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Venous outflow system in rabbit gastric mucosa

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The purpose of this work was to compare the organisation of the gastric mucosal venous system in larger animals, exemplified by rabbits, with that of the rat and the hamster which we have described previously. Rabbits were given atropine and hexamethonium followed by intravital ligation of all veins draining the stomach, causing strong hyperaemia. The distribution of vessels was studied in the non-mounted mucosa, in mounts of mucosa cleared in light mineral oil and in paraffin or semi-thin plastic sections. We found that blood from rabbit gastric mucosa is drained by collecting venules, running from the subepithelial layer towards the muscularis mucosae. The collecting venules join the paramuscular vessels parallel and adjacent to the muscularis mucosae. Neighbouring venules form numerous arcade-like connections and gradually enlarge. Two venules and an arteriole form triplets initially situated at the luminal face of the muscularis mucosae and gradually passing onto its abluminal surface. In rats vascular triplets were absent and the collecting venules drained into paramuscular vessels joining submucosal veins. In hamsters both connections between paramuscular vessels and submucosal veins and the passing of vascular triplets across muscularis mucosae were observed. Contraction/relaxation of the muscularis mucosae may regulate the amount of blood in the venous system of the mucosa and change the intramucosal pressure, affecting movement of the tissue fluid and, indirectly, the function of the gastric cells.

Key words: venules in rabbit stomach, portal vein ligation, hyperaemia, muscularis mucosae

INTRODUCTION

Descriptions of gastric mucosa vascularisation are usually based on scanning electron microscopy analysis of vascular corrosion casts [1, 5–8, 15]. This approach, however, while providing a beautiful picture of arterioles, capillary networks and veins, does not enable the spatial relationship between the venous outflow system and muscularis mucosae to be established. Indeed, both in rats and hamsters, venules collecting blood from the glandular mucosa have been supposed to drain into submucosal veins [5, 7], while histological preparations of hyperaemic mucosa demonstrated that in both species they discharge into intramucosal venules parallel to the muscularis mucosae. In rats these paramuscular venules were short and formed connections with the submucosal veins through a gap in the muscularis mucosae [9, 10]. Similar connections between paramuscular and submucosal venules were also observed in the hamster. Moreover, in this species, numerous collecting vessels discharged into venules belonging to vascular triplets composed of two venules and an arteriole. These triplets were also parallel to the muscularis mucosae.

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They did not form connections with the submucosal veins but gradually passed onto the abluminal side of the muscularis mucosae. Thus some structural elements involved in the venous outflow from the glandular gastric mucosa differed in rats and hamsters.

The functional significance of paramuscular venules has not been established. They appear, particularly in rats, to be too large to serve only as a passage for outflowing blood. They could serve as a reservoir for blood in the gastric mucosa. Conceivably, it would be easier to understand their function if more information on venous outflow from gastric mucosa in other species was available. In this respect the rabbit was chosen as an object of study, since it is an easily accessible laboratory animal, it is much larger than the animals studied previously, and there is already a considerable body of information on the vascularisation of its stomach, both on a microscopic and a gross morphological level.

Ohtsuka and Ohtani [12], studying vascular casts, observed that submucosal arterioles in rabbit gastric mucosa gave off short arterioles which connected with the capillary network at the base of the fundic glands and the long arterioles supplying the capillary network beneath the surface epithelium. Surface epithelial and glandular capillary systems formed numerous connections in the glandular neck region. Both of these capillary networks drained into collecting venules descending along the glands. The authors suggested that the collecting venules emptied into submucosal veins, an explanation which, in the light our finding of paramuscular venules in rats and hamsters, may be rather imprecise.

Piasecki and Wyatt [14], describing the arterial vessels of the rabbit stomach, found that the left gastric artery supplied the central portion of the greater curvature, the lesser curvature and the pylorus. The left gastroepiploic artery supplied the left half of the greater curvature. The right gastric artery was very small and usually did not reach the lesser curvature, terminating by supplying the duodenum. The main branches of these vessels (*vasa recta*), after piercing the tunica muscularis, gave out radiating branches to form the submucosal plexus located within or deep into the muscularis mucosae.

In their studies of venous outflow Artico et al. [2] found that the left gastric vein collected blood from the anterior and posterior wall of the stomach along the minor curvature. The right gastroepiploic vein drained the greater curvature, pylorus and proximal part of the duodenum. The left gastroepiploic vein received *vv. gastricae breves* from the greater curvature and could form anastomoses with the pancreatic vein. Blood from the fundus outflowed through the tributaries of the splenic vein.

