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Histological and histochemical changes in the digestive tract of white sturgeon larvae during ontogeny

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Abstract

Ontogenetic changes in digestive tract histology and digestive enzyme histochemistry were investigated 11 to 36 days post-hatch in white sturgeon *Acipenser transmontanus* larvae. From initiation of exogenous feeding (12 days post-hatch), larvae were fed a commercial salmonid diet for the ensuing 24 days. The digestive system of white sturgeon displayed a high degree of morphologic organization and functionality at the onset of exogenous feeding. An enhancement of digestive capacities occurred with transition to active feeding. On day 2 of feeding, there was a clear increase of alkaline phosphatase, aminopeptidase M, dipeptidyl peptidase IV, and γ-glutamyl transpeptidase activity in the brush border of the spiral intestine. This strong activity is an apparent confirmation of the importance of this segment of the intestine for protein digestion and nutrient absorption. The functional development of the pyloric intestine occurred on day 4 and was concomitant with an increase in the activity of brush border and cytoplasmic enzymes such as acetylcholinesterase, dipeptidyl peptidase II, α- and β-galactosidases. The absence of acetylcholinesterase, lactase, nonspecific esterase, and weak activity of exopeptidases and alkaline phosphatase in the anterior intestine suggests that this segment of the intestine may be less important in nutrient absorption than the pyloric and spiral intestines. The observed quantitative and qualitative differences in enzyme activity along the intestine indicate a high degree of specialization of each segment for specific digestive and absorptive processes.

Introduction

Knowledge of gastrointestinal tract (GIT) developmental changes associated with the process of food assimilation is essential for understanding the nutritional physiology of larval fish (Segner *et al.* 1993). White sturgeon, *Acipenser transmontanus,* the phylogenetically oldest and largest freshwater fish (Lane 1991), is a promising aquaculture species (Hung 1991). Unlike other teleost fishes, sturgeon develop holoblastically and the resultant intraembryonic yolk directly participates in alimentary system formation (Shmalgauzen 1968). In addition, the GIT of sturgeon is unique, with the presence of a spiral intestine, as well as a ciliated gastric and intestinal mucosa retained throughout adult life (Buddington 1991). When sturgeon larvae initiate feeding they possess an anatomically complete digestive tract (Buddington and Doroshov 1986c), and an enzyme complement which is similar to that

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of juveniles and adults (Buddington and Doroshov 1986a, b). As a consequence, white sturgeon larvae can be reared from the first feeding using formulated diets exclusively (Buddington and Doroshov 1984).

To data, only one biochemical study has been conducted to elucidate the possible relationship between the GIT morphological organization of larval white sturgeon and digestive physiology (Buddington and Doroshov 1986a). This previous study focused on alterations in the activity of proteases, lipases, and amylase. Unfortunately, the enzymatic assays, conducted on homogenized segments of digestive tracts, contained tissues from other organs as well as the GIT, thereby distorting the cellular sites of enzymatic activity. Furthermore, assays were performed on homogenates of pyloric and anterior intestines together obscuring possible functional differences between those two segments. Finally, enzymes associated with the brush border and cytoplasm of enterocytes, and their role in digestion and absorption, have not been investigated in white sturgeon larvae.

The importance of enterocyte enzymes in the final stages of digestion has been clearly demonstrated using histochemical methods in other teleost larvae (Evans and Ford 1976; Cousin *et al.* 1987; Segner *et al.* 1989; Verreth *et al.* 1992; Sarasquete *et al.* 1993). The principal advantage of enzyme histochemistry is that it allows precise tissue and cellular localization of activity while maintaining spatial tissue relationships. From initiation of exogenous feeding, the GIT histological development is followed and the role of different intestinal segments in digestive and absorptive processes is elucidated. Brush border and cytoplasmic enzymes are located, and ontogenetic changes, in both histology and enzyme activity, are described.

Materials and methods

Sturgeon larvae, produced after spawning induction (Doroshov *et al.* 1983) of a nine year old domestic female at the University of California, Davis, were used. Two days after hatching, 3,000 yolk-sac larvae were randomly transferred into three experimental tanks (1000 larvae per tank; Hung and Lutes 1987). Water temperature was kept between 15 and 17°C. Twelve days after hatching, larvae defecated melanin plugs and began to ingest Biodiet (BioProducts, Inc., Buhl, ID). This commercial semi-moist salmonid starter feed was used in the previous biochemical study on gastric and pancreatic enzyme secretions (Buddington and Doroshov 1986a). Biodiet (20% moisture, 43% crude protein, 17% lipid and 10% ash) was delivered automatically with rotating disc feeders at 2h intervals (Double A Brand, Dallas, TX). Larvae were fed at a rate of 20–25% of the tank's total biomass every 24h (Conte *et al.* 1988). Mortality was recorded daily and survival was 74.4% after the last sampling. Body mass increased from 34.2 ± 1.8 mg (11 days post-hatch) to 250.8 ± 84.5 mg at day 24 of exogenous feeding (36 days post-hatch).

Twenty larvae were sampled from each tank one day before initiation of exogenous feeding (day −1), and again on days 2, 4, 8, 16, and 24 of feeding. For high resolution light microscopy examination, 10 larvae were euthanized with tricaine methanesulfonate (MS 222, 90 mg l^{-1}), fixed in 10% buffered formalin, dehydrated in graded ethanol, and embedded in glycol methacrylate (GMA, JB-4 solution A, Polysciences Inc., Warrington, PA, USA). A total of 25 serial sections, 4 μm thick, were cut from each block using a LKB Historange microtome. Sections were mounted on glass slides (5 serial per slide), air dried, and stained with Mayer's alum hematoxylin and eosin (H&E).

