# Smooth muscle in the hepatic artery, portal vein and hepatic vein within the liver of the raccoon and guinea pig

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*Summary.* The amount and arrangement of smooth muscle in the intrahepatic vessels suggests that the guinea pig would be a good animal model for studying mechanisms controlling intrahepatic portal vein blood flow, while the raccoon would be good for studying hepatic vein mechanisms of control.
*Key words.* Hepatic artery; portal vein; hepatic vein; liver.

Carlson[4] states that the hagfish has a portal heart. Others have suggested that the contractility of the veins, especially those of the liver, serve as an auxiliary heart[1,8]. Since cardiac output is dependent upon input, control of venous return is very important. Extensive reviews published in 1963[2], 1971[5], and 1983[9] point to the importance of the liver and its circulation but present a meager number of proven mechanisms which control it. Brauer[2] says that physiological and pharmacological evidence shows that active hepatic vein constriction can occur, that the hepatic artery readily responds to vasoconstrictor stimuli, and that active vasoconstriction of the portal vein can take place, indicating that the hepatic circulation has extraordinary plasticity with a multiplicity of mechanisms. However, Greenway and Stark[5] state that our knowledge of the hepatic vascular bed is clearly incomplete and that detailed knowledge of the mechanisms of drug action on the hepatic vascular bed is almost nonexistent.

Blood enters the liver through the hepatic artery and portal vein and leaves through the hepatic veins. The blood flow, blood volume, and fluid exchange of the liver, as with other vascular beds, are controlled by the smooth muscle activity of its vasculature[5]. If the mechanisms controlling hepatic vascular flow act through the smooth muscle in the hepatic vessels, then it should follow that the best means of testing for mechanisms of control would be to use animal models which are best equipped to show strong responses in one or more of the three vascular routes.

The purpose of this paper is to promote the use of the guinea pig and raccoon as animal models for the study of the regulation of blood flow within the liver.

*Materials and methods.* Seven raccoons and seven guinea pigs were used in this study. Four of the raccoons were juvenile and the rest of the animals were adult. Liver tissue from the three adult raccoons and two guinea pigs were fixed by immersion in 10% buffered (pH 7.4) formalin. The remaining animals were perfused through the aorta, portal vein, or hepatic vein with physiological saline followed by 10% buffered formalin at a pressure of 110–120 mm mercury.

Samples of liver were taken from various areas of the livers so that vessels of all sizes could be studied. Samples were embedded in paraffin, sectioned at 7 and 10 µm, and stained by a variety of stains to accent smooth muscle cells, collagen fibers, elastic fibers, or reticular fibers. These included hematoxylin and eosin, Masson stain for collagen, resorcin fuchsin for elastic fibers, Snook's[11] ammoniacal silver nitrate for reticular fibers, alcian blue 8GX and phloxine B, and Feulgen's stain with a sirius supra blue FGL-CF counterstain.

To determine whether or not the smooth muscle in the hepatic vein of the raccoon were circularly or obliquely arranged in spirals, some vessels were dissected free of liver tissue, stained in toto with Harris' hematoxylin, and mounted whole.

*Observations.* No differences in the amount or arrangement of the smooth muscle were noted between the juvenile and adult raccoons or between the specimens fixed by immersion and perfusion.

*Guinea pig.* The hepatic artery of the guinea pig contains a variable amount of smooth muscle. In some regions, the artery is quite muscular, while in others the vessel consists merely of endothelium and a poorly developed collagenous adventitia (figs 1, 2). The development of the muscle layer in the arterial wall does not appear to be dependent upon the size of the vessel. In the smaller tributaries of the portal triad, the portal vein appears to be more important than the artery in controlling blood flow, judged by the morphology of their walls.

The portal vein of the guinea pig is well supplied with smooth muscle (figs 1, 2). The muscle cells are circularly arranged, and they constitute a layer of varying thickness which is located immediately external to the endothelium. Longitudinal sections of the portal vein show that the muscle layer gradually thins and widens along the length of the vessel, and even disappears completely at some points. The terminal branches of the portal vein which distribute to the sinusoids are totally devoid of smooth muscle.

