Microvascularization of the Pancreas in Larval and Adult *Xenopus laevis* – Histomorphology and Scanning Electron Microscopy of Vascular Corrosion Casts

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Summary. The microvascularization of the pancreas of larval and adult South African Clawed Toads *Xenopus laevis*, was studied by scanning electron microscopy of vascular corrosion casts and light microscopy of paraplast embedded Goldner-stained serial tissue sections. We showed that branches of left and right gastric artery, hepatic artery and anterior intestinal artery, namely anterior pancreatic, anterior middle pancreatic, posterior middle pancreatic and caudal pancreatic arteries supply and pancreatic veins drain the adult pancreas into hepatic portal vein, anterior and middle gastric vein, gastroduodenal vein, and anterior duodenal vein. In premetamorphosis the pancreas showed a dense but immature vascular bed with signs of ongoing sprouting and non-sprouting angiogenesis (=intussusceptive microvascular growth; IMG). During metamorphic climax the pancreas shrunk dramatically paralleled by vascular regression.

The larval pancreas had an underdeveloped ductal system which in the course of pancreas remodeling during metamorphic climax developed into a complex ductal system. In adult *Xenopus laevis* the pancreas showed intralobular islets of Langerhans only and an insulo-acinar portal vessel system as described so far in reptiles, birds and mammals. Islets in *Xenopus* located superficially and within deeper regions and emitted both insulo-acinar portal vessels and insulo-venous efferent vessels. Intrainsular microvascular patterns found suggest that in *Xenopus* islets blood flows first to β-cells and subsequently to the other endocrine cells present in the endocrine pancreas.

Key words: *Xenopus*, pancreas, microvascularization, vascular casts, histomorphology

Introduction

Since the first report on the inductive role of signals from blood vessels in pancreas differentiation (Lammert et al., 2001) further knowledge has accumulated that stresses the importance of blood vessels in development, differentiation and growth of the pancreas (Magenheim et al., 2011; Villasenor and Cleaver, 2012; Cleaver and Dor, 2012). Pancreas development, structure, function and blood supply are predominantly studied in mammals (Böck et al., 1997; Wayland, 1997; Murakami et al., 1997a, 1997b). In amphibians
where the pancreas like that in mammals comprises exocrine and endocrine portions development and differentiation of exocrine and endocrine portions are studied at the light microscopic and electron microscopic level (Leone et al., 1976; Milano and Chimenti, 1995; Wiechmann and Wirsig-Wiechmann, 2003; Mukhi et al., 2008, 2009; Heller, 2010; Cleaver and Dor, 2012). Gross arterial supply and venous drainage are well described in few species only (Rana esculenta; Gaupp, 1899; Xenopus laevis; Millard, 1940, 1945, 1949; Rana catesbeiana; Ichimura et al., 2001). Little, however, is known on its microvascularization (Rana temporaria, Rana esculenta; Syed Ali, 1989; Ohtani, 1983).

According to previous authors branches of left and right gastric artery, hepatic artery and anterior duodenal artery supply the pancreas. In Xenopus Millard (1940) supposed that also the anterior intestinal artery sends a branch to the pancreas. Pancreatic veins drain into hepatic portal vein, anterior and middle gastric vein, gastroduodenal vein, and the anterior duodenal vein (Millard, 1940, 1945, 1949). Here we demonstrate microvascular patterns of the larval and adult pancreas of Xenopus laevis Daudin and document the changes they undergo from larval stages 52 (Nieuwkopp and Faber, 1994) to the adult stage. In detail, we (i) verify and refine the results of Millard relating to the gross arterial supply and venous drainage of the adult pancreas (Millard, 1940, 1945, 1949) by scanning electron microscopy of vascular corrosion casts (Aharinejad and Lametschwandtner, 1992; Lametschwandtner et al., 1990; Motta et al., 1992; Murakami, 1971), (ii) demonstrate microvascular changes of the pancreas when the herbivorous tadpoles metamorphize to become carnivorous juveniles, (iii) look into mode(s) of angiogenesis, i.e. sprouting and/or non-sprouting angiogenesis (=intussusceptive microvascular growth; IMG; Djonov et al., 2003) which are active in larval and adult pancreas, (iv) test how the dramatic shrinkage of the pancreas during metamorphic climax is associated with vascular regression reported to occur (Mukhi et al., 2008), and (v) finally compare the vascularization of the islets of Langerhans in Xenopus laevis with those of other vertebrate species described so far in order to demonstrate differences and similarities between vertebrate species (Ali et al., 1991; Murakami et al., 1992a, b; 1993, 1997a, 1997b; Syed Ali, 1987).

