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Incomplete Vascular Casting for a Scanning Electron Microscope Study of the Microcirculatory Patterns in the Rat Pancreas*

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Summary. **Scanning electron microscopy of resin casts prepared by incomplete arterial injections showed that in the rat pancreas, the casting medium fills blood capillaries in the endocrine islets more promptly than those in the exocrine lobules and secretory ducts. Furthermore, the exocrine lobules containing endocrine islets allowed a more rapid resin flow through the insulo-acinar portal route than those lobules lacking an islet. The secretory ducts were the last portions to be filled with resin. Since the resin medium used in this study was as viscous as blood and injected under a physiological pressure, the microcirculatory modes demonstrated by the present method suggest the physiological flow of blood in the rat pancreas.**

Thorough injection of low viscosity casting media into arteries (complete arterial injection) reproduces the whole extent of blood vascular beds, including thick venous portions (MURAKAMI, 1971; MURAKAMI et al., 1973). Small amounts of injection of the media into arteries (incomplete arterial injection) partially reproduce the vascular beds; the capillary-venular or venular system remains unfilled (MURAKAMI et al., 1983; 1987). Similar injection into veins (incomplete venous injection) can reproduce the venous or veno-capillary system (MURAKAMI et al., 1983). A thorough injection into veins is not suited for casting a whole blood vascular bed, since it causes ruptures in capillaries (MURAKAMI et al., 1983). A combination of different, adequate casting methods, both complete and partial, thus allows a precise analysis of the fine vascular connections and arrangement of an organ (MURAKAMI, 1971; MURAKAMI et al., 1983, 1987).

This paper demonstrates findings by scanning electron microscopy of such cast specimens, especially those reproduced by complete and incomplete arterial injections. The rat pancreas is the target organ of the present study, since its microcirculatory pattern has caused much dispute (BONNER-WEIR and ORCI, 1982), including a complicated insulo-acinar portal system which has additional venous drainages (OHTANI et al., 1986).

MATERIALS AND METHODS

Twelve adult male Wistar rats weighing 350-360 g were anesthetized with ethyl ether, and their thoracic aorta was cannulated at a level of the seventh thoracic vertebra. The animals were then irrigated through the cannulated arota with Ringer solution (15-20 ml), and perfused through this aorta with a semi-polymerized and diluted low viscosity methacrylate casting medium (about 4.0 centipoise, i.e., as viscous as blood) (MURAKAMI, 1971; MURAKAMI et al., 1973) at an injection pressure of 120-130 mmHg.

The amount of the casting medium was changed for each animal: 17-20 ml of the casting medium was perfused in four rats; 9-12 ml in four other rats; 2-5 ml in the remaining four rats. In some animals of the latter two groups, the hepatic portal vein was cut off or opened closely below the portal valve (portal spiral, BOOZ, 1964) prior to Ringer perfusion or resin injection.

The resin-injected animals were placed for 1 h in a hot water bath (60°C), and their pancreas was isolated together with the liver, stomach, duodenum, spleen and kidney. These isolated organs were immersed

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in a hot 10% NaOH solution (60°C) overnight, and washed for 8 h in running tap water. This NaOH treatment followed by washing was repeated several times.

The blood vascular casts of the pancreas and other abdominal organs thus prepared were air-dried or freeze-dried, and dissected with forceps or needles to isolate the cast of the pancreas. This isolated pancreatic cast was microdissected with sharpened forceps and needles under a binocular light microscope, coated with gold in a vacuum evaporator, and observed with a scanning electron microscope (S-2300, Hitachi) using an accelation voltage of 5 kV.

RESULTS

An injection of 17–20 ml resin produced a dense and complete vascular cast of the kidney, stomach, duodenum, spleen, pancreas and liver (Fig. 1). Incomplete injection caused a coarse and incomplete cast (Fig. 2). In general, an injection of 2–5 ml resin reproduced only the arterial systems in the spleen, duodenum and spleen, though in the stomach, it reproduced the arterial system and basal capillary plexus of the gastric mucosa. In the kidneys, the glomeruli and their efferent vessels were completely reproduced by the 2–5 ml resin injection. With the 9–12 ml resin injection, the vascular plexuses of the stomach and the kidneys were thoroughly reproduced, though those of the duodenum and spleen were insufficiently replicated.

In the thoroughly injected specimens (dense casts, see above), it was clearly confirmed that the rat pancreas contains a rich blood vascular bed which mainly consists of a lobular capillary network supplying the exocrine lobules, insular capillary networks supplying the endocrine islets of Langerhans, and ductal capillary networks supplying the pancreatic (secretory) ducts (Fig. 3).

