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Aquat. Living Resour. 15 (2002) 169–178

Aquatic Living Resources

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Cytological detection of the main phases of lipid metabolism during early post-embryonic development in three teleost species: *Dicentrarchus labrax*, *Sparus aurata* and *Stizostedion lucioperca*

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Received 13 July 2001; accepted 1 March 2002

Abstract

The aim of this report is the synthetic presentation of observations related to ultrastructural aspects of the lipid metabolism during the ontogenesis of trophic mechanisms in three teleost species, the sea bass, *Dicentrarchus labrax*, the sea bream, *Sparus aurata* and the pike-perch, *Stizostedion lucioperca*. The results have shown the respective roles of the vitelline syncytium, the intestine, the liver and the exocrine pancreas during the change from an endogenous lipid source, the yolk vesicle, to an exogenous lipid one, food. They make it possible to stress the close correlation between the development of the biliary function, that of the exocrine function of the pancreas and that of the intestinal absorption of lipids. These activities established during the endotrophic stage are slight at the beginning of trophic life and then increase at the end of the endo-exotrophic stage. Analysis of the results suggests the occurrence of four physiological phases characteristic in early lipid metabolism establishing the main heads in acquisition of the trophic autonomy according to a pattern similar to that of mammals. © 2002 Ifremer/CNRS/Inra/IRD/Cemagref/Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Résumé

Détection cytologique des principales phases du métabolisme des lipides au cours du développement post-embryonnaire précoce de trois espèces de téléostéens: *Dicentrarchus labrax, Sparus aurata* et *Stizostedion lucioperca*. L'objectif de cette note est de présenter de manière synthétique les observations concernant les aspects ultrastructuraux du métabolisme des lipides au cours de l'ontogenèse des mécanismes trophiques de trois espèces de poissons téléostéens, le loup, la daurade et le sandre. Ces travaux font ressortir les rôles respectifs du syncytium vitellin, de l'intestin, du foie et du pancréas exocrine, lors du passage d'une source lipidique endogène, la vésicule vitelline, à une source lipidique exogène, l'alimentation. Ils mettent en évidence une étroite corrélation entre le développement de la synthèse biliaire hépatique, la production de zymogène par le pancréas et l'absorption intestinale des lipides. Ces activités démarrent en fin de période endotrophe, restent limitées au début de la période endo-exotrophe et augmentent à la fin de celle-ci. L'analyse des résultats suggère l'existence de phases physiologiques caractéristiques dans le métabolisme précoce des lipides, jalonnant l'acquisition de l'autonomie trophique selon un schéma comparable à celui des mammifères. © 2002 Ifremer/CNRS/Inra/Cemagref/Éditions scientifiques et médicales Elsevier SAS. Tous droits réservés.

Keywords: Teleost; Ontogenesis; Lipid; Functional morphology; Cytochemistry; Ultrastructure

1. Introduction

Despite a number of studies based on observations by light microscopy, data on ultrastructural detection of the lipid metabolism during fish post-embryonnic development are scarce. Thus, the resorption of yolk reserves by means of a yolk syncytial layer has been studied in trout (Vernier and Sire, 1977a; Walzer and Schönenberger, 1979b), and in turbot (Poupard et al., 2000). Studies performed on trout (Vernier and Sire, 1977a; Sire and Vernier, 1979), on sea bream (Calzada et al., 1998) and on spinfish (Gallagher et al., 2001) have concerned intestinal lipid absorption. More-

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PII: S0990-7440(02)01169-5

over, in trout, Vernier and Sire (1977a) have shown the involvement of liver in the lipoprotein secretion.

