A Comparison of Endocrine Pancreatic Tissue in Adults of Four Species of Lampreys in British Columbia: A Morphological and Immunohistochemical Study

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Histological techniques and immunohistochemistry with antisera against insulin and somatostatin were used in a study of the identification and the distribution of endocrine pancreatic tissue in adults of four species of lampreys in British Columbia. The fine structure of the pancreatic cells of one of the species was also examined. The four species (*Lampetra ayresi, Lampetra tridentata, Lampetra macrostoma,* and *Lampetra richardsoni*) all possess cranial and caudal pancreatic masses and an intervening intermediate cord, but there are variations in amount and deposition of the pancreatic tissue with respect to the alimentary canal and the liver which appear to be species specific. There is also some variation in the distribution of pancreatic tissue between *L. richardsoni* of different stream populations. A newly identified parasitic variety of *L. richardsoni* in Morrison Creek, called *L. richardsoni* var. *marifuga*, usually has no cranial pancreas but has an extensive intermediate cord. The distribution of pancreatic tissue in these lampreys is of relevance to taxonomy and to variations in development of the endocrine pancreas during lamprey metamorphosis. The pancreatic tissue of all species and var. *marifuga* is composed of cells which are immunoreactive to either insulin or somatostatin antisera. The fine structure of cells from the cranial and caudal pancreatic tissue from *L. ayresi* does not vary with saltwater and freshwater acclimation. Insulin-containing B cells and somatostatin-containing D cells are present in equal numbers in both pancreatic regions and the cells each have characteristic cytoplasmic granules. A third cell type is most abundant in the cranial pancreas but it is of unknown function.

The pancreatic tissue of lampreys consists of exocrine secretory cells within the epithelium of the anterior intestine and islets and follicles of endocrine cells within the connective tissue underlying the esophageal and intestinal epithelium (Barrington, 1972; Youson, 1981). In larval lampreys (ammocoetes) the endocrine tissue is located at the junction of the bile duct, esophagus, and anterior intestine. In most adults there is a cranial endocrine pancreas at the junction of the esophagus, anterior intestine, and the intestinal diverticulum and a caudal endocrine pancreas, associated with the intestinal typhlosole and approximately at the midpoint of the liver. Early studies had suggested that there was a direct relationship between the disappearance at metamorphosis of the bile ducts and other components of the ammocoete biliary tree and the appearance of the adult endocrine pancreatic tissue (Boenig, 1927). The position of the extrahepatic bile duct in ammocoetes of the Southern Hemisphere lamprey, *Geotria australis*, is also believed to be responsible for the concentration of all endocrine tissue in a large cranial mass in adults of this species (Hilliard *et al.*, 1985). Recently, immunohistochemistry was used to provide definitive documentation of the transformation of the bile duct of ammocoetes of the sea lamprey, *Petromyzon*

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marinus, into the caudal endocrine pancreas of adults and to follow the development of the cranial endocrine pancreas (Elliott and Youson, 1987). These latter two studies have clearly shown that there are marked differences in the distribution of the endocrine pancreatic tissue in adults of at least two species of lampreys.

We have been investigating morphological differences in the organs of adults of several species of lampreys in British Columbia (Youson and Beamish, 1986) as supportive evidence for species separation (Beamish and Withler, 1986). Of particular interest is the relationship between a nonparasitic species, *Lampetra richardsoni*, and what appears to be a parasitic variety of this species, *L. richardsoni* var. *marifuga* (Beamish, 1985; Beamish and Withler, 1986). As part of this investigation we examined the distribution of the endocrine pancreatic tissue in adults of four species. Although it was our initial and primary objective to provide evidence to support taxonomic characterization, this study has also yielded data which confirm the role of larval bile ducts in the development of the endocrine pancreas. In the present study, histochemistry and immunohistochemistry are utilized to show distribution of the endocrine pancreas in adults of four species of lampreys in British Columbia. In addition, the fine structure of cells in the endocrine pancreas of adults of *Lampetra ayresi* was examined and compared to that of cells from the endocrine pancreas of adult sea lamprey, *P. marinus* (Elliott and Youson, 1988).

MATERIALS AND METHODS

Juveniles of *L. ayresi* Gunther (8.5–16.4 cm, 0.7–5.6 g) were captured during dredging operations in the lower Fraser River (Beamish and Youson, 1987) and of *Lampetra tridentata* Richardson by electroshocking and of *Lampetra macrostoma* Beamish (13.1–15.5 cm, 3.1–5.3 g) by wire traps on Vancouver Island in Big Qualicum River and Mesachie Lake, respectively. All parasitic species, except *L. tridentata*, were allowed to feed *ad libitum* either on freshly killed herring* in fresh water (*L. ayresi*, *L. macrostoma*) (live herring, *Clupea harengus pallasi*, introduced to fresh water died from the osmotic imbalance with 30–60 min and lampeys fed immediately upon the introduction of the host to the tank and long after their death) or on live herring in salt water (*L. ayresi*). Nonparasitic *L. richardsoni* Vladykov and Follett (12.2–14.6 cm, 2.9–6.1 g) were obtained during electro-fishing in Puntledge River and were reaching sexual maturity. A second group of *L. richardsoni* (10.1–11.5 cm, 1.6–2.2 g) were collected in Morrison Creek, Vancouver Island where they were electroshocked with a variety, *var. marifuga* (9.7–12.3 cm, 1.3–1.8 g), which fed on live and freshly killed herring in a freshwater tank in the laboratory (Beamish, 1985; Beamish and Withler, 1986). Both animals from Morrison Creek showed advanced development of gonads. However, only males of var. *marifuga* were observed and their intestines were large and they contained recently ingested food which was available in the laboratory.