For histochemical examination, 10 euthanized larvae were divided into three pieces with a new razor blade. Tissues were freeze-dried and processed for GMA embedding (Teh and Hinton 1993). Ten slides (3 sections of 4 μm thickness per slide) were used for histochemical tests of enzyme activities. Optimal conditions of incubation (substrate, temperature, pH, time) were adapted from Lojda *et al.* (1979), except for γ-glutamyl transpeptidase which was assayed according to Teh and Hinton (1993), and are presented in Table 1. Incubations were carried out at room temperature, except for lactase which was incubated at 4°C to prevent precipitation of the incubation medium. All substrates were purchased from Sigma (St. Louis,

Table 1. Summary of incubation conditions for enzyme histochemistry of the digestive tract of white sturgeon

Enzyme	Substrate	pH	Time (min)
Acetylcholinesterase (E.C. 3.1.1.7)	Acetylthiocholine iodide	5.5	60
Alkaline phosphatase (E.C. 3.1.3.1)	Naphthol-AS	9.1	45
Aminopeptidase M (E.C. 3.4.11.2)	L-Leucyl-MN*	6.5	60
Dipeptidyl peptidase II (E.C. 3.4.14.2)	Lysyl-alanyl-MN*	5.3	60
Dipeptidyl peptidase IV (E.C. 3.4.14.5)	Glycyl-prolyl-MN*	7.2	120
α-Galactosidase (E.C. 3.2.1.22)	1-Naphthyl-α-D-galactoside	5.0	120
β-Galactosidase (E.C. 3.2.1.23)	1-Naphthyl-β-D-galactoside	5.0	60
γ-Glutamyl transpeptidase (E.C. 2.3.2.2)	γ-L-Glutamyl-1-naphthylamide	7.4	30
Lactase (E.C. 3.2.1.23)	1-Naphthyl-β-glucoside	6.0	overnight
Nonspecific esterases (3.1.1)	1-Naphthyl acetate	7.3	60

*MN: 4-methoxy-2-naphthylamide

MO). After air drying, enzyme preparations for alkaline phosphatase and nonspecific esterase were counterstained with 1% Nuclear Fast Red for 40 s, while those for the other eight enzymes were stained with 1% Mayer's hematoxylin for 1 min. Controls included active sections incubated with substrate-free media and heat-inactivated sections reacted in complete medium. All controls proved to free of enzymatic activity.

Enzyme activity, evaluated using a semi-quantitative ranking of staining intensity, was recorded as weakly or strongly positive. The intensity of lipid absorption in the GIT was estimated by presence and size of unstained by H&E vacuoles, considered as fat lost during alcoholic dehydration and infiltration in GMA, a potent lipid solvent (Litwin 1985; Murray 1992). Our preliminary studies (data not presented) showed that the total extraction of lipids from GMA embedded sections caused a negative staining pattern of vacuoles with oil red O and PAS while in cryostat sections the same vacuoles showed positive reaction for oil red O and negative for PAS.

Results

Histomorphology

Our findings on general histological developmental features of sturgeon GIT closely resemble those previously described by Shmalgauzen (1968) and Buddington and Doroshov (1986c). However, as will become apparent, finer details of GIT development are observed and recorded in the present study.

On day −1, the GIT consists of an esophagus, intraembryonic yolk sac, and intestine (Fig. 1). The intestine is differentiated into three segments: pyloric, anterior, and spiral. There are multicellular gastric glands and large vacuoles in the yolk endoderm. Vacuoles of variable size are also present in the epithelium of the esophagus, anterior and distal spiral intestine, and liver. Enterocytes are mitotically active and the lumen of the spiral intestine is filled with a melanin plug. The mucosa of the pyloric intestine is organized into short, thick villi. Lightly eosinophilic zymogen granules are present in the exocrine pancreas.

On day 2, yolk is completely resorbed and the melanin plug is absent from the lumen of the spiral intestine (Fig. 2). The loop-shaped stomach is differentiated into two parts: a glandular portion with gastric glands and a nonglandular part with a prominent tunica muscularis. The latter is separated from the pyloric intestine by a muscular sphincter. Intestinal mucosal folds are lined by simple columnar epithelium with scattered goblet cells. Villi are short and large in the pyloric intestine, but only shallow rugae are seen in the anterior and spiral intestine. Enterocytes lining the pyloric intestine are tall and narrow, with basally-located nuclei and small supranuclear spaces while those in the anterior intestine are highly vacuolated and appear

