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Distribution of ferric iron in larval lampreys, *Petromyzon marinus L*

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Summary. The distribution and abundance of ferric iron in larval lampreys *(Petromyzon marinus L.)* were investigated using light microscopy and the Prussian blue stain. Animals from various watersheds contained different concentrations of iron, although the sites of deposition were the same for all animals. A major portion of iron is within adipose tissue, while the liver, and cartilage contain predominantly low to trace amounts of iron, respectively. Iron is associated with fibrous connective tissue in several places in the body, and this association may have particular significance in the inner ear. Iron is also located in cells of the meninges. The presence of iron in the epithelial cells of the posterior intestine may reflect elimination of the metal through the extrusion of iron-loaded cells into the intestinal lumen. Iron within mucous cells of the epidermis, suggest elimination of iron during mucous secretion. Iron-loaded cells of bipolar shape are also present in the epidermis, but are particularly prominent around the branchiopore. Low concentrations of iron are observed within in melanin-containing macrophages (melano-macrophages) in regions of iron absorption, erythrophagocytosis, and haemopoiesis. High levels of iron in the epithelia and lumina of pronephric tubules are concomitant with degeneration of this organ. These data are evidence of the wide spread distribution of iron in lamprey tissues and additional evidence for the potential value of lampreys for the study of iron metabolism in vertebrates.

Key words: Iron - Larval lampreys

Introduction

Lampreys *(Petromyzontiformes)* are one of the two extant representatives of the most primitive vertebrates, the agnathan or jawless fishes (Hubbs and Potter, 1971).

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Despite their primitive origins, living lampreys have many specialized morphological and physiological features (Youson, 1985). One of these features is their ability to tolerate high concentrations of serum and tissue iron at most periods of the life cycle. Even during the extended larval (ammocoete) period iron is already present in high concentrations. In the southern hemisphere lampreys, *Geotria australis* and *Mordacia mordax,* serum iron concentrations are >19.000 μg 100 ml^{-1} (Macey et al., 1982a, 1985) compared to only 127μg $100ml^{-1}$ in an average normal human male (Underwood, 1977). Similarly, a histological investigation of the above species revealed that ammocoetes contain large amounts of iron located within the intestinal mucosa and in other body sites, such as the fat column and nephric fold, both of which have abundant adipose tissue (Macey et al., 1982b). Biochemical analysis has also shown that iron concentrations within the ammocoete liver of *G. australis* and *M. mordax* are approximately two to three times greater than those normally found in humans (Macey et al., 1985).

Preliminary analyses suggest that iron concentration and sites of deposition may vary among lamprey species (Macey et al., 1982b; Macey et al., 1985; Macey and Potter, 1986). For example, larvae of *Petromyzon marinus* which were from Ocqueoc in the United States, do not possess any histologically-demonstrable iron (Macey et al., 1982b). However, biochemical experiments with ammocoetes of *P. marinus* captured in south-western England, revealed that the intestine contains relatively large amounts of non-haem iron which are slightly lower than values found for *M. mordax* (Macey et al., 1985). Iron is also present in substantial concentrations in the skin, alimentary canal, nephric folds, and liver of ammocoetes of *P. marinus* from New Brunswick, Canada (Sargent and Youson, 1986). Iron has not been reported in the skin of ammocoetes of other species, but is highly conspicuous in *P. marinus* (Youson and Sargent, 1984). The above results suggest that there may be a variable rate of uptake and tolerance of iron among lamprey species

and also that the environment in which the ammocoetes were found may have a profound influence on the iron levels within the body.

We are presently using ammocoetes from New Brunswick, Canada as an animal model for studying iron absorption, transport, and storage in vertebrates. Essential to our interpretation of data resulting from the experimental studies, is a thorough knowledge of normal sites of iron deposition. Although Macey et al. (1982b) have provided some documentation of the sites of iron accumulation within *P. marinus* from Ocqueoc in the United States, a thorough histological examination of all the iron storage sites within the entire body of ammocoetes of *P. marinus* from New Brunswick, Canada is absent from the literature. In the present study we used histological techniques to describe in detail all areas of iron deposition within ammocoetes of Nashwaaksis Stream in New Brunswick. Also provided is a qualitative evaluation of iron concentration in the most prominent sites and a comparison with sites of deposition of ammocoetes of other populations in New Brunswick.

Materials and methods

Larval lampreys (ammocoetes) of *Petromyzon marinus L.* were captured using an electroshocking device from the Nashwaaksis Stream, Dennis Stream, Jones Forks, and Keswick River of New Brunswick, Canada in May of 1982 and 1986. Larval lamprey were then transported to the laboratory at Scarborough Campus, University of Toronto and stored in fresh-water fiberglass aquaria with substrate from their sites of capture. A total of 19 ammocoetes of various weights (0.1 to 2.5g) and lengths (64-121 mm) were first anaesthetized in a 0.05% solution of tricaine methanesulphonate and then sacrificed by decapitation. The entire bodies of the ammocoetes were cut into 7 equal segments, immersed in Bouin's fluid or neutral buffered formalin for 24 hr, and then stored in 70% ethanol for various periods of time. There were no observable differences in iron distribution between the two fixatives, although Bouin's fixative yielded superior tissue preservation. Tissue samples were dehydrated in ethanol and cleared in Histoclear (Fisher Scientific), and then vacuum infiltrated and embedded in Tissue-Prep. Body segments were embedded so they would be transversely, serially sectioned at a thickness of 10μm. After mounting of the sections on glass slides they were stained for ferric iron with Perl's Prussian blue stain and counter stained with 3% eosin (Gomori, 1936). A control for ferric iron consisted of treating a tissue section known to have iron from previous observations with either 10% sulphuric acid or 5% oxalic acid (Lillie et al., 1963). The treated section was subsequently stained with Perl's Prussian blue. The amount of ferric iron present within the various parts of the body of ammocoetes was qualitatively evaluated in the light microscope using a scale from -to ++++, with -being nil, + low, ++moderate, +++high, and ++++ very high. Where individual variations were pronounced, a range of concentrations was provided.

Results

A comparison of the ammocoetes of varying weights and lengths revealed no pronounced differences in the abundance and distribution of iron within comparable tissues. Samples that were immersed in Bouin's fluid were compared with tissues preserved using 10% neutral buffered formalin. No differences in iron levels and locations were observed, and since tissue sections fixed within Bouin's yielded superior preservation, the remainder of samples were preserved in this fixative. No iron was observed in tissue sections after treatment with either 10% sulphuric acid or 5% oxalic acid and subsequent staining with Perl's Prussian blue. A comparison of tissue sections of ammocoetes from various watersheds in New Brunswick, Canada revealed similar sites of iron deposition. Of the ammocoetes sampled from Dennis Stream, Jones Forks, and Keswick and Nashwaaksis Stream, those from Nashwaaksis Stream showed the highest iron concentrations. Our subjective evaluation of iron concentration and deposition in ammocoetes of *P. marinus* was performed on animals from Nashwaaksis Stream.

Adipose Tissue

The major sites of deposition of adipose tissue in ammocoetes are the fat column, nephric fold, fat triangles, ventral connective tissue and the subcutaneous adipose tissue, while minor fat deposits are located lateral to the nerve cord and notochord, a ventral triangle, and within the myosepta (Youson et al., 1979). Ammocoetes contained adipose tissue in variable amounts, but in all samples iron was associated with this tissue (Figs. 1-5). Ferric iron was stained as a diffuse Prussian blue or as distinct iron granules. Adipose tissue was one type of tissue in which predominantly a granular form of iron was present and the distribution of the metal was dictated by the lipid content. For instance, iron levels within both the trunk and the tail regions were greater than those of the branchial-head region and this was mainly due to the higher concentration of adipose tissue in the former two regions. When the adipocytes had large lipid vacuoles, the iron granules were situated in the peripheral cytoplasm. If the lipid was present in small amounts, the iron granules were observed scattered throughout the cytoplasm (Fig. 1).

The fat column is a triangular deposit of adipose tissue located dorsal to the nerve cord. Originating in the cranial portion of the branchial cavity, the fat column extends in a posterior direction for the remaining length of the body. The amount of granular iron within the fat column was consistent throughout its length within each individual animal, but levels ranged from low to moderate in the ammocoetes examined (Table 1, Fig. 2). Another two triangular-shaped deposits of adipose tissue were located dorso-lateral to the posterior cardinal veins. These deposits in the dorso-lateral triangles

contained moderate levels of granular iron, and were continuous with narrow bands of adipocytes which encircled the notochord, nerve cord, and to a lesser extent, the fat column. These bands of adipose tissue contained low to moderate levels of iron. The iron content of a small amount of adipose tissue around the ventral half of the dorsal aorta was similar to that found in the dorso-lateral triangles.

