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Vessels involved in the venous outflow from glandular mucosa of hamster stomach

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Blood from the glandular part of hamster gastric mucosa is drained by collecting venules, running from the subepithelial layer towards lamina muscularis mucosae. To visualise vessels involved in the venous outflow, hamsters were exposed to atropine and subjected to intravital ligation of portal vein, causing strong hyperaemia. Distribution of vessels and their connections were studied a) in translucent, flat preparations of the glandular stomach, b) in thick sections of glandular mucosa cleared in the mineral oil, and c) in semi-thin plastic or paraffin sections. Collecting venules were drained by single vessels running parallel to the lamina muscularis mucosae (paramuscular venules), which, in turn, joined submucosal veins through openings in muscularis mucosae. Moreover, some collecting and paramuscular venules discharged into venules belonging to vascular triples composed of two venules and arteriole. The triplets were also parallel to muscularis mucosae. Triplets did not form connections with submucosal veins but passed on the abluminal surface of muscularis mucosae. Thus, some structural elements involved in venous outflow from glandular gastric mucosa differ from those in rats, in which vascular triplets were absent and all collecting venules drained into single paramuscular vessels. Contraction/relaxation of muscularis mucosae may regulate the amount of blood present in the venous system of the mucosa and the diameter of venules. Rhythmical changes of the latter could cause changes in intramucosal pressure, affecting movement of tissue fluid in the mucosa and thus the function of gastric cells.

key words: stomach mucosa, hyperaemia, collecting venules, paramuscular venules, muscularis mucosae

INTRODUCTION

The hamster stomach consists of three parts: the forestomach, the corpus and the antrum [6], named according to the terminology introduced earlier by Roberts [11] for rat stomach. The last two parts form the glandular stomach. The blood supply of the hamster stomach is provided by branches of the coeliac artery. The left gastric artery is the main branch supplying the corpus of the stomach. The right gastroepiploic artery runs along the greater curvature of the glandular stomach. Its branch, the right gastric artery, supplies the antrum [8, 12]. In the corpus of hamster stomach the submucosal arteries enter the lamina propria to become ascending capillaries,

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which project toward the top of the lamina propria extending along the glands and gastric pits. They anastomose to create a capillary network beneath the mucosal epithelium. Subepithelial capillaries have a larger diameter than the ascending ones, and drain into wide venules which are less numerous that the ascending capillaries [6]. This description corresponds to that concerning vascularisation of the lamina propria mucosae of rat stomach [1, 5].

We have recently described in rat stomach mucosa the hitherto unrecognised venules running parallel to muscularis mucosae and draining collecting venules [9, 10]. These vessels, called paramuscular venules, could be best visualised creating hyperaemia of the stomach by atropine administration and intravital ligation of portal vein. Paramuscular venules drained into submucosal veins passing through muscularis mucosae.

The question arises whether paramuscular venules occur also in other mammals, and if so, whether their occurrence is related to the species or size of animal. In this work we have analysed the venous system of hamster gastric mucosa. Hamster has been chosen since it belongs to the same order as rat, its size is closest to that of the rat among easily available laboratory animals and, according to previous studies, possesses collecting venules similarly organised as in rats [1, 5, 6]. In spite of these similarities, considerable differences in the outflow of blood from the gastric mucosa between rat and hamster have been observed. Some hamster paramuscular venules discharged into submucosal veins similarly as in rat. Others, however, occurred within mucosa in triplets composed of arteriole and two venules and passed from the mucosa as a whole group, becoming submucosal vessels.

MATERIAL AND METHODS

Experimental animals and surgical procedure

Syrian (golden) hamsters (*Mesocricetus auratus* W.) of both sexes weighing 125–150 g were obtained from the Animal Unit of the Medical University of Warsaw and were housed under standard conditions. Before the experiment, the content of their cheek pouches was mechanically removed and animals were fasted overnight in cages with a double floor to prevent consumption of faeces. Atropine sulphate (Warszawskie Zakłady Farmaceutyczne Polfa, Warsaw, Poland) was administered i.m. in a dose of 1.5 μg per 100 g of body weight, approximately one hour before surgery. Animals were anaesthetised by ketamine and xylazine. The abdomen was opened in a midline. Portal vein was closed with a single ligature as close to the liver porta as technically possible. After 5 min the coeliac trunk was ligated and the animal was killed by cervical dislocation. Oesophagus and duodenum were closed with a ligature and the stomach was distended by injection of 3 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