Our works carried out on the ontogeny of trophic mechanisms in three teleost species of aquacultural interest (the sea bass *Dicentrarchus labrax*, the sea bream *Sparus aurata* and the pike-perch *Stizostedion lucioperca)* have shown the respective roles of the yolk vesicle (Guyot et al., 1993; Mani-Ponset et al., 1994, 1996), the intestine (Deplano et al., 1991; Mani-Ponset et al., 1994, 1996; Diaz et al., 1997b), the liver (Diaz and Connes 1991; Diaz et al., 1997a, 1998; Guyot et al., 1995) and the pancreas (Beccaria et al., 1991, Diaz et al., 1992) in the early lipid metabolism. The aim of this report is to review the observations that are common to these three species. The results from these fish revealed considerable similarity in the phenomena observed, and their comparison emphasises the close correlation between activities of the four studied organs. The illustrations are chosen for their demonstration value, whatever the species. A number of phases in fish post-embryonic lipid metabolism are defined. These phases are superimposed on the three periods established according to the trophic source: the endotrophic period during which the pre-larva lives only on vitelline reserves, the endo-exotrophic period during which the larva still uses its reserves but begins to feed and the exotrophic period during which feeding is the only trophic source. The data are discussed and the comparison with knowledge from higher vertebrates revealed some analogies in the onset of lipid metabolism.

2. Materials and methods

2.1. Fish larvae and rearing conditions

Two stages of postembryonic development were considered in sea bass *Dicentrarchus labrax*, sea bream *Sparus aurata*, and pike-perch *Stizostedion lucioperca*: pre-larva from hatching (day 0) to mouth-opening (around days 5, 3 and 5, respectively), and larva after mouth-opening. With regard to diet, three stages can be distinguished: (1) an endotrophic stage, when the pre-larva lives on its yolk reserves; (2) an endo-exotrophic stage starting at mouth-opening, characterised by depletion of the yolk reserves and beginning of feeding; (3) an exotrophic stage, when feeding is the only source of energy, starting from day 15 to day 17 depending on species.

Sea bream, sea bass and pike-perch pre-larvae and larvae, from eggs spawned by captive broodstock, were supplied by Palavas Ifremer Centre (Hérault, France), Brest Ifremer Centre (Finistère, France) and Montpellier Cemagref Centre (Hérault, France), respectively. The larvae were reared in cylindrical–conical recycled water tanks containing 500 l (sea bream), 35 l (sea bass), 50 l (pike-perch) of water at a density of 100 individual l^{-1}, except for pike-perch (50 ind. 1^{-1}) The sea water was maintained at a salinity of 38 (sea bream) or 35 (sea bass) and at 21 °C (sea bream) or 18 °C (sea bass) during the 20 days of experimentation. Pike-perch was reared in fresh water at 22 °C.

Pre-larvae were kept in darkness and the subsequent larvae under continuous light (sea bream and sea bass) or in natural light and photoperiod (pike-perch).

As standard, larvae were fed ad libitum with live lipid-enriched *Artemia* sp. (sea bass), rotifers (sea bream) and zooplankton, including rotifers, cladocers and copepodes (pike-perch). In some experiments, synthetic feed (Kyowa, 'Aqualarvae' manufactured by Aqualim, France) and glucose dissolved in sea water (5 g l^{-1}) were used and groups of each species were fasted.

2.2. Preparation of larvae

Five larvae of each species group were sampled daily from days 0 to 10, and every 2 day after day 10. Larvae were anaesthetised by cooling (2–4 °C) the water and then treated by double fixation with 2.5% glutaraldehyde and 1% osmium tetroxide in cacodylate buffer at pH 7.2 (450 mOsM for sea bream and sea bass; 350 mOsM for pike-perch). The larvae were then embedded in Epon 812 and ultrathin sections were contrasted with uranyl acetate and bismuth subnitrate.

Lipid detection was carried out on semithin sections with Sudan black (Sire and Vernier, 1980) and on ultrathin sections with thiocarbohydrazide and osmium tetroxide (OTO method of Seligman et al., 1966).

3. Results

3.1. The first lipoprotein synthesis in the yolk vesicle

The yolk vesicle located beneath the digestive tract comprises two kinds of reserve, the external vitellus and the internal oil globule, surrounded and separated from each other by the periblast, a syncytial envelope. During the endotrophic period, vitelline circulation becomes established as a vast blood sinus surrounding the vesicle and closely linked with the liver circulation network. The periblast uses the yolk and a large part of the oil globule from hatching until the first days after mouth-opening. It secretes via the endoplasmic reticulum and the Golgi apparatus (Fig. 1a,b) particles revealed by lipid-specific stains and which can be considered as lipoprotein particles by virtue of their location and size. These lipoproteins are released into the perivitelline circulatory space and are found in the hepatic sinusoids (Fig. 1b) and the general blood circulation system.