Large deposits of adipose tissue are present in the nephric folds within the trunk region (Youson et al., 1979). In the anterior region of the trunk the adipose tissue is mainly concentrated in the dorsal part of the folds due to the presence of the ventrally-located opisthonephric kidneys. The amount of adipose tissue gradually increases posteriorly and eventually takes up the entire fold. The nephric fold contained moderate amounts of Prussian blue-positive granules throughout its length (Fig. 3, Table 1).

Adipose tissue in the subcutaneous layer below the dermis also contained iron (Fig. 5). In the branchial region, iron was present in this tissue at low to moderate levels. In some ammocoetes, iron within subcutaneous adipose tissue increased from low levels in the branchial region to moderate concentrations in more posterior regions (Table 1). Iron granules were also located within the adipose and connective tissues of the myosepta at low to moderate concentrations in all regions but the branchial region (Fig. 4, Table1). Cross sections of the tail revealed moderate levels of iron associated with the adipose tissue of the ventral connective tissue (Fig. 1).

Epidermis

There was a trend for greater amount of ferric iron within the epidermis in areas posterior to the branchial region of ammocoetes (Table 1). In all body regions with the exception of the tail, ferric iron deposits were observed within the dorsal and lateral epidermis but not the ventral epidermis (Table 1). However, posterior to the cloaca iron was present in the epidermis at all surfaces (Table 1). Within the posterior trunk and tail areas, iron staining within the ventral epidermis was never above the moderate level but high levels were present within the dorsal epidermis. The mucous cells, and not the granular and skein cells, contained iron (Fig. 5) and iron-containing mucous cells were predominantly located within the base of the epithelium. Only a few superficial mucous cells contained the metal. The collagenous dermis, immediately below the epidermis, did not contain iron in any of the ammocoetes sampled.

High concentrations of Prussian blue stain were detected in an unknown cell type which was randomly distributed in the epidermis. These epidermal cells were bipolar to oval in shape and varied in size but were large enough to extend the entire height of the epidermis (Fig. 6a,b). The majority of the bipolar-shaped cells were located within the epidermis surrounding and between the branchiopores. These cells were less frequently encountered within other regions of the ammocoetes, but when found, were equally distributed among dorsal and ventral epidermis.

Organs of the anterior and posterior trunk

Heart. The heart is suspended in the cranial portion of the coelom, and consists of a sinus venosus, atrium (auricle), ventricle, and bulbus arteriosus (Fänge, 1972). Diffuse ferric iron of low concentrations was associated with the cardiac muscle of the atrium (Fig. 7, Table 1). It could not be ascertained whether iron deposition was located internal or external to the sarcolemma of cardiac muscle fibres. Iron was also present in melanin-containing macrophages which appeared to be associated with the walls of the atrium (Fig. 7). The more muscular ventricle of the heart contained only trace amounts of iron.

Pronephric and opisthonephric kidneys. The pronephros, located in the anterior portion of the coelomic cavity, contained high to very high levels of iron within both the epithelia and lumina of the majority of pronephric tubules (Fig. 8, Table 1). A few tubules possessed iron of nil to moderate concentrations within their epithelia and lumina. Often the granular iron deposits were large enough to completely occlude the lumina of some pronephric tubules which appeared to be in a state of degeneration. Tubules which did not have occluded lumina, had large granules of iron within their epithelial cells. The most proximal regions of nephrostomes occasionally showed trace amounts of diffuse iron on the apical portion of their many cilia.

The larval opisthonephric kidneys were located in the nephric folds posterior to the pronephros. There was no iron associated with opisthonephric tubules or renal corpuscles (Fig. 3, Table 1), although occasionally a low amount of diffuse iron was located in macrophages containing melanin observed with the sinusoids of the intertubular areas (Fig. 11).

Liver. Unlike the adult lampreys where bile ducts are absent, the liver of ammocoetes is equipped with a biliary tree for the efficient elimination of bile (Youson, 1981a). The hepatocytes are concentrically arranged around bile canaliculi, forming tubules of liver parenchymal cells. The livers of ammocoetes contained variable amounts of ferric iron (Table 1). Ammocoetes demonstrated nil to low iron concentrations with most of the metal restricted to a small number of hepatocytes. Diffuse Prussian blue staining of the portion of the cytoplasm of hepatocytes which bordered a canaliculus was detected in one ammocoete with moderate iron levels. In this latter situation, a larger proportion of the hepatocytes contained iron (Fig. 9). The Kupffer cells (macrophages) within the sinusoids, and the epithelia and lumina of the gallbladder and the bile ducts contained no stainable iron. However, Prussian blue staining of the connective tissue surrounding the common bile duct was observed (Fig. 10).

Alimentary canal. The alimentary canal of ammocoetes

consists of the branchial cavity or pharynx, an oesophagus, an anterior intestine, a posterior intestine, and a hindgut which terminates at the cloaca (Youson, 1981b). At the junction of the oesophagus and the anterior intestine near the caudal tip of the liver a large lateral intestinal fold arises, and is referred to as the typhlosole. This structure is present until the end of the posterior intestine. Within the typhlosole there is haemopoietic tissue. The levels of iron varied dramatically throughout the length of the alimentary canal.

Iron was not detected in the mucosa and the connective tissues of either the lamina propria and submucosa of the oesophagus (Figs. 8,10). No iron was observed within the mucosa of the anterior intestine, but iron containing macrophages, which contained melanin pigment as well, were observed within the haemopoietic tissue of the typhlosole. These macrophages possessed low levels of iron (Table 1) and were also found within the lumina of blood vessels in the serosa. In the transitional region, between the anterior and posterior intestines, small amounts of granular iron were located within the mucosal cells at the top of the typhlosole and in the opposing intestinal wall (Fig. 11). The distal regions of the posterior intestine contained more iron within the mucosa than that observed in the more proximal locations, and iron levels were maximal in the area of the posterior intestine which was located at a point in the trunk where the opisthonephric kidneys had terminated (Fig. 12). Most ammocoetes contained high iron levels within epithelium at the top of the typhlosole and in the opposing wall of the distal region of the posterior intestine (Table 1). Iron was not observed in areas near the base of the typhlosole, but there was a slight gradation of staining intensity from most intense at this top of the fold to less intense along the sides (Fig. 12). A similar gradation was present on the opposing wall. Also, there was a very high Prussian blue staining of feces within the lumen of the posterior intestine (Fig. 11). Macrophages containing pigment were located in the typhlosolar haemopoietic tissue, in similar arrangement and frequency as observed in the anterior intestine (Fig. 11). In the transition zone between the posterior intestine and the hindgut, the typhlosole and its associated haemopoietic tissue was reduced in size, until within the region of the hindgut, neither structure was present. With the gradual reduction of the typhlosole, there was usually a concomitant loss of iron within the intestinal mucosa on all surfaces. Some ammocoetes possessed low levels of iron within the mucosa in the most anterior locations of the hindgut (Table 1).

Head and branchial regions

Gills. Mucus-containing, goblet cells at the base and top of the gill filaments and lining the pharynx possessed trace amounts of diffuse iron (Fig. 13). Medial to these goblet cells, nil to low levels of diffuse ferric iron were observed in melanin-containing macrophages within the cavernous body of gill filaments (Table 1, Fig. 13).

Fibrous connective tissue. A muscular and highly vascularized velum is located at the opening into the branchial cavity. A few macrophages and adipocytes within the velum contained granular iron deposits, while a diffuse iron stain of moderate intensity was also observed associated with loose fibrous connective tissue (Table 1, Fig. 14). Other areas in which connective tissues contained iron were below the dermis in regions which bridge the branchiopores, and in the perichondrial layer surrounding some branchial cartilage. Trace amounts of iron were present within chondrocytes of some cartilage located in the branchial region and also the dorsal fin (Fig. 15). The connective tissue which surrounds the blood vessels of the endostyle also possessed a low amount of iron in some, but not all, ammocoetes.