Numerous reticular fibers are randomly arranged among the smooth muscle cells in the portal vein but are generally longitudinally directed when found between the endothelium and muscle layer. A well developed internal elastic membrane is found when muscle is present but becomes very delicate or absent in small branches which are devoid of muscle. The connective tissue surrounding the structures in the portal triad of the guinea pig is a delicate areolar connective tissue.

Smooth muscle is present in the larger hepatic veins of the guinea pig as a continuous circular tunic of varying thickness

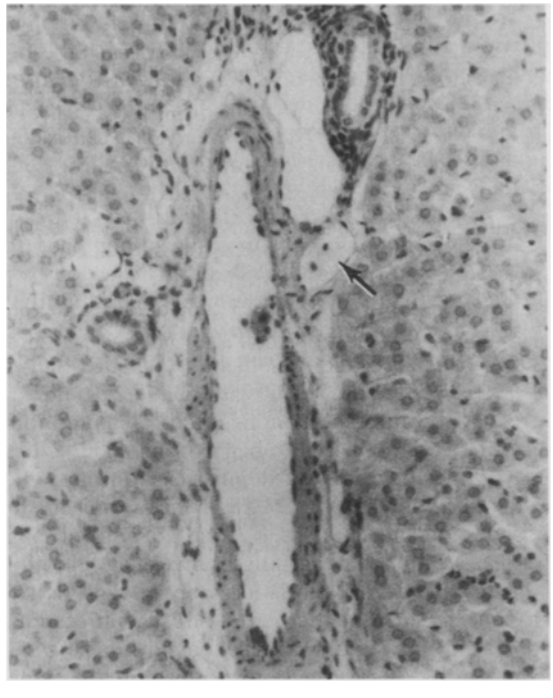

Figure 1. Longitudinal section through a guinea pig portal vein. Note the amount of smooth muscle present and its variation in thickness. A recognizable artery is not present, although the smaller vessel (arrow) adjacent to the vein was seen on adjacent sections stained for elastic fibers to have an internal elastic membrane similar to arteries. Hematoxylin and eosin. ×150.

located immediately external to the endothelium but, in general, the hepatic veins are thin walled. Smooth muscle in the more peripheral interlobular veins is only occasionally present as a single layer of cells and is absent at the level of the central vein (fig. 3). Even in the largest veins, the collagenous adventitia is quite thin, while in the smaller vessels it is not apparent at the light microscopic level. Reticular fibers in the muscular layer run both parallel and perpendicular to the orientation of the smooth muscle cells. Elastic fibers frequently are associated with the smooth muscle cells and are oriented in the same direction. A poorly developed internal elastic membrane is present, and scattered elastic fibers are mixed with the adventitial collagen.

*Raccoon.* The hepatic artery of the raccoon is a typical muscular artery (figs 4, 5). It is well supplied with smooth muscle, and the thickness of the media tapers gradually along the course of the vessel. The contour of the lumen is generally smooth.

The intrahepatic portal vein of the raccoon possesses very little smooth muscle (figs 4, 5). Immediately external to the endothelium, there is a single thickness of smooth muscle cells forming a continuous sheet which extends along most of the length of the vessel and its branches. However, at the level of the terminal branches of the portal vein, the continuity of the smooth muscle sheet is periodically interrupted, resulting in a series of bundles composed of only a few smooth muscle cells each. Circular reticular fibers are prominent in the smooth muscle layer. The many large elastic fibers distributed throughout the smooth muscle layer are oriented along the long axis of the vessel, and a fairly complete internal elastic lamina is present. Longitudinal elastic fibers are intermittently spread throughout the adventitia. The portal triad in the raccoon is embedded in abundant connective tissue.