### Materials and Methods

#### Animals

Adults and tadpoles of the South African Clawed Toad *Xenopus laevis* Daudin housed under standard conditions were studied.

#### Histomorphology

**Tadpoles**

Tadpoles in developmental stages 52, 58 and 62 (staging according to Nieuwkopp and Faber, 1994) were killed by immersion in an overdose of an aqueous solution (0.03%) of tricaine methanesulfonate (MS 222: 3-Aminobenzoic acid ethyl ester, Sigma Chemicals, St. Louis). After recording weight (mg), body length (mm) and total length (mm) tadpoles were fixed by perfusion with Bouin’s solution (Adam and Czihak, 1964), dehydrated, embedded in paraplast, sectioned (7 μm), and stained according to Goldner (Romeis, 1968). Images were taken with a light microscope (Olympus BX 51) equipped with a digital camera (Color View III; Soft Imaging Systems; FRG). Brightness and contrast of recorded images were adjusted according to needs. For details see Lametschwandtner et al. (2012).

**Adults**

Two adult animals (male: body weight 77 grams; body length 80 mm; female: body weight 79 grams; body length 90 mm) were killed by immersion into an aqueous solution of MS 222 (0.5%). Whole animals were fixed by vascular perfusion with 10 ml Bouins solution. The fixed pancreas was excised, dehydrated, and embedded in paraplast. 7 μm thick transverse sections were stained according to Goldner. Further steps were as described for tadpoles.

**Vascular corrosion casting**

A total of 12 adults (7 males and 5 females; body weight 19–70 g; body length 60–85 mm) and 15 tadpoles (body weight 1.5–7.3 g; total length 21–50 mm) was used for vascular corrosion casting. Preparatory steps including...
Pancreas microvasculature in larval and adult *Xenopus*

exposing the heart and flushing the vascular system with Amphibian Ringer solution were the same as described under “Histomorphology”. Once clear reflux escaped from the opened sinus venosus 1 ml (tadpoles) respectively 10 ml (adults) of the polymerizing resin Mercox-Cl-2B (Ladd Res Inc., Burlington, Vermont USA) diluted with methylmethacrylate (4 + 1, v + v, 10 ml monomeric methylmethacrylate contained 0,85 g initiator paste MA) was injected by a syringe pump (Sky Electronics, PSA 50; Grenoble, France) (flow rate: tadpoles: 3–7 ml/h; adults: 40 ml/h). The injection was finished as soon as the effluent Mercox became highly viscous. Injected whole animals were kept for at least 30 minutes at room temperature (about 20°C) to allow the resin to polymerize. Specimens then were tempered, macerated, decalcified (adults), cleaned, frozen in distilled water, and freeze-dried. Finally, vascular beds of abdominal organs overlaying the pancreas were removed, and vascular casts were mounted onto stubs using the “conductive bridge method” (Lametschwandtner et al., 1980). After evaporation with carbon and gold specimens were examined with a scanning electron microscope XL-30 (FEI, Endthoven, Netherlands) at an accelerating voltage of 10 kV. Brightness and contrast of recorded images were adjusted using Photoshop 7.0 whenever necessary. For further details see Lametschwandtner et al., 2010, 2012).

After a thorough photographic documentation of microvascular patterns of exposed pancreas surfaces specimens were demounted, submerged in distilled water and oriented frozen therein. Ice blocks containing casts were frozen onto wooden plates and sectioned transversely or longitudinally with a mini-wheel saw placed inside a cryomicrotome (Lametschwandner and Lametschwandtner, 1992) to enable SEM inspection of the internal pancreatic microvasculature. Sections were thawed in distilled water, cleaned, frozen in distilled water, freeze-dried, mounted onto specimen stubs, sputtered with gold, and reanalyzed in the SEM.