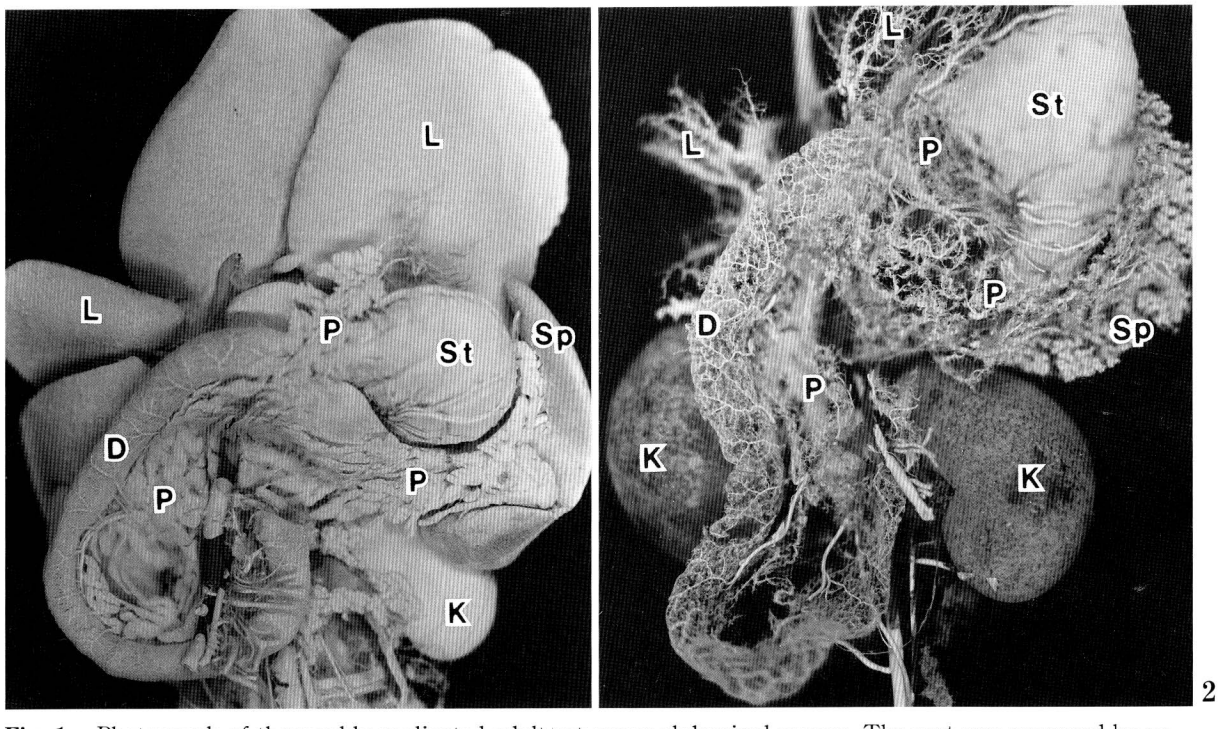

Fig. 1. Photograph of thoroughly replicated adult rat upper abdominal organs. The cast was prepared by an injection of 20 ml resin through the thoracic aorta without incision of the hepatic portal vein (see text). *D* duodenum, *K* kidney, *L* liver, *P* pancreas, *Sp* spleen, *St* stomach. ×1.3

Fig. 2. Photograph of incompletely replicated adult rat upper abdominal organs. The cast was prepared by an injection of 3 ml resin through the thoracic aorta without incision of the hepatic portal vein (see text). Note that the blood bed in the stomach (*St*) is especially well reproduced with resin as compared with other organs (see text). For other abbreviations, see the legends for Figure 1. ×1.5

The capillary networks of the endocrine islets were more or less markedly conglomerated. The islets thus identifiable in the cast were observed as intralobular islets embedded in the lobular capillary network (Figs. 3, 3 Inset, 4) or as extralobular (interlobular) islets located among the lobular capillary networks or along a ductal capillary plexus (Figs. 3, 4 Inset).

The intralobular islets issued insulo-acinar portal vessels draining into the lobular capillary networks (Figs. 3 Inset, 4). These intralobular islets were distributed in relatively small or thin lobules, so that they usually issued some venous efferent vessels directly draining into the lobular veins (Figs. 3 Inset, 4). The interlobular islets issued only venous efferent vessels which drained into the ductal or interlobular

