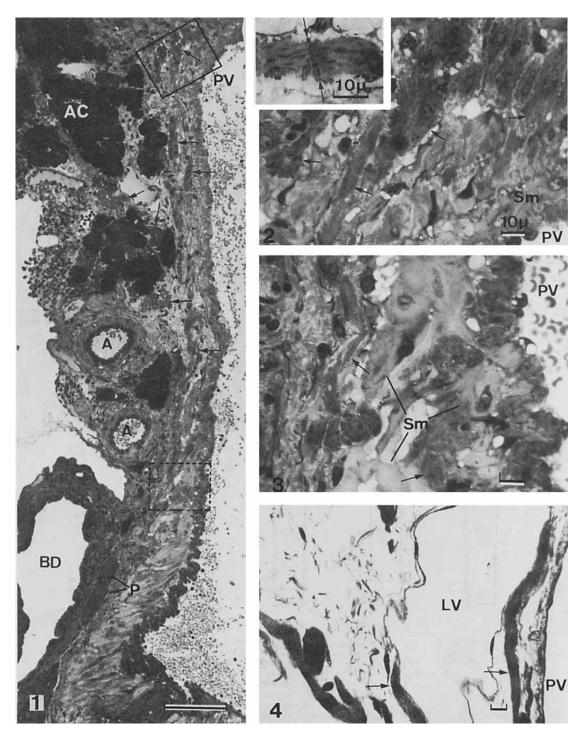


Fig. 1. Light micrograph showing the location of cardiac myocytes (arrows) in the wall of mouse hepatic portal vein (PV). The area boxed by the solid line is enlarged in Figure 2, and an adjacent section of the area boxed by the broken line is shown in Figure 3. AC: Adipose cells; BD: small bile duct; A: arterioles; P: pancreatic parenchymal cells. Epon section stained by toluidine blue. Scale represents 100 μm .

Fig. 2. In this micrograph, striations of cardiac myocytes (arrows) are demonstrated. PV: Lumen of the portal vein; Sm: smooth muscle cells. Inset shows the intercalated disk (between arrows).

Fig. 3. Micrograph showing cardiac myocytes (arrows) intermingled with the smooth muscle cells (Sm) in the wall of mouse portal vein. Scale represents 10 μm .

Fig. 4. Light micrograph of cardiac myocytes (arrows) associated with a lymphatic vessel (LV) in the mouse portal vein wall. PV: Lumen of the portal vein. Epon section stained by toluidine blue. Scale represents 10 μm .

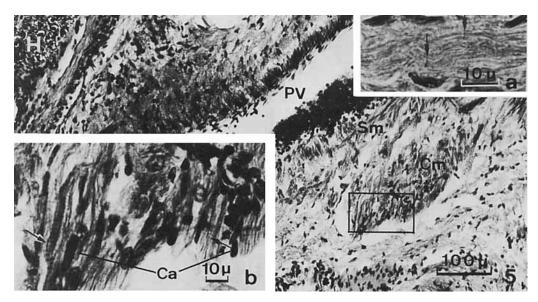
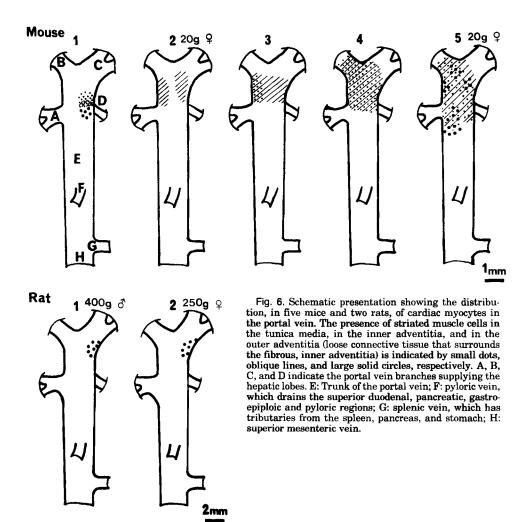


Fig. 5. Light micrograph of iron-hematoxylin-stained cardiac myocytes (Cm) in the wall of rat hepatic portal vein (PV). Intercalated disk (double-head arrows) and myofibrillar striation (arrows) in the rat

specimens are shown in the inset a (Masson-Goldner's stain) and inset b (iron-hematoxylin stain), respectively. Sm: Smooth muscle cells; H: hepatic parenchymal cells; Ca: blood capillaries.