Dissected stomachs were rinsed in water and cleared of the adhering tissues. The forestomach was cut off. The glandular stomach was cut along lesser curvature and then along greater curvature, beginning from the oesophagus area and continuing until its wall could be flattened on a glass plate. Stomach preparations were dehydrated in ethanol, cleared in light mineral oil [2, 10] and mounted with the mucosal surface down in Mounting Medium DPX (Polskie Odczynniki Chemiczne, Gliwice) in a lid of a Petri dish. The bottom of the dish served as a cover slip. Moreover, from fragments of glandular stomach wall cleared in mineral oil, sections perpendicular to the mucosa surface were cut with a sharp blade (further referred to as thick sections). All preparations were inspected under ECLIPSE E800 microscope (Nikon). After inspection, sections containing collecting venules were passed through absolute ethanol to remove oil, transferred to propylene oxide and embedded in low viscosity epoxy resin [14]. Semithin sections were cut with diamond knife and stained with 1% toluidine blue. Fragments of glandular stomach wall were also embedded in paraffin (MEDIM-Plast, MEDIM, Giessen, Germany) and serially sectioned at 10 μm, perpendicularly to the surface (paraffin sections). Sections were stained with haematoxylin and eosin.

RESULTS

The glandular part of the stomach, particularly in the corpus, was strongly hyperaemic. In preparations mounted *in toto* numerous venules running parallel to the stomach surface were visible. Some of them were decorated with dark spots (Fig. 1a). Comparison of *in toto* preparations with sectioned material confirmed that decorated venules corresponded to paramuscular vessels, and dark spots to

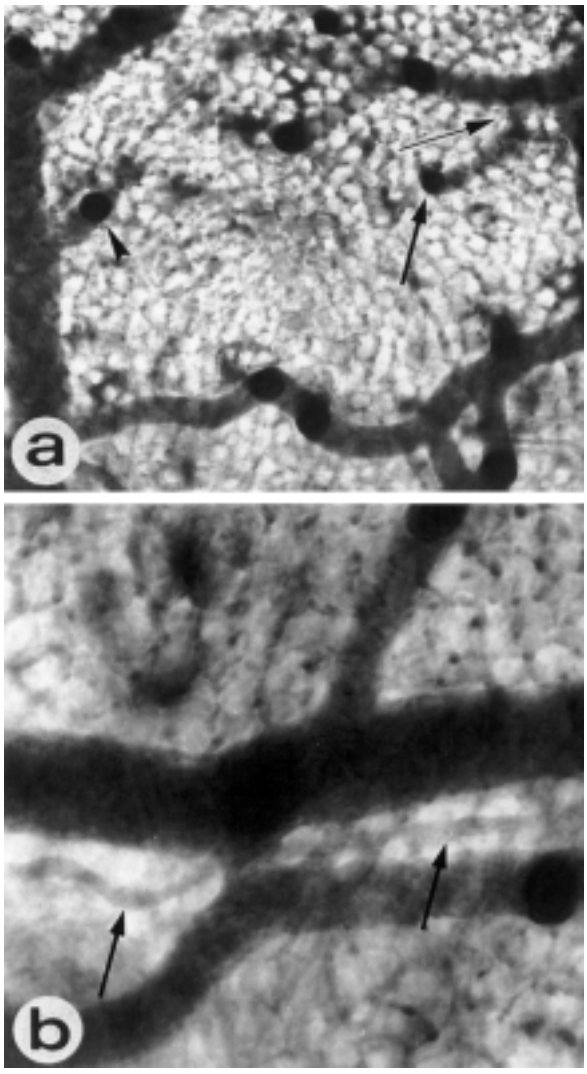

Figure 1. Preparation *in toto* of glandular stomach wall cleared in paraffin oil. Photograph taken from the corpus region. Dark spots correspond to the outlets of collecting venules; **a**) Thick arrow points to collecting venule joining paramuscular venule at its end. Arrowhead indicates collecting venule emptying into paramuscular venule in its middle part. The latter venule presumably enters submucosal vein. Connection between two paramuscular venules is marked by thin arrow. Magnification — 100 ×; **b**) Vascular triplet. Arrows point to arteriole. Magnification — 240 ×.