After anesthetization in a dilute solution of tricaine methanesulfonate, all animals to be utilized for light microscopic examination received an abdominal incision to expose the organs of the coelomic cavity. Entire animals were then placed in Bouin's fluid for 24 hr and were stored for up to 1 year in 70% ethanol. A portion of each animal which contained the most posterior branchiopore and the entire liver was severed from the rest of the body and was dehydrated in a graded series of ethanols, cleared in Histoclear (National Diagnostics), and embedded in paraffin. Serial sections, 8 μm thick, were obtained and placed on precleaned glass slides and were stained for routine microscopy with either (i) hematoxylin and eosin (HE), (ii) periodic acid–Schiff, acid hemalum, and orange G (PAS), or (iii) the aldehyde–fuchsin trichrome (AFT) stain of Epple (1967) with the modifications suggested by Mowry (1983).

Sections were also stained using the peroxidase–anti-peroxidase, unlabeled antibody technique as described previously (Elliott and Youson, 1986). The primary antisera used were (i) anti-bovine insulin prepared in guinea pig (gift of Dr. C. Yip, Banting and Best Institute, University of Toronto) diluted 1:10,000 in 0.01 *M* phosphate-buffered saline (PBS), pH 7.4, and containing 1% normal goat serum and (ii) anti-synthetic somatostatin-14 prepared in rabbit (Elliott and Youson, 1987) and diluted 1:750 in PBS. Negative controls consisted of replacement of the primary antisera with either (i) preimmune rabbit or guinea pig serum, (ii) antisera absorbed with excess insulin or somatostatin, or (iii) PBS alone. A positive control for the antisera prepared as above was the periodic immunostaining of pancreatic sections from adults of the sea lamprey, *Petromyzon marinus* L. (Elliott and Youson, 1986, 1987).

The pancreatic tissue from three juvenile *L. ayresi* in fresh water and three in salt water (28‰) were prepared for electron microscopy by first exposing the

entire coelomic cavity and then flooding the cavity with ice-cold 2% glutaraldehyde in 0.1 *M* phosphate (Millonig, 1961) at pH 7.3. The cranial and caudal pancreatic tissues were excised from the animals and were trimmed of extraneous tissues and diced into small pieces while immersed in fresh fixative. The small pieces of each pancreatic region were placed in separate vials where fixation continued for an additional 2 hr at 4°. After several washes in the phosphate buffer, the tissues were postfixed in 1% OSO_4 in phosphate buffer, dehydrated in ethanol and propylene oxide, and embedded in Epon–araldite. Ultrathin sections were cut on a Reichert OM-U3 ultramicrotome with glass knives, placed on uncoated copper grids, stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiskop 102 electron microscope.

RESULTS

General Morphology of the Esophageal–Intestinal Junction

The alimentary canal of adult lampreys is a straight tube (Youson, 1981) and consists of an esophagus completely separate from the pharynx, an anterior intestine, a posterior intestine, and a hindgut which leads into the cloaca. The junction of the esophagus and the anterior intestine occurs dorsal to the pericardial cartilage and at this point there is usually an anteriorly directed diverticulum of the intestine (Figs. 1 and 2). Although longitudinal mucosal folds are found in the esophagus, diverticulum, and anterior intestine, a secondary ridge of submucosal connective tissue and folds extends into the lumen of the anterior intestine as the typhlosole. The typhlosole in all species but *L. richardsoni* var. *marifuga* began at the esophageal–anterior intestine junction. In var. *marifuga* the typhlosole commenced at a position far posterior to that observed in other lampreys (Fig. 1). The diverticula of the species under investigation were of variable size but the diverticulum was particularly small in var. *marifuga* (Fig. 1; J. H. Youson and R. J. Beamish, unpublished observations). The submucosae of the diverticulum and the esophagus were continuous and transverse sections of the region dorsal to the pericardial cartilage revealed two tubes which were intimately apposed and connected by a particularly thick band of fibrous connective tissue (Fig. 2). The ventral, and part of the lateral, surface of the anterior intestine was positioned within a concavity of the dorsal surface of the liver (Fig. 1) and there was a bridge of submucosal connective tissue from the typhlosole between these two organs. The fibrous connective tissue of the intestinal submucosa was much thinner than that of the diverticulum and the esophagus.

Histology and Immunohistochemistry of the Pancreatic Tissue

Endocrine pancreatic tissue appeared as follicles or islets of epithelial cells grouped into masses of variable dimension in the submucosal connective tissue of the esophagus, diverticulum, and anterior intestine. A large mass of islets in the diverticulum–esophageal region is termed the *cranial pancreas* (Fig. 2) whereas tthe mass of islets in the submucosa of the anterior intestine is the *caudal pancreas* (Fig. 3). Small collections of islets independent of the two major masses are the *pancreatic intermediate cord* (Fig. 1). The cells of the islets of all regions were pyramidal to columnar in shape and they were intimately associated with the surrounding blood vessels. Routine staining methods revealed some differential staining between the islets and indicated that some may have lumina and therefore were more follicular in their form. Most follicles and islets were composed of cells of the same staining reaction (Fig. 4). When the aldehyde–fuchsin–trichrome (AFT) stain was applied to the sections, two cell types were clearly identified in all pancreatic regions of all specimens. A dark-staining reaction recognized the B cells and a lighter stain the D cells (Epple, 1967). These B and D cells were present in approximately equal numbers in all collections of pancreatic tissue and, although