In one vascular cast of the pancreas of adult *Xenopus* superficial vessels were ripped off by a fine tipped insect pin under binocular control to enable SEM inspection of the vascular beds of islets of Langerhans located inside the organ as well as to convincingly display insulo-acinar vascular relations.

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**Results**

**Histomorphology**

**Tadpoles**

At late premetamorphosis (stage 52) the pancreas locates between duodenum (lies dorsally), liver (lies medially), stomach (lies laterally and ventro-laterally) and the large intestine (lies medially to ventro-medially). In transverse sections it reveals a squared profile with one extension towards the liver and one towards the small intestine (Fig. 1). Most exocrine acinar cells already form clusters with centroacinar cells in the center. Acini are not yet clearly separated from each other. Several small one-layered epithelial pancreatic ducts without any fibrous tissue cover or embracing periductal capillaries form the simple ductal system (Fig. 2). Main pancreatic ducts own a fibrous tissue cover and are surrounded by blood vessels. Sinusoidal capillaries and larger vessels which run between acini clearly show that the pancreas is already well vascularized (Figs. 1 and 2).

At early metamorphic climax (stage 58) position and profile of the pancreas are nearly the same as in stage 52 (Fig. 3). Acini are well developed and are now clearly separated from each other by connective tissue septa. They connect to a simple system of small ducts formed by a one-layered cuboidal epithelium (Fig. 4). Main pancreatic ducts are surrounded by fibrous tissue (Fig. 3).

At mid metamorphic climax (stage 62) the pancreas shrinks and changes its position together with the stomach.

**Colour-coding of arteries and veins in scanning electron micrographs**

For an easy identification of arteries and veins displayed in scanning electron micrographs of vascular corrosion casts arteries were colored red and veins were colored blue with Photoshop 7.0 (Adobe Inc., Redwood, CA, US). The characteristic endothelial cell nuclei imprints at the surfaces of microvascular corrosion casts which are longish in arteries and orientate parallel to the long axis of arteries, and which are round to oval and orientate randomly in veins, were used to identify arteries and veins (Miodonski et al., 1976).
Fig. 1. Topography of abdominal organs in larval *Xenopus* at Nieuwkoop and Faber stage 52. Transverse section (7 μm) through whole tadpole at the level of the abdomen. Light microscopy. Goldner staining. Note the two extensions (arrows) of the pancreas (pa) towards the liver (lv) and the small intestine (si). bd bile duct, dd duodenum, li large intestine, st stomach.

Fig. 2. Histomorphology of the pancreas in larval *Xenopus* at stage 52. Detail of Fig. 1 (box). Note the loose clusters of acinar cells (ac) which are not yet clearly separated by connective tissue septa from each other. Note also the intercalated duct outlined by dashed line. c capillary, ca centroacinar cells, dd duodenum, v vein, vv venule.

Fig. 3. Topography of abdominal organs in larval *Xenopus* at stage 58. Transverse section (7 μm) through whole tadpole at the level of the abdomen. Light microscopy. Goldner staining. bd bile duct, li large intestine, lv liver, pa pancreas, si small intestine, st stomach.
and the duodenum (Fig. 5). The pancreas parenchyma degenerates and shows many signs of vacuolization. Many lacunae filled with degenerating cells are present. Exocrine cells become disorganized and are no longer arranged in acini. Pancreatic ducts do still exist (Fig. 6). The pancreas is well vascularized whereby arterioles and venules dominate (Fig. 6).

**Microvascularization**

In stage 52 vascular casts of the pancreas locally reveal endothelial cell nuclei imprints and arteries and veins have to be differentiated primarily by their origin, gross morphology and branching patterns. Venous vessels by far outnumber arterial ones (Fig. 7). Capillaries exhibit signs of intussusceptive microvascular growth (IMG) and sprouting (Fig. 8). They impose as immature and vessels lack a clear hierarchy. Capillaries form a dense meshwork with short intervals between branchings (Figs. 7 and 8). The main pancreatic duct reveals periductal capillaries (Fig. 7). Though islets of Langerhans are already present they do not yet show a distinct vascular pattern and thus cannot be differentiated in casted specimens.