Miscellaneous. Iron was associated with meningeal tissue which covered the external surface of the brain and the dorsal surface of the spinal cord. However, there were morphological differences between these two regions of meningeal tissue. Lateral to the brain, low to moderate levels of diffuse and granular iron were located within cells of the meningeal tissue (Table 1, Fig. 16). Occasionally, large iron granules within the meningeal tissue were observed surrounding nerve fibres which originated within the brain (Fig. 16). Dorsal to the spinal cord, meningeal cells contained slightly higher levels of iron than the equivalent cells within the meningeal tissue of the brain area (Fig. 2, Table 1). The iron was in both a diffuse and granular form (Fig. 17).

The ear of the ammocoete is surrounded by a cartilaginous labyrinth in which the sensory structures can be found. This cartilaginous auditory capsule is lined by a ciliated epithelium and is partially subdivided into the anterior and posterior ciliated chambers. Connected to these ciliated chambers are the anterior and posterior ampullae, in which the neuroepithelium, of the cristae, are found. The sensory cells lie on a basement membrane and their ciliary processes are embedded into the jelly-like cupula (Lowenstein et al., 1968). Small iron granules were intertwined with the cilia of the ciliated chambers (Fig. 18). Also low Prussian blue staining of the connective tissue, in which the sensory cells of the cristae are embedded, was usually detected (Fig. 18). A single macula, located at the base of the labyrinth and covered by a mass of calcareous otolithic crystals, showed no Prussian blue staining.

Fig. 1. In the ventral connective tissue iron granules (arrows) are present in the peripheral cytoplasm of adipocytes with large vacuoles (V). Caudal vein (cv); M, muscle; P, pigment. × 600. Inset: iron granules (arrowheads) within the cytoplasm of adipocytes with no pronounced vacuoles. × 595.

Fig. 2. Both the fat column (F) and cells in the meninges (arrows) contain iron. Spinal cord (Sc); pigment (P); muscle (M). × 170. Inset: granular iron deposits are distributed in the cytoplasm of adipocytes of the fat column. × 595.

Fig. 3. Dorsal region of the opithonephric kidney showing granules of iron in the adipose tissue (arrows), but no iron in kidney tubules (T) and the intertubular sinusoids (S). Pigment (P). × 525.

Fig. 4. Iron (arrows) in the connective tissue of the myosepta within musculature (M) of the body wall. × 600.

Fig. 5. Dorsolateral epidermis in the anterior trunk region. Ferric iron is predominantly located within basal mucous cells (Mu), although some superficial mucous cells contain iron (arrowhead). No iron is associated with granular (G) or skein cells (S), and the collagen of the dermis (D). Pigment (P) and iron granules (arrows) are located in the subcutaneous adipose tissue. × 480.

Fig. 6. Bipolar cells in the epidermis possessing abundant iron but absence of the metal in mucous (Mu) and granular (G) cells and in the collagenous dermis (D). **a.** The lateral epidermis in the bridge between adjacent branchiopores. P, pigment. × 410. **b.** Ventral epidermis of anterior trunk region with iron granules (arrowheads) in subcutaneous adipose tissue. × 1030.

Fig. 7. The heart contains low levels of diffuse iron associated with the cardiac muscle (M) and with melanin-containing macrophages (arrowheads) of the atrium (A). × 130.

Fig. 8. Some pronephric kidney tubules (T) contain high amounts of iron globules within the epithelia. No iron is associated with the oesophagus (O), nephrostome (N), and intertubular sinusoids (S). Pigment (P). × 285. Inset: high concentrations of iron occlude the lumina of kidney tubules (T) which appear to be in a state of degeneration. × 220.

Fig. 9. Hepatocytes of the liver contain moderate amounts of iron (arrows) around the bile canaliculus (arrowhead). Bile ducts (B) do not contain iron. Pigment (P). × 430.

Fig. 10. Ferric iron of moderate concentrations is present within fibrous connective tissue surrounding the common bile duct (B) but no iron is located within tissues of the oesophagus (O) or the anterior mesenteric artery (A). × 350.

Fig. 11. Iron within the mucosal cells (large arrow) of the top of the typhlosole (T) and the opposing intestinal wall (arrowhead) in the proximal region of the posterior intestine. Intense Prussian blue staining of feces is observed in the intestinal lumen (L). Low levels of iron (small arrows) are present within melanin-containing macrophages of haemopoietic tissue (H) of the typhlosole and sinusoids of the opisthonephric kidney (K). Pigment (P). × 120.

Fig. 12. Mucosa (Mu) of the distal region of the posterior intestine at the top of the typhlosole (T). The concentrations of iron within mucosal cells reduces in areas lateral to the top of the typhlosole (between two large arrows). Note iron granules within the intestinal lumen (arrowheads) and their association with debris × 1160.

Fig. 13. Low levels of diffuse iron in mucus-containing goblet cells (G) at the top of the gill filament. Iron (arrows) is also associated with melanin-containing macrophages within the cavernous body. × 470.

Fig. 14. Diffuse iron stain is associated with the fibrous connective tissue of the velum (V) while granular iron deposits are found in macrophages (arrows) in close proximity to a blood vessel (BV). × 115.

Fig. 15. Fibrous connective tissue (F) containing diffuse iron is located adjacent to branchial cartilage (Bc). Melanin pigment (P) and iron granules (arrows) within adipose tissue of low lipid content are also observed. × 295. Inset: iron granules in perichondrium (arrow) and chondrocytes (arrowhead) of branchial cartilage. × 135.

Fig. 16. Iron granules (arrowheads) and diffuse iron stain (small arrow) are present in cells of the meninges lateral to the brain (B). Iron granules are also associated with a nerve which is connected to the brain (large arrow). The space between the brain and the meninges is an artifact. Pigment (P). × 460.

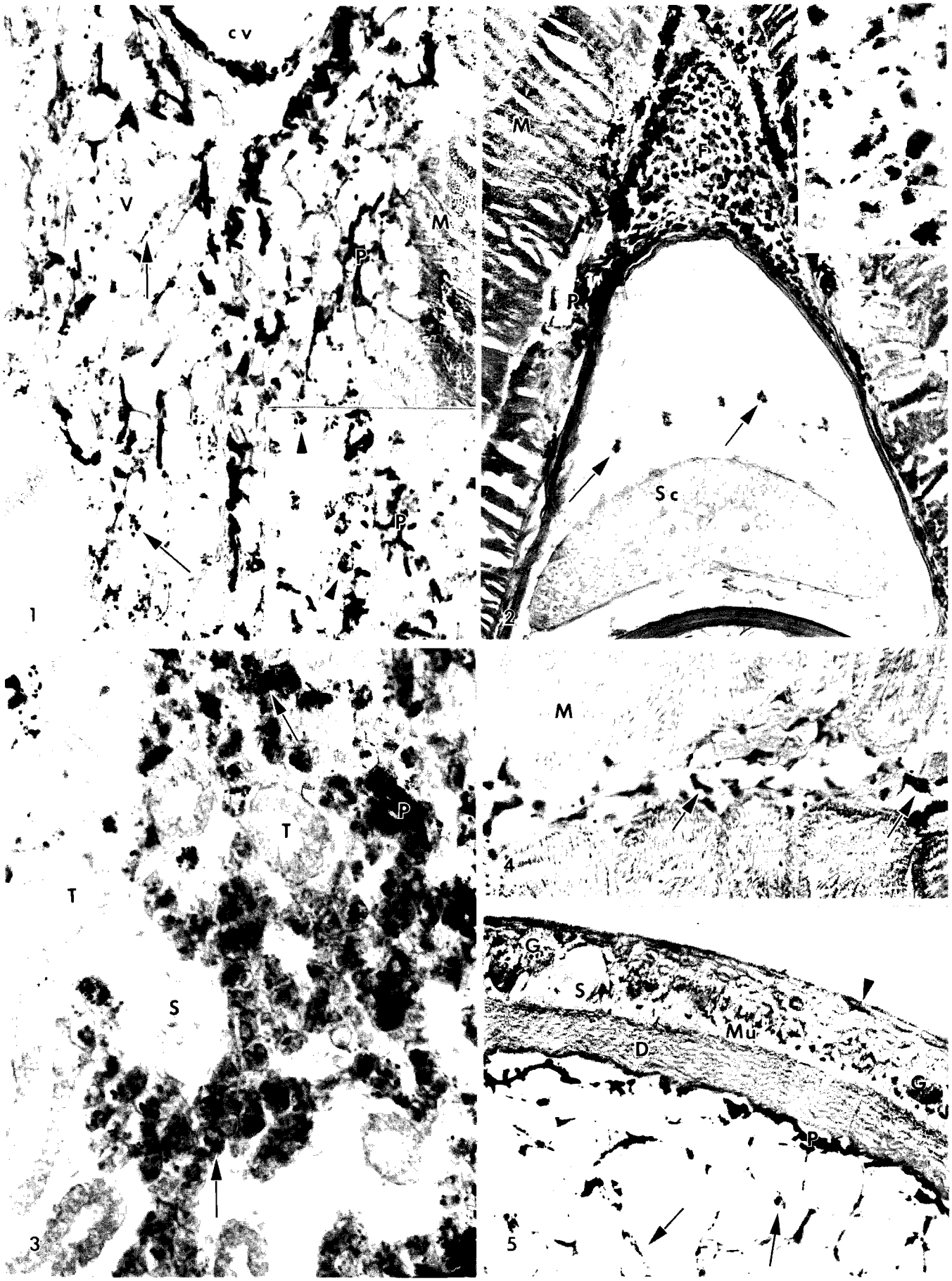
Fig. 17. Dorsal to the spinal cord (Sc), granular and diffuse iron (arrows) are located in cells of the meninges. The fibrous connective tissue (cT) does not stain for iron. × 460.