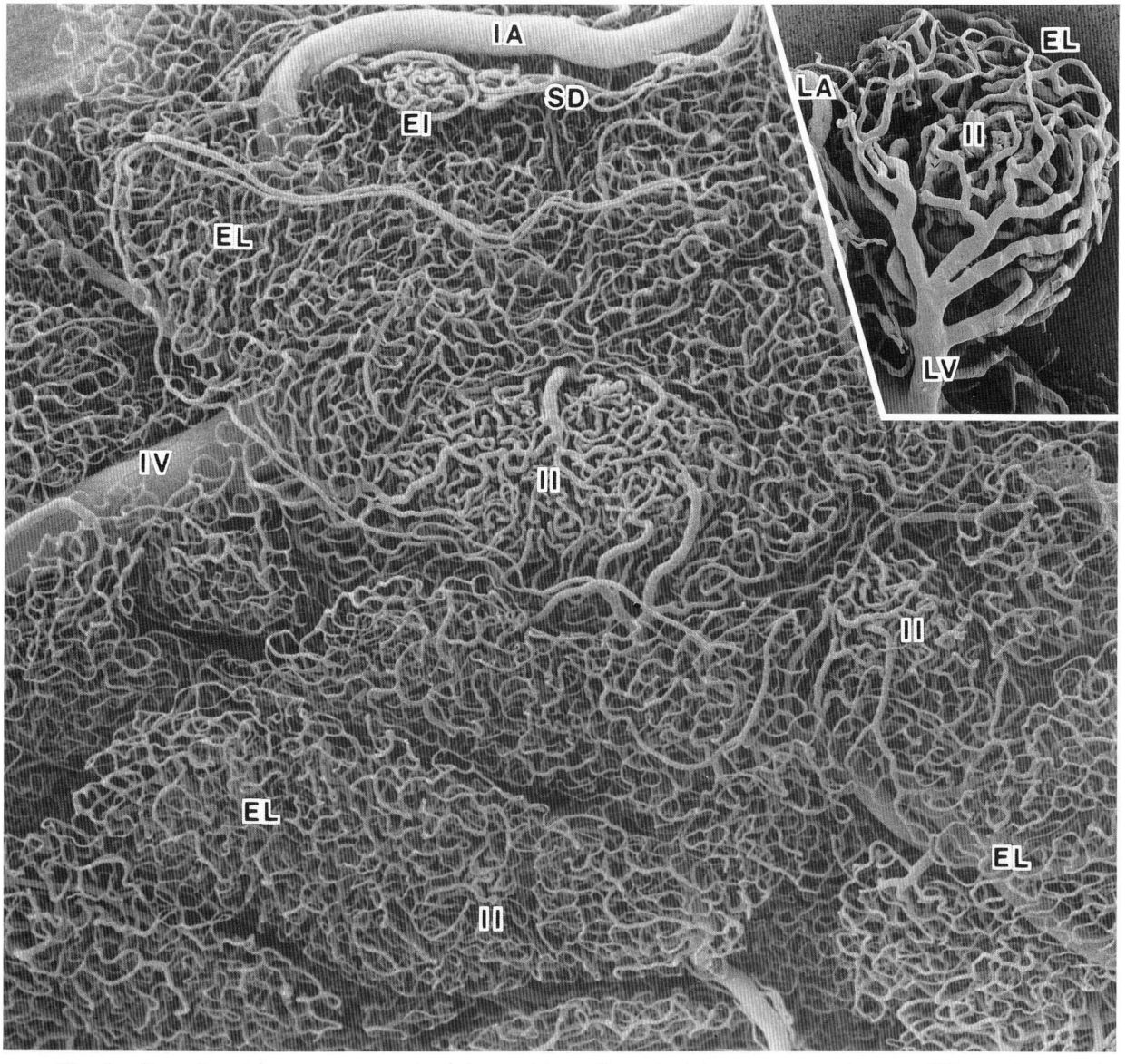

Fig. 3. Scanning electron micrograph of thoroughly replicated adult rat pancreas (a part of Figure 1). Note the intralobular islets (*I I*) in exocine lobules (*EL*) and the extralobular (interlobular) islets (*EI*) among lobules or along secretory (pancreatic) ducts (*SD*). The **Inset** shows an isolated exocrine lobule (*EL*) containing an intralobular islet (*I I*). *IA* interlobular artery, *IV* interlobular vein, *LA* lobular artery, *LV* lobular vein. ×75, Inset: ×135

veins (Fig. 4 Inset). Thus, many exocrine lobules contained one or more well-developed islets, and received dual blood supply from the islets via the insulo-acinar portal vessels and from the acinar arteries (lobular branches of the lobular arteries) (Figs. 3, 4).

Noteworthy is that with the 2-5 ml resin injection, the insular capillary networks were sufficiently reproduced, whereas the capillary networks of exocrine lobules and ducts remained unfilled (Fig. 5). The