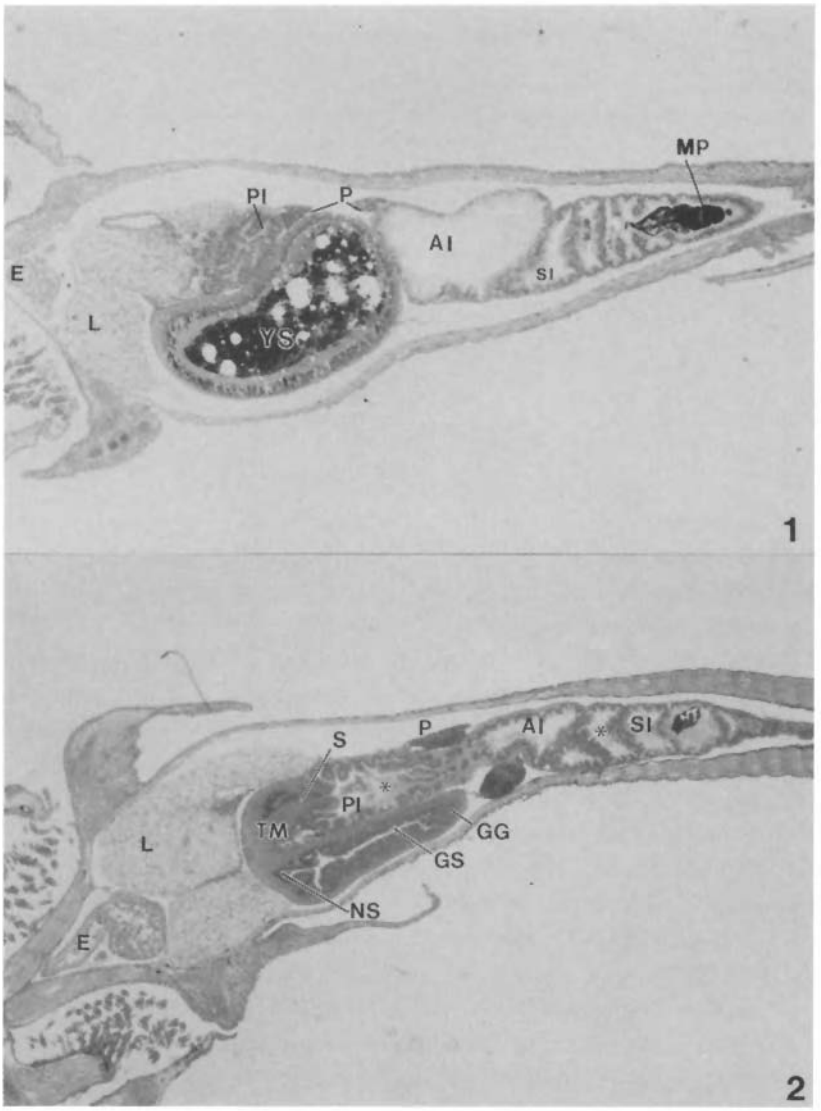

Fig. 1. Digestive system of white sturgeon larvae one day before initiation of exogenous feeding (day −1). Lumen of the stomach is filled with an intraembryonic yolk sac (YS) and that of the spiral intestine (SI) with a melanin plug (MP). Short mucosal folds are present in the pyloric (PI) and anterior (AI) intestines. Liver (L) is highly vacuolated. (E) Esophagus, (P) pancreas. H&E (×25).

Fig. 2. Digestive system of white sturgeon larvae on day 2 of exogenous feeding. Differentiation of the glandular stomach (GS) with gastric glands (GG) and nonglandular stomach (NS) with a large tunica muscularis (TM) is completed. Liver (L) remains vacuolated. Feed particles (*) fill the lumen of pyloric (PI), anterior (A) and spiral intestines (SI). (E) Esophagus, (P) pancreas, (S) sphincter. H&E (×25).

less differentiated. Enterocytes in the proximal spiral intestine are similar to those in the pyloric intestine, but possess prominent larger supranuclear vacuoles. Enterocytes in the distal spiral intestine are short with central nuclei. The liver parenchymal tissue is compact with poorly defined tubulation and highly vacuolated hepatocytes. Observed clear vacuoles resemble those of enterocytes and contain lipids as indicates positive oil red O staining done on cryostat section (data not presented). Prominent zymogen granules are observed in exocrine pancreatic cells.

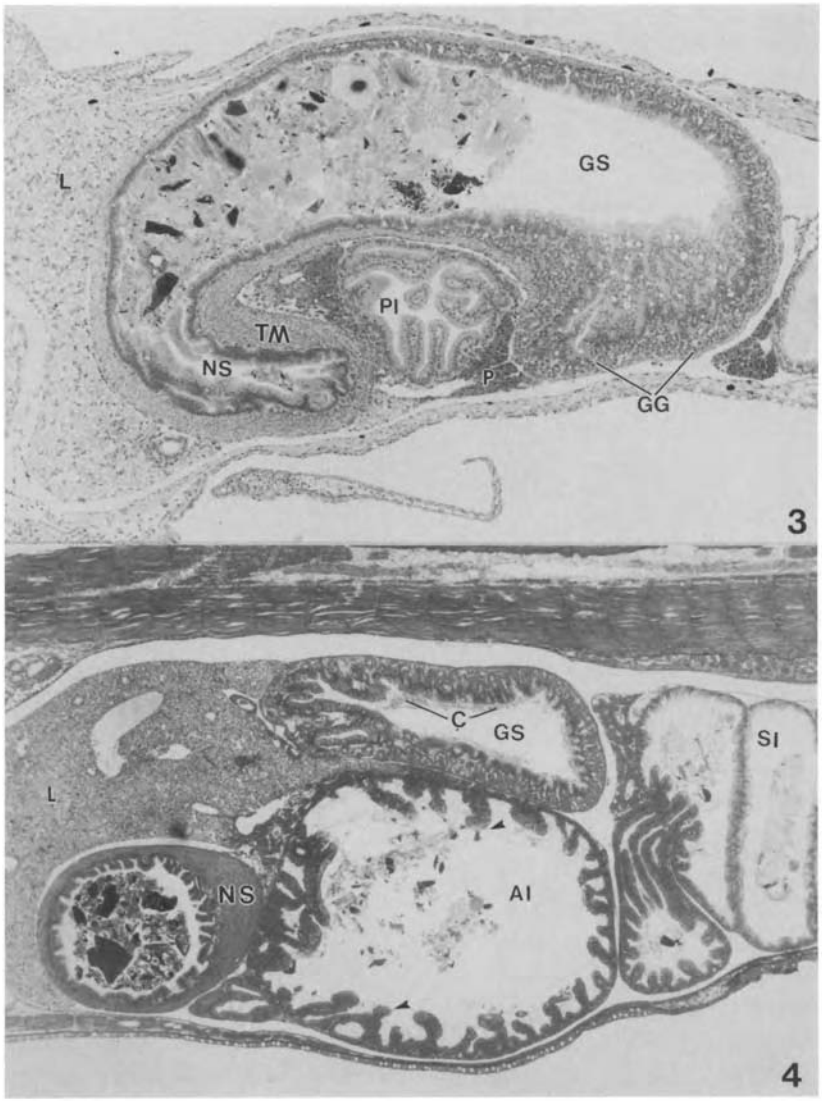

Fig. 3. Proximal portion of the digestive tract of white sturgeon larvae on day 4 of exogenous feeding. Gastric glands (GG) in the glandular stomach (GS) are more numerous and muscle layers (TM) of the nonglandular stomach (NS) are thicker. Mucosal folds in the pyloric intestine (PI) form blind sac. (L) Liver, (P) pancreas. H&E (×50).

Fig. 4. Proximal portion of the GIT of white sturgeon larvae after 24 days of feeding. Note secondary folds (arrowheads) in the anterior intestine (AI) and cilia (C) in the epithelium of glandular stomach (GS). Liver (L) achieves normal structure with less vacuolated hepatocytes. (NS) Nonglandular stomach, (SI) spiral intestine. H&E (×25).

