Japanese Journal of Ichthyology
Vol. 31, No. 2 1984

魚類学雑誌
31巻2号 1984年

Histological Changes of Several Organs during Growth of the Brook Lamprey *Lampetra reissneri*

Kazuhiko Tsuneki and Masami Ouji

(Received October 20, 1983)

Abstract The gonad, buccal cavity, gill, esophagus, salivary gland, intestine, liver, kidney, fat column, and eye were studied histologically during growth of the brook lamprey, *Lampetra reissneri*. Gonads of all individuals showed ovary-like structures at about 70 to 90 mm in total length. In females, oocytes continued to grow. In males, oocytes degenerated, and a tiny testis originated from a small number of residual undifferentiated germ cells. Upon histological observation, the sex was easily determined in individuals larger than 120 mm in total length. Initial tooth development (intraepidermal keratinization) occurred at stage 5 of metamorphosis. Esophagus of the adult type was distinguishable by stage 5. The salivary gland and its duct were also formed by stage 5. Intestine and kidney degenerated during metamorphosis. The liver of tubular gland type changed to the adult type by stage 3. In ammocoetes larvae, main hematopoietic tissues were located in intestine and kidney, but in adults they were found in the fat column.

In a previous paper (Tsuneki and Ouji, 1984), we reported external changes of the brook lamprey, *Lampetra reissneri* (Dybowski), during growth from ammocoetes to adults. In this paper, histological changes of some internal organs, especially those showing profound changes during growth, are described. Gonadal differentiation of the lamprey has long been a subject of extensive study (Okkelberg, 1921; Hardisty, 1965, 1971, 1979). Histological changes of teeth, gill, esophagus, intestine, liver, kidney, fat column (supraneural myeloid body), and eye have also been extensively studied, especially in terms of metamorphosis (George and Beamish, 1974; Percy and Potter, 1977; Manion and Piavis, 1977; Youson, 1980, 1981a,b,c; Dickson and Graves 1981; Lethbridge and Potter, 1981). However, all these studies are concerned with European, North American, or Australian lampreys, and mainly with parasitic species. In the Far Eastern region, Yoshie and Honma (1979) studied the change of the buccal cavity of the parasitic *Lampetra japonica*, and Fukayama and Takahashi (1982) examined sex differentiation of the same species. However, systematic study of histological changes of internal organs of the non-parasitic lamprey has been scarce in this region. The present study may partly fill this gap in knowledge.

Materials and methods

The brook lamprey, *Lampetra reissneri*, were collected throughout a year (1982) at the Hosshoji River, Tottori Prefecture, Japan. Ammocoetes of varying sizes (n=213), metamorphosing individuals representing different stages (n=22), and adults (n=17) were obtained. Stages of metamorphosis were determined mainly according to criteria given for *Lampetra planeri* (Bird and Potter, 1979). Fully mature adults were not studied. For further details, see the previous paper (Tsuneki and Ouji, 1984).

In order to know whether a regional difference exists in the gonad, various parts of the gonads were examined in five individuals (total length= TL: 43, 54, 98, 108, and 122 mm). The gonad extended anteriorly to the posterior part of the liver, and posteriorly to the region slightly anterior to the cloaca. However, the development was poor both at the anterior and the posterior extremity, especially at the latter. The gonad was relatively well-developed about the middle of the abdominal cavity, and this regional development was parallel to that of the kidney (mesonephros or opisthonephros). Therefore, only the middle part of the gonad was studied in the other individuals.

The organs histologically studied were: gonad (in all individuals), buccal cavity (in ammocoetes of 166 mm in TL, metamorphosing animals

from stage 2 to 5, and two adults), branchial region at the level of about the fourth branchial pore (in ammocoetes of 36, 43, 56, 65, 73, 89, 98, 109, 111, 113, 121, 137, 143, 158, 166 and 179 mm in TL, metamorphosing animals from stage 2 to 5, and two adults), intestine at the mid-trunk region (in all ammocoetes, metamorphosing animals from stage 2 to 5, and seven adults), liver (in ammocoetes of 36, 43, 45, 49, 54, 56, 57, 60, 63, 70, 98, 109, 119, 125, 138, 146, 152 and 179 mm in TL, metamorphosing animals from stage 2 to 5, and all adults), kidney at the level of the mid-trunk region (in all ammocoetes, metamorphosing animals from stage 2 to 5, and four adults), fat column at the level of the mid-trunk region (in all ammocoetes, metamorphosing animals from stage 2 to 5, and five adults), and eye (in ammocoetes of 36, 45, 56, 64, 70, 82, 98, 109, 119, 125, 138, 146, 152 and 179 mm in TL, metamorphosing animals from stage 2 to 5, and two adults). The tissues were fixed with Bouin's fluid and embedded in paraffin. They were cut sagittally (buccal cavity) or transversely (the other tissues) at 6 to 7 μm, and stained with Masson-Goldner's method.