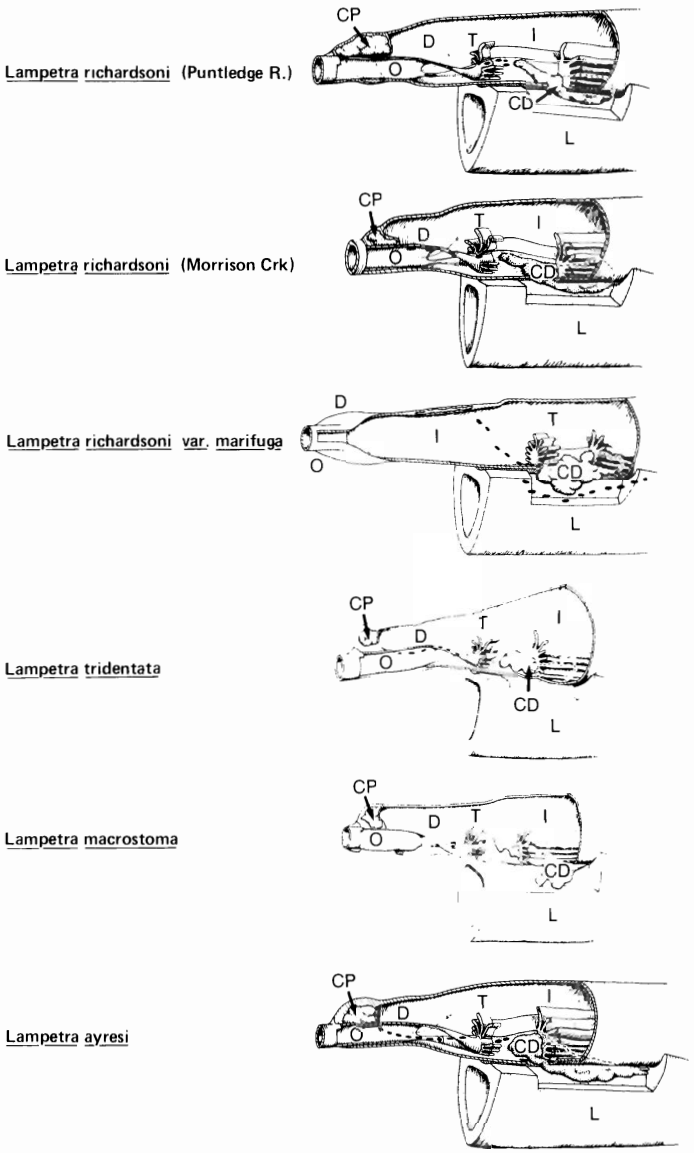

FIG. 1. Diagrammatic representation of the distribution of the cranial (CP) and caudal (CD) pancreases and the intermediate cord (dark patches) with respect to the esophagus (O), diverticulum (D), liver (L), anterior intestine (I), and the typhlosole (T) in adults of four species of lampreys from British Columbia. Also included are a comparison of a species from two different habitats and a newly described variety of this species. The heart is not represented and portions of the liver, intestine, diverticulum, esophagus, and typhlosole have been removed to provide internal detail. Longitudinal intestinal folds are only drawn on the typhlosole but are found throughout the mucosal wall of the esophagus, intestine, and diverticulum.

some follicles and islets contained both cell types, most were composed of only a single type.

The cells of the islets and follicles of all animals showed differential immunoreactivity to antisera against insulin and somatostatin. Adjacent sections stained with anti-insulin, anti-somatostatin, and PAS

(Figs. 5–7) showed that insulin-(IR) and somatostatin-immunoreactive (SR) responses occurred in different cell types and that the IR cells corresponded to B cells and the SR cells corresponded to D cells. Immunostaining also revealed that pancreatic follicles and islets were not composed of a single cell type. Negative controls showed no immunostaining whereas the primary antisera stained sections of cranial and caudal pancreatic tissue of adult *P. marinus*.

Distribution

Cranial pancreas. All species except var. *marifuga* consistently possessed a cranial mass of pancreatic tissue which was roughly spherical and located dorsal or dorsolateral to the esophagus and anterior to the diverticulum (Fig. 1). A small patch (100 μm long) of cranial pancreas was found in only one of five var. *marifuga*. The individual with the cranial pancreas also had a large diverticulum which was not a feature of other var. *marifuga*. *L. richardsoni* from the Puntledge River had the largest (970 μm long) cranial pancreas at the anterior end of the diverticulum and it also extended around one-half the circumference of the esophagus (Fig. 8). In contrast, *L. richardsoni* from Morrison Creek had a cranial pancreas only up to 470 μm long. The cranial pancreas of *L. ayresi* was up to 600 μm long but was usually divided into several small clumps due to the presence of several horns of the diverticulum. A similar division of the 530-μm-long cranial pancreas was noted in *L. macrostoma* (Fig. 9). *L. tridentata* had the most extensive diverticulum of all species but the anterior end was capped by a relatively small (420 μm long) cranial pancreas which was subdivided by a horn of the diverticulum.

Intermediate cords. In all species, small, discontinuous clumps of submucosal islets and follicles were found in a region extending from the cranial pancreas to the caudal pancreas. They were also present in the liver of var. *marifuga*. Immunohistochemistry was particularly useful in the identification of these small clumps of cells (Fig. 6, 7, 10). Only a few patches of intermediate islets were present in *L. macrostoma* and in *L. richardsoni* from both localities and these were more highly concentrated posterior to the esophageal–intestinal junction, but in the submucosa of the esophageal side. In contrast, *L. ayresi*, *L. tridentata*, and var. *marifuga* showed an extensive distribution of intermediate cords. In *L. ayresi* islet tissue first appeared as isolated cords seemingly continuous with the cranial pancreas and extending between the esophagus and diverticulum. However, more posteriorly the islets became positioned in the submucosa directly beneath the esophageal epithelium where they were located as a discontinuous cord until they entered the typhlosole of the anterior intestine. The intermediate cord of *L. tridentata* was of similar volume to that of *L. ayresi* but it was more widely distributed between the esophagus and the diverticulum and only was positioned to the esophageal side near the junction with the anterior intestine. It extended into the typhlosole and eventually was replaced by the caudal pancreas. Var. *marifuga* had the most widely distributed intermediate cord and in one animal it first appeared as a small diameter, continuous collection of islets in the dorsal submucosa of the anterior intestine. A cord of unconnected islets were located in the submucosa along the right lateral side of the anterior intestine and more posteriorly took up a position within the typhlosole, which in this animal began much more caudal than was seen in other species (Fig. 1). Islets were located among the liver parenchymal cells as intrahepatic islets (Fig. 10), even after the caudal pancreas had disappeared (Fig. 1).

