THE MICROVASCULAR ARCHITECTURE OF RAT PROXIMAL DUODENUM, WITH PARTICULAR REFERENCE TO BRUNNER'S GLANDS¹

Jay BROWNING and Bren GANNON

Human Morphology Department, School of Medicine, the Flinders University of South Australia, Bedford Park, S. A. 5042, Australia

ABSTRACT

The microvascular architecture of rat duodenal mucosa was investigated by scanning electron microscopy of vascular corrosion casts, and of fractured tissue specimens in which the interstitial matrix had been digested by acid/enzyme treatment. Light microscopy of cleared tissues, in which the vasculature had been injected with silicone elastomer, was used to define the spatial organization of the larger supply/drainage vessels. Within the mass of Brunner's glands, possibly because of its compact nature, it proved impossible to define differing microvascular networks related to particular functional regions of the glands (acini and ducts). The microvascular bed of Brunner's glands is, however, clearly a separate vascular bed from that of the rest of the duodenal mucosa, and is arranged essentially in parallel to it; it is argued that this separation of venous outflow from the glands may be important in maintaining the HCO_3^- protection of the duodenal surface from acid erosion. Microvascular architectural differences in the villi and intestinal gland regions, compared to more distal rat small intestine, include an apparently denser villus subepithelial capillary network origin of villus venules closer to the villus tip, and frequent direct drainage of the pericryptal network into adjacent venules.

As part of a continuing study relating the microvascular organization to differing regional functions of the gut wall, we have investigated the microvascular architecture of the proximal duodenum in the rat, with particular attention to that of the duodenal (Brunner's) glands. As a result of recent studies of gastric microvascular architecture, we have recently proposed an intramucosal vascular transfer of HCO_3^- in oxyntic mucosa

which might assist in protection of the gastric surface from back-diffusing H^+ ions (6, 7). In the duodenum, secretion of HCO_3^- by Brunner's glands must be associated with acidification of the adjacent interstitial fluid: one might predict that venous blood effluent from this region would not pass towards the surface epithelium of the duodenum (in contrast to the equivalent situation in the stomach), since acidified blood is unlikely to do other than compromise duodenal mucosal protective mechanisms against luminal acid.

Thus the current study paid particular attention to the venous drainage pattern of Brunner's glands, and the possible blood supply routes to the duodenal surface, in order to test whether such predictions, based on functional considerations, might prove correct.

¹This paper is dedicated to the memory of Ms Jan Nowell, who died recently. Ms Nowell, formerly of the Electron Microscope Laboratory, University of California, Santa Cruz, was a pioneer in the application of the corrosion vascular casting-SEM technique, and a personal friend of B.G. Her contributions in teaching and research will be sorely missed.

MATERIALS AND METHODS

Adult male Porton rats, 250 to 350 g, fed *ad libitum* with rat pellets, were used throughout.

Vascular Casting

Rats were narcotised with ether and then anaesthetized with pentobarbitone (Nembutal: 30 mg/kg intraperitoneal injection). The thoracic aorta was exposed by a left thoracotomy and cannulated with polyethylene tubing (PE 160, Intramedic, Clay-Adams, NJ). Blood was washed out with a colloid containing saline (23), perfused via the aortic cannula with an air pressure head of approximately 100 mm Hg; the thoracic posterior vena cava was cut to permit outflow of blood and saline. An acrylic casting medium was then infused, usually via the aortic cannula, using an air pressure head of approximately 200 mm Hg. For complete casts ($n=6$), a partially polymerized methacrylate mixture (prepared according to reference 5) was used. In order to deliberately make partial casts, the commercially available, blue dyed resin Mercor CL2B (Vilene Hospital Ink and Chemical, Tokyo) was used. Arterial partial casts ($n=3$), in which the infusion mass is largely limited to the arterial and arteriolar tree, were made by mixing the Mercor resin with its catalyst, then waiting several minutes until the mixture began to thicken, before injecting it by hand pressure via the aortic cannula; the viscous Mercor so prepared penetrates the capillaries only with difficulty (see 6, 7). Observation of the duodenum, with a binocular dissecting microscope, permitted observation of the degree of penetration of the blue injectate into the blanched tissue. Venous partial casts were similarly prepared ($n=3$), except that Mercor injection was retrograde via a cannula in the hepatic portal vein, with the mesenteric vein tied off a little distally to limit the area of the injection. Hardening of the casts, tissue digestion, preparation for scanning electron microscopy (SEM), and stereo-photomicrography were as previously described (6, 23).

Microfil Studies

Rats ($n=4$) were anaesthetized by ether vapor. The thoracic aorta was cannulated and blood washed out as for the vascular casting above.