Fig. 18. Cilia (cl) of the ciliated chambers of the ear are coated with iron granules of variable sizes (arrow). No iron is present within cartilage of the auditory capsule (Ac). × 300. Inset: the connective tissue of the crista contains iron, but not the neuroepithelium (NE). × 650.

Table 1. Relative abundance and distribution of ferric iron within the head-branchial, anterior trunk, posterior trunk-tail regions of ammocoetes, *Petromyzon marinus* L.

Tissues sampled	Head - branchial	Anterior trunk	Posterior trunk - tail
Epithelium			
–dorsal epidermis	+/++	+/+++	++/+++
–ventral epidermis	–	–	+
–anterior intestine	–	–	
–posterior intestine			++/+++
–hindgut			–/+
–pronephros		+++/++++	
–opisthonephros		–	
–liver		–/++	
Macrophages			
–cavernous body	–/+		
–heart		+	
–liver		–	
–opisthonephros		+	+
–typhlosole		+	+
Adipose tissue			
–subcutaneous	+/++	++	++
–myosepta		+/++	+/++
–fat column		+/++	+/++
–dorso-lateral		+/++	+/++
–nephric fold		++	++
–ventral connective			++
Fibrous connective tissue			
–velum	++		
–endostyle	–/+		
–bile duct		++	
Miscellaneous			
–meninges	+/++	++	++
–ear	+		

– nil
+ low
++ moderate
+++ high
++++ very high
/ range of values within group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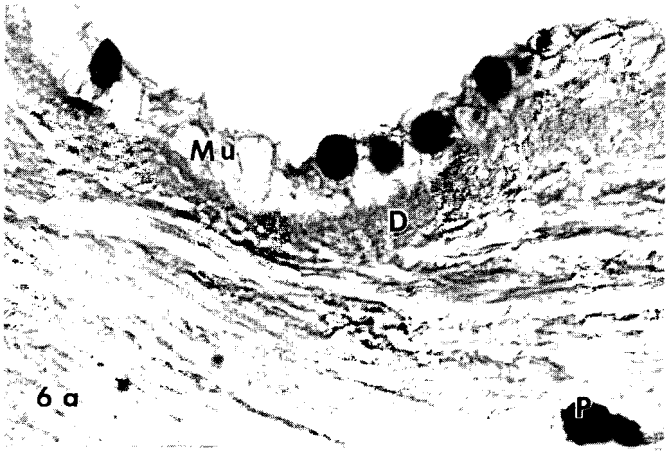

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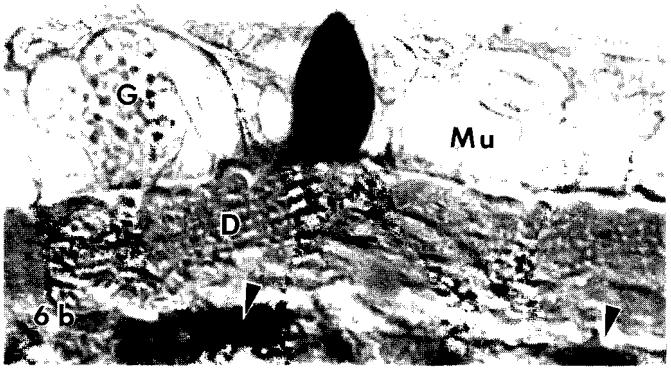

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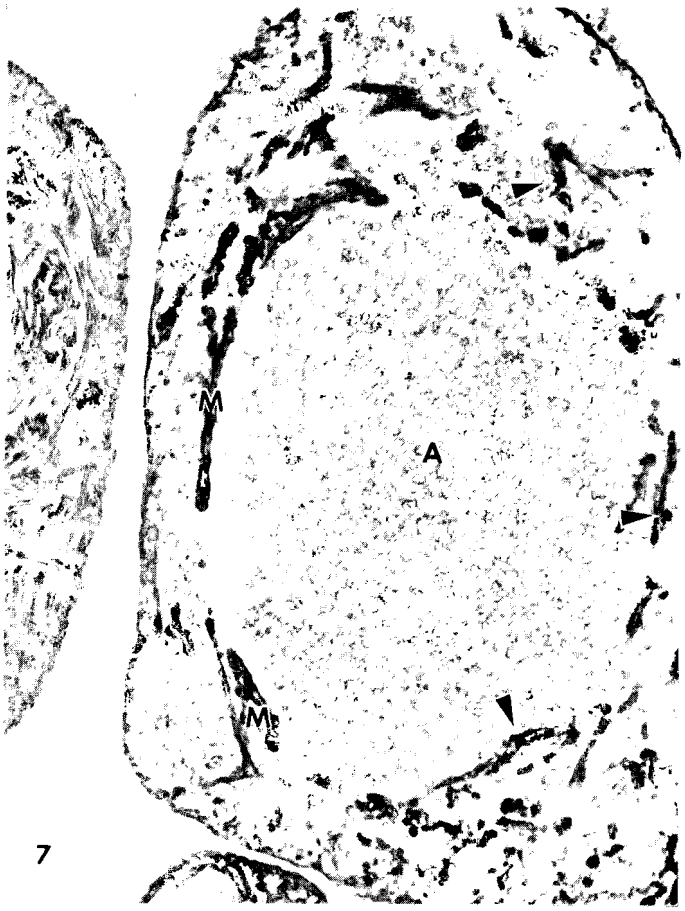

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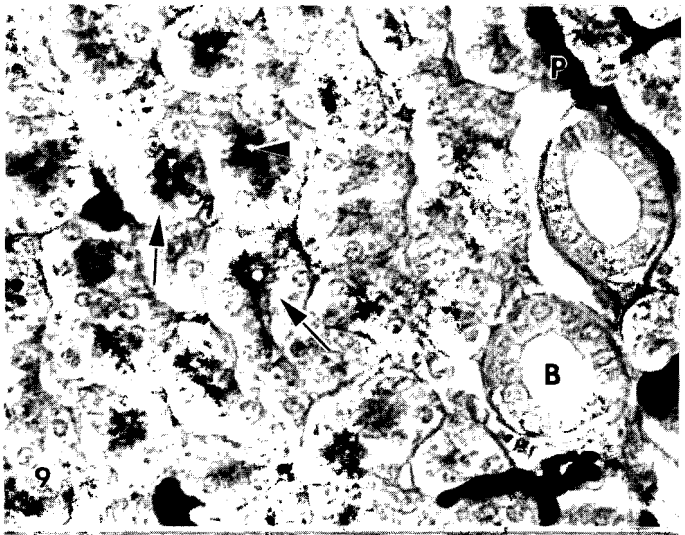