The purpose of our study was to establish whether rabbit gastric mucosa contains paramuscular veins and, if so, whether they are similar to those described in rats, occur predominantly in vascular triplets, as in the hamster, or form another pattern.

MATERIAL AND METHODS

Experimental animals and surgical procedure

Twelve male New Zealand White rabbits weighing 2500–3000 g were obtained from the Institute of Zootechnics in Cracow, Poland and were housed under standard conditions. Before the experiment the animals were fasted overnight in cages with a double floor to prevent the consumption of faeces. Two rabbits were subjected to surgical procedure without pharmacological pretreatment. Ten rabbits received i.m. atropine sulphate (Warszawskie Zakłady Farmaceutyczne Polfa, Warsaw, Poland) in a dose of 0.5 mg/kg of body weight, one hour before surgery. Five of these rabbits also received an i.v. injection of hexamethonium chloride (Sigma-Aldrich Chemie, D-82041 Diesenhofen, Germany) in a dose of 2 mg/kg, 5–10 min before surgery. The animals were anaesthetised by an i.m. injection of ketamine and xylazine. The abdomen was opened midline. The portal vein was closed with a single ligature. The branches of the left gastric and left and right gastroepiploic and splenic veins were ligated as close to the stomach wall as possible. After 15 min the coeliac trunk was ligated and the animal was killed by cervical dislocation. The oesophagus and duodenum were closed with a ligature and the stomach was distended by an injection of 50 ml of 10% formaldehyde. Then the whole animal was submerged in formaldehyde for 24 h and the stomach dissected.

The study was approved by the Animal Ethical Committee of the Medical University of Warsaw.

Tissue preparation

The dissected stomachs were rinsed in water and cleared of the adhering tissues. The stomach was cut along the lesser and greater curvatures. It was then placed in a beaker filled with water and the remnants of food were removed with a fine brush. Next the muscle layer was dissected away, leaving the submucosa and mucosa. During dissection arteries piercing the muscularis mucosae

and veins emerging from it were cut [14]. The mucosa/submucosa was inspected and photographed under an Olympus Zoom stereo microscope (Olympus Optical Co, Ltd, Germany) with epi-illumination and trans-illumination. Large pieces of mucosa were dehydrated in ethanol, cleared in light mineral oil [3] and mounted with the mucosal surface down in Mounting Medium DPX (Polskie Odczynniki Chemiczne, Gliwice) in the lid of a Petri dish. The bottom of the dish served as a cover slip. Some fragments of mucosa with collecting veins and submucosal veins emerging from the tissue were dehydrated, embedded in paraffin (MEDIM-Plast, MEDIM, Giessen, Germany) and serially sectioned at 10 μm, perpendicularly to the mucosal surface. The sections were stained with haematoxylin and eosin. Other fragments were embedded in low viscosity epoxy resin [19]. Semi-thin sections were cut with a diamond knife and stained with 1% toluidine blue.

RESULTS

Only moderate hyperaemia was obtained in the stomachs of rabbits with ligated veins without pharmacological pre-treatment. After administration of atropine or a combination of atropine and hexamethonium, the fundus and the body viewed from the serosa side were strongly hyperaemic, while the vessels in the pyloric part were narrow and inconspicuous. No distinct difference was noted between rabbits exposed to atropine or a combination of atropine and hexamethonium. After dissection at the level of the submucosa, 10 prominent groups of vessels emerging from the mucosa of the body and fundus were usually visible (Fig. 1A). In most cases such groups were composed of two veins and an artery. The veins were connected by short thick anastomoses (Fig. 1B). In preparations of the stomach body cleared with mineral oil the mucosa tributaries of neighbouring venules were seen to form arcade-like connections. The larger vessels, grouped into triplets, subsequently passed into the submucosa. The sites of connection of the collecting venules and the paramuscular vessels appeared as dark spots (Fig. 1C). The vessels in the pyloric part and partially in the antrum were less prominent. In the mucosal preparations *in toto* they were visible as a network of narrow vessels (Fig. 1D). In the paraffin sections perpendicular to the mucosa from the stomach body, numerous capillaries, collecting venules perpendicular to the muscularis mucosae and paramuscular venules parallel and adjacent to its luminal side were present. All these vessels were distended with blood. The collecting venules either bent close to the muscularis mucosae, passing into paramuscular venules, or discharged into the latter at various points. The collecting venules were usually irregular in shape and frequently contained two or three chambers, partially separated by a rim of tissue. At the outlet of the collecting vessel a triangular protrusion usually occurred, possibly directing blood flow (Fig. 2A–C).