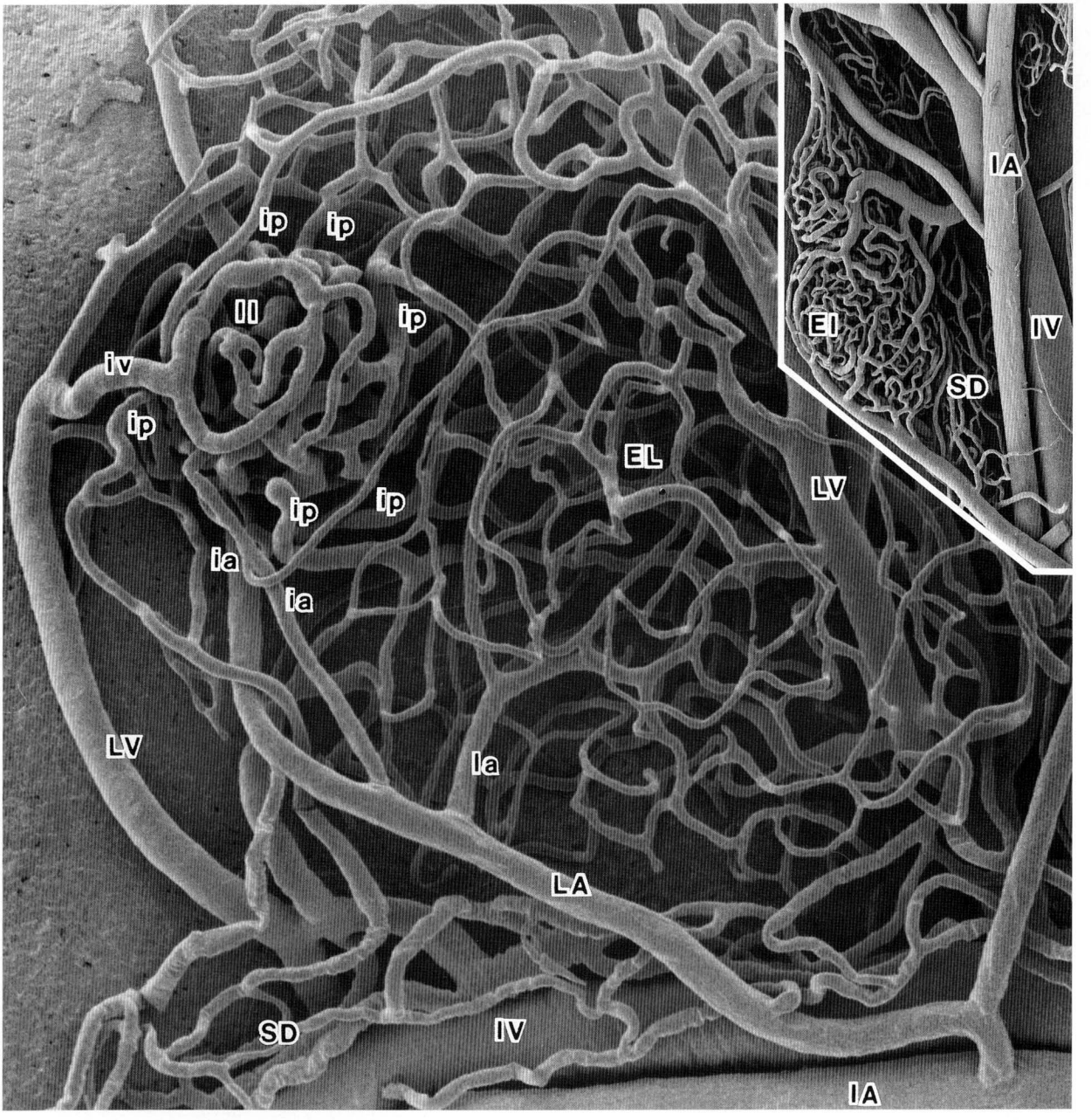

Fig. 4. An exocrine lobule (*EL*) containing an intralobular islet (*II*). Note that the lobule is dually supplied by six or more insulo-acinar portal vessels (*ip*) of the islet and by a branch (acinar artery, *la*) of a lobular artery (*LA*), and that the islet additionally issues a venous efferent vessel (*iv*) continuous with the lobular vein (*LV*). The **Inset** shows an extralobular islet (*EI*). *IA* interlobular artery, *IV* interlobular vein, *SD* ductal capillary network, *ia* islet afferent vessel. ×290, Inset: ×75

venous efferent and insulo-acinar portal vessels of the islets were also replicated with the 2–5 ml resin injection (Fig. 6). It was further noted with this injection that the islets received their afferent rootlets from their superficial aspect and emitted their efferent rootlets from both their deep and superficial aspects, and that the efferent rootlets continued either into the venous draining vessels or into insulo-acinar portal vessels (Fig. 6). Moreover, it was confirmed, particularly in a 2 ml resin-injected rat, that the superficial or cortical capillaries of the islets were more promptly filled with resin than were the deep capillaries of the islets; in this rat, the deep capillaries of many islets were scarcely reproduced or remained unfilled.

With the 9–12 ml injection, the islets and their venous efferent and insulo-acinar portal vessels were reproduced together with the lobular capillaries connecting with the insulo-acinar portal vessels (Figs. 7–10). This injection showed that the lobular capillaries, derived from the acinar arteries (see above), were partially replicated (Fig. 7). The venous efferent vessels of the islets contained larger amounts of resin than insulo-acinar portal vessels, being sufficiently reproduced together with their connecting intralobular or interlobular veins (Figs. 8, 9). In these veins, some other peripheral branches were usually reproduced by the retrograde flow of resin (Fig. 9). This back flow of resin into the peripheral veins typically occurred in cases where the hepatic portal vein was neither cut nor opened (Figs. 7, 9).

Replication of the ductal capillary networks was insufficient with the 2–5 ml and 9–12 ml resin injections (Fig. 12). Sufficient casting of these ductal networks was noted for the 17–20 ml resin injections (Figs. 3, 4, 4 Inset).