High viscosity Microfil¹ silicone elastomer (a catalysed 1:1 mixture of MV-122 yellow elastomer with HV diluent) was then syringe injected by hand pressure via the aortic cannula in order that only the arterial side of the circulation would be filled. The degree of infusion was constantly observed with a binocular dissecting microscope. The stomach and duodenum were excised together as a block after setting of the Microfil (24 h); these specimens were dehydrated in an ascending alcohol series (70%, 80%, 90%, $2 \times 100\%$) for 24 h at each concentration, and then cleared *en masse* by immersion in methyl salicylate, with 3 changes over 14 days.

The cleared specimens were then viewed and photographed in a Wild M400 Photomakroskop. After observation of whole specimens from the serosal aspect, the specimens were bisected by cutting along the greater and lesser curvatures of the stomach and attached duodenum to permit study of the cut edge and of the luminal aspect of the specimen.

Tissue Morphology

Animals ($n=4$), prepared similarly to those for vascular casting, were perfusion fixed via the aortic cannula with 0.1 M phosphate-buffered 4% glutaraldehyde for 5 min. A tissue mass comprised of the gastric antral region and attached proximal duodenum was excised, further fixed for 1 h by immersion in the same fixative, rinsed in saline, then placed in a 25:75 mixture of dimethylsulphoxide (DMSO) with normal (0.9%) saline for 1 h, followed by immersion in 50:50 DMSO/saline (1 h). The cryoprotected sample was slowly frozen on a metal plate cooled to liquid nitrogen temperature (Eiko model TF1 tissue freezing chamber) and then cleaved with a hammer blow to a chisel-type blade; appropriate orientation of specimen and blade ensured that the fracture plane passed along the duodenum and through the gastro-duodenal junction. The cleaved specimens were thawed in 25:75 DMSO/saline, dehydrated in an ascending alcohol series, critical point dried from absolute ethanol, then gold/palladium sputter coated and viewed in the SEM.

Acid-Digested Tissue

Other perfusion fixed segments of pyloro-duodenal junctions plus proximal duodenum were placed in 6N HCl at 55°C in a shaking water bath for 30–50 min, until the tissues were judged subjectively to be optimally digested (26). The par-

¹Canton Biomedical Products, P. O. Box 2017, Boulder, CO, 80302

Fig. 1 SEM of vascular corrosion casts of rat proximal duodenum, sectioned longitudinally. a: The pyloric region (Py) and the most proximal duodenal villi (DV) are indicated. The dotted outline defines the region containing the Brunner's glands; the bulk of this tissue lies adjacent, or proximal, to the most oral duodenal villi. Gut lumen, L; serosal aspect, S. Bar, 0.5 mm. b and c: Arteries supplying the proximal bulb of Brunner's glands are indicated (A); however their detailed branching pattern cannot be clearly discerned. Drainage of the periglandular capillary network into venules (V) is obvious (arrows). Note that in this area the prominent venules lie abluminal to the submucosal position more usual in the intestine. Gut lumen, L; serosal aspect, S. Bar: 2 mm (b); 0.15 mm (c)

tially digested specimens were then rinsed in water, dehydrated in an ascending alcohol series, and prepared for SEM as indicated above.

In addition, fixed duodenal specimens were prepared for conventional histological examination of haematoxyline-eosin stained, $\sim 7 \mu\text{m}$ paraffin sections.

RESULTS

Brunner's Glands

The location of the coma shaped mass, or bulb, of Brunner's glands in rats (see *e.g.* 25), also typical of mice (18), was first verified by conventional light microscopy. The bulk of the glandular tissue lies adjacent to the gastro-duodenal junction and tails off in the proximal duodenal segment. Identification of the corresponding region in the vascular casts was thereby facilitated (Fig. 1). Much of the Brunner's glands bulb lies slightly proximal to the junction of the pyloric and duodenal mucosae. However, as indicated by the casts (Fig. 1), the glandular tissue lies serosally in a discrete mass and appears somewhat isolated from the pyloric tissue. Attempts to fracture the Brunner's gland mass in the critical-point-dried whole tissue and also in acid-digested preparations (Fig. 4) often caused the whole gland mass

to break off as a relatively complete and discrete block of tissue.