Caudal pancreas. All species and var. *marifuga* (Fig. 10) possessed a prominent mass of endocrine pancreatic tissue in the submucosal connective tissue of the anterior intestine (Fig. 1). However, the deposition of this caudal pancreas with respect to


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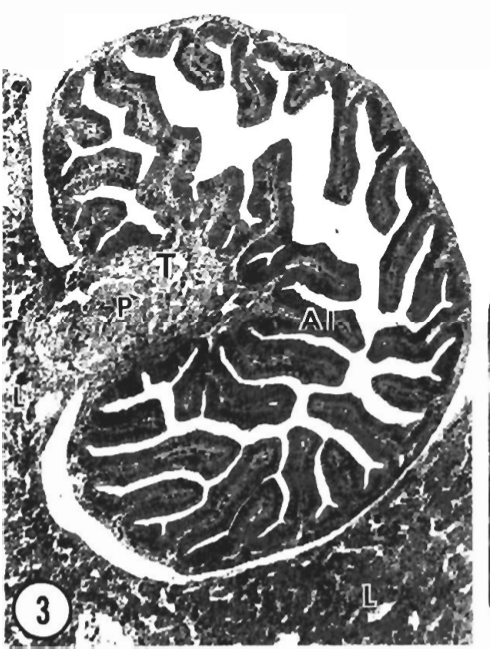
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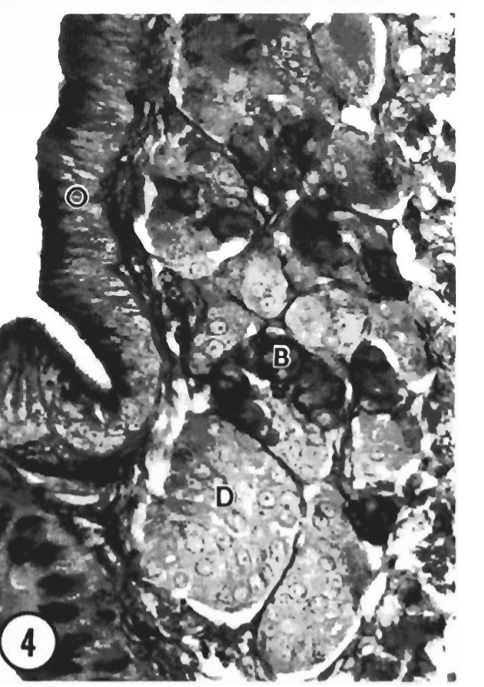
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the typhlosole and the liver showed interspecific variation. *L. tridentata* was unique in having all but the posterior tip of the caudal pancreas (1200 μm long) confined within the submucosa of the typhlosole, whereas all other animals possessed a continuous pancreatic mass which at some point was located in both the typhlosole and the liver. Although there was some slight individual variation in deposition of the caudal pancreas among *L. richardsoni* (670–1400 μm long), it was characteristic that most of this tissue was located in submucosal connective tissue within the liver with only the thin, tapered anterior end in the typhlosole. As the intestine was markedly reduced in size due to the advanced state of sexual maturation of *L. richardsoni*, the relationship with the liver may have been somewhat misleading. The caudal pancreas of var. *marifuga* (up to 620 μm long) was wedge-shaped and was present in both the liver and typhlosole throughout most of its length (Fig. 10). There were numerous intrahepatic, isolated islets (intermediate cord?) closely associated to the main pancreatic mass. Toward the posterior end, the main pancreatic mass in this variety came to reside within the typhlosole but there were still many isolated, intrahepatic islets (Fig. 10). In *L. ayresi* the anterior end of the caudal pancreas was exclusively confined to the typhlosole but as it extended posteriorly (930 μm) it eventually was seen as a narrow strip of tissue in the liver. There was a small diameter, anterior section of the caudal pancreas of *L. macrostoma* embedded deep within the typhlosole but more posteriorly it widened and then narrowed before finally increasing to a large diameter mass mostly confined to the liver. The most posterior region was a small diameter cord. The caudal pancreas extended over 1600 μm.

Fine Structure

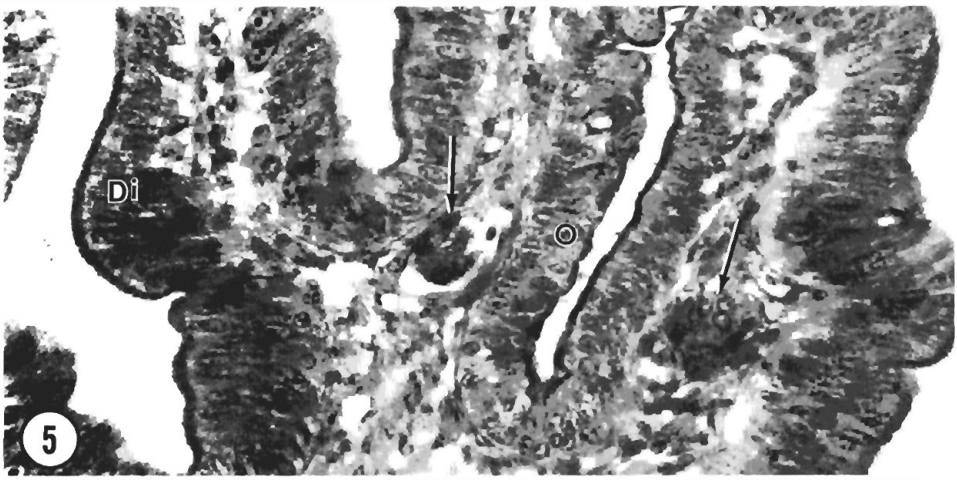
Ultrastructure observations, particularly on cytoplasmic granule morphology, revealed that the cranial and caudal pancreatic tissue of *L. ayresi* possessed three distinct cell types. Two of these cells were of roughly oval shape, they were in equal numbers, and they made up the majority of the pancreas in both regions. These two major cells were found isolated within separate islets or mixed within the same islets. A rare third cell was more commonly found in the cranial pancreas. It was variable in shape and often was located within the center of an islet with a thin cytoplasmic process extending toward the basal lamina (Fig. 11). The cells of the islets had an intimate association with endothelial cells of the surrounding capillaries and with nerve terminals of small nerve plexi. The islets of the cranial pancreas were separated from the epithelial cells of the diverticulum and esophagus by only a narrow band of fibrous connective tissue (Fig. 11), whereas the islets of the caudal pancreas had less intimacy with hepatocytes and with intestinal epithelial cells due to the presence of a thick band of connective tissue separating them from the latter. There were no differences in the fine structure or the apparent frequency of cells in saltwater-and freshwater-adapted animals.