In several specimens of adults, length of the liver (from the posterior tip of heart cartilage to the posterior tip of the liver) was measured before fixation.

Results

Gonad. The gonad occupied the mid-dorsal region of the abdominal cavity. It was a median structure, although slight bilaterality was noticed in some gonads. The gonad consisted of solid cell nest except for the differentiated ovary.

The lamprey spawn in April and May (see Tsuneki and Ouji, 1984). The gonads of the new hatchlings caught in September and October (about 40 to 50 mm in TL, half year old) were very small and contained a few nests with undifferentiated germ cells. The gonads of new hatchlings caught in November to March (about 55 to 70 mm in TL) contained the nests of germ cells initiating meiotic prophase (Fig. 1). In the following April to June, ammocoetes measured 70 to 90 mm in TL and were about one year old. Their gonads consisted of nests full of oocytes in diplotene stage (Figs. 2, 3). Somatic cells were also frequently found in the nest. Somatic cell nuclei were irregular in outline in contrast to the round nuclei of germ cells. From summer to autumn of the second year, sex differentiation occurred. The TL of these ammocoetes were 90 to 120 mm. In females, preexisting oocytes continued to grow. In individuals possibly to become males, oocyte degeneration proceeded, and residual germ cells became spermatogonia. In some individuals, oocyte degeneration was noted as early as June (Fig. 4). The incipient testis was very small. After winter of the second year, sex was easily determined on the histological preparations. The TL of these ammocoetes was more than 120 mm. The testis consisted of several nests containing spermatogonia, but was always small in volume compared with the ovary (Fig. 5). The testicular epithelium consisted of an especially dense array of cuboidal cells. In the ovary, oocytes grew continuously (Fig. 6).

Here, it must be noted that the above statements are generalizations of the observations and neglect extreme individual variations. A few individuals could not be categorized into the framework above with regard to gonadal development, TL, or season.

During metamorphosis (stage 4 and 5), the testis became much larger, but testicular nests still contained spermatogonia exclusively. The testis of the individual caught in November (macrophthalmia stage) contained nests with both spermatogonia and spermatocytes. In adults caught later than December, the testis consisted of nests with spermatocytes. In some individuals caught in January, a few testicular nests contained spermatids.

In females, egg degeneration (formation of atretic follicles) occurred in some individuals of earlier metamorphic stages. Yolk granules were found in eggs of individuals older than stage 3 of metamorphosis. Morphometric changes of egg growth was described in the previous paper (Tsuneki and Ouji, 1984).

Teeth. At stage 3 of metamorphosis, the epithelium of the regions where supraoral, infraoral, and lingual teeth develop became thicker than the surrounding epithelium (Fig. 7). Flare-like epithelial protrusions were evident around the sites where supraoral and infraoral teeth develop. Germinal layer cells of the epithelium of the tooth region frequently divided. At stage 5, the epithelium initiated intraepidermal