The vascular casts show that the arterial vessels supplying the bulb of Brunner's gland tissue branch at its outer surface, serosally and proximally (Fig. 1). The casts also suggest that in this proximal region, arterial vessels in the submucosa diverge to send branches lumenally to the pyloric tissue, and additional branches ablu-menally (or serosally) to the Brunner's glands. The casts are complicated here because of the adjacent pyloric vasculature, and the branching pattern of larger arterial vessels was further elucidated with Microfil preparations. The arterial branching pattern shown diagrammatically in Fig. 5 represents information collated from the corrosion casts and Microfil preparations (Fig. 3). The arterial vessels to the more distal 'tail' of Brunner's glands also originate largely from a plexus of arterial vessels at its serosal aspect (Fig. 2). Capillaries of the Brunner's glands, as seen in the casts (Figs. 1 and 2) and in acid-digest preparations (Fig. 4), are arranged in a typical honey-comb-like periglandular plexus.

Capillaries of the proximal bulb of the Brunner's gland mass drain into one of several large venous vessels (Fig. 1), which are centrally placed in the glandular tissue and so lie serosally, unlike

Fig. 2 SEM of vascular corrosion cast of rat proximal duodenum, sectioned longitudinally. The distal region (tail) of Brunner's glands is outlined (see also Fig. 1). The arterial supply (A) is located serosally, while venules (V) are found within the gland areas, just serosal to the submucosal vascular plexus (SM). Gut lumen, L; serosal aspects, S. Bar, 0.1 mm

Fig. 3 Photomicrograph of proximal rat duodenum, longitudinal section of Microfil vascular injection in cleared tissue. Elastomer is limited to the arterial tree. The pyloric region is indicated (Py). Arterial vessels traverse the glandular bulb of Brunner's glands to branch submucosally both into the glandular tissue and into the gut mucosa (arrowheads). The arterial supply (As) branching at the serosal aspect of the glandular tissue appears to be its major supply. Note arterial supply from submucosa to villi (V) and blind ending marginal villus distending arterioles (arrows). Gut lumen, L; serosal aspect, S. Bar, 0.5 mm

the submucosal position typical of the gastrointestinal venous plexus generally. Distal to these large vessels, there are other smaller, increasing less frequent venous vessels within the glandular tissue, until at the tail of the Brunner's gland mass, capillaries drain inwardly, directly into the submucosal venous plexus (Figs. 2 and 6).

Duodenal Villi

The villi of the proximal duodenum are flattened, leaf shaped structures oriented at right angles to the long axis of the intestinal lumen (see Fig. 1a). Their microvascular architecture is, in general, similar to that described previously for rat intestine (e.g. 19). An arteriolar vessel proceeds, without branching, from the submucous arterial plexus to the central tip region of each villus, there branching at a T-junction to provide marginal arterioles which pass around the margins of the particular villus (Figs. 3 and 8). The apparently dense subepithelial capillary plexus of

the villus (Fig. 9c) is supplied from these marginal arterioles, (Fig. 9b), which become indistinguishable from capillaries as they approach the villus base (Fig. 9a). The subepithelial capillary network of the villus near its base is continuous with the capillary plexus surrounding the adjacent intestinal glands (Fig. 8).

Venous drainage of each villus is by one or two venules which commence at about 2/3 of villus height above the villus base. These venules receive capillary tributaries principally within the upper region of the villus (Figs. 7 and 9b); however it was apparent that, below the level of the base of the villus, the venules receive further direct capillary tributaries from the capillary plexus surrounding the intestinal glands (Figs. 6 and 7). In addition, direct capillary and small venular tributaries to the submucous venous plexus from the pericryptal capillary network were commonly observed (Figs. 6 and 7).

Large, ridge-like villi, apparently formed by fusion of the lateral edges of adjacent villi, were fairly frequently observed (Fig. 9c).

Fig. 4 SEM of an acid-digest tissue preparation of Brunner's glands, viewed from the abluminal (serosal) aspect. A capillary (C) network ramifies over the serosal aspect of, and between, gland acini. Bar, 15 μ m

From both the Microfil studies and from observation of casts at low magnification it was apparent that, to a large extent, the vascular beds of the villi plus intestinal glands and that of the compact Brunner's gland mass are distinct, separate beds arranged in parallel to each other, having clearly distinct arterial supplies, and for the most part distinct initial venous drainage pathways (Fig. 10).

DISCUSSION

Brunner's gland secretions are thought important in protecting the duodenal mucosa against the acidic digesta released from the stomach. A primary difficulty in identification of the specific secretory component responsible for this protection lies in the inability to readily separate Brunner's gland secretions from those of the adjacent intestinal glands. Nevertheless the drugs cincho-

phen, 3-4 toluendiamine and cysteamine, which cause duodenal ulcers, also affect the Brunner's glands; secretory rates are decreased and the histological appearance of the glands is changed (9, 15, 21, 22). Pentagastrin, on the other hand, while a potent stimulator of gastric H^+ secretion, does not readily cause duodenal ulcers nor does it noticeably affect the structure and function of the Brunner's glands (15).