the outlets of collecting venules filled with blood. The diameter of the largest outlets reached 20 μm in the region of stomach's corpus. Some collecting venules joined paramuscular venule at its end, others in its middle part. Paramuscular venules formed also a connection with each other (Fig. 1a). Moreover, vascular triplets composed of two paramuscular venules and an arteriole were observed (Fig. 1b). Outlets of connecting venules were most conspicuous in the proximal part of the corpus, along the greater curvature. In the antrum the outlets were smaller and less numerous and vascular triplets were not observed.

In thick sections of glandular stomach cleared in mineral oil, most of the vessels including capillary network were filled with blood. Collecting venules were usually straight and ran perpendicularly to the muscularis mucosae. Some collecting venules bent at a right angle and smoothly (without dilatation) passed into the paramuscular vessel. Other collecting venules joined the paramuscular vessel in its middle part or close to its end (Fig. 2).

Semi-thin and paraffin sections revealed two distinct types of venous outflow from the mucosa. Collecting venules emptied either into single paramuscular vessels according to the pattern observed in thick sections (Fig. 3a) or into venules belonging to the vascular triplets. In some cases the wall of the collecting venule within the outlet had a triangular protrusion probably directing blood flow (Fig. 3b). Single paramuscular vessels joined submucosal veins through gaps in muscularis mucosae (Fig. 4). Vascular triplets, however, passed on the submucosal side of muscularis mucosae as a whole unit (Figs. 5a–j). The venules belonging to the triplet were joined not only by collecting venules but also by single paramuscular venules. Occasionally, triplet venules formed connections with each other. After separation, one venule usually increased in size, evidently taking blood from the other. The artery belonging to the triplet had a considerably larger diameter within submucosa than at the beginning of observations within mucosa. After passing into submucosa, veins of the triplets collected blood from single paramuscular venules through a gap in muscularis mucosae.

DISCUSSION

The architecture of vessels participating in the venous outflow from glandular stomach mucosa in hamster is more complex than that in the rat. In the latter species, collecting venules drain into paramuscular vessels, which, in turn, convey blood into submucosal veins [9, 10]. In the hamster, in addition to the system existing in the rat, another type of vessels, occurring in triplets, is present. The triplet consists of long venules formed within mucosa and accompanying intramucosal arteriole. These paired paramuscular venules drain blood both from collecting venules and single paramuscular vessels, until the whole triplet passes into submucosa. There they act as submucosal veins receiving blood from paramuscular venules piercing muscularis mucosae.

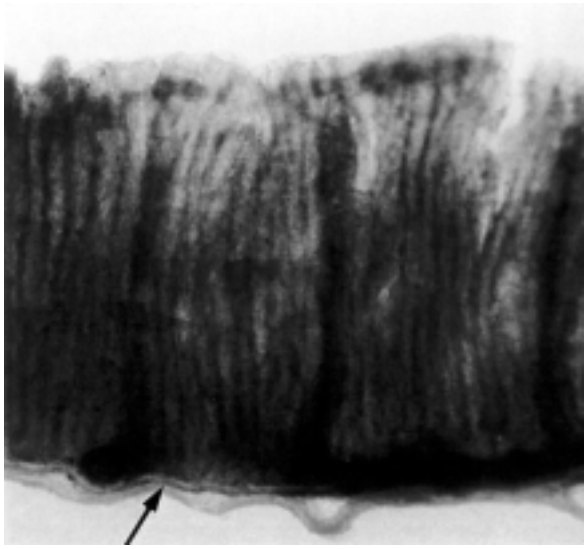

Figure 2. Thick section of glandular stomach cleared in paraffin oil. Collecting venules pass at a right angle into paramuscular venule. Arrow indicates muscularis mucosae. Magnification — 240 ×.