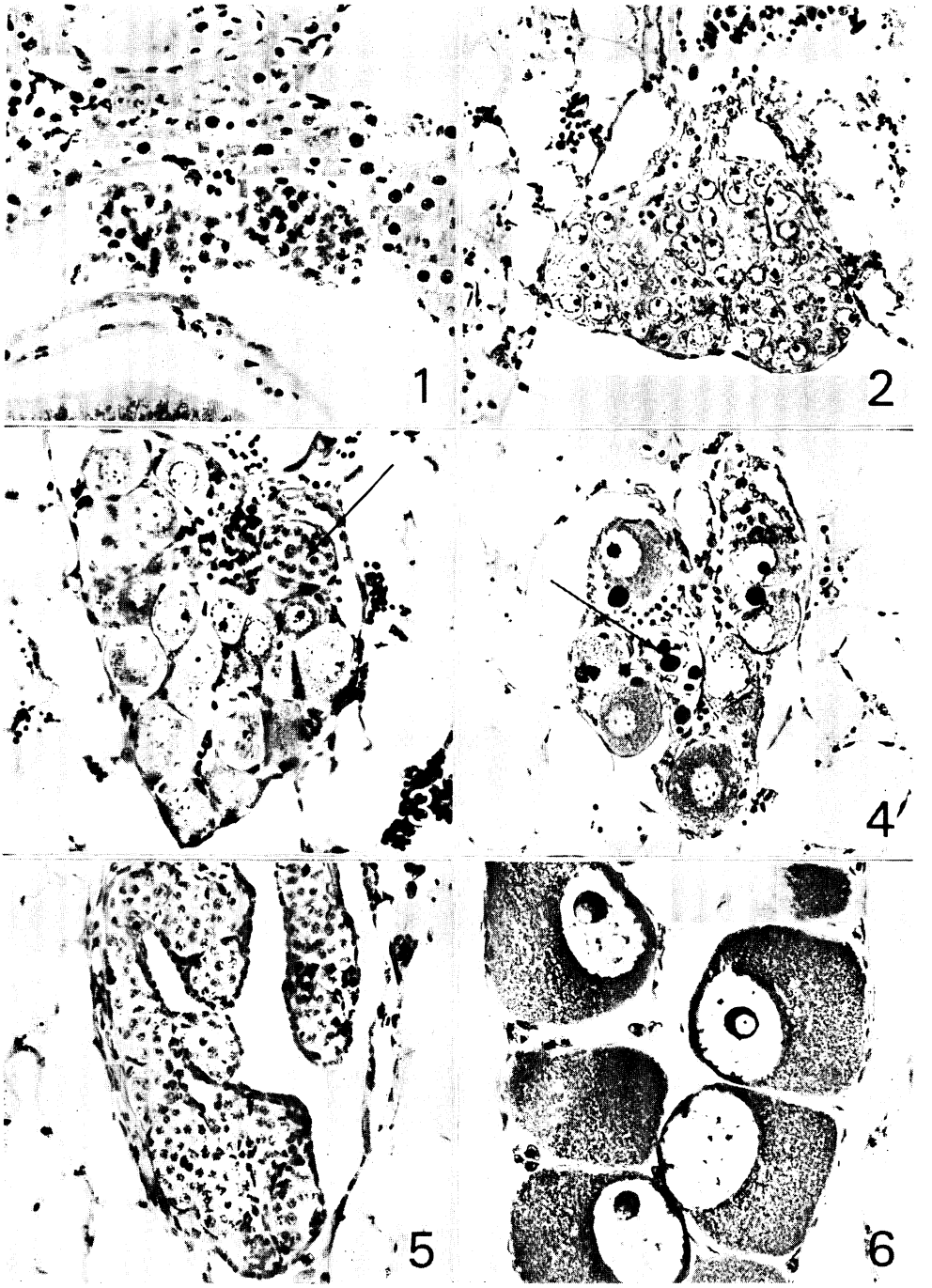

Fig. 1. Tiny gonad of 43 mm-long ammocoetes caught in January. Germ cells initiate meiosis except for those on the right. ×370.
Fig. 2. Ovary-like gonad of 75 mm-long ammocoetes caught in April. The cells are in diplotene stage of meiotic prophase. ×280.
Fig. 3. Ovary-like gonad of 82 mm-long ammocoetes caught in May. In addition to many oocytes, a nest of undifferentiated germ cells is seen (arrow). ×280.
Fig. 4. Ovary-like gonad of 95 mm-long ammocoetes caught in June. Some oocytes are degenerating (arrow). ×280.
Fig. 5. Testis of 154 mm-long ammocoetes caught in June. ×280.
Fig. 6. Ovary of 122 mm-long ammocoetes caught in December. ×280.