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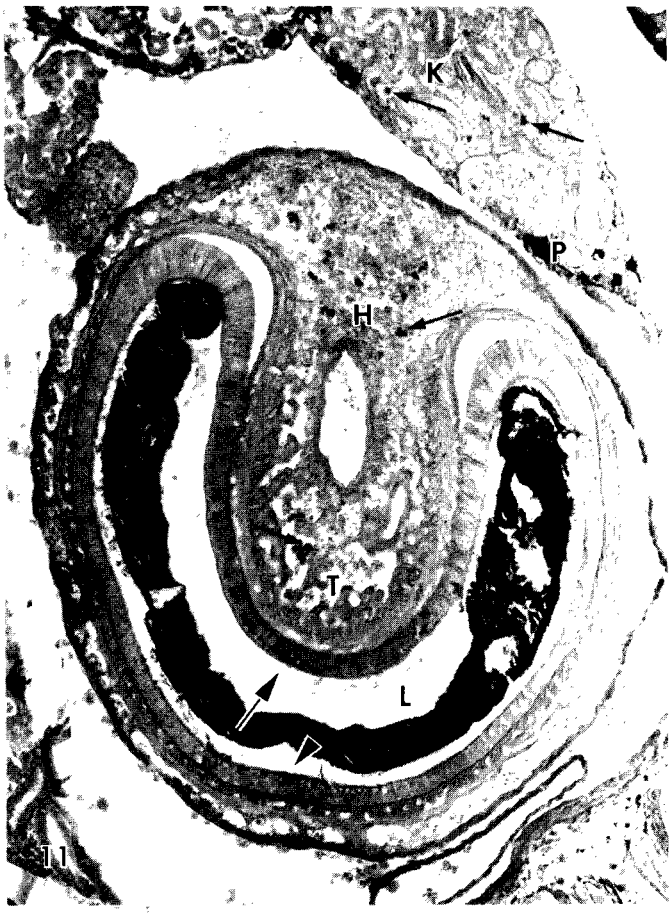

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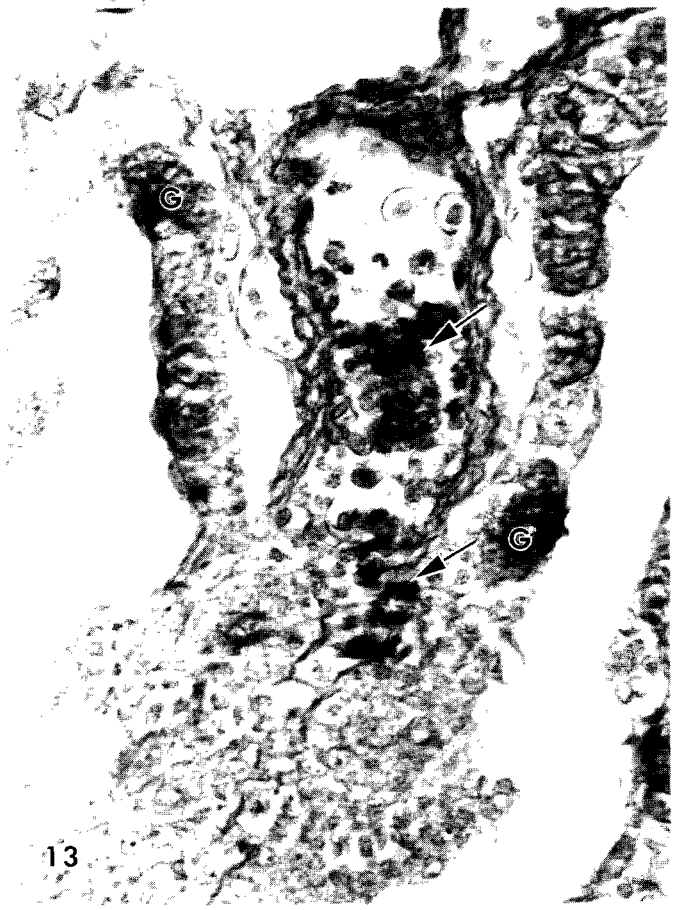

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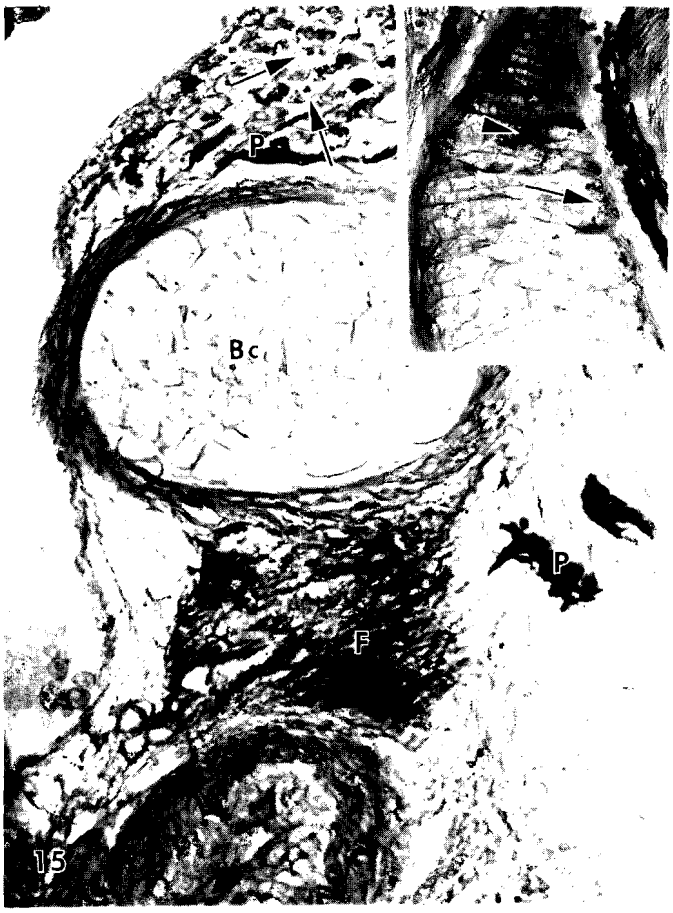

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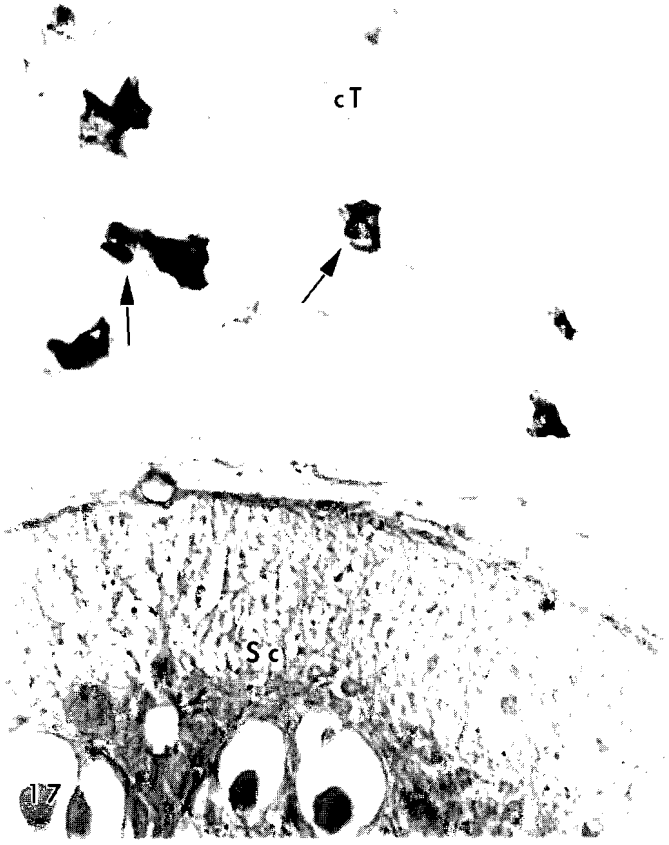