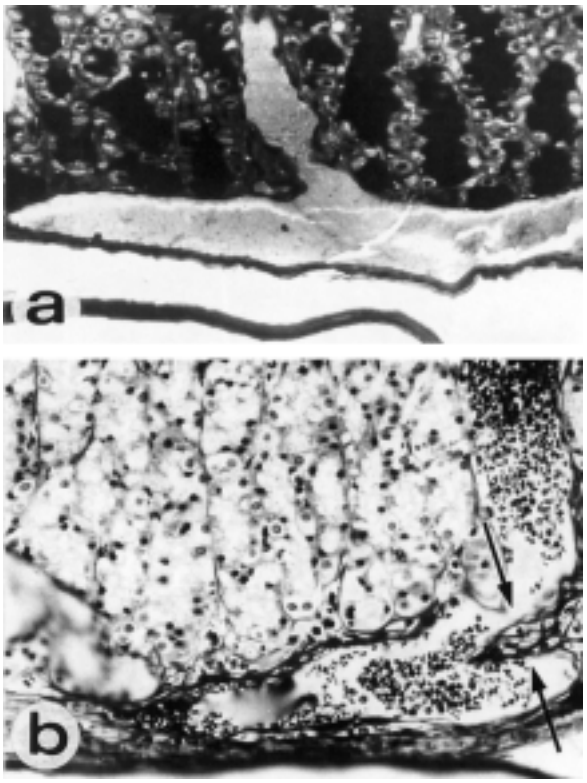

Figure 3 Semi-thin sections stained with toluidine blue; **a)** Collecting venule joins paramuscular venule; **b)** Collecting venule passes at a right angle into paramuscular vessel. In the outlet triangular protrusion (arrows), probably directing blood flow, is present. Magnification — 240 ×.

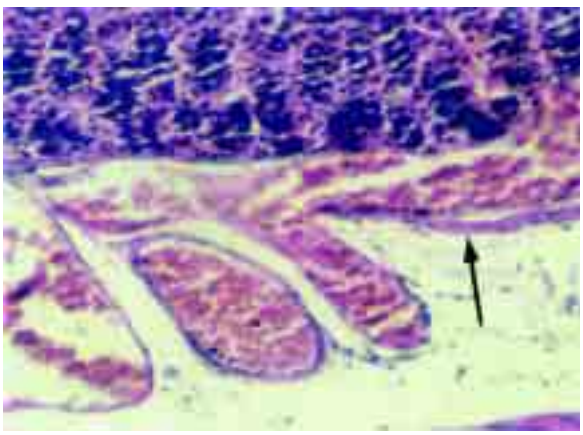

Figure 4. Paraffin section. Connection of paramuscular venule with submucosal vein. Arrow indicates muscularis mucosae. H.E. × 240.

The mechanism of venous outflow from the gastric mucosa is not, as yet, fully elucidated. Seelig et al. [13], in a three-dimensional ultrastructural study of opossum stomach mucosa, described the system of the smooth muscle strands that emanate from the muscularis mucosae, run in the lamina propria and are associated in a unique manner with the gastric glands. The muscle strands arise from the muscularis mucosae at regular intervals and become branched to form an intricate wrapping around a series of gastric glands that empty into one gastric pit. In another study, involving rat, dog and human gastric mucosa, similar strands of smooth muscle cells were demonstrated by actin staining. Rhythmic contractions of muscularis mucosae and its branches were found to be responsible for oscillations in glandular pressure [16]. Muscularis mucosae is contracted by acetylcholine and relaxed by atropine [18], which is a non-selective antimuscarinic, inhibiting smooth muscle activity [15]. In our previous study we found that atropine, even without intravital ligation of portal vein, very effectively relaxed collecting venules [10]. It is, therefore, possible that strands of smooth muscle cells present in the mucosa not only affect glandular action but also help to convey blood from the mucosa into submucosal vessels. In this manner, the intramucosal smooth muscle cells would represent the first link in the long chain of such cells present in veins of the portal vein system [4, 7, 17]. Involvement of these smooth muscle cells is necessary to increase local blood pressure and to force blood through liver sinusoids [3, 7].