On day 4, taller mucosal folds and proliferated gastric glands are observed in the gastric mucosa (Fig. 3). Muscle layers in the nonglandular stomach are thicker. Mucosal folds within the pyloric intestine are organized into blind sacs. The large vacuoles in enterocytes of the anterior and spiral intestines are no longer present, but are replaced by small supranuclear vacuoles. On day 8, stomach and liver are increased in size, and secondary folds are forming in the pyloric and spiral intestines. The mucosa of the pyloric and spiral intestines has a well-marked striated border and cilia. By day 16, liver parenchyma achieves normal tubular architecture. Hepatocytes reveal discrete cell margins, are

Table 2. Ontogenetic changes in histochemically demonstrated enzyme activities, as estimated by semi-quantitative ranking of staining intensity*, in the brush border and cytoplasm of enterocytes in the spiral, pyloric and anterior intestines of white sturgeon larvae

	Days of feeding					
	−1	2	4	8	16	24
Brush border enzymes in the spiral intestine:						
Alkaline phosphatase	w	s	s	s	s	s
Aminopeptidase M	w	s	s	s	s	s
Dipeptidyl peptidase IV	w	s	s	s	s	s
γ-Glutamyl transpeptidase	w	s	s	s	s	s
Lactase	w	w	w	w	w	s
Nonspecific esterase	w	s	s	s	s	s
Cytoplasmic enzymes in the pyloric intestine:						
Acetylcholinesterase	w	w	s	s	s	s
Dipeptidyl peptidase II	w	w	s	s	s	s
α-Galactosidase	w	w	s	s	s	s
β-Galactosidase	w	w	s	s	s	s
Nonspecific esterase	w	s	s	s	s	s
Brush border and cytoplasmic enzymes in the anterior intestine:						
Acetylcholinesterase	A	A	A	A	A	A
Lactase	A	A	A	A	A	A
Nonspecific esterase	A	A	A	A	A	A
Alkaline phosphatase	w	w	w	w	w	w
Aminopeptidase M	w	w	w	w	w	w
Dipeptidyl peptidase IV	w	w	w	w	w	w
γ-Glutamyl transpeptidase	w	w	w	w	w	w

*staining intensity ranking: A, absent; w, weak; s, strong

less vacuolated, and have centrally located nuclei. At this stage, the microanatomy of the GIT is identical to that observed on day 24 (Fig. 4).

Enzyme histochemistry

All ten enzymes show accurate localization of the final reaction product without apparent artifacts and diffusion of the final reaction product. Ontogenetic changes in enzyme activity in the brush border and cytoplasm of enterocytes in the spiral, pyloric and anterior intestines, as evaluated by their staining intensity, are summarized in Table 2. On day −1, weak activity of alkaline phosphatase, aminopeptidase M, dipeptidyl peptidase IV, γ-glutamyl transpeptidase, nonspecific esterase and faint activity of lactase are observed in the brush border of enterocytes in the proximal part of the spiral intestine (Fig. 5, 7). Enterocytes distal to the melanin plug remain unstained. An apparent increase in all brush border enzyme activities, except lactase, is noticed throughout the spiral intestine on day 2 (Fig. 6, 8), and in the pyloric intestine on day 4 (Fig. 9). Lactase activity remains weak until day 24, when it increases in the spiral intestine (Fig. 8b). At all stages, the anterior intestine shows weaker activity of brush border and cytoplasmic enzymes in comparison to the pyloric and spiral intestines (Table 2), and completely lacks acetylcholinesterase, nonspecific esterase (Fig. 9c), and lactase activity.

Activity of cytoplasmic nonspecific esterase increases on day 2 in the spiral intestine (Fig. 8c) while that of cytoplasmic acetylcholinesterase, dipeptidyl peptidase II, α- and β-galactosidases increases on day 4 mainly in the pyloric intestine (Fig. 10). In addition to mucosal intestinal activity, acetylcho-

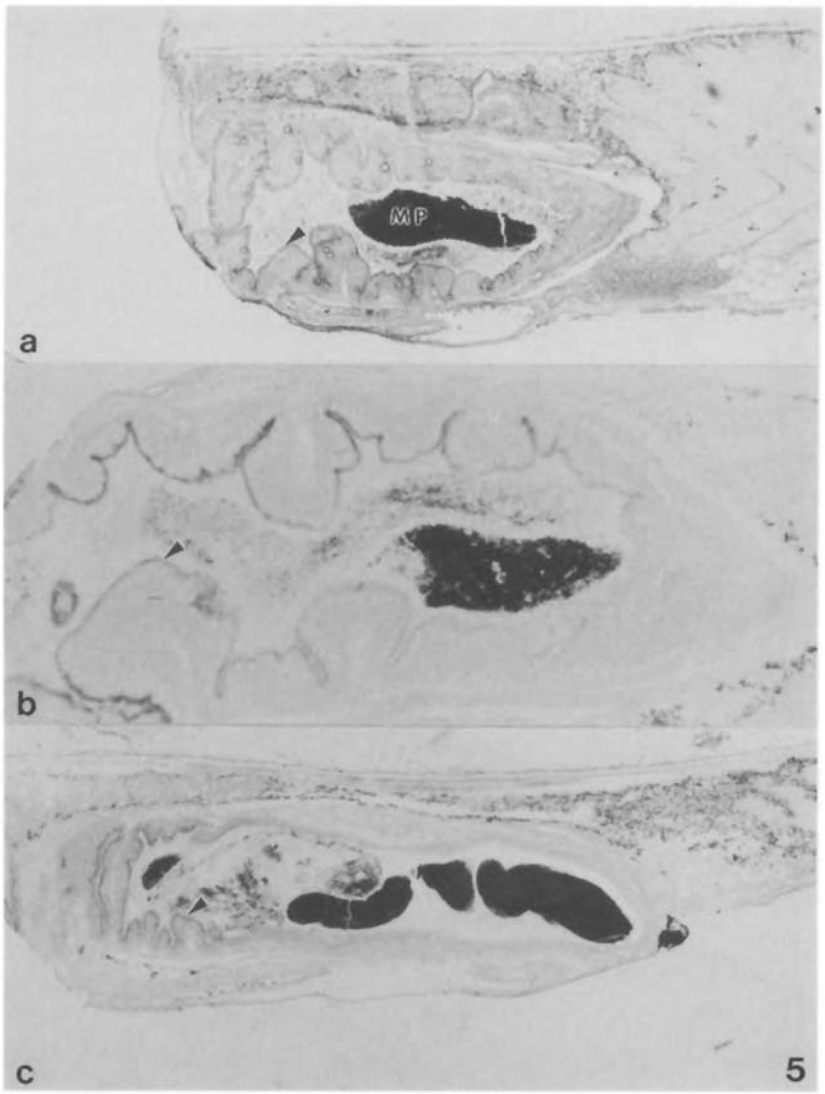

Fig. 5. One day before initiation of exogenous feeding (day −1) reaction products of: **a** – alkaline phosphatase (× 50), **b** – aminopeptidase M (× 125), **c** – dipeptidyl peptidase IV (× 50) is present as a narrow band (arrowheads) in the brush border of the proximal spiral intestine of white sturgeon larvae. (MP) Melanin plug.