The most striking feature of the raccoon hepatic vasculature is the presence of projections from the walls of the hepatic vein into the lumen, giving the wall a scalloped appearance in longitudinal sections (fig. 6). These projections contain discrete rings of smooth muscle in the smaller interlobular veins (fig. 7), but in the larger hepatic veins, the muscle bundles run as a continuous spiral which is interrupted periodically by the entrance of small tributaries into the larger vessel. At such sites, the spiral frequently terminates and forms a discrete circular ring. The

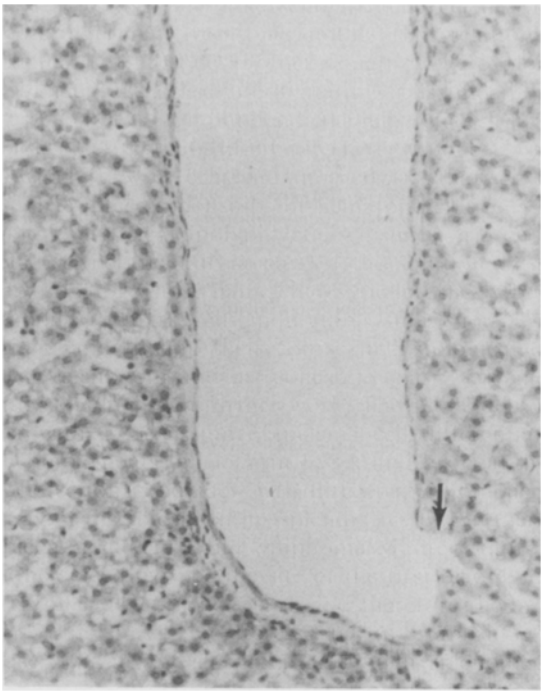

Figure 3. Longitudinal section of guinea pig hepatic vein showing small amount of muscle and collagen making up its wall. Note central vein entering at arrow. Hematoxylin and eosin. ×134.

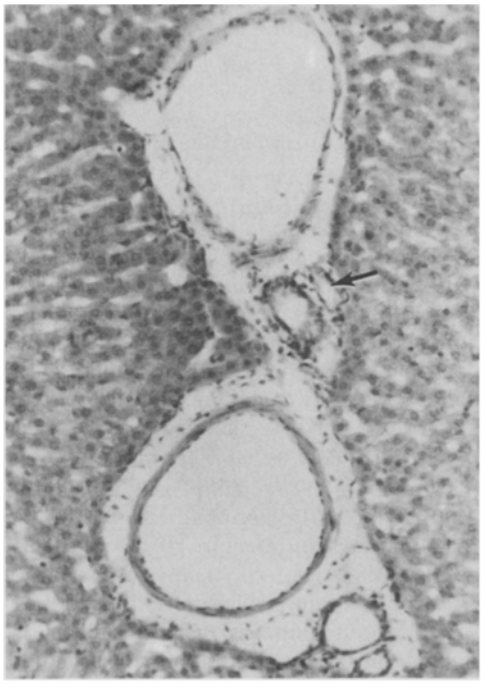

Figure 2. Cross section of guinea pig portal triad near a bifurcation. Smooth muscle layer in the portal vein is thicker than in the raccoon. Branches of hepatic artery (arrow) have little or no smooth muscle at this level. Hematoxylin and eosin. ×146.

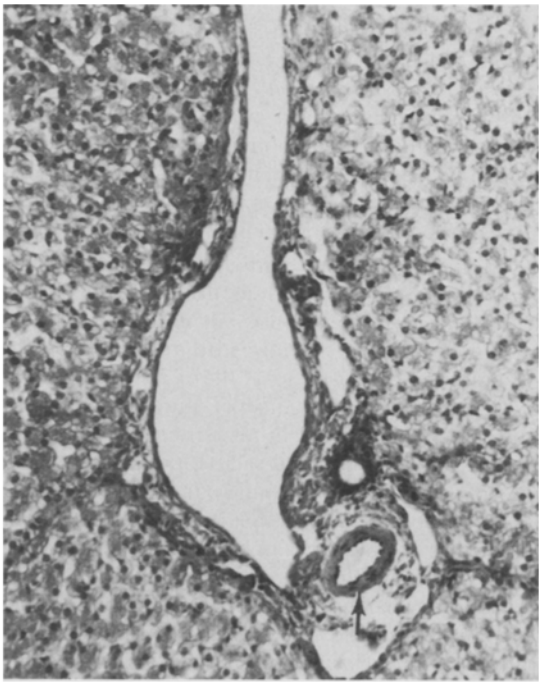

Figure 4. Longitudinal section of raccoon portal vein. Smooth muscle layer is only one cell thick in most regions. Artery with typical muscle layer (arrow) is present. Alcian blue 8GX and phloxine B. ×164.