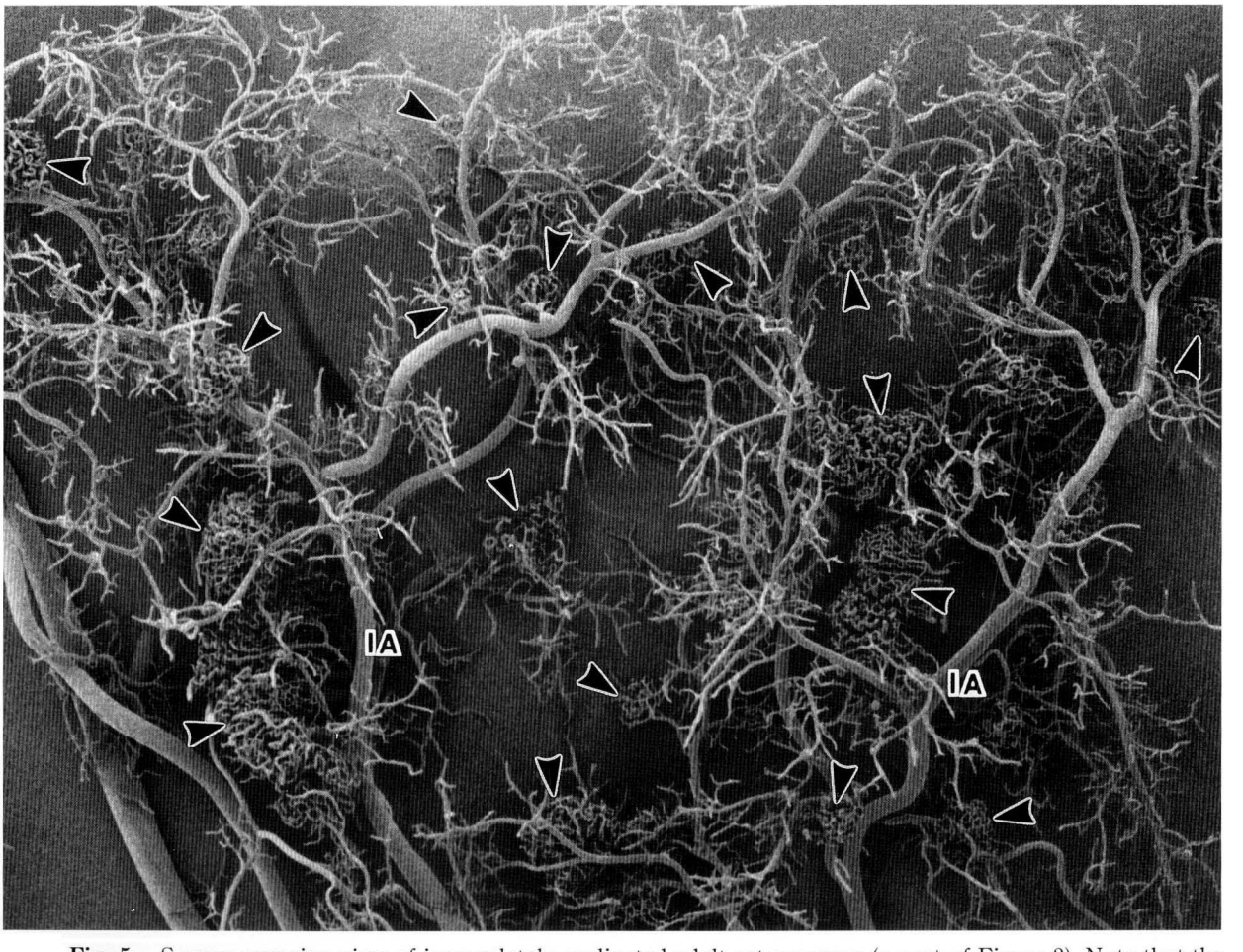

Fig. 5. Survey scanning view of incompletely replicated adult rat pancreas (a part of Figure 2). Note that the capillary networks of the islets (*arrowheads*) are reproduced, whereas those of the lobules are not replicated. *IA* interlobular artery. ×40