After the first days following mouth-opening, the periblast does not synthesise or release lipoproteins, while the resorption of the oil globule continues. The complete disappearance of the yolk vesicle marks the end of the endo-exotrophic period.

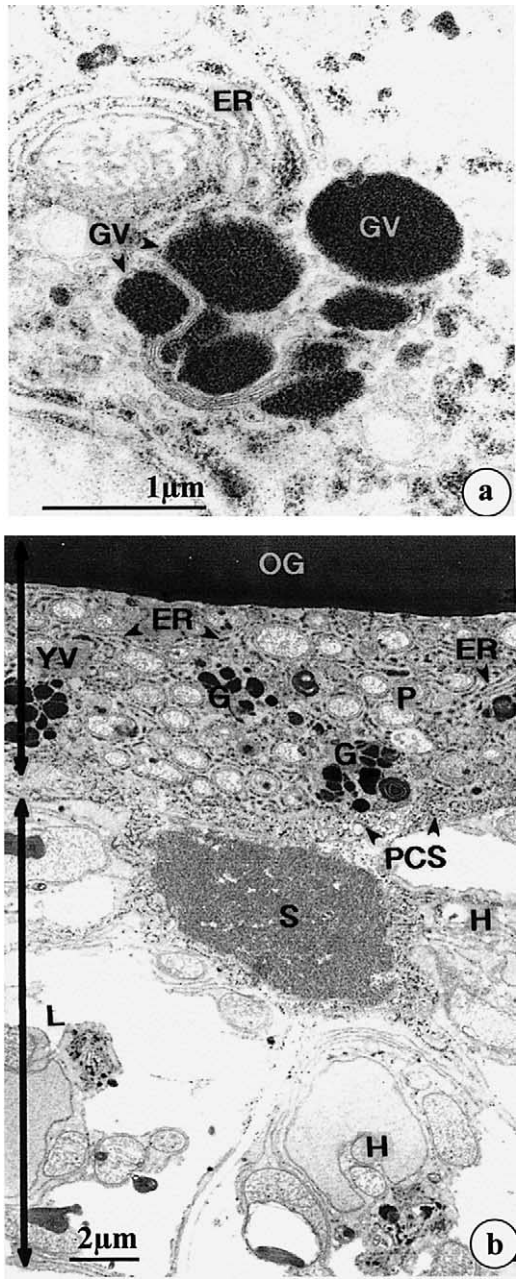

Fig. 1. Display of lipids in yolk vesicle during periblast lipoprotein synthesis and release (OTO method). (a) High magnification of the periblast in a sea bass pre-larva 1 d before mouth-opening, showing lipoprotein particles within the endoplasm reticulum and the Golgi vesicles. ER: endoplasmic reticulum; GV: Golgi vesicles. (b) Yolk vesicle and liver in a sea bass pre-larva 1 d before mouth-opening. Lipoprotein particles are localized in the endoplasmic reticulum and in the Golgi apparatus of the periblast and are released in the perivitelline circulatory space and in hepatic sinusoid. ER: endoplasmic reticulum; G: Golgi apparatus; H: hepatocyte; L: liver; OG: oil globule; P: periblast; PCS: perivitelline circulatory space; S: sinusoid; YV: yolk vesicle.

3.2. Lipid absorption in the gut

After hatching, differentiation of the gut is completed during the endotrophic period. The intestine possesses an anterior and a mid region specialised in lipid absorption and separated by a valvula from a posterior region specialised in the absorption of macromolecular proteins.

Intestinal absorption of lipids is shown during development by the presence of two sorts of lipid inclusions in the enterocytes. The first are particles whose dimensions and location in the endoplasmic reticulum and the Golgi apparatus show that they are very low-density lipoproteins (VLDL) lipoproteins (20–70 nm) or chylomicrons (70–500 nm). The second are droplets of amorphous material—probably triglycerides—that can be up to 6 µm in diameter.