linesterase displays strong activity in the motor end plates of the striated musculature around the pyloric intestine (Fig. 10a). Goblet cells are lactase (Fig. 7b, 8b) and dipeptidyl peptidase II positive (Fig. 10b). In the gastric region, weak activity of alkaline phosphatase is observed in the lamina propria and muscularis, while nonspecific esterase is strongly positive in the cytoplasm of both epithelial and gastric gland cells (Fig. 9c). Hepatocytes are negatively stained for γ-glutamyl transpeptidase, but positively for nonspecific esterase (Fig. 9c). The endothelium of sinusoids and large blood vessels shows alkaline phosphatase and acetylcholinesterase activity (data not shown). Exocrine pancreatic cells show strong activity of nonspecific esterase (Fig. 9c), but display only weak activity for alkaline phosphatase and lactase (data not shown).

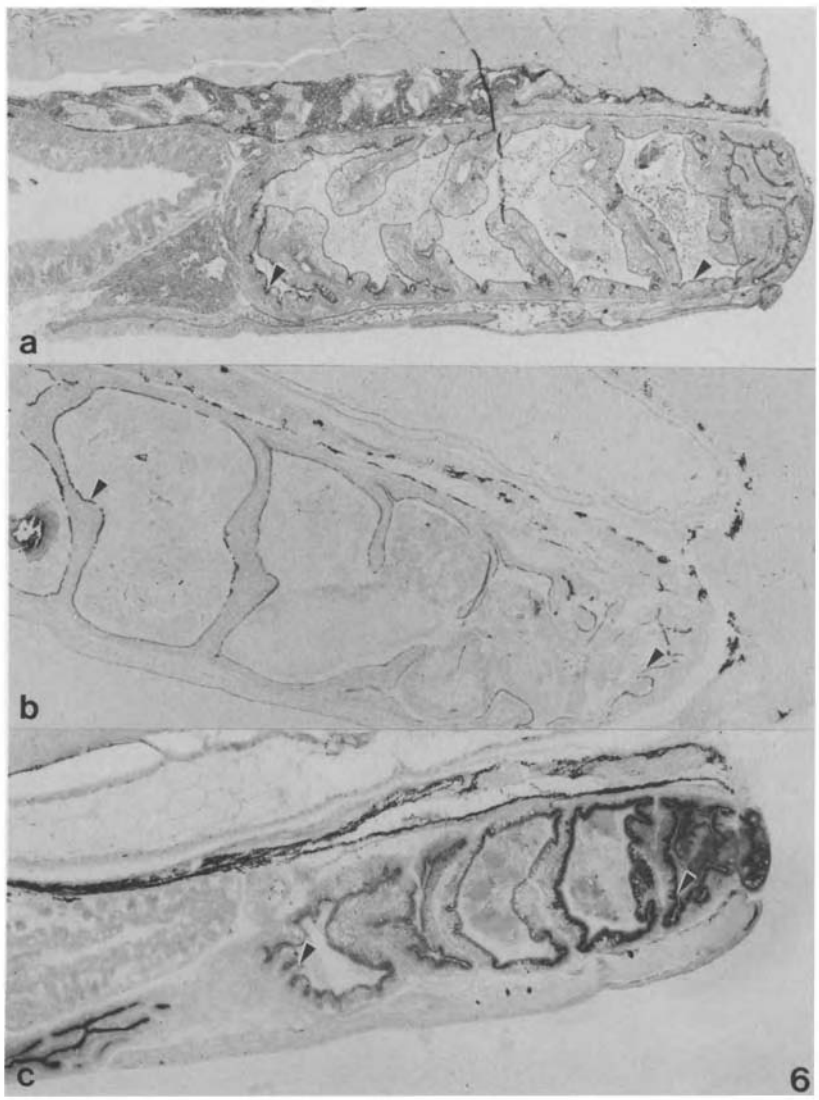

Fig. 6. On day 2 activity of brush-border membrane bound: **a** – alkaline phosphatase, **b** – aminopeptidase M, **c** – dipeptidyl peptidase IV is positively enhanced throughout the entire spiral intestine (arrowheads) (× 50).

Discussion

Our results suggest that 1 day prior to the onset of exogenous feeding, larvae were still using endogenous reserves. Vacuoles within endodermal cells of these 11-day old larvae lined the yolk sac and were in the mucosal epithelium of the esophagus, anterior and distal spiral intestines. This morphology is indicative of endocytotic absorption of yolk sac material (Heming and Buddington 1988). We believe, based on our findings, that the end of the endogenous phase is realized when extrusion of the melanin plug has occurred and full resorption of the yolk sac is apparent. When larval sturgeon initiate food ingestion, gastric glands are present and secreting pepsinogen and hydrochloric acid (Buddington and Doroshov 1986a). This gastric development, plus the numerous vacuoles in the GIT mucosa and differentiating histologic features of the pancreas and liver, suggest a functional alimen-