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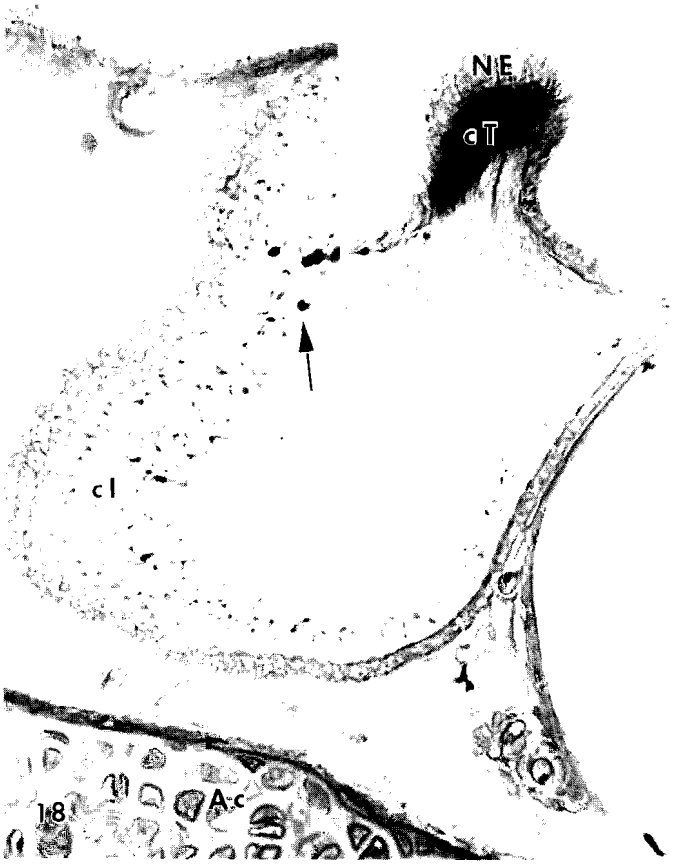

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Discussion

It is apparent from the data presented, that ammocoetes of *Petromyzon marinus* from New Brunswick, Canada contain Prussian blue iron within various organs and tissues, and that many of the sites of deposition, and levels of iron accumulaton are different from those reported in a similar study on ammocoetes of various northern and southern hemisphere species (Macey et al., 1982b). Most notable among these differences is the complete absence of stainable ferric iron in the tissues of ammocoetes of *P. marinus* from Ocqueoc, U.S.A. It has been proposed that iron levels in the habitats in which lampreys are captured, may influence the iron concentrations within these animals (Macey et al., 1982). It is possible that environmental factors, such as the levels of iron within the water and substrate would affect the amount of iron present in ammocoetes from remote watersheds. Further information is required in order to assess the bioavailability of environmental iron, and its relationship to tissue iron levels within ammocoetes.

Biochemical studies using ammocoetes of *Geotria australis* have shown that once a body weight of 0.1-0.2g is obtained, there is only a slow and small rise in concentrations of total non-haem and haemosiderin iron, while ferritin iron concentrations remain similar throughout larval life. Prior to the above body weights, a rapid increase in the concentration of ferritin, total non-haem and haemosiderin iron occurs (Macey et al., 1985). Our histological observations on *P. marinus* support the above data, since very little variation in iron levels were observed in ammocoetes ranging in weight from 0.1-2.5g.

Adipose Tissue

It has been shown in the present study that all adipose tissue within ammocoetes of *P. marinus* contains iron granules, and that this component represents a large proportion of total body iron. As a result of their size, the fat column and nephric folds are significant regions of iron accumulation, even though their concentrations of iron are never above moderate levels. This contrasts with the situation present in some ammocoetes of *G. australis* in which both the nephric folds and the fat column contained high concentrations of iron (Macey et al., 1982b), so much so that the nephric folds alone comprised 40% of the overall body content of non-haem iron (Smalley et al., 1986). The majority of iron within this region of *G. australis* and *P. marinus* is in the form of haemosiderin (Macey and Potter, 1986). Large deposits of iron are also associated with adipose tissue within juvenile lampreys of *P. marinus* from New Brunswick, and represents a major site of deposition of the metal (Youson and Cheung, 1987). It is clear that adipose tissue within lampreys is a substantial site of iron location, and that levels of iron within this tissue vary between different species and stages of the lamprey life cycle. The adaptive and physiological significance of the iron-adipose tissue association is not known, but it has been suggested that iron within adipose tissue in remote areas of the body of ammocoete may function to protect various organs which are susceptible to tissue damage by the toxic effects of large quantities of iron (Macey et al., 1982a; Macey and Potter, 1986).

Liver

It is of interest that the capacity of the liver to withstand extremely large amounts of iron, without tissue damage, exists in metamorphosing and adult lampreys (Macey et al., 1982b; Youson et al., 1983a,b: Sargent and Youson, 1986; Smalley et al., 1986; Youson and Cheung, 1987). Since ammocoetes in this study do not contain large amounts of iron within their livers, it is not known whether they possess the ability to tolerate high iron concentrations, or whether this ability is acquired during metamorphosis when bile ducts are lost. The presence of a functioning biliary tree in ammocoetes (Peek et al., 1979), may represent a pathway for the elimination of iron in the intestine through bile.

Epidermis

It has been suggested that iron within the mucous cells of the dorsal epidermis of *P. marinus* could contribute to body pigmentation, and may represent a pathway for the potential elimination of iron through the excretion of this metal along with mucus (Youson and Sargent, 1984). Prussian blue-positive granules in the more basally-located mucous cells of ammocoetes, and the presence of most of the iron in superficial epidermal cells of adults (Youson and Sargent, 1984; Youson and Cheung, 1987), may be a reflection of the fact that cell turnover of the epidermis is slow in lampreys and occurs over these two periods of the life cycle. However, it may be of relevance to our interpretation that during the early stages of metamorphosis in *Lampetra fluviatilis* and *Lampetra planeri* the numbers of mucous cells and the thickness of the epidermis increases (Lethbridge and Potter, 1981). It is possible that the major upward migration of iron-containing, basal, mucous cells occurs during metamorphosis and results in the beginning of iron elimination at this site.

The present investigation has revealed the existence of an iron-loaded epidermal cell, which does not appear to be of the mucous, granular, or skein cell types. This cell is bipolar to oval in shape, and is particularly located around the branchiopore, but is also scattered throughout the epidermis of the body and tail. The identify of this cell is not known, but three types of bipolar-shaped cells have been described in *Lampetra* spp. which are possible candidates (Whitear and Lane, 1983a,b,c). One of these, a so called multivillous cell, is the most likely cell to stain with iron, for it contains a photo-labile cytoplasmic pigment in the form of cylinders arranged in tetrads (Whitear and Lane, 1983c). These cytoplasmic inclusions or sinnets are similar to those found in invertebrate eyes and are known

to contain the respiratory pigment, haemocyanin (Wigglesworth and Baum, 1980). In future studies we will attempt to isolate and to characterize the fine structure of these iron-positive, bipolar cells.

Pronephric and opisthonephric kidneys

The first kidneys to develop in the prolarval stage of lampreys are the paired pronephroi or head kidneys (Youson, 1981c). These kidneys function as the sole excretory organ until the animal reaches 13mm in length, at which time the larval opisthonephric kidney commences development (Ooi and Youson, 1976). Subsequently, a gradual degeneration of the pronephric tubules occurs with the eventual occlusion of these tubules and a loss in connection between the pronephric duct and the cloaca (Torrey, 1938). Unique to ammocoetes of *P. marinus* from easter Canada, is the presence of extensive globular deposits of iron within the epithelium and the lumina of the pronephric tubules. The amount of iron accumulation within pronephric tubules appears to be related to the state of degeneration, for the largest globular deposits of iron were associated with degenerated tubules showing the most extensive degeneration. The source of iron for deposition within the pronephric tubules is not known, although it may be related to the very high plasma iron levels (Macey et al 1982b; Macey and Potter, 1986), and perhaps tissue fluid levels of ammocoetes. Iron may be present in the coelomic fluid and eventually pass into the ciliated nephrostome which leads into the proximal and distal tubules, where the absorption or elimination of the metal may occur. In young ammocoetes, excess iron can be eliminated through the pronephric duct, but eventually there is a loss in the connection between the pronephric duct and the cloaca (Torrey, 1938). Consequently, iron would accumulate in the lumina of tubules. Atrophy of tubules may lead to the deposition of iron-loaded epithelium within the lumina as well. Further information is required to fully understand the relationship between pronephric kidney and iron excretion in early larval life.

No iron staining was detected in the opisthonephros of ammocoetes of *P. marinus* , although iron is present in kidneys of some juvenile adults of this species (Youson and Cheung, 1987), and in larvae of *G. australis* (Macey et al., 1982b). It is possible that kidney tubules are involved in the reabsorption and/or excretion of iron, which in the latter case would result in the loss of iron from the body via the archinephric ducts (Smalley et al., 1986).