No trace of lipids is visible in the intestinal epithelium during the first few days after hatching. At the end of the endotrophic period and at the beginning of larval life, before the first feeding, lipoprotein synthesis is detectable in the enterocytes (Fig. 2a). Particles not larger than 70 nm appear in the still poorly developed endoplasmic reticulum and Golgi apparatus (Fig. 2b) and are transferred to inter-enterocyte spaces. Lipids are also present in the intestinal lumen (Fig. 2c) at mouth-opening.

Enterocyte lipoproteinogenesis remains fairly slight when feeding starts. VLDL or chylomicron particles are visible in the endoplasmic reticulum and the Golgi vesicles. Lipid droplets appear from day 7 onwards. Small quantities of lipoprotein particles are transferred to the intercellular spaces but are rare or totally absent in larval blood vessels around day 7 to day 9.

Lipid absorption intensifies from day 9 onwards. It was substantial from the end of the endo-exotrophic period until the end of the experiment. It is characterised by the presence of numerous lipoprotein particles in the cavities of the endoplasmic reticulum, which has developed considerably, and in the Golgi apparatus vesicles (Fig. 2d), whose siting is generally perinuclear (Fig. 2e). Numerous triglyceride droplets also accumulate in the enterocytes.

Transfer of enterocyte lipoproteins to the larval circulation is extremely intense at the end of the endo-exotrophic period. The particles can accumulate in intercellular spaces, cross the basal lamina (Fig. 2e) and concentrate strongly in the intestinal vessels and in the blood circulation.

3.3. Biliary lipid synthesis in the liver

The liver forms from hatching onwards by a thickening of the gut wall and develops in contact with the yolk vesicle. Hepatocytes differentiate during the endotrophic period while the sinusoidal blood system and the biliary excretion system are formed.

The secretion of biliary lipids involves the endoplasmic reticulum and the Golgi apparatus in synthesis, their transport by vesicular pathways and excretion into the bile canaliculi by exocytosis. Lipids are detected in the various hepatic compartments. VLDL-type particles are observed in the endoplasmic reticulum and in certain Golgi vesicles (Fig. 3a). Other vesicles located in the Golgi areas and around the bile canaliculi contain lipids in the form of more