It is suggested, from study of human tissue, that basal granule cells in the Brunner's glands may be involved in hormonal control over duodenal protective mechanisms (13). It is also reported that Brunner's glands contain large amounts of epidermal growth factor which is secreted into the duodenal luminal surface layer (10), thereby enhancing replacement of damaged mucosa.

However, in regard to Brunner's gland secretion, mucus and HCO_3^- remain the most obvi-

Fig. 5 Diagrammatic representation of major supply and drainage patterns to Brunner's gland tissue in the proximal duodenum of the rat. Inset: Horizontal section of the stomach illustrating location of Brunner's glands

ous candidates. Secretion of mucus must occur, as judged by histological evidence (reviewed in 2), but the importance of this mucus to duodenal protection is not established. There is good evidence to suggest that HCO_3^- secretion by Brunner's glands is important here. Florey and Harding (4) found that the first part of the duodenum, into which the Brunner's glands secrete, is more resistant to acid erosion than the more aboral

proximal small intestine. In addition, it was found that following acid stimulation, intestinal luminal HCO_3^- concentration increased more in that portion of the duodenum containing Brunner's glands than it did in the adjacent duodenal region (4). The comparatively high rate, and importance of HCO_3^- secretion in the Brunner's gland region, is supported by various, more recent studies (see *e.g.* 2, 11, 14).

Fig. 6 SEM of vascular corrosion cast of rat proximal duodenum viewed from the gut lumen; venous partial cast with plastic restricted to venous vessels and their immediate capillary tributaries. The villus venules, originating high in the villi (Vi) drain to a submucous venous plexus (SM), which also receives direct capillary and small venular tributaries from the pericryptal plexus (arrows). Below the submucosal plexus (*i.e.* serosally) the periglandular capillary plexus of Brunner's glands is evident (BG). Bar, 0.1 mm

Duodenal mucosa, without any Brunner's glands, can also secrete HCO_3^- (3); the relative contribution of the mucosa associated with the Brunner's glands to HCO_3^- production has not been assessed.

The movement of pancreatic juice (rich in HCO_3^-) or bile, by reverse peristalsis, is undoubtedly important in neutralization of H^+ in the proximal duodenum (see *e.g.* 27). Indeed it has been argued that such reverse peristalsis

could account for the neutralization of all the incoming H^+ (12), although there are opposing views (14). It seems most improbable that such retrograde movement of fluid could occur simultaneously with the normal peristaltic action that results in gastric emptying. That is, it seems unlikely that the most proximal duodenal mucosa could have access to HCO_3^- rich pancreatic juices immediately following gastric emptying, when luminal H^+ are in highest concentration. It

Fig. 7 SEM of vascular corrosion cast of rat proximal duodenum, just distal to the region containing Brunner's glands; venous partial cast viewed from the gut lumen. Capillaries of the villus (C) drain to a villus venule, which passes to the submucosal venous plexus (SM). Note the separate drainage sites of the pericryptal capillary plexus into the villus venule and the submucosal venules (arrows). Bar, 30 μ m

should be noted here that Brunner's glands are almost invariably only found proximal to the entry of the pancreatic and bile ducts into the duodenum, and are usually most concentrated in a region immediately adjacent to the pylorus (2, 25).

On the available evidence it can be concluded that Brunner's gland secretions are of primary importance in the prevention of acid damage to the proximal duodenum, and it appears likely that the HCO_3^- in this secretion is one of the

more important components of duodenal defense.

In a previous study, we established that the vasculature of the oxyntic mucosa of the stomach is so arranged as to maximise the possibility that HCO_3^- will be transferred toward the surface mucous cells, where protection against back-diffusing H^+ is required (5, 6). The HCO_3^- is produced in equimolar quantities to, and as a consequence of, H^+ secretion by the parietal cells. The H^+ ions are secreted into the gastric

Fig. 8 SEM of vascular corrosion cast of rat proximal duodenal mucosa at the distal boundary of Brunner's glands; longitudinal section. The extent of the villi (1) and pericryptal (2) plexi, and plexus of the tail of Brunner's glands (3) are indicated. A submucosal arteriole supplies a villus arteriole (A) and also directly supplies the pericryptal capillary plexus (arrowheads). Villus venules (V) originate high in the villi, draining the subepithelial capillary network; additional direct venous drainage from the pericryptal capillary network is also evident (arrows). Bar, 0.1 mm