Conceivably, contractions of muscularis mucosae, narrowing and opening connections between paramuscular and submucosal vessels, could regu-

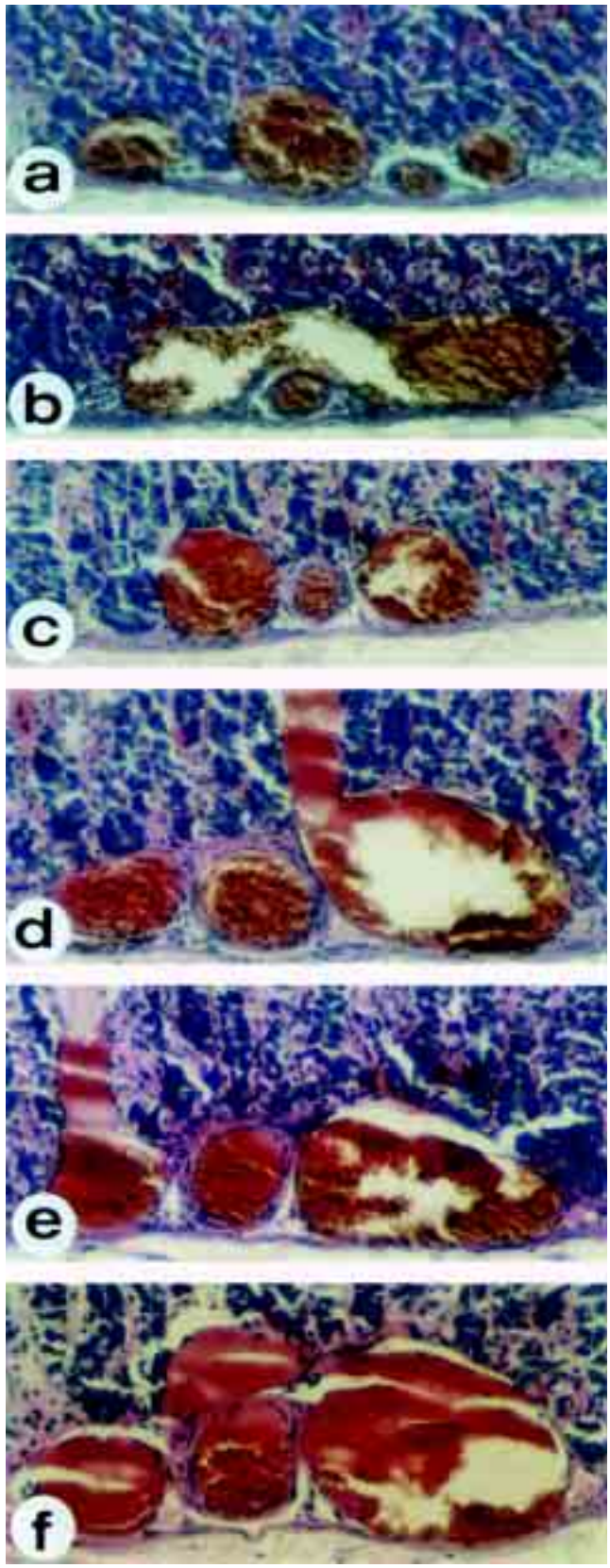

Figure 5. Vascular triplet and its relationship to other mucosal venules followed in serial paraffin sections for a distance of 6,880 μm (calculated from the number of inspected sections); **a** — beginning of observations, **b** — 90, **c** — 1520, **d** — 2960, **e**— 3440, **f** — 4130, **g** — 5360, **h** — 5520, **i** — 6530 and **j** — 6880 μm from the starting point; **a**) Vascular triplet located close to muscularis mucosae, venules differ in size. On the left side of the triplet single paramuscular venule in cross-section is present; **b**) Fusion of venules; **c**) Separated venules have similar dimensions; **d**) Collecting venule joins enlarged right venule of the triplet; **e**) Collecting venule joins left venule of the triplet; **f**) Connection between venules (incomplete, but traceable in serial sections).