At stage 58 capillaries are not as wide and impose as less immature as at stage 52, but still show a high number of extravasates. As in stage 52 the vascular bed shows many blind ending vessels with smooth and rounded endings. Venous vessels still predominate. Arterioles and venules now can be partially distinguished by their endothelial cell nuclei imprints but mainly still by their morphology and origins. They can now be better distinguished from capillaries as they run over longer distances without bifurcating. The capillary meshwork is no longer as dense as at stage 52 and intervals between ramifications are longer. In areas where capillaries look mature as indicated by their smaller diameter, many sprouts are found (Fig. 9). Areas with wide and immature appearing capillaries show signs of sprouting and intussusceptive microvascular growth (Figs. 9 and 10). Outside the pancreatic parenchyma main pancreatic ducts are surrounded by a network of periductal capillaries (Fig. 9). Islets of Langerhans do not reveal distinct microvascular patterns yet and thus still cannot be identified in vascular corrosion casts.

At stage 62 the pancreas decreases in size and presents a miniaturized capillary meshwork (Figs. 11 and 12). Arterioles and venules dominate; arteriovenous transition distances are shorter than in preceeding stages. Locally, arteriovenous anastomoses with no interposed capillaries are found. The vascular bed shows signs of vascular regression particularly on the ventral surface of the pancreas. There are many blind ending vessels with round or tapered endings and the course of some capillaries, arterioles and venules is tortuous. Some vessels look flaccid and porous others display bulges and extravasations. The vascular bed of main pancreatic ducts which join the common bile duct shows few signs of vascular regression (Fig. 11). The vascular bed of the islets of Langerhans displays still no specific patterns and thus cannot be differentiated from that of the exocrine portions.

**Adults**

**Histomorphology**

The pancreas of adult *Xenopus* is an elongated flat organ whose main part overlies the stomach. It has a hepatic lobe adjacent to liver and spleen, a duodenal lobe adjacent to the duodenum and the dorsal side of the stomach and a gastric lobe facing the ventral side of the stomach and duodenum (Fig. 13). In a transverse section it exhibits a triangular profile. A thin connective tissue capsule encloses the organ (Fig. 14). Exocrine tissue makes up the largest part of the organ. Fine capillaries wriggle between the acini and surround...
Fig. 7. Microvasculature of the pancreas in larval *Xenopus* at stage 52. Vascular corrosion cast (VCC). Scanning electron micrograph (SEM). Lateral view at the pancreas (pa). Cranial is at right, ventral is at the top. Note the dense meshwork of immature vessels. a artery, aa arteriole, lv liver, md main pancreatic duct, v vein, vv venule.

Fig. 8. Microvasculature of the larval pancreas at stage 52. Note the signs of intussusceptive microvascular growth (IMG) (arrows). v vein.

Fig. 9. Microvascular anatomy of the larval pancreas at stage 58. Cranial is at left, ventral is at top. Note extravasates (black arrows) and blind ending vessels (white arrows). aa arteriole, md main pancreatic duct, v vein, vv venule.

Fig. 10. Microangioarchitecture of the larval pancreas at stage 58. Note the many signs of ongoing intussusceptive microvascular growth (IMG) (arrows). vv venule.

Fig. 11. Microvascular pattern of the larval pancreas at stage 62. Note the small vascular plexus of the pancreas (pa) with a miniaturized capillary meshwork. Arterioles and venules dominate. aa abdominal aorta, av abdominal vein, cma coeliac-mesenteric artery, lv liver, md main pancreatic duct, st stomach.