Fig. 9 SEM of a vascular corrosion cast of rat proximal duodenum, distal to the region containing Brunner's glands. Villi are flattened across the long axis of the gut. a: View of the dense subepithelial capillary plexus of the villi (C), submucosal arterioles (A) and venules (V), and the pericryptal capillary network (PC). Bar, 0.1 mm. b: A single villus receives a villus arteriole (A), which distributes via marginal arterioles (MA) to the subepithelial capillary network. A single venule (V) originates high in the villus core, draining the villus capillaries, and also receives venous tributaries from the pericryptal network (PC). Bar, 0.1 mm. c: A compound villus with a dense subepithelial capillary network. The capillaries appear to converge on a point half way down the villus—presumably at the origin of the villus venule (V). Bar, 0.1 mm

Fig. 9

Fig. 10 Block diagram of the rat gastro-duodenal junction, illustrating the pattern of intramural blood supply and drainage of intestinal mucosa and Brunner's glands in the proximal duodenum

gland; the HCO_3^- moves ablu-menally, into the lamina propria, there to be picked up by capillaries *en route* to the basal aspects of the gastric surface mucous cells.

In that portion of the duodenum containing Brunner's glands, the opposite situation with regard to H^+ and HCO_3^- movements must pertain. That is, HCO_3^- secretions into the gland lumen will be associated with interstitial release of H^+ . However, in the duodenal wall, the interstitial by-product (H^+) is not required for defence at the mucosal lining. On the contrary, endogenously produced interstitial H^+ ions are presumably a liability in a mucosal wall which requires protective mechanisms to cope with an exogenous acid load applied at its luminal surface. As a consequence, the vasculature of the Brunner's glands and adjacent duodenal surface mucosa (the villi), is unlikely, for functional reasons, to be arranged as seen in the stomach wall. Our results clearly show this prediction to be correct; there are essentially parallel circulations to the Brunner's glands and duodenal villi (Fig. 10). There can be little significant vascular transfer from the region of the Brunner's glands to the villi: in particular, interstitially released H^+ (produced in equimolar amounts and as a consequence of HCO_3^- secretion from the Brunner's

glands) cannot be transferred via capillaries to the mucosal region of the duodenum.

As indicated earlier, the relative contributions of Brunner's glands and of the crypts of Lieberkühn, into which they empty, to the combined HCO_3^- secretion of this region is unknown. However, the pericryptal microvascular plexus of the proximal duodenum also appears to be largely separate from the villus microvascular bed; frequent drainage sites from this pericryptal capillary plexus into the submucosal venous plexus and into villus venules *en route* to that plexus were observed. This indicates that any interstitially released H^+ produced in the duodenal crypts is similarly denied vascular access to the luminal mucosal lining.

Study of the microvascular architectures of other glandular tissue (20) has enabled description of detailed small vascular units which are associated with acini, initial and larger ducts etc. In the current study, we were unable to discern discrete microvascular modules related to the acinal and ductal portions of the Brunner's gland system. This difficulty presumably arises because of the compact nature of the gland mass, and the absence of any apparent polarity of gland acini and ducts in space. In this regard, study of species with a more diffuse distribution of Brunner's

glands may be more revealing.

The enlarged, ridge-like compound villi observed occasionally in the duodenum have been reported previously (1, 16, 17), arising perhaps by fusion of the edges of adjacent villi. The incidence of such villi in man increases with age. Similar compound villi have been observed in more distal human small intestine (24, 8). There are reported geographic differences in incidence of such villi, which some consider due to differing diet or prevalence of intestinal disease (24).

The microvascular architecture of the individual duodenal villi differed but slightly from that described for the more distal regions of rat small intestine (19). The major differences seem to be firstly, a relatively higher density of subepithelial capillaries, possibly a reflection of higher activity of enterocytes here, and secondly, the level of origin of the villus venule(s) being further up towards the tip of each villus (approximately 2/3) than is usual distally (<1/3) in the rat. The capillary network of the lower portion of the villus is continuous with that surrounding the openings of adjacent intestinal glands, as is seen elsewhere in the rat, and in other species (8, 24). In the more distal small intestine, the direction of blood flow in the portion of the capillary network between the intestinal glands and the villus venule has been found to be from intestinal glands up the sides of the adjacent villus to the villus venule (8). However, it has been noted that there are few connections of the pericryptal capillary plexus to veins at the crypt level in those more distal intestinal regions. The existence of frequent direct venous drainage routes from the pericryptal plexus in the duodenum argues that a significant 'local portal' type of blood flow from the crypts to the villi, as has been reported for the more distal intestine (8), is unlikely to be significant in the duodenum. The actual direction of blood flow in the capillaries lying between the level of origin of the villus venule and the capillary rings surrounding the necks of the adjacent intestinal glands of the duodenum remains to be established.

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