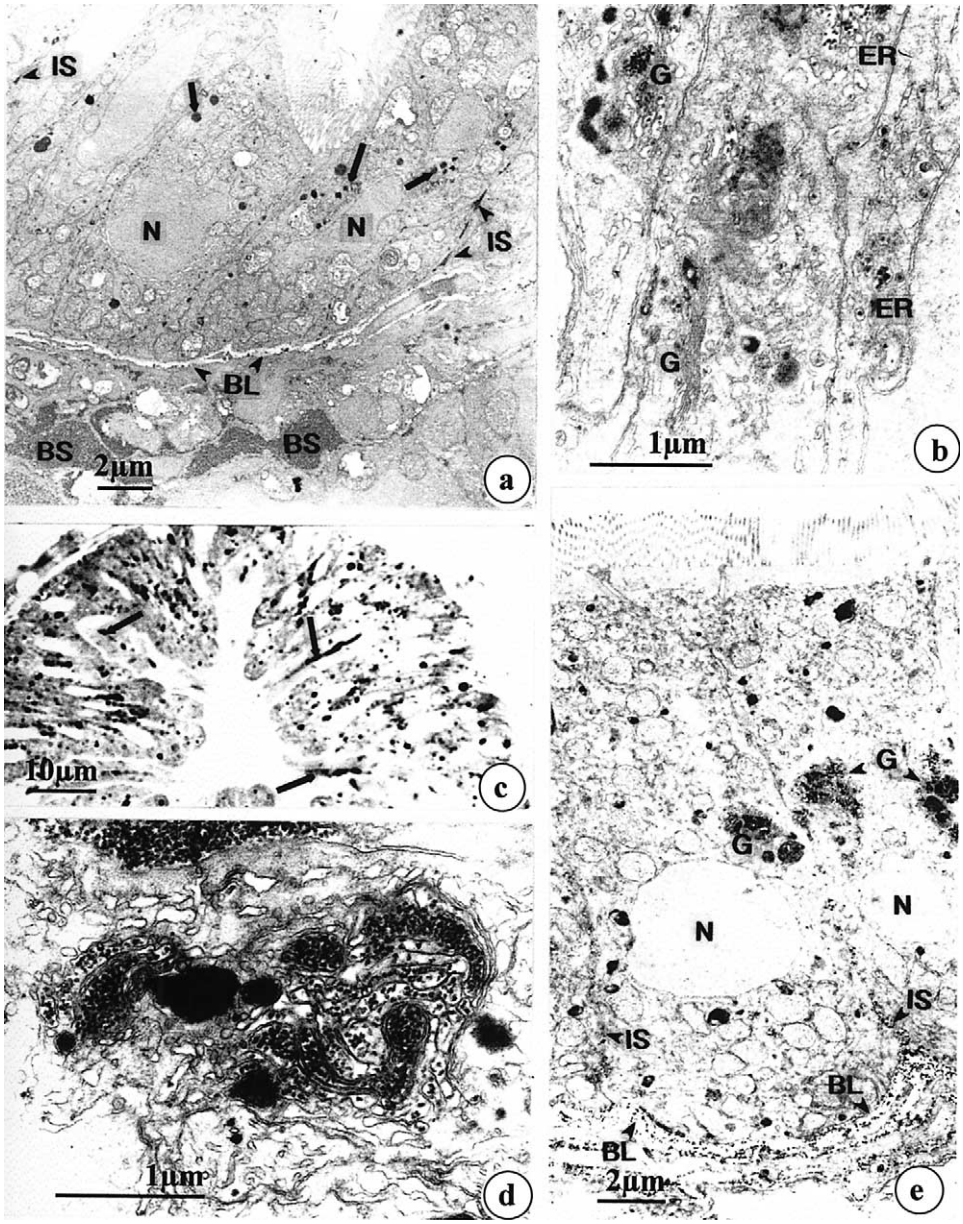

Fig. 2. Lipid absorption in the gut (OTO method). (a) Part of intestinal wall of a sea bass larva at mouth-opening showing enterocytes containing lipid inclusions (arrows). Lipoprotein particles can be seen in inter-enterocyte spaces, across the basal lamina and within blood spaces of the chorion. BL: basal lamina; BS: blood spaces; IS: interenterocyte spaces; N: nucleus. (b) Appearance of lipoprotein particles within poorly developed endoplasmic reticulum and Golgi apparatus of enterocytes in sea bass larva at mouth-opening. ER: endoplasmic reticulum; G: Golgi apparatus. (c) Presence of Sudan-stained material (arrows) in the intestinal lumen, between adjacent mucosa folds, of a sea bass larva at mouth-opening. (d) Lipoprotein particles within highly developed Golgi apparatus in an enterocyte of 18-d-old pike-perch larva. (e) Intensified lipid absorption in intestine of 16-d-old pike-perch larva. This is characterized by the presence of numerous lipoprotein particles within perinuclear Golgi apparatus, in interenterocyte spaces and across the basal lamina. BL: basal lamina; G: Golgi apparatus; IS: interenterocyte spaces; N, nucleus.

finely granular and homogeneous material (Fig. 3b). Lipids are also detected in the lumen of the bile canaliculi (Fig. 3c) of the cholangioles, the cystic duct and the gall bladder.

The Golgi activity associated with the production of bile lipids develops during hepatic organogenesis and leads to significant production of pericanalicular vesicles at mouth-opening. During the next 4 to 5 d, this activity decreases strongly (pike-perch) or ceases totally (sea bass and sea bream). It then resumes and intensifies at the end of the endo-exotrophic period.

3.4. Pancreas zymogen production

Like the liver, the pancreas is formed by a thickening of the gut wall. Most of its organogenesis takes place during the endotrophic period.