Fig. 12. Microvascularization of the larval pancreas at stage 62. Note signs of vascular regression (white arrows). Arteriovenous transition distances are reduced and locally arteriovenous anastomoses with no interposed capillaries are found (black arrow). a artery, aa arteriole, pa pancreas, v vein, vv venule.
Pancreas microvasculature in larval and adult *Xenopus*

the pancreatic ducts. Acinar cells arrange in berry-like clusters with centroacinar cells in the center. Intercalated ducts which consist of a thin epithelium only start from the acinar lumen (Figs. 15 and 16). Intercalated ducts merge and form intralobular ducts. These ducts have a wider lumen, own a cuboidal epithelium and are surrounded by fibrous connective...
tissue (Fig. 16). Intralobular ducts merge and form outside the lobules wider interlobular ducts which have the same structure as the intralobular ducts (Figs. 14). The endocrine islets of Langerhans scatter throughout the exocrine tissue. They are richly capillarized whereby capillaries have a wider lumen than those in the surrounding exocrine tissue (Fig. 15).

**Gross arterial supply and venous drainage**

The gross arterial supply of the pancreas is via branches of gastric, hepatic and anterior intestinal arteries (Figs. 17–21). In detail, anterior, anterior middle, posterior middle and caudal pancreatic arteries supply the pancreatic lobes.

The anterior pancreatic arteries supply the hepatic lobe. The most rostral and thicker pancreatic artery arises from the hepatic artery shortly after the hepatic artery originates from the right gastric artery (Figs. 17 and 18). The thicker anterior pancreatic artery runs in close vicinity to the right gastric artery for a short distance caudally. It then bifurcates and sends branches towards rostral and caudal. The hepatic lobe also receives a rostrally directed branch from the anterior middle pancreatic artery (see below). The thinner and slightly more caudally located anterior pancreatic artery arises from the anterior intestinal artery (Figs. 17 and 18). The anterior middle pancreatic artery arises either from the anterior intestinal artery (Figs. 17 and 18) or from the right gastric...
Pancreas microvasculature in larval and adult *Xenopus*

artery (Fig. 19). It supplies the rostral portions of the gastric and duodenal lobes and abuts a branch to the bypassing common bile duct (Fig. 18). The posterior middle pancreatic artery arises from the right gastric artery (Figs. 20–22). Shortly after its origin it splits up into several smaller arteries which either ascend towards the dorsal surface of the pancreas (Figs. 20 and 21) or remain ventrally (Fig. 22). Middle and caudal portions of the duodenal lobe receive also branches from the left gastric artery (Fig. 20).

At the dorsal surfaces of the pancreatic lobes arteries and arterioles run often rather superficially before they pierce the dense superficial capillary network to course within deeper layers of the pancreas.

The adult pancreas drains via pancreatic veins into hepatic portal vein, anterior and middle gastric vein, gastroduodenal vein, and anterior duodenal vein (Figs. 17–22). The anterior gastric vein receives a few small veins from the anterior portion and the duodenal lobe of the pancreas (Fig. 17). It drains into the hepatic portal vein which itself receives small venules from the hepatic lobe of the pancreas (Fig. 20). The anterior duodenal vein drains the posterior region of the duodenal lobe into the gastroduodenal vein which also receives a vein from the anterior part of the pancreas. The gastroduodenal vein joins the medial gastric vein to finally drain into the left branch of the abdominal vein (Figs. 17 and 20). Gastric lobe and middle part of the pancreas drain into the middle gastric vein (Figs. 17 and 20).
Microvascularization

Superficially running pancreatic arterioles issue small side branches which either (i) capillarize superficially to supply the capillary bed of superficially located exocrine acini (Fig. 21, inset), (ii) descend to capillarize intraparenchymally to feed deeper located acini (Fig. 21, inset; Figs. 22–26) or (iii) issue afferent arterioles into the core of the endocrine islets of Langerhans (Figs. 27–29).

Small lobular ducts are embraced by a one-layered meshwork of periductal capillaries, bigger lobular ducts own a second vascular layer consisting of arterioles feeding and venules draining the ductal subepithelial capillary layer (Fig. 30).