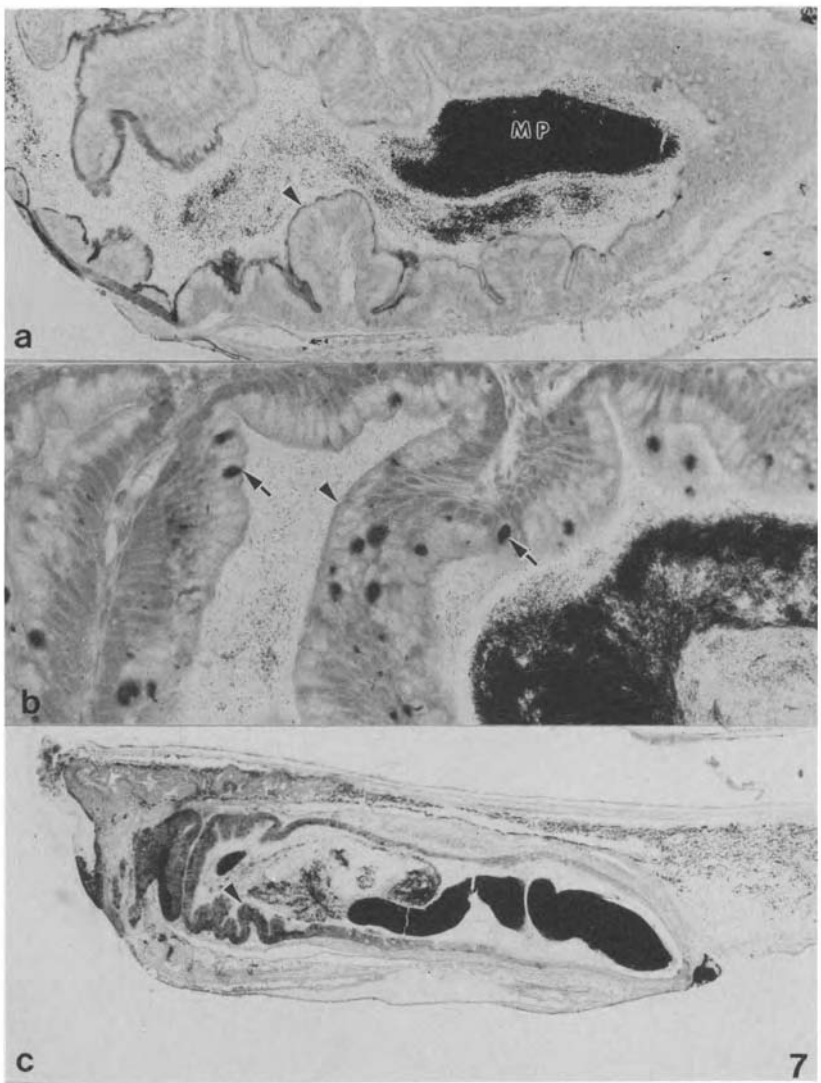

Fig. 7. The spiral intestine brush border on day −1 is weakly positive for: **a** − γ-glutamyl transpeptidase (×125), **b** − lactase (×250) and **c** − nonspecific esterase (×50). Arrows point to the goblet cell which are lactase positive. (MP) Melanin plug.

tary system at the time of first feeding. At the intracellular level, the functional development of the stomach is further demonstrated by the strong intensity of nonspecific esterase in the stomach epithelium (Ferraris *et al.* 1987; Verreth *et al.* 1992) which occurs before the onset of exogenous feeding.

Subsequently, the liver expands rapidly and is active in storage. Decreased hepatocyte vacuolation, observed from day 16, indicates possible functional changes in lipid metabolism or requirements. This observation is consistent with the decreased lipase activity reported to occur at the same stage of development in both white (Buddington and Doroshov 1986a) and lake sturgeon (Buddington 1985). The reduction in lipase occurred independently of the type of diet, and was probably determined by a decreased lipid requirement (Buddington and Doroshov. 1986a). The observed changes in the liver, however, may also be related to alteration in glycogen storage and utilization. Studies are in progress to elucidate the relationship between the lipid and glycogen utilization and deposition, and

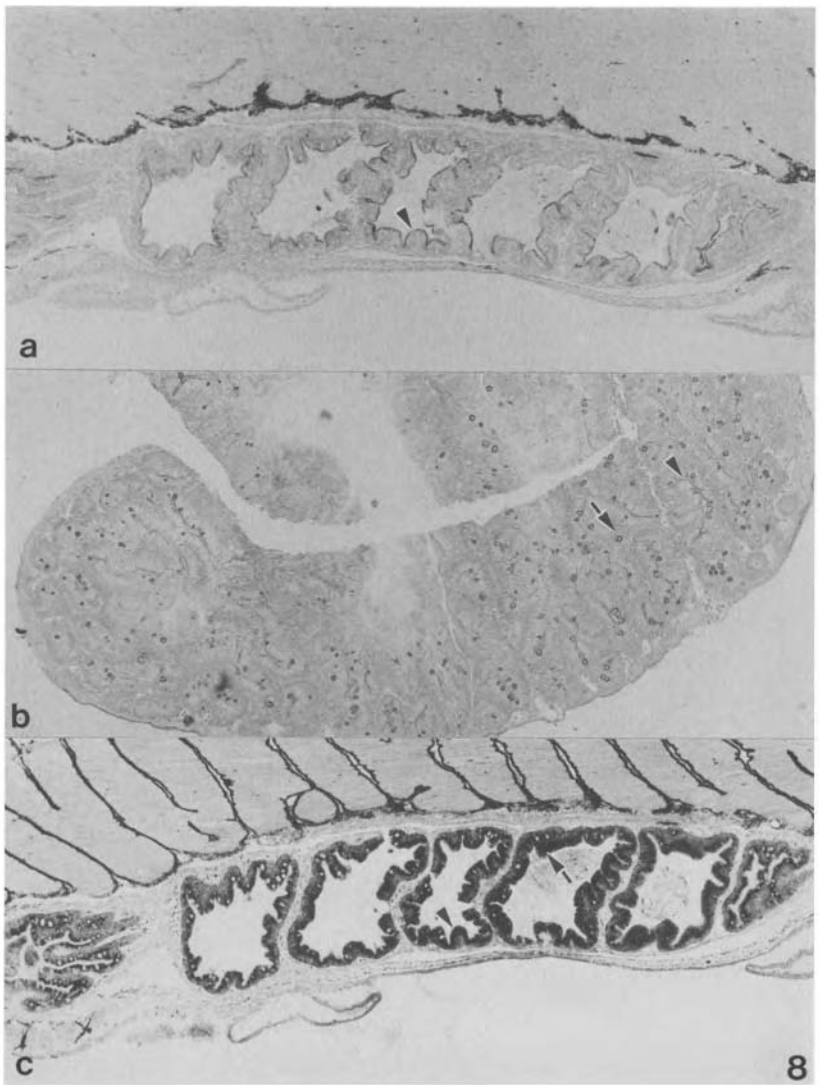

Fig. 8. An increased activity of: **a** – γ-glutamyl transpeptidase on day 2, **b** – lactase on day 24 and **c** – nonspecific esterase on day 2 is evidenced by the presence of stronger stained bound along the brush border of the spiral intestine (arrowheads). Arrows indicate lactase active sites in goblet cells and diffuse nonspecific esterase positive reaction in the apical portion of enterocytes (×50).

hepatic enzyme histochemistry with respect to different levels of dietary lipids.