mouth of the tributary vessel is also the site of a complete ring projecting into the lumen. The invaginations of the vessel wall consist mostly of smooth muscle covered internally by the endothelial cell layer. The smooth muscle cells are circularly or obliquely oriented and are concentrated into a fairly compact bundle at the tip of the elevation. Within the adventitial collagen external to the projection, there are additional small bundles of smooth muscle cells which run in the same direction as the other smooth muscle. The large terminal hepatic veins have some longitudinally oriented smooth muscle fibers scattered in the adventitia some distance from the endothelium. A circular bundle of collagen is included in each projection containing smooth muscle, but the bulk of adventitial collagen is longitudinally arranged. The collagenous adventitia is thick and contains numerous endothelium-lined spaces, as well as small branches of the hepatic artery.

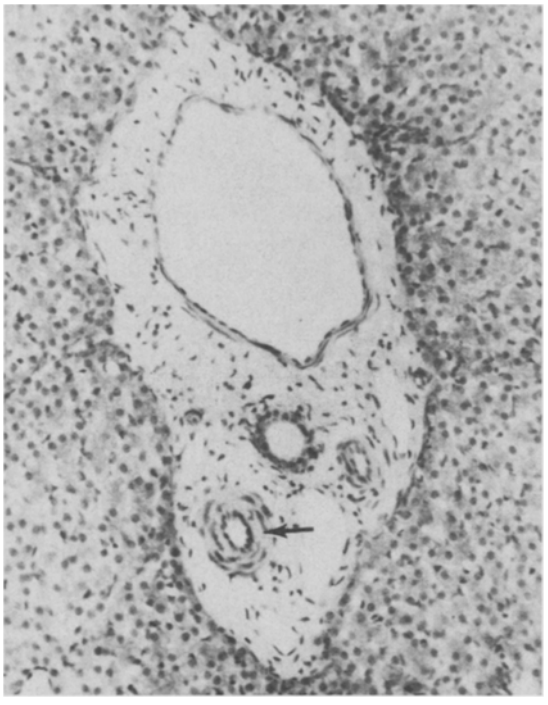

Figure 5. Cross section of raccoon portal triad. Note the small amount of smooth muscle in the wall of the portal vein and the typical small artery (arrow) nearby. Hematoxylin and eosin. ×179.

*Discussion.* In recent years, the extrahepatic portal vein has become a popular structure for morphological, physiological, and pharmacological studies. The extrahepatic portal vein has an outer longitudinal and an inner circular layer of smooth muscle, and each layer has its own characteristics. Komuro and Burnstock[6] demonstrated by electron microscopy that in the rabbit portal vein the muscle cells of the outer longitudinal layer were spindle-shaped with no branching, whereas the muscle of the inner circular layer showed much branching of cytoplasmic processes which often made membrane contacts with neighboring cells. Shirakata[10] studied enzymes in the portal vein of five different mammals and found differences between the outer longitudinal layer of smooth muscle and the inner circular layer within the same species. Brown et al.[3] suggest that the outer and inner layers of muscle of the rabbit portal vein respond equally to acetylcholine but not to histamine. Mathison[7] showed that longitudinal muscle was more sensitive than circular muscle to acetylcholine, 5-hydroxytryptamine and angiotensin II, both muscle layers were equally sensitive to noradrenaline and substance P, and the circular muscle was generally unresponsive to neurotensin. These studies show that the two layers of smooth muscle in the extrahepatic portal vein differ in morphology, physiology, and pharmacology. This suggests that muscle in the different intrahepatic vessels might also differ in their characteristics. Animal models which have distinctive intrahepatic vessels should markedly aid in the