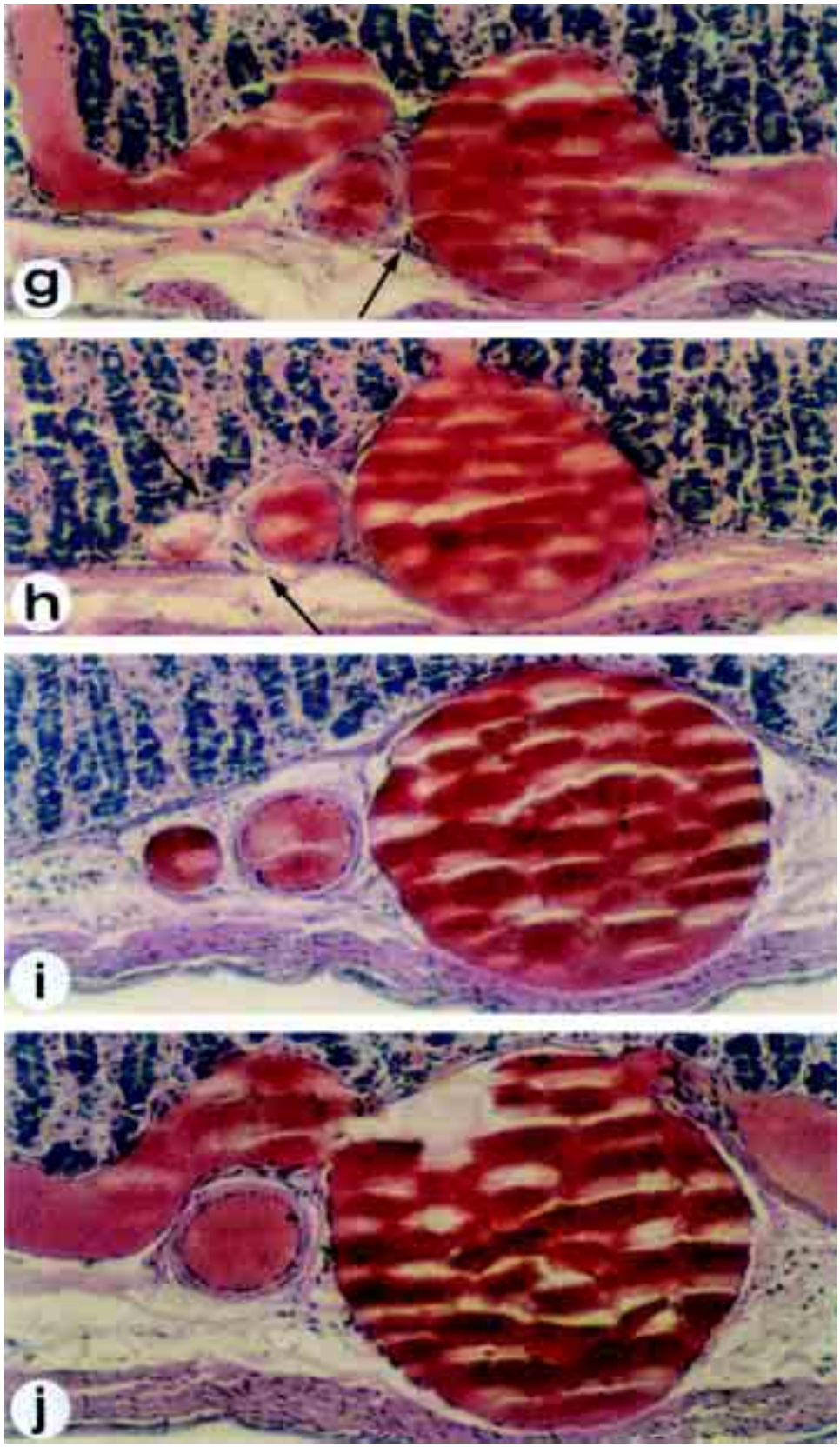

Figure 5. g) Large right venule connected with paramuscular venule. Left venule of the triplet is connected with collecting venule by a short paramuscular vessel. Triplet is still separated from the submucosa by muscularis mucosae (arrow) but right venule bulges into it; **h**) Right venule connected with collecting vessel. Left venule considerably decreased in size. Muscularis mucosae is split into two layers encompassing triplet (arrows); **i**) Triplet passed on the other side of muscularis mucosae and is located in submucosa; **j**) Right venule of the triplet is joined by paramuscular venule, crossing muscularis mucosae. Left venule is incorporated into paramuscular venule and joins enlarged right vessel. Note that arteriole in (**j**) is considerably larger than in (**a**). Haematoxylin — eosin. Magnification — 240 ×.

late blood flow within mucosa, changing the diameter of paramuscular and collecting venules. This, in turn, would change the intramucosal pressure and thus facilitate, for example, movement of the tissue fluid within mucosa. Inclusion of paramuscular venules as a connecting link between collecting and submucosal vessels would increase the length of mucosal venules and the area of their interaction with other mucosal structures.

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