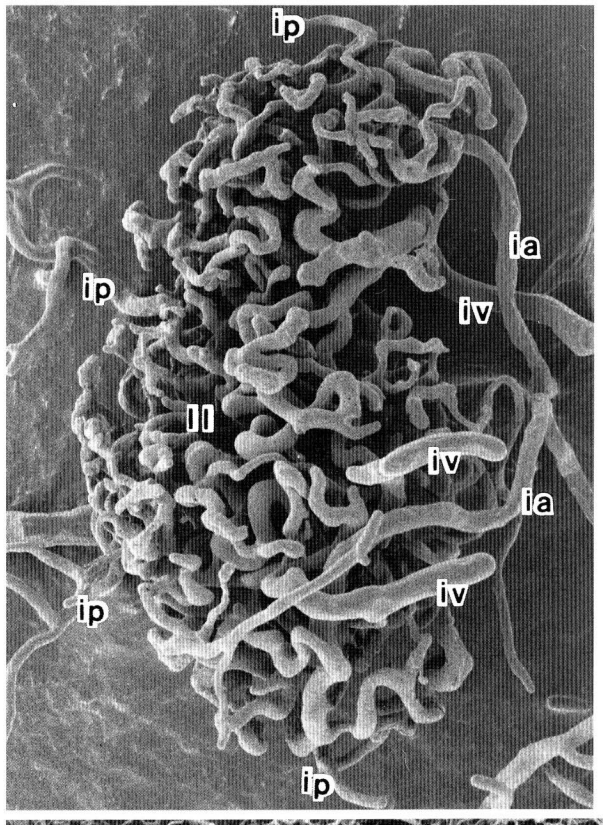

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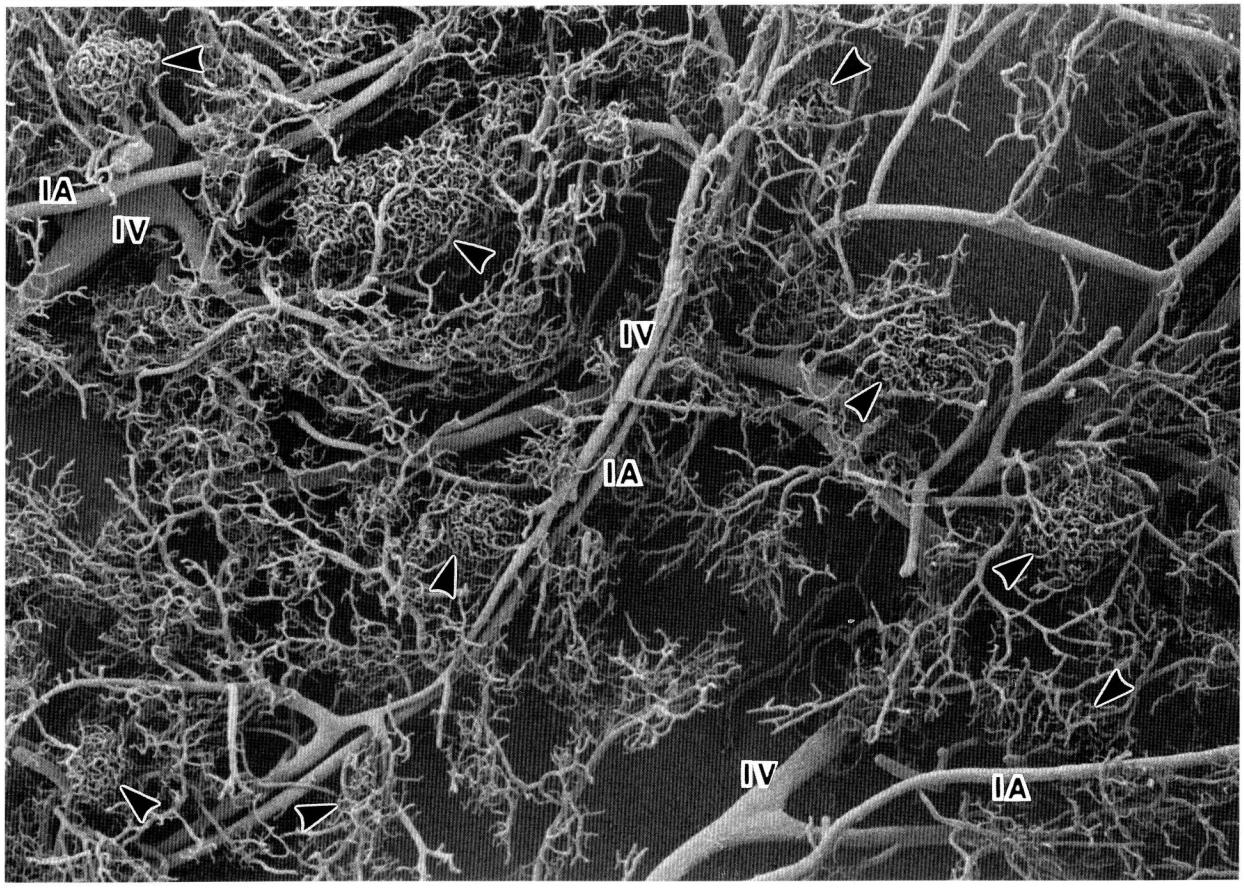
Fig. 6. Intralobular islet (*II*) isolated from the cast prepared by a 3 ml resin injection (the same cast as shown in Figure 5). Note that main (afferent and efferent) vessels of the islet are sufficiently filled with resin, though some insignificant discontinuities of capillaries are recognized within the islet. *ia* Islet afferent vessels, *ip* insulo-acinar portal vessels, *iv* islet venous drainage. ×200

Fig. 7. Incompletely replicated adult rat pancreas. The cast was prepared by an injection of 9 ml resin through the thoracic aorta with incision of the hepatic portal vein (see text). Note that the islets (*arrowheads*) as well as their portal vessels and venous efferent vessels are sufficiently reproduced. Also note that resin flows into the interlobular veins (*IV*) *via* the islets and their venous efferent vessels. The lobular capillary bed derived from the lobular arterial branches (acinar arteries, see text) is still insufficiently reproduced. *IA* interlobular artery. ×55



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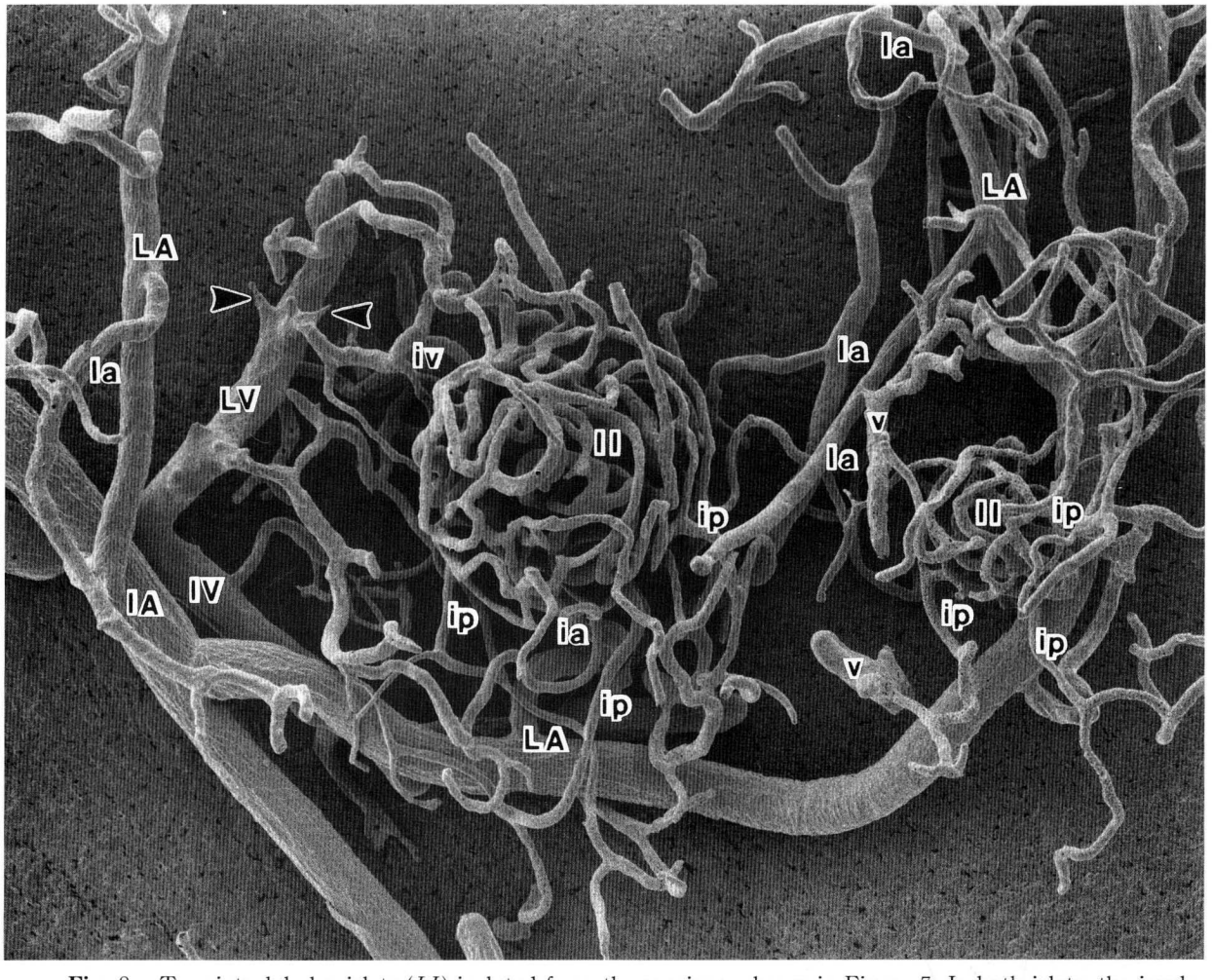