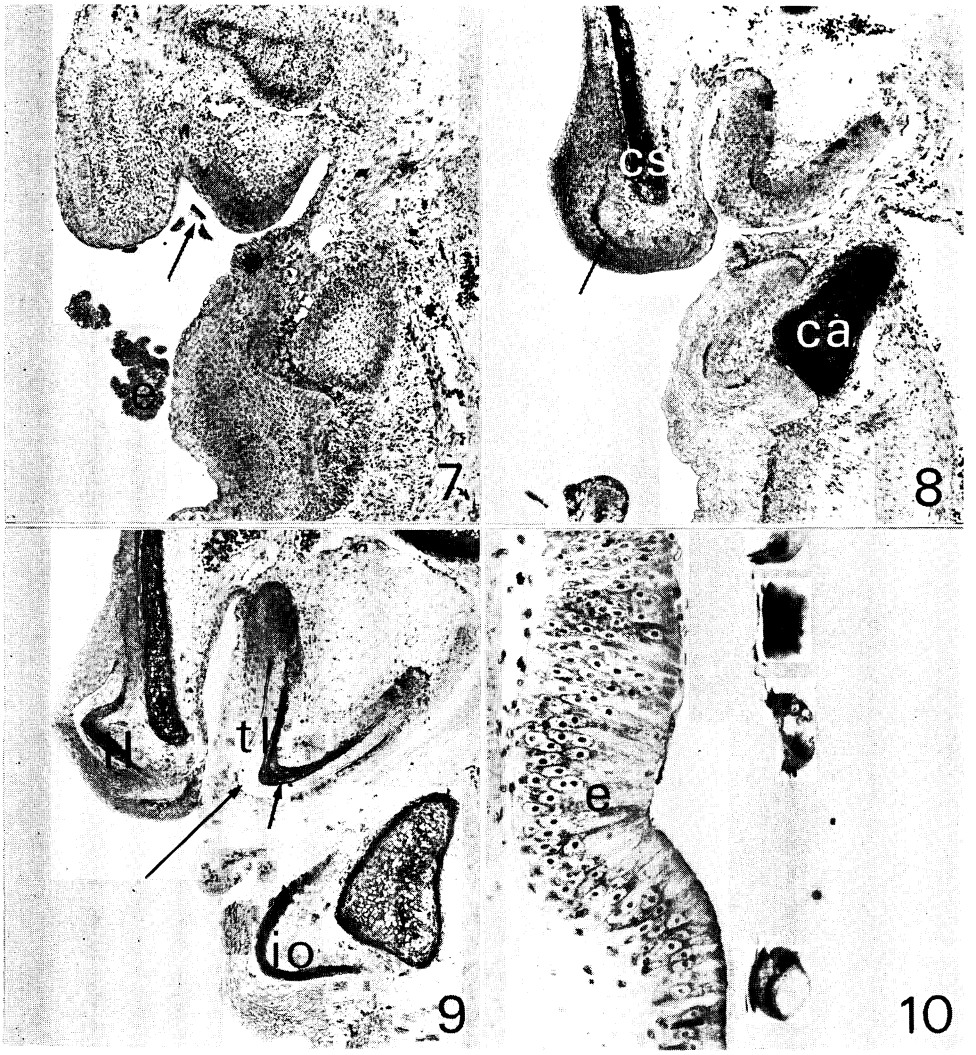

Fig. 7. Sagittal section of the lower buccal cavity of stage 3 lamprey caught in October. An arrow indicates parasites. e, epidermal protrusion. ×70.

Fig. 8. Sagittal section of the lower buccal cavity of stage 5 lamprey caught in November. Intraepidermal keratinization starts (arrow). ca, cartilago apicalis; cs, carlilago supraapicalis. ×70.

Fig. 9. Sagittal section of the lower buccal cavity of immature adult caught in November. Note the first generation of tooth (long arrow) and the second generation of tooth (short arrow) of longitudinal (l1) and transverse lingual (tl). io, infraoral. ×70.

Fig. 10. Parasites in the pharyngeal chamber of 56 mm-long ammocoetes caught in February. e, epithelium of epipharyngeal "uvula". ×280.

keratinization between germinal layer and prickle cell layer (Fig. 8). Such keratinization was especially prominent at the supraoral site. In immature adults caught in November, two successive generations of keratinized teeth were observed in the epidermis of lingual teeth (Fig. 9). The first generation was located more apically and devoid of cell nuclei. The cells situated between these two generations of teeth were highly vacuolated. Two generations of teeth were not separated in supraoral and infraoral teeth of immature adults caught in November. In an adult caught in February, however, two generations were clearly separated