The islets of Langerhans greatly vary in size, always lie intralobularly and distribute randomly over the pancreas. Islets lie either rather superficially and can be identified without any removal of superficial vessels (Fig. 27) or locate deeply inside the pancreas and can be seen only after removal of overlaying superficial vessels (Figs. 31 and 32). Endocrine islets in general reveal wide sinusoidal capillaries which form round to ovoid glomerular vascular meshworks (Figs. 27–29 and 31). Islet meshworks are much denser than the capillary networks of the exocrine portions. Islet sinusoids are fed by one to three individual afferent arterioles (Figs. 28 and 29). Afferent vessels penetrate deep into the islets where they change into slightly thicker efferent venules (Fig. 29, inset). Efferent vessels either connect with the surrounding capillary bed of exocrine portions forming insulo-acinar portal vessels or continue as insular efferent venules and merge with venules coming from other pancreatic regions (Figs. 28).
**Discussion**

Scanning electron microscopy (SEM) of vascular corrosion casts (VCCs) proofed as a powerful technique to study the microvascularization of the larval and adult pancreas in the model organism *Xenopus laevis* in a very detailed manner. Hitherto this technique was applied to demonstrate the angioarchitecture of the Islets of Langerhans in adult *Rana esculenta* and *Rana temporaria* (Syed Ali, 1989).

Our findings demonstrate that main arteries which supply the pancreas are almost the same in ranids and pipids, whereby in *Xenopus* the left gastric artery additionally contributes to the supply of the pancreas. Millard (1940) was not sure about the branch from the anterior intestinal artery, but our results clearly confirm her assumption. Millard (1940) did not name any individual pancreatic arteries, because each of the main supplying pancreatic arteries gives off several small branches which are difficult to further follow in their individual course towards their terminal portions by binocular observation. This, however, can be easily done by SEM in VCCs and so we named the large branch from the anterior intestinal artery and the branch from the right gastric artery to the more middle and caudal parts of the pancreas the middle and caudal pancreatic arteries.

In respect to the venous drainage of the adult pancreas we broadly confirm Millard’s findings (Millard, 1940) with the exception that she did not recognize the direct drainage into the hepatic portal vein. Moreover, we found pancreatic veins that drained into the middle gastric vein, but not into the...
Fig. 21. Microvascular patterns of caudal portions of gastric lobe (gl) and duodenal lobe (dl) of the pancreas. Dorsal aspect. After removal of overlying vessels the stem of the posterior middle pancreatic artery (pmpa) can be seen to arise from the right gastric artery (rga). The artery gives off rostrally and caudally directed branches. Note that the thicker main branch (arrowhead) descends ventrally. adv anterior duodenal vein, dl duodenal lobe, gl gastric lobe, mgv middle gastric vein. Inset: Small superficially running pancreatic artery (pa) abutting an arteriole (aa) which partially capillarizes into superficial acinar capillaries (c) and descends into deeper portions (arrow).

gastroduodenal vein as described by Millard (1940). In contrast to Millard (1940) who described middle gastric vein and gastroduodenal vein as joining individually the left branch of the abdominal vein, in our specimens the gastroduodenal vein and the middle gastric vein always joined before and thus drained via a common stem into the left branch of the abdominal vein.

The microvascularization of the larval exocrine pancreas differs from that of the adult pancreas in some respects. So blood vessels of the larval pancreas are immature at stage 52 (up to stage 55; Sundqvist and Holmgren, 2004). Capillaries are wide and the vasculature lacks a clear hierarchical structure. Although immature, the pancreas already owns a dense meshwork of capillaries. This network adequately supplies the acinar cells which already function in premetamorphosis (Leone et al., 1976). Our results reveal that in early metamorphic climax diameters of pancreatic capillaries decrease but capillaries still look immature, though all acini are differentiated and connective tissue septa around them are well developed.

It is known that during metamorphic climax the pancreas shrinks to 20% of its initial size, that ~40% of its cells die, and acinar cells lose their zymogen granules and become disorganized (Mukhi et al., 2008). Our findings confirm this and show that coincidently with tissue degradation the vascular bed decreases in size paralleled by a transformation of a previously regularly arranged capillary network into an irregular capillary system.
In adult *Xenopus* the pancreas owns a ductal system comprising intercalated ducts, intra- and extralobular ducts as it is the case in mammals (Pearl et al., 2009). Except from the intercalated ducts all pancreatic ducts show a clear periductal vascular plexus in corrosion casts. This plexus consists of an inner layer made up of a fine capillary meshwork and an outer layer from arterioles and venules that is similar to that described in rat (Murakami and Fujita, 1992b). Ohtani (1983) reported that in the rabbit pancreas sometimes efferent vessels from the islets drain the periductal plexus. Such an “insulo-ductal portal system” was not traceable in the pancreas of adult *Xenopus*.