Alimentary canal

There is no histological evidence that the cells of the anterior intestine of ammocoetes absorb iron. However, iron absorption seems to occur in the anterior intestine of adults *P. marinus* during their feeding on the blood and body fluids of host fish (Youson and Cheung, 1987). High concentrations of iron have been observed in the absorptive cells located at the top of the typhlosole and the opposing intestinal wall of the posterior intestine in ammocoetes of several species (Macey et al., 1982b). Our present qualitative observations are in agreement with quantitative data (Sargent and Youson, 1986) which have shown that ammocoetes of *P. marinus* contain the highest amounts of iron in the intestine of any stage of the life cycle. We also support the suggestion that the posterior intestine is important in regulating iron elimination (Macey et al., 1982b).

Iron present within mucosal cells of the posterior intestine may be the result of excessive iron uptake, and a lack of transfer to blood vessels due to sufficient body iron stores, or could also reflect the deposition of the metal into mucosal cells from the plasma for purposes of elimination. Evidence strongly suggest that iron may be temporarily stored at the top of the typhlosole and the opposing intestinal wall for eventual excretion during cell extrusion (Hansen and Youson, 1978a, b; Macey et al., 1982b). The posterior intestine of adult *P. marinus* is involved in elimination of the metal through its excretion along with mucus (Youson and Cheung, 1987). It is apparent that changes in methods of dealing with intestinal iron between ammocoetes and parasitic adults are related to the transformation which takes place in the alimentary canal during metamorphosis (Youson, 1981b; Youson and Horbert, 1982). These alterations are a preparation for changes in the diet from that of algae, detritus, diatoms and other microorganisms in ammocoetes (Moore and Beamish, 1973: Potter, 1980b), to that of blood and some body fluids in the feeding parasitic adults (Farmer, 1980).

Macrophages

Many of the iron-containing macrophages of the present study also contain the brown pigment, melanin. These macrophages have been termed melano-macrophages in other fish species (Agius and Agbede, 1984). In the ammocoetes of our investigation only trace to low amounts of iron have been observed in macrophages of the alimentary canal, gill, sinusoids of the opisthonephric kidney, and the heart. This contrasts with the situation in juvenile adults of the same species, in which melano-macrophages are engorged with iron, even though they are present in similar locations in the body (Youson and Cheung, 1987). Iron-loaded macrophages are also found in the adult liver and the fat column (Youson and Cheung, 1987). The increase in levels of iron within melano-macrophages of adult lamprey may reflect the generally higher levels of iron in the body and the increase in erythrophagocytosis which occurs during metamorphosis as larval haemoglobins are replaced by adult haemoglobins (Potter and Brown, 1975; Percy and Potter, 1981). Absorbed iron in juvenile adults of *P. marinus* within the anterior intestine may be concentrated in macrophages as a result of iron absorption (Youson and Cheung, 1987), whereas the presence of small amounts of iron in melano-macrophages in the same region in ammocoetes, may be a result of erythrophagocytosis, since most of the

melano-macrophages were located in haemopoietic tissue. Similarly, erythorphagocytosis has also been observed in the cavernous body of the gills (Yamaguchi, 1979), sinusoids of the opisthonephric kidney (Youson, 1971), and in the typhlosole (Percy and Potter, 1981), and would undoubtedly result in iron deposits. It is of interest that melano-macrophages are involved in such process as lipid peroxidation, haemosiderosis, and possibly the immune response in other fish species (Agius and Agbede, 1984).

Heart

In the present investigation, low amounts of iron were associated with cardiac muscle of the atrium and to a lesser extent the ventricle of the heart in ammocoetes. Assuming that iron deposited in the myocardium is from the plasma, and that there is a larger volume of blood in the less muscular atrium compared to the ventricle (Fänge, 1972), there may be more of an opportunity for interaction between the slow moving blood and the macrophages in the atrium and therefore a greater deposition of iron. Also the existence of high plasma iron found in ammocoetes (Macey et al., 1982a; Macey and Potter, 1986; Youson et al., 1987) would possibly increase the change of deposition of the metal within cardiac muscle. The absence of iron in the myocardium of juvenile adults may be related to the lower levels of plasma iron concentrations found in this period of the life cycle, and the efficiency of the liver in filtering and accepting iron from the plasma (Smalley et al., 1986). It is of interest that the deposition of iron in the myocardium of the ventricle of the heart in patients with pathological iron-loading diseases, such as transfusional siderosis and idiopathic haemochromatosis, eventually leads to cardiac arrhythmias and death if treatment is not instituted (Powell and Halliday, 1980; Hoffbrand, 1980).

Fibrous connective tissues

The deposition of iron within fibrous connective tissues of ammocoetes, may represent an additional region of iron storage in connective tissue. Since iron has been observed in connective tissue of juvenile adults (Youson and Cheung, 1987), this site is perhaps significant. Iron in connective tissue of the velum and endostyle were always in close proximity to blood vessels and it is tempting to speculate that this form of iron storage would be readily mobilized in times of increased iron demands. The subepithelial connective tissue of the common bile duct contained iron which may be stored or transported to the lumen of the bile duct for subsequent elimination into the lumen of the intestine.

The presence of trace amounts of iron in branchial and dorsal fin cartilages of ammocoetes of *P. marinus* likely represents the first stages of iron accumulation within this hard connective tissue, since the amount of iron within chondrocytes has increased to low levels in juvenile adults of the same species (Youson and Cheung, 1987). Iron observed in the perichondrium of branchial cartilage in ammocoetes may eventually become incorporated into chondrocytes located deep in the matrix of cartilage which persists into adult life (Johnels, 1948).

Miscellaneous

In humans with idiopathic haemochromatosis iron deposition is found to some degree in all organs, except the brain and nervous tissue (Jacob, 1980). In ammocoetes the brain was free of iron, but there was an ammocoete which contained iron granules associated with a cranial nerve. Iron was also present in cells of the meninges of the brain and spinal cord. The meninges appears opaque and yellowish in the dissecting microscope and this has been attributed to the abundance of fat within these regions (Rovainen et al., 1971). This observation is of interest in light of our previous discussion on the relationship between fat and iron. The meninges of juvenile adults of *P. marinus*, also contain iron-loaded cells (Youson and Cheung, 1987).

The relationship between iron, and the connective tissue at the base of the neuroepithelium in the inner ear of both ammocoetes and juvenile adults of *P. marinus* (Youson and Cheung, 1987) is not fully understood. The occurrence of iron in the meninges, and nervous tissue also defies explanation, although it is interesting that iron in the form of magnitite is found in the heads of vertebrates and may have some function in magnetic field detection in navigation (Walcott et al., 1979; Zoeger et al., 1981; Beason and Nichols, 1984; Hanson et al., 1984). Currently little is known about the migratory habits of lampreys.

In conclusion, this investigation has documented the sites in which iron is present in larvae of *P. marinus* from New Brunswick. Many sites which had previously not been known to contain demonstratable iron have been revealed. This vital information will be utilized to interpret experimental data pertaining to iron deposition, storage, and elimination in this organism.

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References

Agius C. and Agbede S.A. (1984). An electron microscopical study on the genesis of lipofuscin, melanin and haemosiderin in the haemopoietic tissue of fish. J. Fish. Biol. 24, 471-488.

Beason R.C. and Nichols J.E. (1984). Magnetic orientation and magnetically sensitive material in the transequitorial migratory bird. Nature 309, 151-153.

Fänge R. (1972). The Circulation System. In: The Biology of Lampreys. vol. 2. Hardisty H.W. and Potter I.C. (eds). Academic Press. London. pp. 241-259.

Farmer G.J. (1980). Biology and physiology of feeding in adult lampreys. Can J. Fish Aquat. Sci. 37, 1751-1761.

Gomori G. (1936). Microtechnical demonstration of iron. Am. J. Pathol. 12, 655-663.

Hansen S.J. and Youson J.H. (1978a). Morphology of the epithelium in the alimentary tract of the larval lamprey, *Petromyzon marinus* L. J. Morph. 155, 193-218.

Hansen S.J. and Youson J.H. (1978b). Cell renewal in the epithelium of the alimentary tract of the larval lamprey, *Petromyzon marinus* L.J. Morph. 155, 219-236.

Hanson M., Karlsson L. and Westerberg H. (1984). Magnetic material in European eel (Anguilla anguilla L.). Comp. Biochem. Physiol. 77, 221-224.

Hoffbrand A.V. (1980). Transfusional Siderosis and Chelation Therapy. In: Iron in Biochemistry and Medicine. Jacobs A. and Worwood M. (eds). Academic Press. London. pp 499-527.