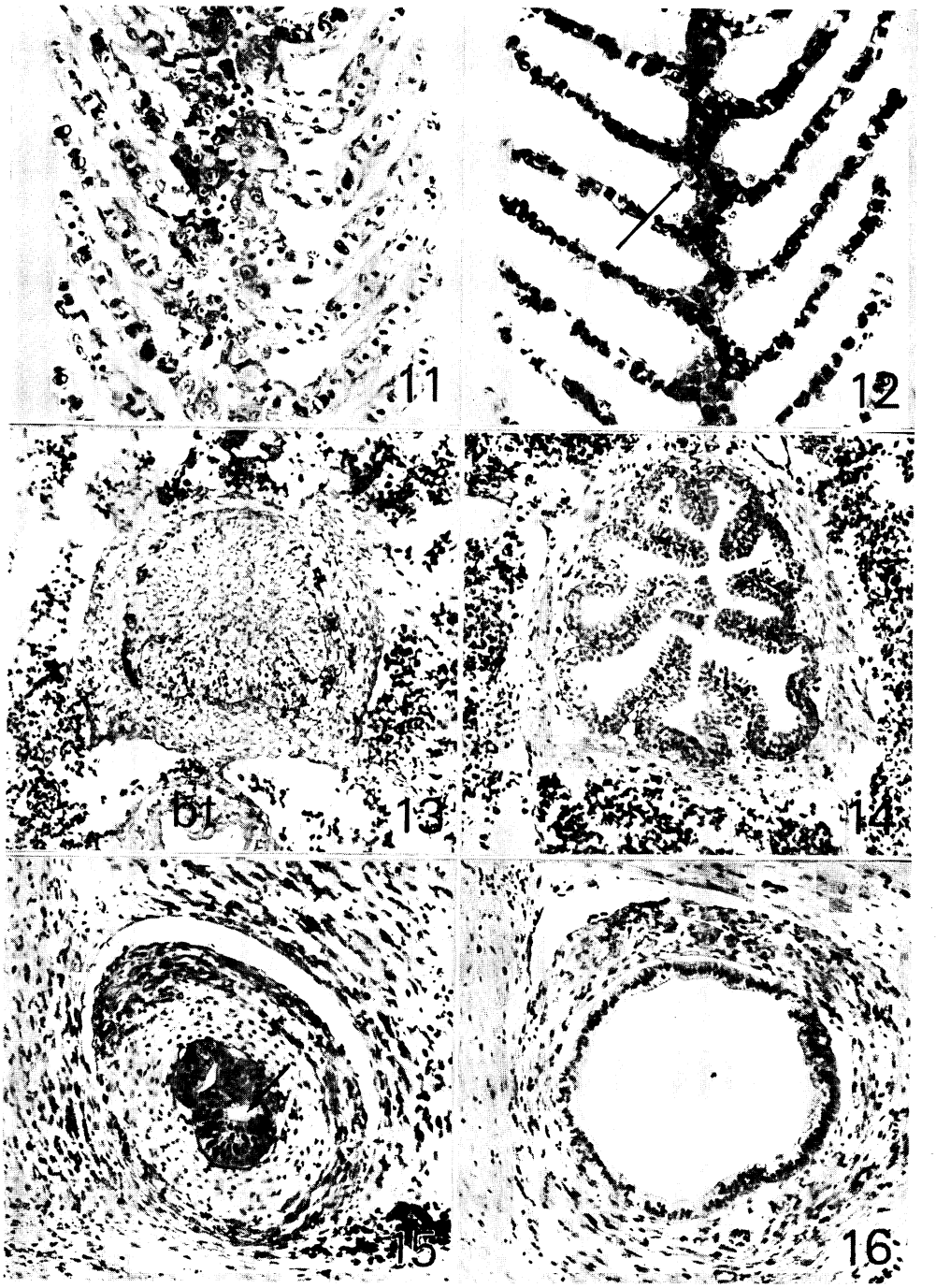

Fig. 11. Gill lamella of 89 mm-long ammocoetes caught in February. Note a mass of chromophilic cells between two successive secondary lamellae. ×280.
Fig. 12. Gill lamella of immature adult caught in November. An arrow indicates "adult light cell". ×280.
Fig. 13. Solid esophagus of stage 3 lamprey caught in October. bt, branchial tube. ×140.
Fig. 14. Patent esophagus of stage 5 lamprey caught in October. Note epithelial folding. ×140.
Fig. 15. Salivary gland of stage 3 lamprey caught in October. Lumen is forming (arrow). ×140.
Fig. 16. Patent salivary gland of stage 5 lamprey caught in October. Flocculent material is seen in the lumen. ×140.

even in supraoral and infraoral teeth. Furthermore, the third generation adhered to the undersurface of the second generation.