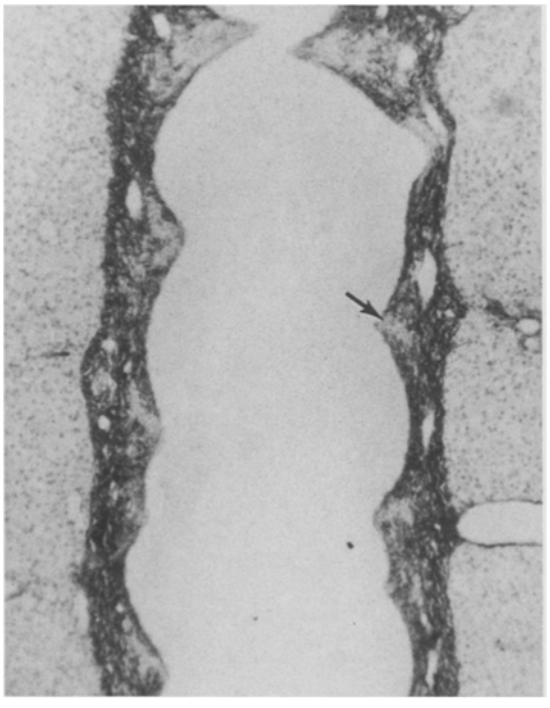

Figure 6. Longitudinal section of raccoon hepatic vein showing scalloped appearance due to the smooth muscle sphincters (arrow). Feulgen and Sirius Supra Blue FGL-CF. ×106.

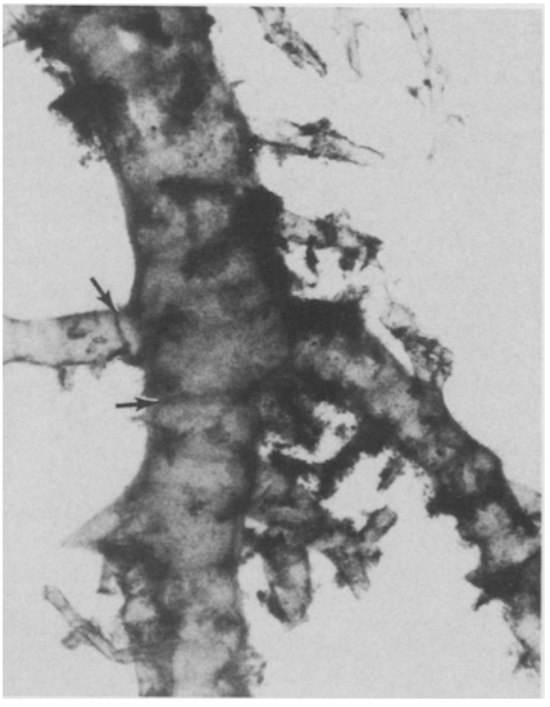

Figure 7. Whole mount of raccoon hepatic vein. The dark bands (arrows) are bundles of smooth muscle. Hematoxylin. ×32.

study of mechanisms which control each vessel. We suggest that the vascular system with the greatest amount of smooth muscle will give the greatest response to a stimulus, other things being equal, and thus responses and mechanisms should be easier to identify.

Based purely on morphological evidence, the guinea pig should be a good animal model for studying the mechanisms of control of the intrahepatic portal vein. Since the artery has little muscle in its wall, the portal vein must be more important for controlling inflow into the liver. The hepatic veins have little muscle except near the termination of the veins into the inferior vena cava and, thus, control of hepatic outflow must be poor.

The raccoon has a very interesting arrangement of muscle on the hepatic vein which must be important in controlling intrahepatic distribution or hepatic outflow.

The most interesting hepatic vessels seen by this author are the portal veins of the monitor lizard. These vessels have numerous remarkable doughnut-shaped sphincters which should be capable of intricate control of blood flow through the liver[12].