Fig. 8. Two intralobular islets (*II*) isolated from the specimen shown in Figure 7. In both islets, the insulo-acinar portal (*ip*) and venous draining vessels (*iv*) are sufficiently reproduced, whereas the acinar arteries (*la*) are replicated only partially. Note the retrograde flow of resin (*arrowheads*) in the lobular vein (*LV*). *IA* interlobular artery, *IV* interlobular vein, *LA* lobular artery, *v* lobular veins partially filled *via* the insulo-acinar portal routes of the smaller right-hand islet, *ia* islet afferent vessel. ×260

DISCUSSION

This paper confirms that thoroughly replicated specimens are most important for a precise morphological analysis of blood vascular beds under the scanning electron microscope. It further indicates that arterially and incompletely replicated specimens are additionally useful to study the inflow modes of the casting medium into the blood vascular beds and also the outflow modes of the medium from the capillary beds into the veins, as well as the running modes of the medium in the capillary beds.

Our casting medium was as viscous as blood and perfused under a physiological pressure (see above). Our preliminary experiments by intravital microscopy using the mesenterium of ethyl ether-anesthetized adult rats have shown that our medium runs through mesenterial capillaries in fashion similar to that of blood (data, not shown). These data indicate that our vascular casts prepared by incomplete arterial injections properly reflect the actual blood flow patterns or provide important, additional information concerning the dynamic flow of blood.

It is noteworthy that in the abdominal organs, the stomach, especially its mucosa, is perfused as