Hubbs C.L. and Potter I.C. (1971). Distribution, Phylogeny and Taxonomy. In: The Biology of Lampreys. vol 1. Hardisty H.W. and Potter I.C. (eds). Academic Press. London. pp1-65.

Jacobs A. (1980). The Pathology of Iron Overload. In: Iron in Biochemistry and Medicine. Jacobs A. and Worwood M. (eds). Academic Press. London. pp 427-459.

Johnels A.G. (1948). On the development and morphology of the skeleton of the head of *Petromyzon*. Acta Zool. (Stockh.). 29, 140-277.

Lethbridge R.C. and Potter I.C. (1981). The Skin. In: The Biology of Lampreys. vol. 3. Hardisty M.W. and Potter I.C. (eds). Academic Press. London. pp 377-448.

Lillie R.D., Geer J.C. and Gatierez A. (1983). The removal of histochemically demonstrable iron from tissue sections by brief exposure to sodium dithionite solution. J. Histochem. Cytochem. 11, 662-664.

Lowenstein O., Osborne M.P. and Thornhill R.A. (1968). The anatomy and ultrastructure of the labyrinth of the lamprey (*Lampetra fluvialitis* L.). Proc R. Soc. (B). 170, 113-134.

Macey D.J. and Potter I.C. (1986). Concetrations of non-haem iron in ammocoetes of species representing the three extant lamprey families. Comp. Biochem. Physiol. 84A, 77-79.

Macey D.J., Smalley S.R., Potter I.C. and Cake M.H. (1985). The relationship between total non-haem, ferritin and haemosiderin iron in larvae of Southern Hemisphere lampreys (*Geotria australis* and *Mordacia mordax*). J. Comp. Physiol. 156B, 269-276.

Macey D.J., Webb J. and Potter I.C. (1982a). Iron levels and major iron binding proteins in the plasma of ammocoetes and adults of the Southern Hemisphere lamprey *Geotria australis* Gray. Comp. Biochem. Physiol. 72A, 307-312.

Macey D.J., Webb J. and Potter I.C. (1982b). Distribution of iron-containing granules in lampreys, with particular reference to the Southern Hemisphere species *Geotria australis* Gray. Acta Zool. (Stockh.) 63, 91-99.

Moore J.W. and Beamish F.W.H. (1973). Food of larval sea lamprey (*Petromyzon marinus*) and American brook lamprey (*Lampetra lamottei*). J. Fish. Res. Board Can. 30, 7-15.

Ooi E.C. and Youson J.H. (1976). Growth of the opisthonephric kidney during larval life in the anadromous sea lamprey, *Petromyzon marinus* L. Can. J. Zool. 54, 1449-1458.

Peek W.D. Sidon E.W., Youson J.H. and Fisher M.M. (1979). Fine structure of the liver of the larval lamprey, *Petromyzon marinus* L; hepatocytes and sinusoids. Am. J. Anat. 156, 231-250.

Percy R. and Potter I.C. (1981). Further observations on the development and destruction of lamprey blood cells. J. Zool. (Lond.) 193, 239-251.

Potter I.C. (1980). Ecology of larval and metamorphosing lampreys. Can. J. Fish Aquat. Sci. 37, 1641-1657.

Potter I.C. and Brown I.D. (1975). Changes in haemoglobin electropherograms during the life cycle of two closely related lampreys. Comp. Biochem. Physiol. 51B, 517-519.

Powell L.W. and Halliday J.W. (1980). Idiopathic Haemochomatosis. In: Iron in Biochemistry and Medicine. Jacobs A. and Worwood M. (eds). Academic Press. London. pp 461-498.

Rovainen C.M. Lemcoe G.E. and Peterson A. (1971). Structure and chemistry of glucose-producing cells in meningeal tissue of the lamprey. Brain. Res. 30, 99-118.

Sargent P.A. and Youson J.H. (1986). Quantification of iron deposits in several body tissues of lampreys *(Petromyzon marinus L.)* throughout the life cycle. Comp. Biochem. Physiol. 83A, 573-577.

Smalley S.R., Macey D.J. and Potter I.C. (1986). Changes in the amount of non-haem iron in the plasma, whole body and selected organs during the post-larval life of the lamprey *Geotria australis*. J. Exp. Zool. 237, 149-157.

Torrey T.W. (1938). The absorption of colloidal carbon from the body cavity of ammocoetes. A study of the structure and function of the larval kidneys and blood forming tissues. J. Morph. 63, 163-179.

Underwood E.J. (1977). Iron. In: Trace Elements in Human of Animal Nutrition. 4th ed. Academic Press. New York. pp 13-55.

Walcott C., Gould J.L. and Kirschvink J.K. (1979). Pigeons have magnets. Science 205, 1027-1029.

Whitear M. and Lane E.B. (1983a). Polyvillous cells of the epidermis: Transportive elements of lamprey skin. J. Zool., London. 199, 345-358.

Whitear M. and Lane E.B. (1983b). Oligovillous cells of the epidermis: sensory elements of lamprey skin. J. Zool., Lond. 199, 359-384.

Whitear M. and Lane E.B. (1983c). Multivillous cells: epidermal sensory cells of unknown function in lamprey skin. J. Zool., London. 201, 259-272.

Wrigglesworth J.M. and Baum H. (1980). The Biochemical Functions of Iron. In: Iron in Biochemistry and Medicine. Jacobs A. and Worwood M. (eds.). Academic Press. London. pp 29-86.

Yamaguchi K., Tomonaga S., Ihara K and Awaya K. (1979). Electron microscopic study of phagocytic lining cells in the cavernous body of lamprey gill. J. Electron Microsc. 49, 106-116.

Youson J.H. (1971). Blood cell destruction in the opisthonephric kidney of the sea lamprey, *Petromyzon marinus L.* Can. J. Zool. 49, 962-963.

Youson J.H. (1981a). The Liver. In: The Biology of Lampreys. vol. 3. Hardisty M.W. and Potter I.C. (eds). Academic Press. London. pp 263-262.

Youson J.H. (1981b). The Alimentary Canal. In: The Biology of Lampreys. vol. 3. Hardisty M.W. and Potter I.C. (eds). Academic Press. London. pp 95-198.

Youson J.H. (1981c). The Kidney. In: The Biology of Lampreys. vol. 3. Hardisty M.W. and Potter I.C. (eds). Academic Press. London. pp 191-261.

Youson J.H. (1985). Organ Development and Specialization in Lampey Species. In: Evolutionary biology of primitive fishes. Foreman R.E., Gorbman A., Dodd J.M. and Olsson R. (eds).

Plenum Press. New York pp 141-164.

Youson J.H. and Cheung R. (1987). Distribution of ferric iron in juvenile adults of the lamprey, *Petromyzon marinus L.* Can. J. Zool 65, 1833-1841.

Youson J.H. and Horbert W.R. (1982). Transformation of the intestinal epithelium of the larval anadromous sea lamprey *Petromyzon marinus L.* during metamorphosis. J. Morphol. 171, 89-117.

Youson J.H. and Sargent P.A. (1984). Iron deposition in the integument of lampreys. Anat. Rec. 209, 461-468.

Youson J.H., Lee J. and Potter I.C. (1979). The distribution of fat in larval, metamorphosing, and young adult anadromous sea lampreys, *Petromyzon marinus L.* Can. J. Zool. 57, 237-246.

Youson, J.H., Sargent P.A. and Barrett A. (1987). Serum iron concentration and other blood parameters during the life cycle of the sea lamprey, *Petromyzon marinus* L. Comp. Biochem. Physiol. (in press).

Youson J.H. Sargent P.A. and Sidon E.W. (1983a). Iron loading in the liver of metamorphosing lampreys, *Petromyzon marinus L.* Cell Tissue Res. 234, 109-124.

Youson J.H. and Sargent P.A. and Sidon E.W. (1983b). Iron loading in the liver of parasitic adult lampreys, *Petromyzon marinus L.* Am. J. Anat. 168, 37-49.

Youson J.H., Sidon E.W., Peek W.D. and Shivers R.R. (1985). Ultrastructure of the hepatocytes in a vertebrate without bile ducts. J.Anat. (Lond.). 140, 143-158

Zoeger J., Dunn J.R. and Fuller M. (1981). Magnetic material in the common Pacific dolphin. Science 213, 892-894.

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