Larger paramuscular venules paired and ran together with an arteriole, forming characteristic triplets. These triplets gradually crossed the muscularis mucosae, which usually split into two layers during the passage, encompassing the triplet. The layer separating them from the submucosa then gradually disappeared (Fig. 3A–C). Sometimes, however, single venules passed from the mucosal to the abmucosal side through a gap in the muscularis mucosae, joining the submucosal vessels (Fig. 3D).

In the pyloric regions the muscularis mucosae was considerably thicker than in the body or fundus. The capillaries and collecting venules were only slightly distended. Larger amount of blood accumulated only in the paramuscular venules.

DISCUSSION

Vascularisation of the gastric mucosa was studied in rabbits exposed to relaxing agents and with ligated stomach veins, since the microscopic vascular pattern of the mucosa is difficult to visualise in intact animals [10]. The paramuscular vessels in the rabbit gastric mucosa formed a dense network. They fused into larger vessels and finally formed venules, joining arterioles and grouping into triplets, which gradually passed into submucosa. Only occasionally did single mucosal venules form connections with submucosal vessels. Our observations differ from those of Ohtsuka and Ohtani [12], who suggested a direct connection between the collecting venules and the submucosal vessels.

The neighbouring paramuscular venules were connected by arcade-like vessels, as demonstrated in Figure 1C. After leaving the mucosa, larger veins were also connected by anastomoses (Fig. 1B). Thus the whole venous system of the rabbit gastric mucosa and submucosa in the body and antrum appears to be connected.

Studies of the venous outflow from the rabbit stomach by Artico et al. [2] were limited to general observations. Piasecki and Wyatt [14] found a submucosal plexus of arterial vessels lying between the tunica muscu-