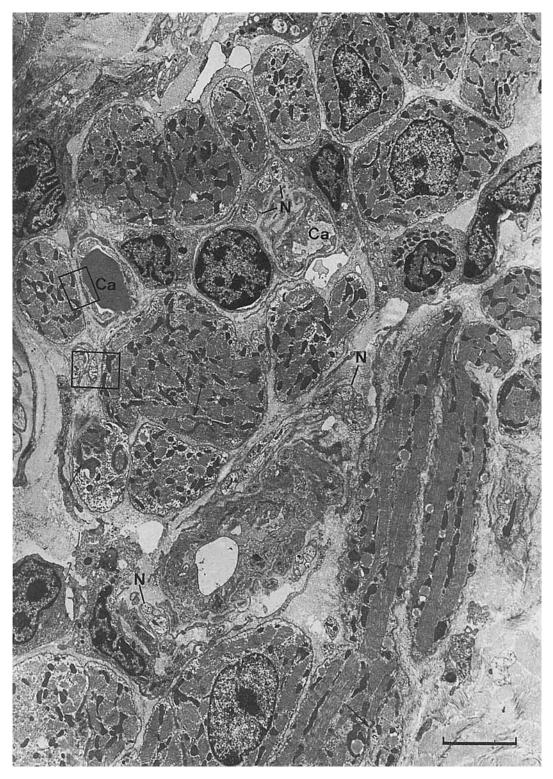
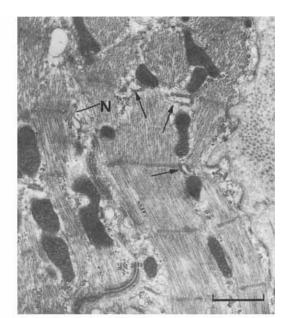


Fig. 7. Low-power electron micrograph showing cardiac myocytes in the outer adventitia of mouse portal vein. Unmyelinated nerve fibers (N) and fenestrated capillaries (Ca) are distributed between the myocytes. The boxed areas are enlarged in Figures 11 and 12. Arrows indicate intercalated disks between the myocytes. Scale represents 5 μm .

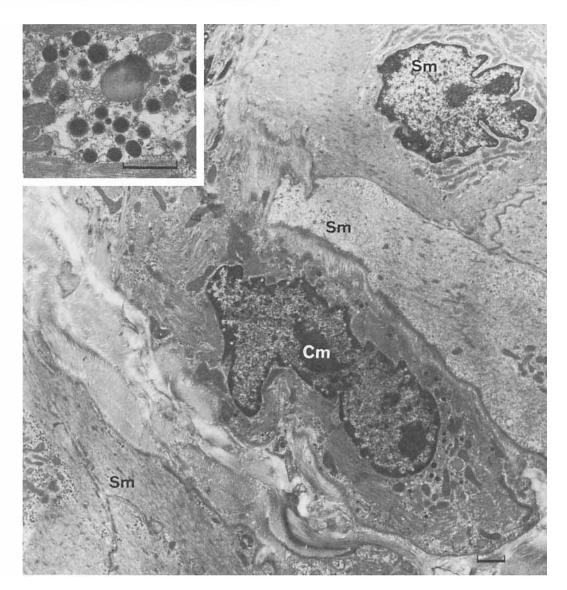


ocytes, the closest neuromuscular apposition being 0.3 μm apart.

DISCUSSION

Light microscopy of serial sections carried out in this study has failed to detect cardiac myocytes in the portal vein of 8 of 13 mice and 2 of 4 rats. This may be taken as indicative of a considerable individual variation existing in the distribution of such a muscular cell type in the portal vein of mice and rats. As to the reason for this variation, very little insight was gained in this study: the two rats positive for cardiomyocytes were of both sexes and there were 1 negative and 2 positive female mice. So, difference in the sex would probably be unimportant as an influencing factor. Other factors such as the age and body weight may remain to be considered. Identification under light microscopy of the striated musculature as the cardiac muscle, based on the detec-

Fig. 8. Transverse tubules (arrows) are seen at Z-line levels in a cardiac myocyte in mouse portal vein. N: Nexus-like structure in the intercalated disk. Scale represents 1 μm .



