Relocalization of membrane enzymes accompanies biliary atresia in lamprey liver*

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Summary. Light- and electron-microscopic histochemical procedures were used to show the distribution of the membrane-bound enzymes alkaline phosphatase (Alp), adenosine triphosphatase (ATPase), and 5'-nucleotidase (5'-nuc) in the livers of lamprey, Petromyzon marinus, throughout the life cycle. In larvae, the three enzymes are located at the biliary pole on the canalicular membranes of microvilli. At metamorphosis the enzymes become localized at all lateral cell surfaces of hepatocytes as bile canaliculi degenerate in the programmed regression of the entire biliary tree. This latter pattern of enzyme distribution persists during the parasitic adult phase but no activity is evident in individuals in the spawning migration. As the timing of the relocalization of enzymatic activity correlates well with a build-up of bile products and iron during metamorphosis, it is suggested that the lateral surface may be the new site for transport of these products.

Key words: Lamprey — Biliary atresia — Hepatocytes — Membrane enzymes

The liver of larval lampreys is composed of hepatocytes arranged in a tubular fashion around the bile canaliculi (Kitada 1970; Shin 1977; Peek et al. 1979). The latter structures are histologically continuous with a bile duct system and gallbladder (Youson and Sidon 1978; Sidon et al. 1980). During metamorphosis, degeneration of bile canaliculi (Sidon and Youson 1983b) and bile ducts takes place and the events of this process closely resemble those in human biliary atresia (Sterling et al. 1967, 1968; Youson and Sidon 1978; Sidon and Youson 1983a). As a consequence of these changes there is progressive accumulation of iron throughout the hepatocytic parenchyma (Macey et al. 1982; Youson et al. 1983a, b) and the development of bile stasis (Bertolini 1965; De Vos et al. 1973; Sidon and Youson 1983b). Yet, the lamprey can be distinguished from other vertebrates by its ability to survive for an extended period of time without a hepatic exocrine system for excretion of biliary products.

Extensive morphological changes of the cell surface are associated with the regression of bile canaliculi and transformation of lamprey hepatocytes. These are observed along canalicular membranes, lateral (intercellular) membranes (cell junctions), and sinusoidal surfaces (Youson 1981; Sidon and Youson 1983b). Since the developmental state of an organism resembles pathological conditions in that it often effects the changes in the functions of the different hepatocyte plasma membrane surfaces (Toda et al. 1975; Phillips et al. 1979), we wished to examine the cell surface of lamprey hepatocytes during various stages of the life cycle. This can be performed using histochemical procedures for identification of membrane-bound enzymes. The only previous cytochemical study of enzyme localization in lampreys was restricted to the larval stage and spawning migrant animals of a non-parasitic species (De Vos et al. 1973). From the results reported it is unclear whether the pattern of enzyme changes is related to the production and release of vitelloproteins (Pickering 1976; Larsen 1978), the fasting state of the animal (Autuori and Bertolini 1965), or structural changes characteristic of cholestasis during metamorphosis. In the current investigation we examine the differential patterns of localization of alkaline phosphatase, adenosine triphosphatase, and 5'-nucleotidase throughout all stages of the life-cycle of the parasitic sea lamprey, Petromyzon marinus. The significance of enzyme distribution is discussed in relation to the secretory pole of hepatocytes following biliary atresia.

Materials and methods

Larval lampreys (ammocoetes) of Petromyzon marinus were collected and maintained in the laboratory as previously described (Sidon and Youson 1983a). Six ammocoetes were sacrificed and prepared for observation as soon as possible after capture but the majority were permitted to enter metamorphosis. Ten metamorphosing individuals were classified as to stage 1 (earliest) through to stage 7 (latest) according to the criteria described by Youson and Potter (1979). Two young parasitic adults (sexually immature juveniles) were caught in Washademoak Lake, New Brunswick by fishermen netting gaspereau (Alosa pseudoharengus). When the young adults were captured they were feeding on the gaspereau and white suckers (Catastomus commersoni). The livers of all ammocoetes, metamorphosing animals and young adults were brown to orange-brown in colour. Three spawning migrants (2 females, 1 male) of landlocked P. marinus were netted in the Humber River, Ontario. The livers of spawning migrants were pale green (male) and
dark green (females) indicating the advanced stage of sexual maturity (Youson 1981). After transport to Scarborough College, University of Toronto, young adults and spawning migrants were maintained at $10 \pm 1^\circ C$ in fibreglass tanks containing either recirculated or continuous-flowing, dechlorinated tap water. Young adults were provided with rainbow trout (Salmo gairdneri), on which they fed.

For the identification of alkaline phosphatase (Alp) in light-microscope sections, livers were removed, placed in Ames O.C.T. embedding compound, and frozen at $-20^\circ C$ for cryostat sectioning. Frozen sections, 12 $\mu$m thick, were cut in an Ames cryostat and mounted on subbed slides. The sections were then treated according to the method of Ackerman (1962). This procedure basically involves the incubation of tissue sections in a medium containing naphthol AS-MX phosphate solution for 25 min at room temperature in darkness and at pH 8.6. After incubation, slides were washed and counterstained with Mayer’s haematoxylin and mounted with immersion oil. Controls were incubated in medium lacking the substrate.

For the demonstration of Alp in the electron microscope thin slices were fixed by immersion in cold ($\approx 0^\circ C$) 6.0% glutaraldehyde and 3.0% paraformaldehyde in 0.07 M sodium cacodylate buffer, pH 7.2 (Toro and Joo 1966) for 2 h. They were then rinsed in the same buffer overnight at 4$^\circ C$ and minced further the following day. Subsequently, the tissues were incubated in freshly-prepared medium containing sodium beta-glycerophosphate (2.0 mg/ml) as substrate in a 0.05 M Tris HCl Buffer, pH 8.2 at room temperature for 45 min. A saturated solution of lead citrate was added as a capture reagent (Saito and Ogawa 1968). Control tissues were incubated in a medium without substrate. Following incubation, tissues were washed in 0.07 M sodium cacodylate buffer, postfixed for $\frac{1}{2}$ h in 1% OsO$_4$ in phosphate.