Histochemical evidences, in the present study, show that the transition to active feeding coincides with an increase in enzymatic activity in the brush border of the spiral intestine. After 2 days of external feeding, nutrient absorption capacities are established as indicated by strong activity of alkaline phosphatase (Evans and Ford 1976; Stroband *et al.* 1979). Moreover, increased activity of aminopeptidase M, dipeptidyl peptidase IV, and γ-glutamyl transpeptidase confirms that sturgeon larvae possess the enzymes required to complete protein digestion (McDonald and Barrette 1980), and transport of amino acids through membrane (Tate and Meister 1974). These brush border enzymes and lactase have long been used as markers of enterocyte differentiation in mammals (Smith 1992). Full expression of these enzymes is indicative of complete differentiation of enterocytes. Thus, increased

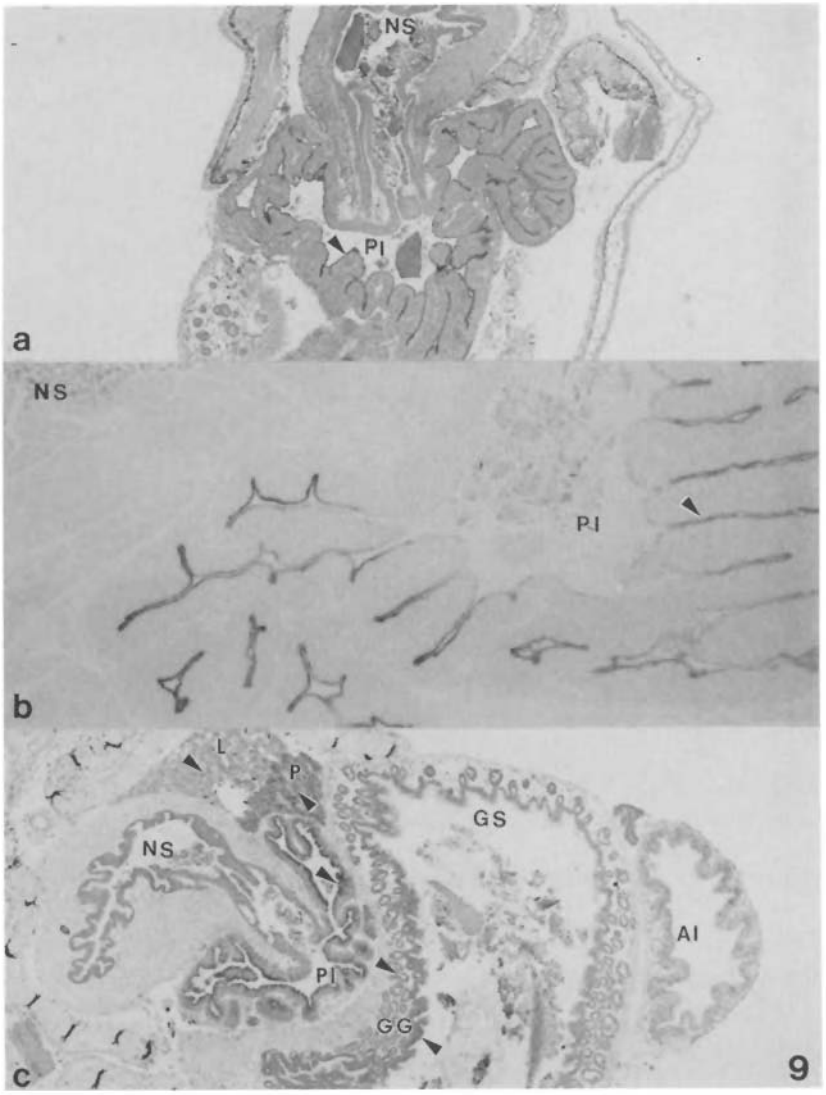

Fig. 9. Sections of GIT showing increased activity (arrowheads) of: **a** – alkaline phosphatase (× 50), **b** – amino peptidase M (× 125) and **c** – nonspecific esterase (× 50) in the brush border of pyloric intestine (PI). Reaction product of nonspecific esterase (**c**) is absent from the brush border of the anterior intestine (AI) and present in the pancreas (P), liver (L), gastric glands (GG) and epithelium of glandular stomach (GS). (NS) Nonglandular stomach.

brush border enzyme activity in the spiral intestine suggests that these enterocytes were functionally more developed than those in the anterior and pyloric intestine. This observation is supported by Shmalgauzen (1968) who reported on the asynchronous development of the digestive tract, and found the distal intestine to be differentiating first.

Deep, blind-ending sacs in the pyloric intestine effectively increase the absorptive surface area of the digestive tract (Buddington and Diamond 1986), and imply an attainment of the fully differentiated state for this part of the GIT. Based on our study, the functional development of the pyloric intestine occurred on day 4. At the same time, the pH of the stomach was reported to drop between 5.5 to 5.0, and the development of the gastric acid secretion was assumed to be complete (Buddington and Doroshov 1986a). The pH optima for the cytoplas-