One cell type was characterized by cytoplasmic granules with an electron-dense central core and a loose-fitting limiting membrane (Fig. 12a). This rather wide elec-

FIG. 2. Cranial pancreas (arrows) in the submucosa of the esophagus (O) and diverticulum (Di) in *Lampetra macrostoma* PAS stain. ×140.

FIG. 3. Caudal pancreas (P) in the submucosal connective tissue extending from the liver (L) into the typhlosole (T) of the anterior intestine (AI) of *Lampetra ayresi*. HE stain. ×95.

FIG. 4. Portions of cranial pancreas of *Lampetra tridentata* showing dark-staining B cells (B) and light-staining D cells (D). O. esophagus. AFT stain. ×440.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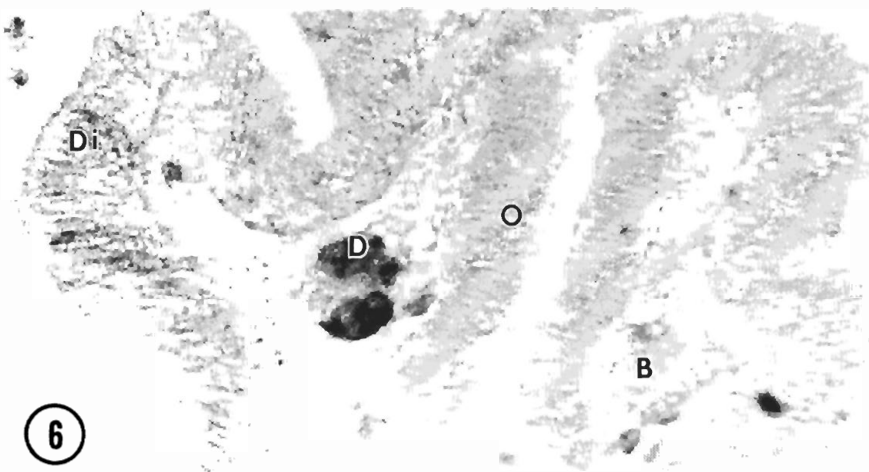
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tron-lucent space appeared as a halo and it surrounded a spherical- to rod-shaped core. As these granules resembled those present in the insulin-containing B cells of other vertebrates, this cell was referred to as the B cell. This cell also possessed numerous large mitochondria, an extensive system of rough endoplasmic reticulum, and glycogen.

The other abundant cell type had granules with an internal matrix of similar electron density to that of the cytoplasmic matrix (Figs. 11, 12b). Compared to the granules of the B cells, the granules of this cell type were present in greater numbers and they lacked the distinct electron-lucent halo between their limiting membrane and their central matrix. This cell had fewer mitochondria than the B cell but had extensive rough endoplasmic reticulum, glycogen, and a prominent Golgi apparatus.

The third, less frequent, cell type lacked the extensive rough endoplasmic reticulum of the other two types and had numerous round to oval granules (Fig. 11, 12c). These granules had some resemblance to those in the second cell type but they were less frequent, their central core was much more electron dense, and there was a slight halo beneath the limiting membrane.

DISCUSSION

The present study provides a further demonstration that endocrine pancreatic tissue is of variable distribution in adults of lamprey species. Hilliard *et al.* (1985) showed that adults of the Southern Hemisphere species, *G. australis*, possess only a large cranial mass of endocrine tissue. The only other lamprey believed to demonstrate this type of deposition of endocrine pancreatic tissue is another Southern Hemisphere species, *Mordacia mordax* (Epple and Brinn, 1986; Potter, 1986). Although there is some interspecific variation, the species in British Columbia generally follow the pattern for other holarctic lampreys, such as *P. marinus* (Epple and Brinn, 1975, 1976; Elliott and Youson, 1986, 1987) and *Lampetra fluviatilis* (Boenig, 1927; Barrington, 1945), in that cranial, intermediate cords, and caudal portions of endocrine pancreatic tissue are present. However, we found one example among lamprey populations in British Columbia where a parasitic variety of nonparasitic *L. richardsoni* possessed little or no cranial pancreas but both intermediate cords and a caudal pancreas. This variety, called variety *marifuga*, has only been located in Morrison Creek on Vancouver Island. The nonparasitic *L. richardsoni* in this Creek has a cranial pancreas but tissue volume is much reduced compared to the cranial pancreas of *L. richardsoni* from Puntledge River. Morrison Creek flows into this latter River. The deposition of pancreatic tissue in lampreys of British Columbia has implications for both taxonomy and for further understanding the development of the pancreas in adult lampreys.