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## Influence of hydrochlorothiazide on the pain threshold and on the antinociceptive activity of morphine, in rats[1]

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*Summary.* Hydrochlorothiazide, acutely injected in rats, has a weak analgesic activity per se and potentiates and prolongs the antinociceptive effect of morphine.

*Key words.* Rat; hydrochlorothiazide; pain threshold; antinociceptive activity; analgesic activity, morphine.

The involvement of $Na^{+2-4}$, as well as of other cations ($Ca^{++}$, $Mg^{++}$, $Mn^{++}$)[5,6], in the actions of narcotic analgesics is definitely established. Pert and Snyder[2] and Simon et al.[3] reported that $Na^+$ selectively affects the stereospecific binding of narcotic analgesics in mouse brain homogenates, an increase in $Na^+$ concentration decreasing the stereospecific binding of agonists and increasing the stereospecific binding of narcotic antagonists. In vivo, Lujan et al.[4] showed that large quantities of NaCl, given i.p. to mice, decrease the antinociceptive activity of morphine.

These findings prompted us to investigate the effects of increased natriuresis on the pain threshold and on the antinociceptive activity of morphine.

*Methods.* Male rats of a Wistar strain (Morini S.Polo d'Enza, Reggio Emilia), weighing 240–280 g, were randomly selected for treatment or for control experiments. Basal pain thresholds and the antinociceptive activity of morphine (morphine sulphate, Carlo Erba, Milan) were determined by the hot-plate test (constant platform temperature: 55 °C), with a cut-off time of 60 sec. The effect of morphine was calculated as a percentage of the maximum possible effect (MPE) according to the formula[7]: (TL − BL/60 − BL) × 100 (TL = test latency; BL = baseline latency; 60 = cut-off time of 60 sec).

1 h before the experiment, rats were randomly assigned to five groups and substances administered by i.p. injection as follows: groups 1, 2 and 3 – hydrochlorotiazide, 1, 5 and 10 mg/

Influence of hydrochlorothiazide on pain threshold and on morphine activity. Hot plate test (55 °C).

| Treatment* (i.p., 1 h before morphine) | Baseline reaction latency (sec ± SE) (immediately before morphine injection)* | % increase in reaction latency (MPE)** at the following times after morphine injection (5 mg/kg i.p.)* | | |
|---|---|---|---|---|
| | | 15 min | 30 min | 60 min |
| Saline (27) | 4.58 ± 0.31 (443.8 ± 15.01) | 10.99 ± 3.74 (437.25 ± 14.35) | 8.70 ± 1.48 (423.9 ± 16.18) | 4.99 ± 1.73 (435.6 ± 16.38) |
| Hydrochlorothiazide, 10 mg/kg (28) | 6.14 ± 0.82● (435.6 ± 15.41) | 25.59 ± 6.13● (396.0 ± 4.66)● | 44.14 ± 6.88●● (394.2 ± 6.18) | 45.80 ± 9.09●● (376.28 ± 4.18)● |
| Hydrochlorothiazide, 5 mg/kg (10) | 6.62 ± 0.81● (424.0 ± 9.80) | 38.17 ± 14.50● (418.0 ± 15.86) | 45.81 ± 16.32● (433.41 ± 17.6) | 24.04 ± 11.58 (399.3 ± 3.30) |
| Hydrochlorothiazide, 1 mg/kg (10) | 5.21 ± 0.53 (429.0 ± 12.34) | 17.05 ± 4.61 (402.6 ± 6.02) | 30.72 ± 9.95● (409.2 ± 6.6) | 23.92 ± 9.82● (396.0 ± 4.66) |
| Mannitol, 2 g/kg (10) | 5.99 ± 1.60 (417.45 ± 11.23) | 5.10 ± 1.55 (427.35 ± 12.16) | 9.91 ± 1.25 (427.35 ± 10.9) | 11.12 ± 4.43 (433.95 ± 8.25) |

* No. of rats in brackets. ** See methods for details. ★ Figures in brackets are serum sodium concentrations (mg/dl). Each value is the mean ± SE for 8 rats. ● $p < 0.05$; ●● $p < 0.001$ (compared with controls at the same times) (Student's t-test).