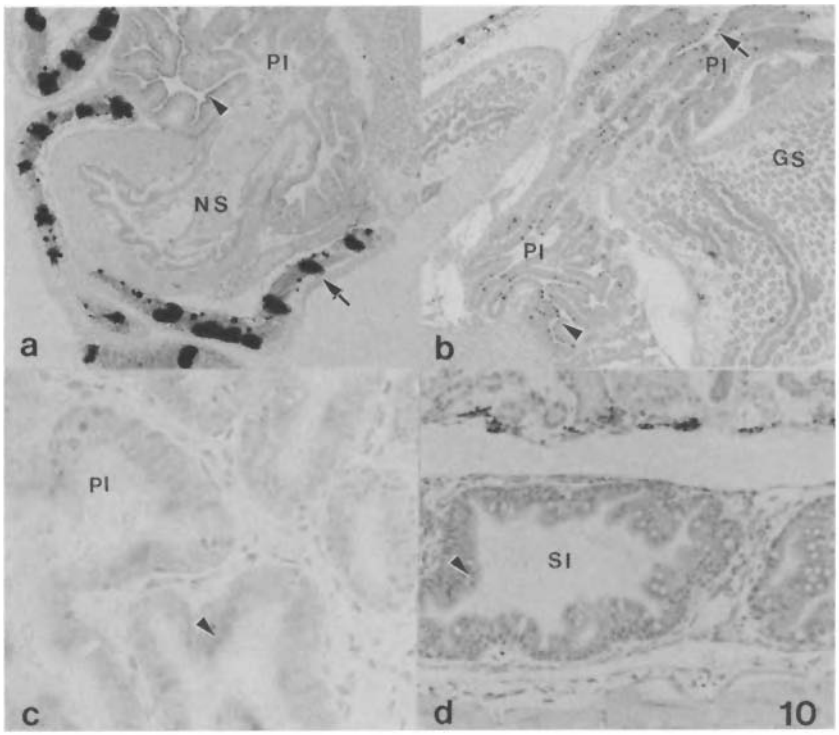

Fig. 10. Cytoplasmic enzyme activity in the pyloric (PI) and spiral (SI) intestines of white sturgeon larvae on day 4 of feeding. Apical cytoplasm of enterocytes shows strong activity (arrowheads) of **a** – acetylcholinesterase (×50), **b** – dipeptidyl peptidase II (×50), **c** – α-galactosidase (×250) and **d** – β-galactosidase (×50). Sites of positive reaction of dipeptidyl peptidase II (**b**) in goblet cells, acetylcholinesterase (**a**) in endplates are indicated by arrows. (GS) Glandular stomach, (NG) nonglandular stomach.

mic enzymes, such as acetylcholinesterase, dipeptidyl peptidase II, α- and β-galactosidases are also between 5.0 and 5.5 (Lojda *et al.* 1979). Thus, the functional development of the pyloric intestine coincides with the decreased pH of the chyme entering the pyloric intestine. The pyloric intestine plays an important role in neutralizing the acid bolus entering the intestine from the stomach (Buddington and Diamond 1987). This fact is supported by the absence of pyloric intestine in stomachless fish. The pleated folding of the pyloric intestinal mucosa may aid in the mixing of chyme with hepatic and pancreatic secretions as well as with mucus secreted by goblet cells. Increased secretion of mucus may be necessary to protect the pyloric wall against the acid chyme as well as to neutralize it (Garrido *et al.* 1993). The increased activity of acetylcholinesterase in the pyloric intestine also suggests intensive mucus secretion by goblet cells (Neutra *et al.* 1984), and coincides with increased activity of α- and β-galactosidases, which are possibly responsible for the degradation of mucopolysaccharides (Furth and Robinson 1965).

The functional development of the pyloric intestine is supported by an increase in the activity of nonspecific esterase associated with microsomes of endoplasmic reticulum as well as with various organelles such as Golgi apparatus, mitochondria and lysosomes (Deimling and Böcking 1976). The strong intensity of the enzyme suggests that this segment of the intestine may be important in the hydrolysis of carboxylic esters, which occurs mainly during lipid and carbohydrate metabolism (Deimling and Böcking 1976). However, these processes may not occur in the anterior intestine where activity of nonspecific esterase is absent. Moreover, low absorptive capacities of the anterior intestine are supported also by the lack of active acetylcholinesterase, lactase, and the presence of only a weak activity of exopeptidases and alkaline phosphatase.

These observations suggest that the anterior intestine of white sturgeon larvae, unlike what has been reported for other teleost fish (Cousin *et al.* 1987; Segner *et al.* 1989; Verreth *et al.* 1992), may be less important in nutrient absorption than the pyloric and spiral intestines.

The most unexpected finding in this study was the discovery of lactase activity in the exocrine pancreas, gastric and intestinal epithelial mucosa of white sturgeon larvae. Although lactase is thought to be specific to mammals, its activity has been detected in the intestine of adult common carp (Kawai and Ikeda 1971) and brook trout (Phillips 1969). The exact site of secretion of lactase has not been found in fish. Kapoor *et al.* (1975) suggest that lactase is secreted by the pancreas, whereas Dahlqvist (1964) indicates that major synthesis of this enzyme occurs in the intestine. In larval sturgeon, lactase appears to be synthesized by both the intestinal mucosa and exocrine pancreas. The role of lactase in the digestive system of fish is currently unclear. Since milk lactose is not a normal part of sturgeon diet, why is lactase present? Only a few studies have been conducted to evaluate the effect of lactose-rich diets on lactase activity in fish, and no data is currently available for sturgeon larvae. Juvenile white sturgeon fed a lactose-rich diet do not respond with increased lactase activity, and may even develop symptoms of lactose intolerance (Hung *et al.* 1989). Based on this study, it seems that lactase activity in sturgeon is insufficient to allow the use of lactose as a source of glucose. It is likely that even with the presence of some lactase activity, lactose is poorly metabolized by sturgeon and this may inhibit enterocyte migration rate as was observed in the neonatal rats (Smith 1992). It is known that lactase is a membrane-bound glycoprotein initially synthesized as a large precursor which is cleaved intracellularly before being anchored to the brush-border membrane (Naim *et al.* 1987). Thus, the time required for complete biosynthesis is considerably longer than that of other brush border enzymes (Smith 1992). This may explain the delayed increase in lactase activity when compared to other brush border enzymes on day 24. The presence of lactase in white sturgeon is intriguing and further studies are required to elucidate its role.

In conclusion, at the onset of exogenous feeding, the digestive system of white sturgeon shows a high degree of functionality. Further changes in activity of the intestinal enzymes follow the asynchronous development pattern with the spiral intestine first being developed. The strong intensity of brush border enzymes observed in the spiral intestine, from day 2 onward, tends to confirm the importance of this segment in nutrient absorption from the beginning of exogenous feeding. Further development is associated with differentiation of the pyloric intestine and is concomitant with an increase in brush border and cytoplasmic enzyme activity on day 4. Lower enzymatic activities in the anterior intestine and the absence of their ontogenetic increase may be indicative that this part of intestine is less involved than spiral and pyloric intestines in the digestive and absorptive processes. The quantitative and qualitative differences between the three segments of the intestine are indicative of a marked specialization of each of them for digestive and absorptive processes at the specific time of the ontogenetic development.

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