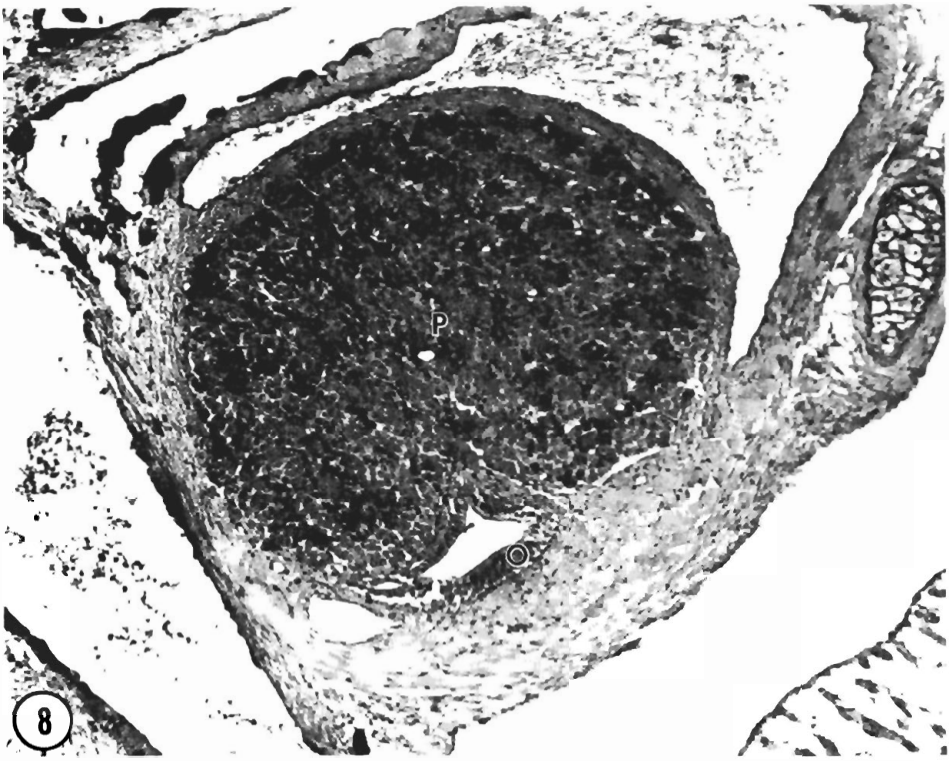
L. tridentata is an anadromous parasitic species which seems to be genetically distinct from the other saltwater parasitic species, *L. ayresi*, and from *L. richardsoni* (Beamish and Withler, 1986). Although the genetic relationship between *L. tridentata* and the freshwater parasitic species, *L. macrostoma*, is not clearly understood, there are marked differences in external characters and the former has great difficulty in osmoregulating and feeding in fresh water (Clarke and Beamish, 1987). The present study provides further evidence for the separation of these latter two species,

FIGS. 5–7. Adjacent transverse sections of the intermediate cords between the diverticular (D) and esophageal (O) epithelia in *Lampetra ayresi*. ×550.

FIG. 5. Intermediate cords (arrows): PAS.

FIG. 6. Somatostatin immunoreactivity in D cells (D) but not B cells (B).

FIG. 7. Insulin immunoreactivity in B cells (B) but not D cells (D) of Fig. 6.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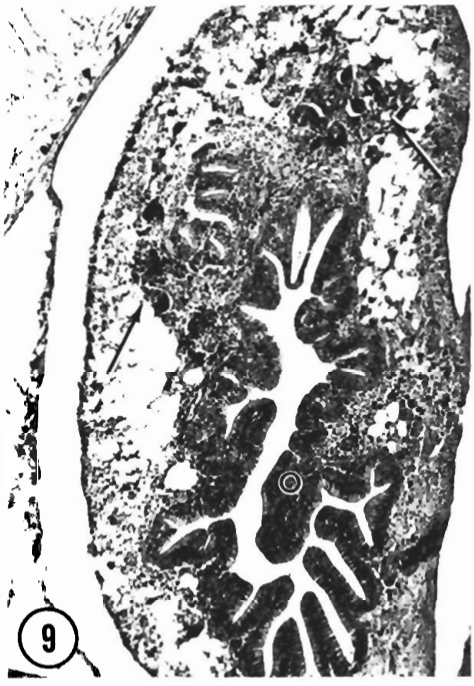
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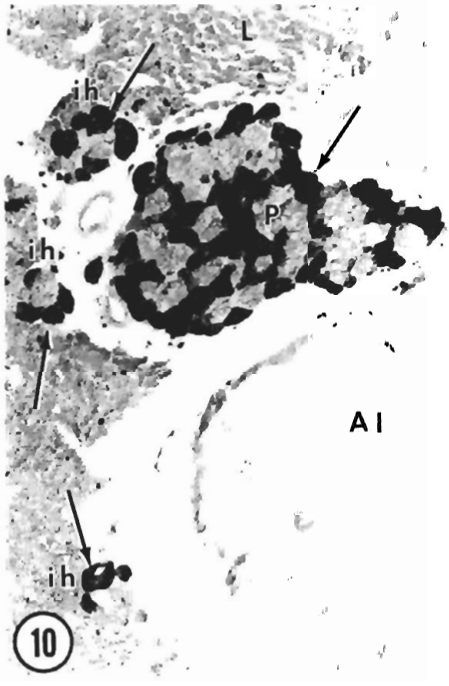
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for there are marked differences in the distribution of their pancreatic tissues. Notable are the larger cranial mass in *L. macrostoma*, the more extensive intermediate cord in *L. tridentata*, and the greater preference for the typhlosole for the caudal pancreas of *L. tridentata*.