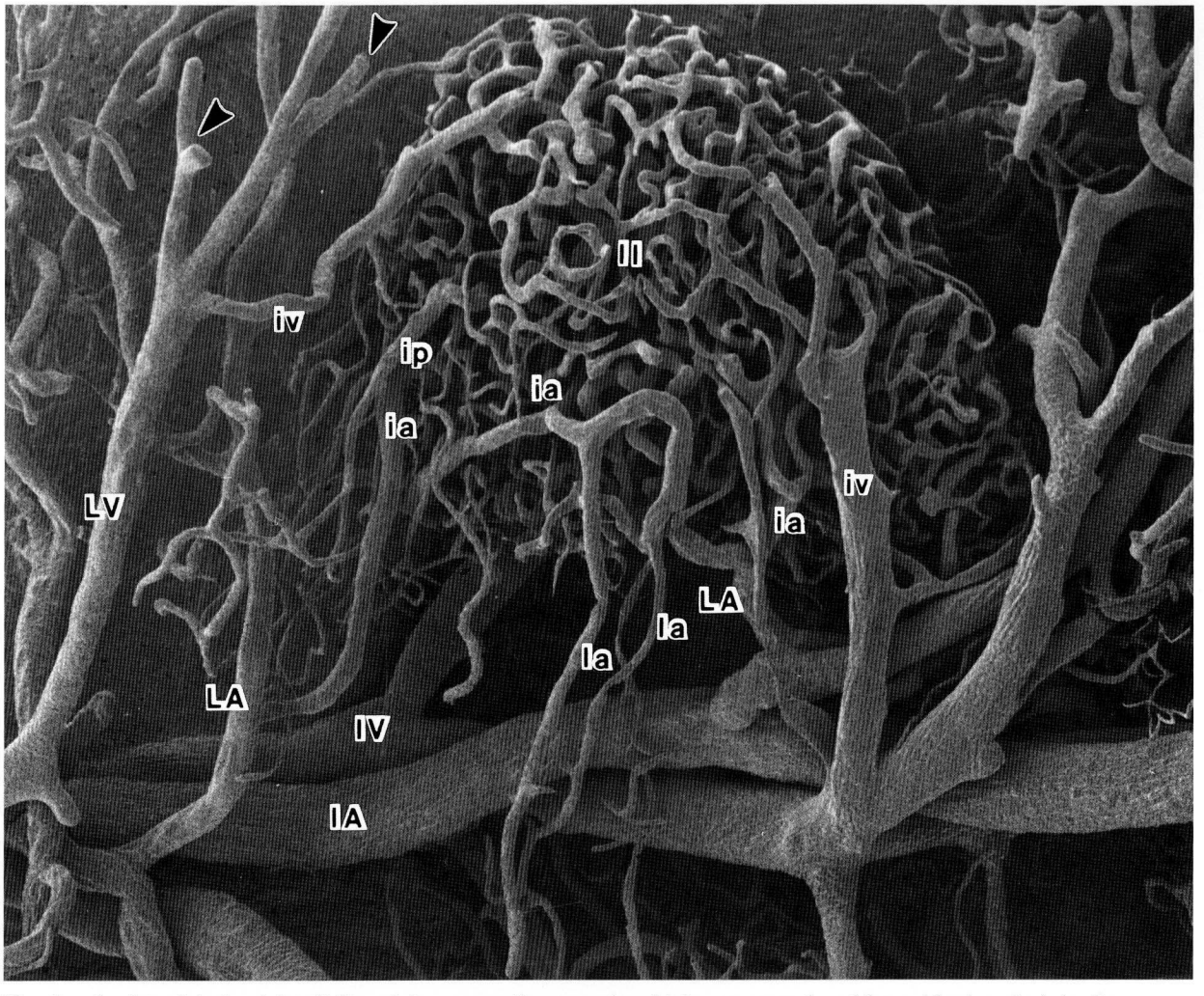

Fig. 9. An intralobular islet (*II*) and its connecting vessels which are reproduced by a 10 ml resin injection through the thoracic aorta; no incision was made on the hepatic portal vein (see text). Note that the islet and its insulo-acinar portal (*ip*) and venous efferent vessels (*iv*) are sufficiently reproduced, whereas the acinar arteries (*la*) are insufficiently reproduced. A marked retrograde flow of resin is observed (*arrowheads*), since the hepatic portal vein was not incised. *IA* interlobular artery, *IV* interlobular vein, *LA* lobular artery, *LV* lobular vein, *ia* islet afferent artery. ×200

promptly as the kidneys with the casting medium. This suggests that the stomach is one of the organs which are most rapidly and richly supplied with blood.

This paper shows that in the rat pancreas, the vascular plexuses of the islets are filled with the casting medium more promptly than those of the lobules and ducts, and that in the lobules containing the islets, the medium runs more rapidly through the insulo-acinar portal routes than through the lobular routes. It also shows that even in the intralobular islets with well-developed insulo-venous efferent vessels, the casting medium prefers to flow into the portal vessels. These facts prove that the arterial blood in the rat pancreas preferentially flows into the insulo-acinar portal routes, and that neither the insulo-venous routes nor the lobular and ductal routes significantly interfere with the insulo-acinar portal routes, which convey high concentrations of insular hormones to the exocrine acini (FUJITA, 1973; FUJITA and MURAKAMI, 1973).

Prompt filling with resin of the cortical capillaries of the islets and later filling of their deep capillaries support the previous findings of our associates that,

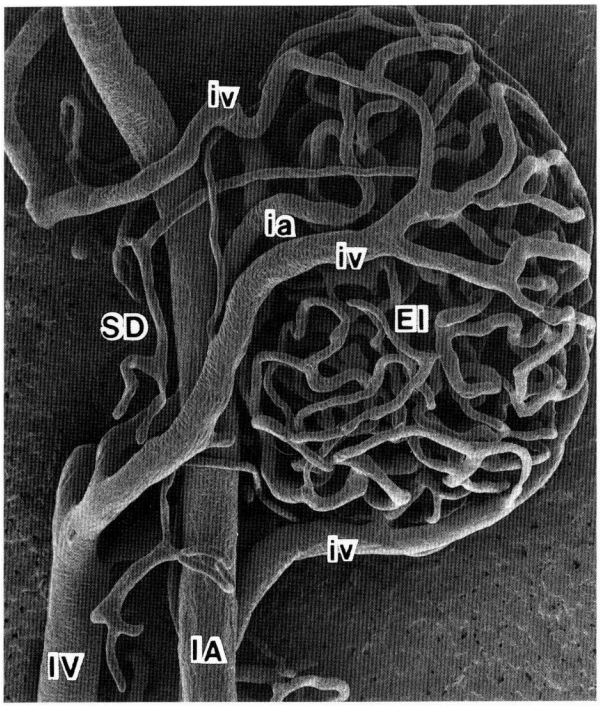

Fig. 10. An extralobular islet (*EI*) located along a pancreatic duct (*SD*). Isolated from a specimen prepared by a 12 ml injection with an incision of the hepatic portal vein. Note that the islet is sufficiently replicated, though the duct (*SD*) is barely reproduced. *IA* interlobular artery, *IV* interlobular vein, *ia* islet afferent vessel, *iv* islet venous drainage. ×230

in the rat islets, the blood mainly flows from the A-D cell mantle to the B cell core (FUJITA, 1973; OHTANI et al., 1986).

Microcirculation patterns of blood have been studied by intravital light microscopy of living tissues or organs which are sometimes injected with India ink, fluorescent dyes, microspheres or other substances (BUNNAG et al., 1963; MCCUSKEY and CHAPMAN, 1969; LIFSON et al., 1980; FRAZER and HENDERSON, 1980; OHTANI, 1983; OHTANI et al., 1986). Although these intravital light microscopic methods are useful for direct observation of blood flow, their use may be limited; deep and wide areas are hardly observed by the intravital methods. Furthermore, the images from the intravital methods are not always clear because of the limited resolution and shallow focus of the light microscope (OHTANI, 1983; OHTANI et al., 1986). This shortage is supplemented by the incomplete casting/scanning technique introduced in the present paper.

Retrograde flow of the injected resin in the veins seems a major problem of this technique. To overcome this problem, complete opening of the efferent vessels of the organs to be examined is useful. The incompletely injected (cast) specimens are so fragile and easily deformed that they should be freeze-dried.

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