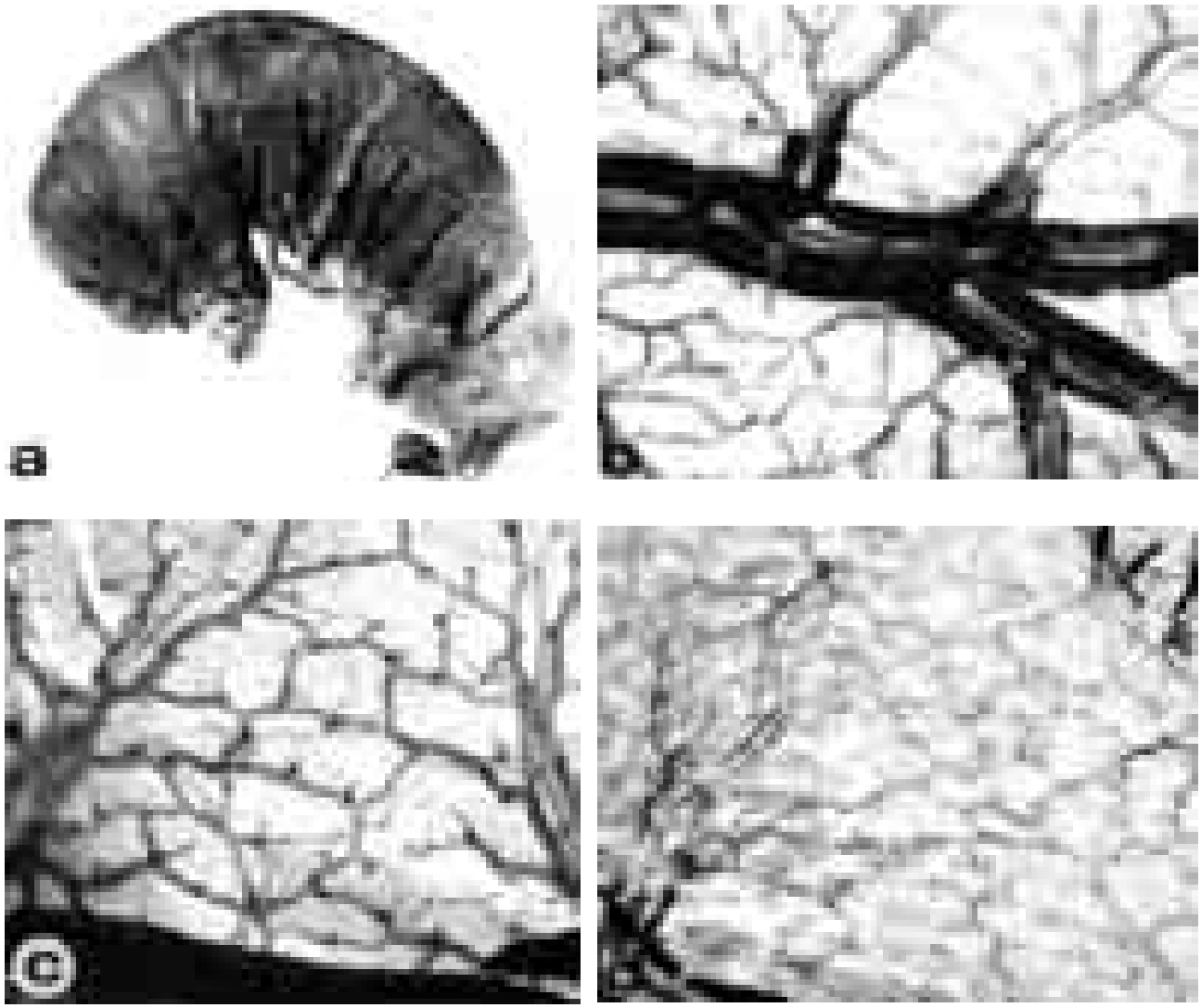

Figure 1 A. Blood vessels in the wall of the stomach photographed from the submucosal side after removal of the muscularis externa. Fundus and body are strongly hyperaemic, while in the pyloric part the vessels are inconspicuous. Unstained preparation, photographed in water; **B.** Two veins connected by short thick anastomoses, and artery present within the submucosa. Unstained preparation, photographed in water. Magnification 10 ×; **C.** Fragment of stomach body mucosa cleared in light mineral oil and observed from the submucosal side. Paramuscular venules (parallel and close to the muscularis mucosae) are filled with blood. Tributaries of the neighbouring venules form arcade-like connections. Dark spots represent the outlets of collecting venules into the paramuscular vessels and have a saccular appearance. The arterioles usually run parallel to the venules but are distinctly thinner; **D.** Similar to **C** preparation of pyloric mucosa. Blood vessels are inconspicuous in comparison with those of the stomach body mucosa. Magnification 10 ×. Figures represent stomach preparations from rabbits exposed to atropine and hexamethonium.

laris and the mucosa. All the main anastomotic vessels of this plexus were embedded within the muscularis mucosae and ramified in the mucous membrane to supply discrete definable areas (cf. Figs. 7 and 8 in their paper [14]). These mucosal or intramuscularis mucosae vessels evidently correspond to the arteries belonging to the vascular triplets which we observed.

We were never been able to obtain fully developed hyperaemia in the pyloric part of the gastric mucosa. The veins draining the stomach form interorgan anastomoses between the stomach and the spleen and the stomach and the pancreas [2]. To prevent the outflow of blood from the vascular bed of the stomach, veins draining the stomach were ligated as close to its wall as possible, but some loss of blood during distension of the stomach by formalin can not be excluded.

Neuroeffector transmission from the excitatory vagal pathway is cholinergic, and is thus blocked by atropine, causing relaxation of the gastric wall, especially evident in the fundus and body [16]. Furthermore, the presence of vagal afferent fibres has