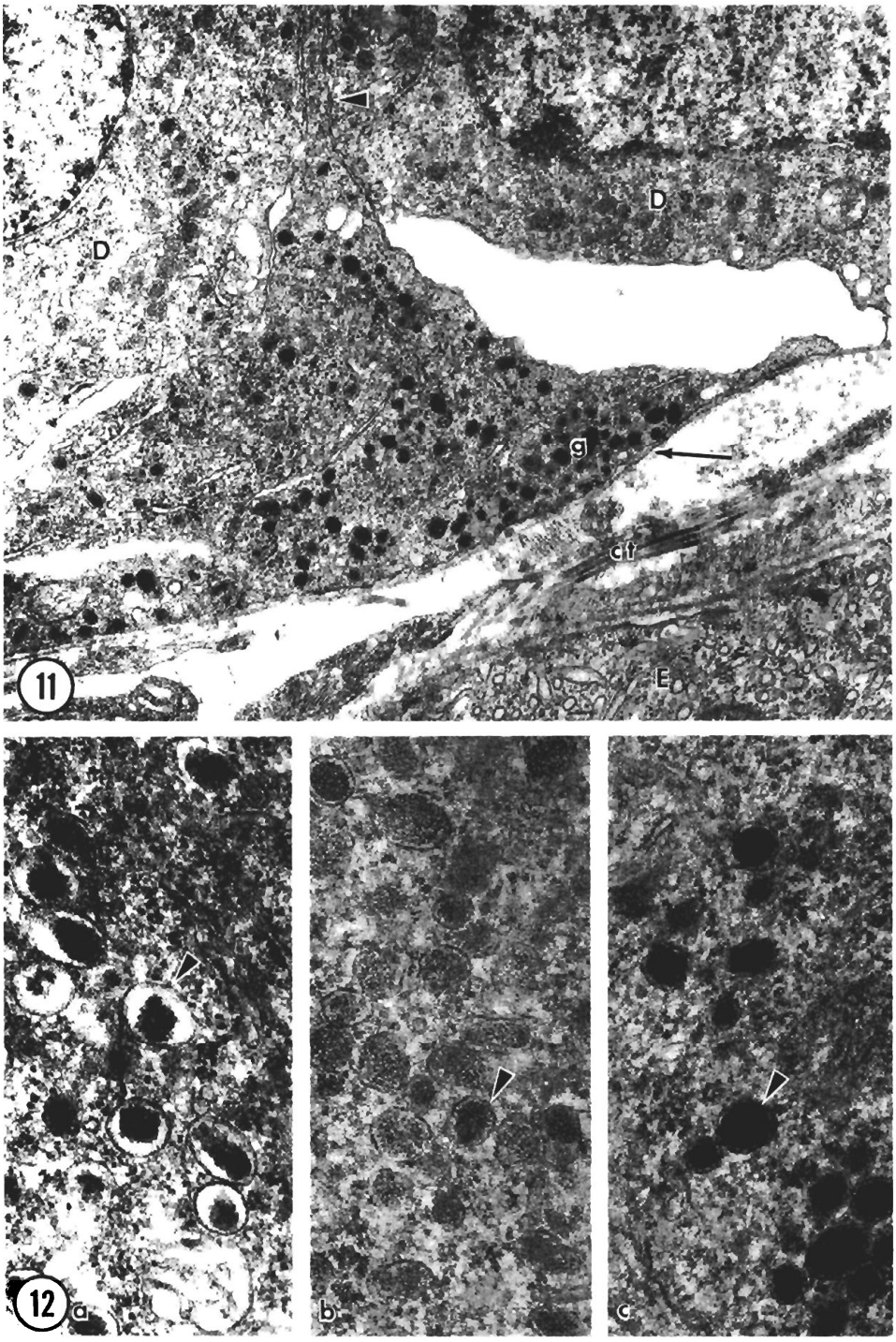
L. ayresi and *L. richardsoni* are distinct species but they are satellite or paired species (Potter, 1980; Vladykov and Kott, 1980) and lack of genetic differentiation suggests a very close relationship (Beamish and Withler, 1986). Comparison of the distribution of the pancreatic tissue in *L. ayresi* from the Fraser River and in *L. richardsoni* from the Puntledge River corroborates this species separation in that the former is characterized by an extensive intermediate cord whereas the latter has the largest cranial pancreas of all the species examined. There seems to be only subtle differences in their caudal pancreases. It is particularly interesting that there should be a marked difference in the distribution of pancreatic tissue in *L. richardsoni* from Morrison Creek and the Puntledge River, for Beamish and Withler (1986) have shown that geographically isolated populations of *L. richardsoni* are as genetically distinct from one another as they are from *L. ayresi*. Perhaps the morphological variation in pancreatic tissue is a manifestation of genetic variation which exists between *L. richardsoni* in different parts of the same watershed. Unfortunately, data is not available on allozymic variation at the 22 enzyme loci for the Puntledge population which would permit a comparison with these data from the Morrison Creek *L. richardsoni*. In the future, it would be of value to examine the distribution of pancreatic tissue in several other populations of *L. richardsoni*.

Although *L. richardsoni* in Morrison Creek can be separated electrophoretically from this species in other watersheds, allelic frequencies at 21 loci do not differ from those of var. *marifuga* in this Creek. Despite this genetic similarity, there are some substantial differences in the distribution of pancreatic tissue in these two lampreys of Morrison Creek. The most significant difference is the almost total absence of a cranial pancreas in var. *marifuga*. Only one of five animals had a cranial pancreas and this animal had other morphological differences from the other four which suggested that it was atypical. Although *L. richardsoni* of this Creek had an intermediate cord it could not compare with the extensive cord of var. *marifuga*. Moreover, it is characteristic of the anterior portion of the caudal pancreas of var. *marifuga* to be positioned mostly with the liver and to have many isolated islets (or cords) closely associated. These features of the pancreas, combined with morphological observations of the anterior intestine and diverticulum, would suggest that var. *marifuga* is a different lamprey from *L. richardsoni* of the same watershed. The derivation of var. *marifuga* is not known, but because the allelic frequencies between nonparasitic *L. richardsoni* and the potentially parasitic var. *marifuga* are similar and distinct spawning populations cannot be identified in Morrison Creek, it is believed that the var. *marifuga* is produced by the nonparasitic *L. richardsoni* population. We do not know if the variety appeared from a previous hybridization between *L. richardsoni* and *L. ayresi*. However, based on the morphology of the

FIG. 8. Large cranial pancreas (P) and atrophied esophagus (O) from *Lampetra richardsoni* of Puntledge River. HE ×150.

FIG. 9. Cranial pancreas of *Lampetra macrostoma* in two masses (arrows) around the esophagus (O). ×100.

FIG. 10. Somatostatin-immunoreactive cells (arrows) in both the caudal pancreas (P) and the intrahepatic islets (ih) of *Lampetra richardsoni* var. *marifuga*. L, liver; AI, anterior intestine. ×100.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pancreas it does not appear that var. *marifuga* is an intermediate of *L. ayresi* and *L. richardsoni*. Instead it is best to think of var. *marifuga* as a trophic representative of a unique population of *L. richardsoni* which is polymorphic. Further morphological study on other organs and tissues may provide an explanation of the origin and the successful perpetuation of this variety in Morrison Creek.