Zymogen synthesis starts 2–3 d after hatching. Secretion granules accumulate in the exocrine cells (Fig. 4a) until mouth-opening, but no excretion is visible in the lumen of the pancreatic ducts. The beginning of larval life is marked by a distinct decrease in the number and volume of the

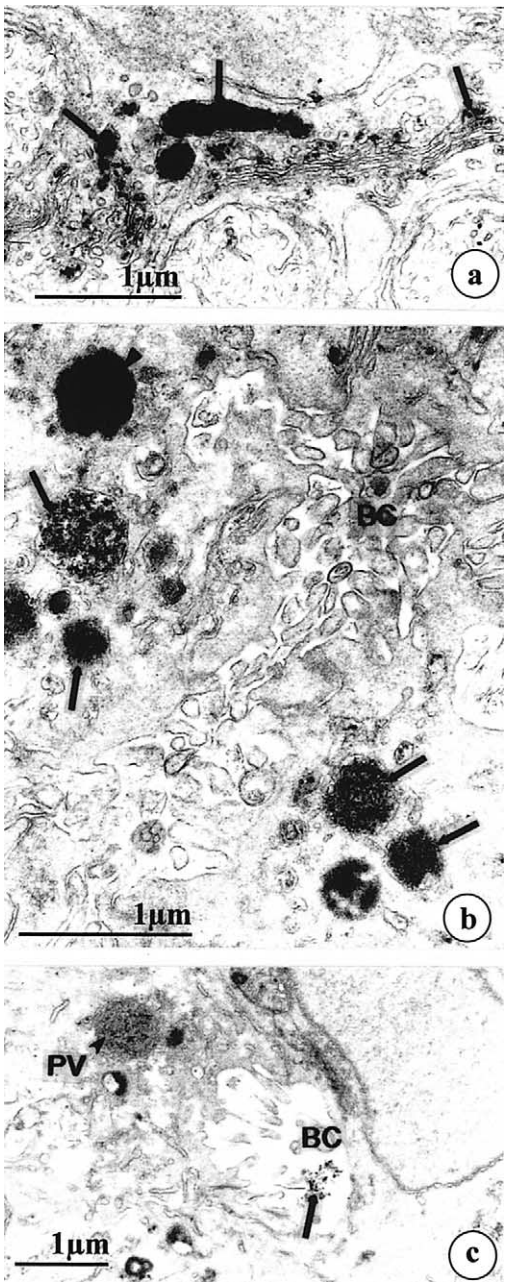

Fig. 3. Display of lipids in hepatocytes during biliary synthesis (OTO method). (a) Golgi apparatus containing VLDL-like particles (arrows) in hepatocyte of 10-d-old sea bass larva. (b) Pericanalicular vesicles containing finely granular (arrows) and homogenous (arrowhead) lipid materials in hepatocyte of 10-d-old sea bass larva (× 26 000). BC, bile canaliculus. (c) Display of lipids in a pericanalicular vesicle and in the lumen (arrow) of a bile canaliculus hepatocyte of 7-d-old sea bass larva. BC: bile canaliculus; PV: pericanalicular vesicle.

secretion granules (Fig. 4b) and by the excretion of the zymogen.

The synthetic activity of the exocrine cells is slight during the endo-exotrophic period. It then increases to attain a level comparable with that of an adult at the beginning of the strictly exotrophic period.

4. Discussion

Two sources of lipids succeed each other during the development of vertebrates. The first depends directly on the maternal organism in viviparous animals and on reserves accumulated in the egg in oviparous animals. The second source is feeding.

As has been shown in work on trout (Vernier and Sire, 1977a,b; Walzer and Schönenberger, 1979a,b), on sea bass, sea bream and pike-perch (Mani-Ponset et al., 1996) and on turbot (Poupard et al., 2000), the resorption of yolk reserves (the vitellus and the oil globule) is by means of a syncytial formation, the periblast, that separates and surrounds these two kinds of reserves. The endoplasmic reticulum and the Golgi apparatus of this syncytium synthesise lipoproteins that are released into the perivitelline sinus and thus reach the blood stream. The work of Lambson (1970) and of Noble and Cocchi (1990) on chicken and that of Plonné et al. (1992) on rat show that lipoprotein particles are also released into the blood stream of the embryo. The syncytial organisation and functioning of the periblast in fish are thus reminiscent of the yolk sac endoderm in the other vertebrates. The two types of structure are analogous and are perfectly suited to the transfer of lipids from egg yolk reserves (fish, reptiles and birds) or from the mother's blood (mammals) to the embryo. Moreover, recent data show that the genes coding for lipid transfer proteins express early in fish as well as in mammals (Babin et al., 1999).