Gill, pharynx, and salivary gland. In the branchial chamber of ammocoetes, an urceolariid ciliate was frequently found (Fig. 10). The ciliate was also observed in the buccal cavity of metamorphosing individuals (Fig. 7). Urceolariid ciliates are known also to occur in *Petromyzon marinus* (Appy and Anderson, 1981) and *Lampetra japonica* (Honma et al., 1982).

Terminology of various parts of the alimentary canal is somewhat confusing in the lamprey. In ammocoetes, the branchial region is termed here as the pharynx, the narrow region adjacent to the liver as the esophagus, and the more posterior region as the intestine. In adults, the branchial tube develops beneath the alimentary canal which is called the esophagus here.

The cell population covering the axial plates of the gill filaments changed profoundly during metamorphosis, but cell identification was difficult at the light microscopical level and must wait for electron microscopical study (see Morris, 1957; Youson and Freeman, 1976; Peek and Youson, 1979). In ammocoetes, the base between two successive secondary lamellae was mainly occupied by a mass of "stout cells" (Fig. 11). These cells possessed acidophilic cytoplasm and an irregularly shaped nucleus with a prominent nucleolus. The superficial position of the lamellar base was occupied occasionally by "dark cells", and by "light cells", which were stained less intensely. The larval light cells might be immigrants from cells covering the secondary lamellae. "Large mucous cells" were restricted to the periphery of the branchial chamber, and were never found between secondary lamellae. In metamorphosing individuals, cells covering the base of secondary lamellae were similar to those covering the secondary lamellae themselves although the cells were cuboidal in the former and flat in the latter. In adults, "adult light cells" (chloride cells?) appeared between successive secondary lamellae (Fig. 12). Large mucous cells were also occasionally seen between successive secondary lamellae.

The branchial tube and the adult esophagus was formed at stage 3 (Fig. 13). The esophagus was still solid at this stage. It was slightly perforated at stage 4, and completely perforated at stage 5 (Fig. 14). The epithelium of the esophagus consisted of two apparent layers of cells. They usually lacked cilia. The subepithelial tissue did not contain smooth muscle cells.

Invagination of the salivary gland had already started at stage 2, but the gland itself was still solid. At stage 3, the initial sign of lumen formation appeared in the gland (Fig. 15). The duct was still solid even at stage 4. At stage 5, both the gland and the duct were completely patent (Fig. 16).

The endostyle was apparently intact at stage 2, but started degeneration at stage 3.

Alimentary canal. The epithelium of larval esophagus was ciliated. The epithelial cells were columnar. The subepithelial tissue was composed of connective tissue and devoid of a distinct muscle layer. The adult esophagus was described above.

The intestinal epithelium of ammocoetes was underlain by layers of smooth muscle cells without intervening mucosal connective tissue. In the mid-trunk region of the intestine, the inner muscular layer consisted of longitudinally

Fig. 17. Transverse section of intestinal wall of 93 mm-long ammocoetes caught in July. Inner muscular layer consists of longitudinal fibers (l) and outer muscular layer circular fibers (c). Brush border is prominent on the apical surface of epithelium. Submuscular space (s) is occupied by capillaries and connective tissue. k, kidney. ×370.

Fig. 18. Intestine of 85 mm-long ammocoetes caught in August. Note hematopoietic typhlosole (t) and typhlosolar artery (ta) in it. ×70.

Fig. 19. Intestine of stage 4 lamprey caught in October. Note epithelial folding and typhlosole (t) reduced in size. ×70.

Fig. 20. Intestine of immature adult caught in November. Note much reduced lumen and loose typhlosole (t). ×70.

Fig. 21. Degenerated intestine of immature adult caught in February. Note that the magnification is twice that of the preceding three figures. ×140.

Fig. 22. Fixed gut contents of 129 mm-long ammocoetes caught in July. Diatoms predominate. ×370.

oriented muscle cells, and the outer muscular layer consisted of circulalry arranged muscle cells (Fig. 17). These arrangements of muscle cells lost their identity at the boundary between regular intestinal wall and typhlosole. Within the typhlosole itself, the inner layer bordering the epithelium was mainly composed of circular muscles and the outer layer of longitudinal muscles. In small ammocoetes, however, such subdivision of the muscle layers was indistinct.