It has been clearly established that the extrahepatic common bile duct and part of the intrahepatic common bile duct of the ammocoete of the sea lamprey, *P. marinus*, contributes a population of cells which will eventually differentiate and expand into the insulin- and somatostatin-secreting cells of the caudal pancreas (Elliott and Youson, 1987). The intrahepatic bile ducts and ductules of the ammocoete of this species (Yamamoto *et al.*, 1986) degenerate and disappear during metamorphosis (Sidon and Youson, 1983). In several of the species under present investigation, small bile ducts are still present in the liver following the completion of metamorphosis (J. H. Youson and R. J. Beamish, unpublished observations). In addition, in var. *marifuga* pancreatic islets are present in the liver in positions formally occupied by small bile ducts. Although intrahepatic groups of islets have been reported in adults of *P. marinus* (Epple and Brinn, 1975; Brinn and Epple, 1976), we conclude from these observations that var. *marifuga* has an even greater contribution of the bile ducts to the adult caudal pancreas than has been previously noted in other lampreys. The observations are further support for the view that the caudal endocrine pancreas of adult lampreys is derived from the epithelium of the biliary tree of ammocoetes during metamorphosis. As the location of the bile duct in larvae of lamprey species may be a determining factor for dictating the presence or absence of a caudal pancreas (Hilliard *et al.*, 1985, Potter, 1986), it can be assumed that larvae of the species under present study have a bile duct which enters the alimentary canal at or near the esophageal–intestinal junction. This may be a common feature of larvae of holarctic species.

An explanation for the absence of a cranial pancreas in most of the var. *marifuga* is not immediately obvious. This portion of the endocrine pancreas forms during metamorphosis in *P. marinus* by budding of islets from the epithelia of the transforming esophagus, the newly differentiated, proximal section of the anterior intestine, and the developing diverticulum (Elliott and Youson, 1987). The epithelium of the diverticulum seems to be particularly important as a source of somatostatin cells during late stages of metamorphosis. The presence of a relatively small diverticulum in var. *marifuga* may be of direct relevance to the absence of a cranial pancreas in this lamprey. However, there does not seem to be any correlation between the size of the diverticulum and the volume of cranial pancreas in other lampreys of British Columbia.

The general histology of the pancreatic tissue of all lampreys in the present study conforms to that observed in adults of other lamprey species. That is, there are islets and follicles of epithelial cells which are surrounded by a rich vascular network. The epithelial cells are predominantly of two

FIG. 11. Electron micrograph of D cells (D) and an unknown cell type with electron-dense granules (g), a broad surface above the basal lamina (arrow), and a narrow neck of cytoplasm (arrowhead) extending toward the center of the islet in the endocrine pancreas of *Lampetra ayresi*. A thin layer of fibrous connective tissue (ct) separates the islet cells from the epithelium (E) of the diverticulum. ×30,000.

FIG. 12. Electron micrographs of granules (arrowheads) in three cell types of endocrine pancreas of *Lampetra ayresi*. (a) B cell; ×47,000. (b) D cell; ×55,000. (c) Third cell type; ×56,000.

types, somatostatin- and insulin-immunoreactive cells, corresponding to the D cells and the B cells, respectively, which are present in the islet tissue of other vertebrates. The homology of lamprey D cells to those of gnathastomes is not without question (Epple and Brinn, 1986). The high proportion of somatostatin-immunoreactive cells (almost 50%) is unique to lamprey pancreatic tissue, yet no physiological significance has been found for this feature. There is also some evidence that additional cell types may appear in the lamprey pancreas as the animals reach sexual maturation (Epple and Brinn, 1975), but we have been unable to confirm this through fine structural and immunocytochemical observations of *P. marinus* (Elliott and Youson, 1988).

The fine structure of cells from the cranial and caudal pancreatic tissue of *L. ayresi* has some resemblance to that described for the pancreas of adults of *L. fluviatillis* (Winbladh, 1966), *Lampetra planeri* (Titbach and Kern, 1969), and *P. marinus* (Epple and Brinn, 1975; Elliott and Youson, 1988) in that all have at least two cells of varying morphology. However, the early studies of *Lampetra spp.* concluded that the two cell types may represent different secretory phases of the insulin-secreting B cell and Epple and Brinn (1975) noted B cells and four other cell types (called P cells) in *P. marinus*. Recently, it has been clearly documented that adults of *P. marinus* have two types of cells which make up the majority of the pancreatic tissue and which have a characteristic fine structure and an immunoreactivity to either insulin or somatostatin antisera (Elliott and Youson, 1988). The insulin-immunoreactive cell is called the B cell and the somatostatin-immunoreactive cell is called the D cell. A third cell type is not immunoreactive to antisera to mammalian insulin, to synthetic somatostatin-14, or to mammalian glucagon and therefore is of unknown hormonal content.

Based on granule morphology and other fine structural features, it can be concluded that the pancreas of *L. ayresi* consists of at least three cell types. The B cell is characterized by granules which are similar to those present in insulin-containing B cells of *P. marinus* (Epple and Brinn, 1975; Elliott and Youson, 1988) and other vertebrates. The second cell is part of the population which immunostains for somatostatin in the light microscope and therefore would correspond to the D cell of *P. marinus*. However, the second cell in *L. ayresi* has granules with a central core which is less electron dense than is seen in the granules of D cells in *P. marinus*. Although the differences could be the result of variable leaching of granule content during tissue preparation, the possibility of species variability in granule morphology cannot be overlooked. On the other hand, it does not seem unreasonable to state that this abundant cell with characteristic fine structure and somatostatin immunoreactivity is the D cell of the pancreas of *L. ayresi*.

We made no quantitative comparison of the frequency of the third cell type in the three pancreatic regions of *L. ayresi* but this cell was more often encountered in the cranial pancreas. It was also more conspicous than we had noted in the pancreas of *P. marinus* (Elliott and Youson, 1988). No functional significance can be placed with this observation until the identity of the third cell is known in this and other species of lamprey.

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