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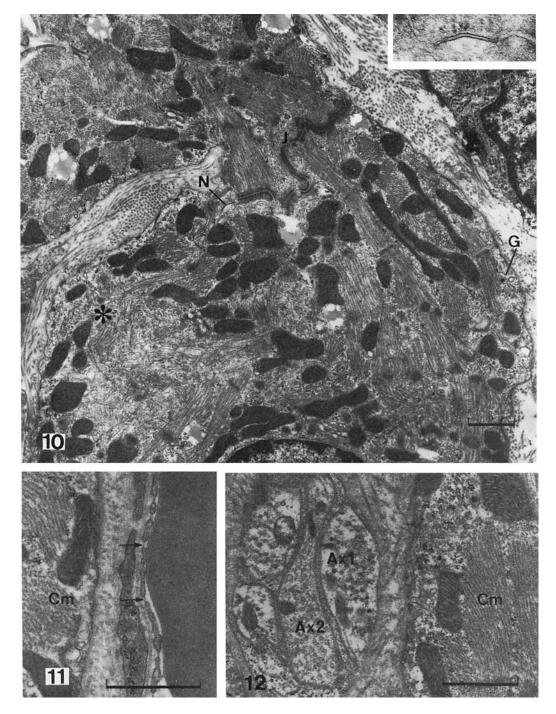


Fig. 10. Two cardiac myocytes connected by an intercalated disk (J). Asterisk shows an area of disordered myofilaments. G: Atrial specific granule (?); N: nexus (enlarged in the inset). Mouse portal vein. Scale represents 1 μ m.

Fig. 11. A fenestrated capillary apposed to the cardiac myocyte (Cm)

in mouse portal vein. Arrows indicate the fenestrae of the endothelial cell. Scale represents 1 $\mu m.$

Fig. 12. Unmyelinated axons lying fairly close to a cardiac myocyte (Cm) in mouse portal vein. They contain small dense cored vesicles (Ax 1), or predominantly small clear vesicles (Ax 2). Scale represents 1 μm .

Fig. 9. A cardiac myocyte (Cm) associated with smooth muscle cells (Sm) in the tunica media of mouse portal vein wall. Note atrial specific granules at the perinuclear region of the cardiac myocyte. Inset: Golgi apparatus and atrial specific granules in another cardiac myocyte in tunica media of the mouse portal vein. Scale represents 1 μm .

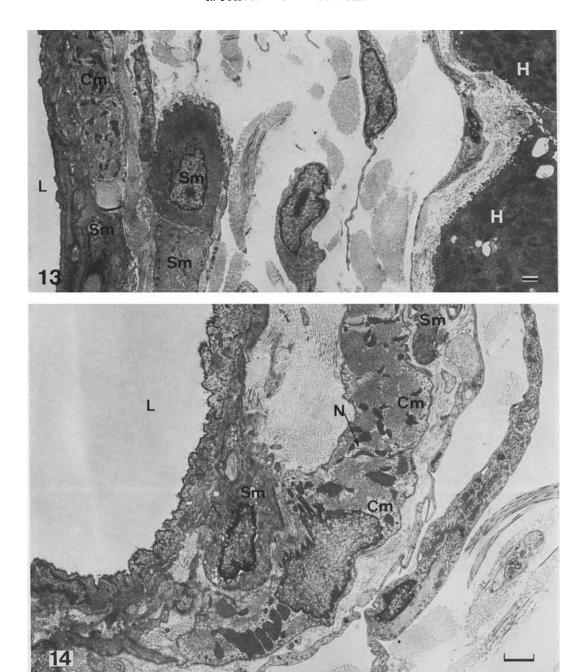


Fig. 13. An intrahepatic branch of the mouse portal vein with a tunica media consisting of cardiac myocytes (Cm) and smooth muscle cells (Sm). H: Hepatic parenchymal cells; L: lumen of the vein. Scale represents 1 μm .

Fig. 14. Cardiac myocytes (Cm) in the same intrahepatic vein as in Figure 13 are seen to be interconnected by a long nexus (N). Sm: Smooth muscle cells; L: lumen of the vein. Scale represents 1 μm .

tion of its intercalated disks, was supported by the electron microscope observations made in the mice. Of the tissue samples from 22 mice and 4 rats processed for the purpose of obtaining thin sections of the cardiac muscle in the portal vein, only those from two mice (sex unknown) were useful in providing the fine structural data in this paper. This outcome should not be accounted for,

nevertheless, in terms of the presence or absence of the striated musculature in the samples from the remaining 20 mice and 4 rats. Also, it follows that the fine structure of striated muscle in the rat portal vein has not been considered in the present study.