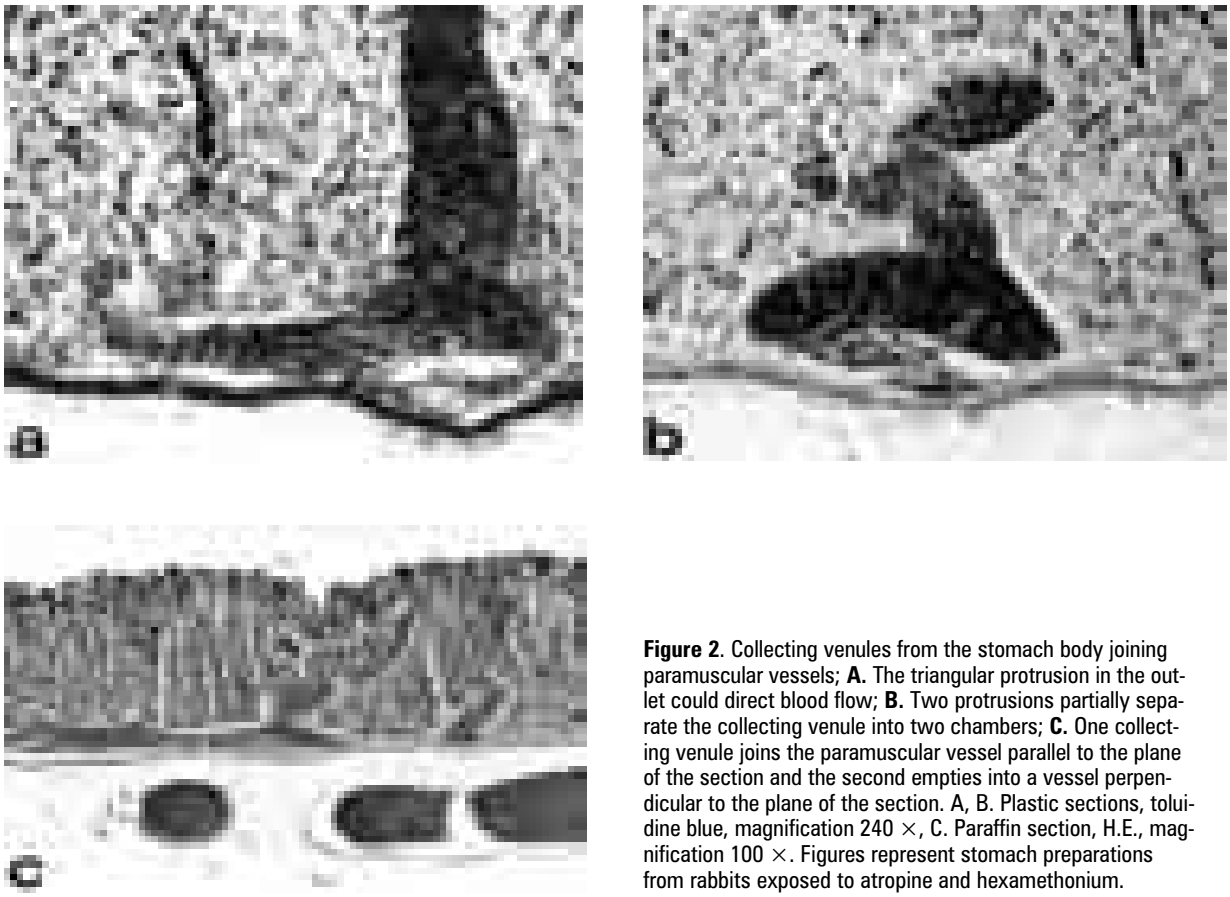

Figure 2. Collecting venules from the stomach body joining paramuscular vessels; **A.** The triangular protrusion in the outlet could direct blood flow; **B.** Two protrusions partially separate the collecting venule into two chambers; **C.** One collecting venule joins the paramuscular vessel parallel to the plane of the section and the second empties into a vessel perpendicular to the plane of the section. A, B. Plastic sections, toluidine blue, magnification 240 ×, C. Paraffin section, H.E., magnification 100 ×. Figures represent stomach preparations from rabbits exposed to atropine and hexamethonium.