Non-sprouting angiogenesis, namely intussusceptive microvascular growth (= IMG), is a process to optimize the vascular growth and remodeling after the formation of the primary capillary plexus (Djonov et al., 2003). In our study we found clear signs of IMG at stages 52 and 58 in the immature vascular bed coincident with sprouting angiogenesis. The prometamorphic pancreas and its vascular bed must develop fast to achieve a high level of organization before the pancreas shrinks at climax. IMG seems to be a very effective mode to adjust the vascular bed of the larval pancreas because this mode can expand a capillary plexus more rapidly than sprouting (Kurz et al., 2003). The signs of IMG prove the importance of non-sprouting angiogenesis in growth and differentiation of pancreatic vessels.

Fig. 22. Same as Fig. 21 but ventral aspect. Note the rostrally and caudally directed branches of the posterior middle pancreatic artery (pmpa). adv anterior duodenal vein, gdv gastroduodenal vein, mgv middle gastric vein, pv pancreatic vein, rga right gastric vein.
Insulin producing β-cells are reported to scatter with other endocrine cells forming very small clusters (islets) in the premetamorphic pancreas (Pearl et al., 2009). These clusters are so small that islet cells obviously get a sufficient oxygen supply by diffusion from nearby capillaries. Consequently, vascular corrosion casts do not display vascular patterns typically found in adult islets of Langerhans.

Histomorphology of Goldner stained tissue sections as well as SEM of vascular corrosion casts clearly showed that islet capillaries are wide and strongly interconnected. These wide sinusoid capillaries most likely lead to an increase in islet blood volume but also to a slow down of blood velocity.
Pancreas microvasculature in larval and adult *Xenopus*

This increases diffusional oxygen delivery from the blood to the islet cells and the transport capacity for hormones discharged into islet blood vessels.

Murakami et al. (1993) hypothesized that microvascular routes inside the islet enable non-β-endocrine-cells to influence the insulin release of β-cells. In different papers it
was suggested that the intrainsular microcirculatory pattern is related to the specific endocrine cell arrangement in the islet, which differs between species, to enable the most efficient signaling inside the islet. Some studies proposed the opposite to Murakami et al. (1993) namely that the β-cells first produce insulin and then the cells in the periphery of the islet modify the response. Comparative studies showed that there is no standard islet formation model and that at present no defined relationship between architecture and microcirculatory pattern of the islet can be demonstrated (Kim et al., 2009). It is hypothesized that - besides variations in developmental mechanisms (Steiner et al., 2010) - the variation in islet structure between species is affected by differing metabolic requirements and physiological conditions. Our results rather indicate that in *Xenopus* - because afferent vessels enter the core of the islet - islet blood flows first to β-cells and subsequently to the other endocrine cells. Moreover, in the adult *Xenopus* pancreas islets consist of insulin producing β-cells only while the remaining endocrine cells are only loosely associated with the islet and are mostly scattered as single cells in the pancreas (Horb and Slack, 2002; Mukhi et al., 2009).

Fig. 30. Microvascular patterns of the duodenal lobe adjacent to the anterior duodenal vein (adv). Note the many differently dimensioned venules (arrows) draining the pancreatic tissue into the large vein. There are not superficially located endocrine islets of Langerhans to be seen.

Fig. 31. Same as Fig. 30 but after removal of the superficial vascular network. Now the deep located endocrine islets of Langerhans (green) can be seen.

Fig. 32. Periductal subepithelial capillary plexus of interlobular ducts (id). Note that two interlobular ducts merge and form a main pancreatic duct (md). a artery, aa arteriole, v vein, vv venule.
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