Previous studies have shown the occurrence of cardiac myocytes in regions other than the heart; i.e., in the

pulmonary vein of 47 species of rodents including deer mouse, rice rat, and prairie dog (Kramer and Marks, 1965), mouse (Karrer, 1959; Best and Heath, 1961), rat (Best and Heath, 1961; Ludatscher, 1968), squirrel (Best and Heath, 1961), dog (Carrow and Calhoun, 1964), and man (Benninghoff, 1930); in the superior vena cava of mouse and rat (McAllister et al., 1963), dog (McAllister et al., 1963; Carrow and Calhoun, 1964), and man (Benninghoff, 1930); in the inferior vena cava of mouse (Karrer, 1959) and dog (Carrow and Calhoun, 1964); and in the azygos vein of mouse and rat (McAllister et al., 1963) and dog (McAllister et al., 1963; Carrow and Calhoun, 1964). Cardiac myocytes found in these thoracic large veins can be considered to be an extension of the sinual, or atrial cardiac muscle.

On the other hand, no cardiac myocytes appear to have been described in the abdominal, including hepatic portal, veins of any mammalian species. The results of the present study are of interest in respect with the peculiar existence, among vertebrates, of the hepatic portal vein heart in Myxinoids, which is endowed with myocardial cells and pumps the venous blood into the liver (Carlson, 1904; Fänge et al., 1963; Yamauchi, 1980). In order to see if cardiac muscles in the murine hepatic portal vein have any phylogenetic relevance to the portal vein heart in Myxinoids, investigations are necessary on the intermediate classes of the vertebrates.

As shown by electron microscopy in this study many of the cardiac muscle cells of the mouse portal vein were much like the atrial myocardial cells in being relatively small and irregular in shape, as well as in possessing the simply formed SR, and a few T-tubules, together with the membrane-bound granules that closely resemble the atrial specific granules (see Jamieson and Palade, 1964; Fawcett and McNutt, 1969; McNutt and Fawcett, 1969; Simpson et al., 1973; Ayettey and Navaratnam, 1978). A striking feature was that some of the cardiac myocytes (classified as Group 2 in this study) in the mouse portal vein wall were supplied by fenestrated capillaries, as in the case of specialized myocytes in the atrioventricular node and bundle in the mouse, rat, rabbit, cat, and primate (tupia) (Weiche and Kalmbach, 1978). In view of the fact that the blood capillaries in the ordinary myocardium are exclusively of the nonfenestrated type (Bruns and Palade, 1968; Simionescu et al., 1974), a possibility arises that the Group 2 cardiac myocytes associated with the fenestrated capillary may share some functional properties with the intracardiac specialized myocytes in the mammalian heart. The multidirectional arrangement of the myofibrils in Group 2 cardiac myocytes of the hepatic portal vein is also conspicuous, and at the same time it coincides with the characteristic myofibrillar feature seen in the Purkinje fibers (Bogush, 1974) and myocardial cells of trabeculae carneae (Lindner, 1968; Appell and Stang-Voss, 1980). Such a myofibrillar pattern has been considered to play a role in the events in which myocytes gain a high rigidity during contraction (Appell and Stang-Voss, 1980), or display a shortening that results in a marked change in the myocyte shape after contraction (Bogush, 1974). It is possible that the myofibril pattern of the portal vein myocytes is of significance for conservation of the spatial integrity of the vessel wall during its contractions.

Spontaneous pulsations of the hepatic portal vein in vivo have been noted in the mouse (Attardi, 1955a,b; Mislin, 1963, 1969), rat (Attardi, 1955a; Booz, 1959; Funaki and Bohr, 1964; Johansson and Ljung, 1967b, 1968), guinea pig (Attardi, 1955a; Booz, 1963), rabbit (Johansson and Ljung, 1967a; Holman et al., 1968), and cat (Johansson and Ljung, 1967a). According to Ljung (1970), the portal vein pulsations are rhythmically induced in the region of the hepatic end, rather than of the mesenteric end, of the portal vein and become propagated along the vessel. It is noteworthy that the present study revealed the cardiac myocytes to be clearly concentrated toward the hepatic end of the main trunk of the vessel. Some of the cardiac myocytes (especially those belonging to Group 2) may possibly participate in the pace-making activity for pulsations of the hepatic portal vein. An apparent lack of nexus junctions between the cardiomyocyte and the smooth muscle cell in the tissues sampled in the present study, however, renders such a possibility unlikely. Further studies are necessary to see threedimensional arrangements of the cardiomyocytes and to evaluate their functional role in the activity of the vessel.

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