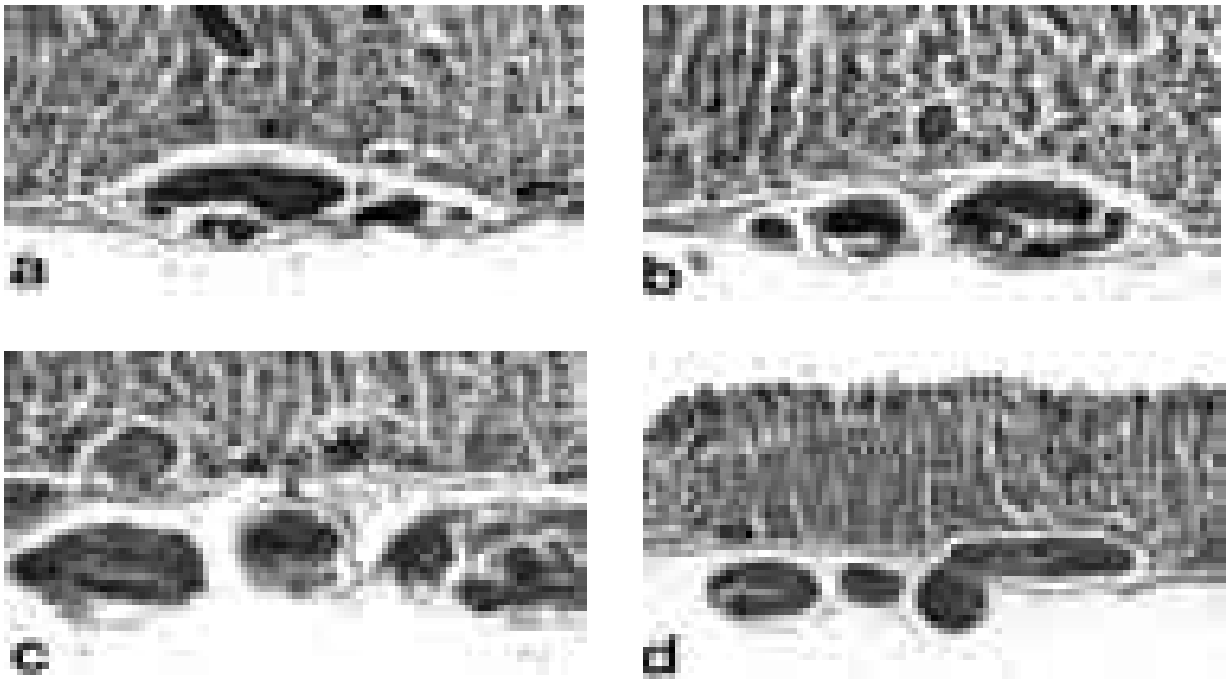

Figure 3. Triplet containing two venules and an arteriole from the stomach body passing through the muscularis mucosae; **A.** The vessels lie within the mucosa. One of the venules is sectioned parallel to its long axis; **B.** The vessels lie within the muscularis mucosae split into two layers; **C.** The vessels lie in the submucosa. H.E., magnification 160 ×; **D.** Paramuscular venule from the stomach body empties into a submucosal vessel through a gap in the muscularis mucosae. H.E., magnification 100 ×. Figures represent stomach preparations from rabbits exposed to atropine and hexamethonium.

been demonstrated in rabbit gastric mucosa [17] and the contraction of the muscularis mucosae from the antral and fundic area of the rabbit stomach triggered by acetylocholine was atropine sensitive [13]. In the opossum atropine induced relaxation of the muscle strands arising from the muscularis mucosae and associated with the gastric glands [18]. Thus relaxation of the gastric wall after the administration of atropine could facilitate access of blood to the submucosa/mucosa.

Contractions of the pyloric area were insensitive to atropine, but could be blocked by hexamethonium, an agent antagonising ganglionic transmission [4, 16]. In view of these data, some rabbits were exposed both to atropine and hexamethonium in order to increase relaxation of stomach and facilitate access of blood into the mucosa. This treatment did not, however, improve the visualisation of venules in the pyloric region. Another possible explanation for poor filling of the pyloric region vessels could involve the properties of the muscularis mucosae. It is a pharmacologically complex tissue that is not homogeneous throughout the stomach and is considerably thicker in the pyloric region than in other regions of the stomach. Walder [20] observed that the response of the muscularis mucosae in the human stomach varied according to the site from which it was taken, as also did it its response to drugs. Differences between the fundic and antral ends of the rabbit gastric corpus in reaction to various stimulatory or relaxing agents have also been noted by Percy et al. [13]. The response of the muscularis mucosae to pharmacological agents in the pyloric region remains, however, to be established.

The poor demonstration of vessels in the pyloric region could also have been caused by a paucity of vascularity in the distal parts of the stomach, as previously observed by Piasecki and Wyatt [14], in various species, including the rabbit. Possibly the amount of blood delivered to the pyloric mucosa was insufficient to achieve filling of the venous bed similar to that occurring in the gastric body.

As suggested in our previous papers [10, 11], the flow of blood in thin-walled venules on their way from the mucosa to the submucosa could be regulated by constriction/relaxation of the muscularis mucosae. A periodical increase in the amount of blood present in the paramuscular and collecting venules could, in turn, help to circulate extracellular fluid within the mucosa.

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