The change from one lipid source to another is a critical phase of development. It corresponds to birth in mammals, hatching in birds and reptiles, and to mouth-opening in fish. Indeed, the periblast continues to produce lipoproteins from hatching to mouth-opening in fish.

The use of food lipids involves their emulsification and hydrolysis under the combined effect of bile and pancreatic enzymes and then absorption of the products of degradation by the intestinal mucusae. The liver, the pancreas and the intestine ensure these functions. In sea bass, sea bream and pike-perch, they differentiate to a considerable extent during the pre-larval period and become functional at the moment when the larva can feed itself.

We described in fish for the first time the synthesis of the lipid components of bile and their release into the bile canaliculi (Diaz et al., 1998). As the secretion of bile lipids is closely linked to that of bile acids (Crawford et al., 1988, 1991), it can be considered that the cytochemical visualisation of the former is the sign of bile secretion as a whole. The bile secretion become obvious from the end of the endotrophic period, as is shown by the swelling of the gall bladder and the presence of lipids in the excretory ducts and in the intestinal lumen (Diaz et al., 1997a). This activity decreases considerably or stops shortly after mouth-opening. It resumes and intensifies at the end of the endo-exotrophic period.

Bile secretion in fishes has many points in common with that of mammals, where bile acids have been studied above

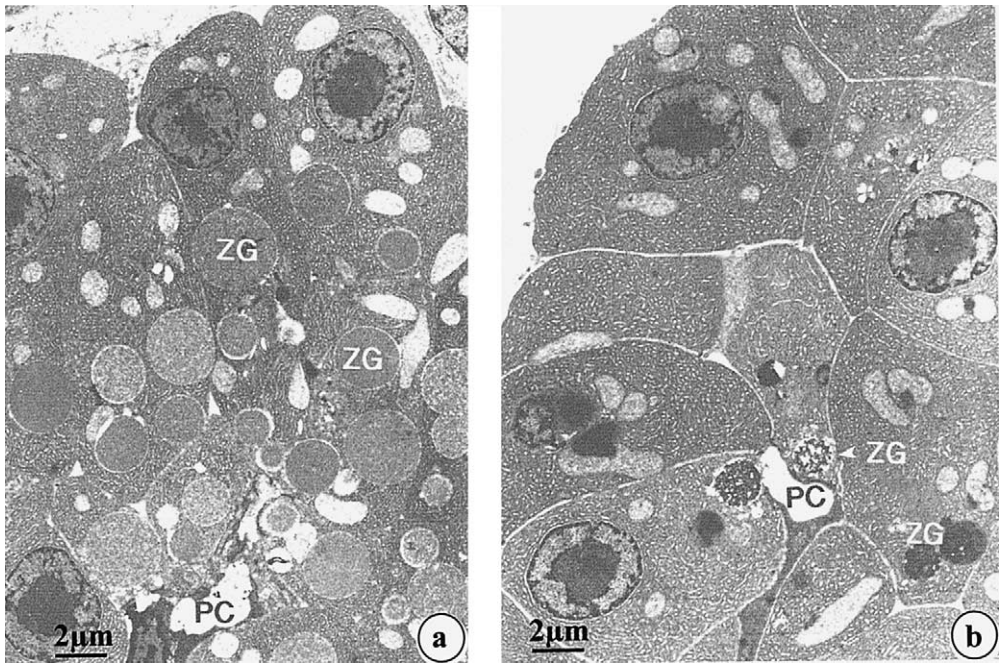

Fig. 4. Pancreas zymogene production during endo-exotrophic stage. (a) Pancreatic cells filled with zymogene granules in a sea bream larva at mouth-opening. ZG: zymogene granules. (b) Pancreatic cells containing scarce zymogene granules of a 10-d-old sea bream larva. PC, pancreatic canaliculus; ZG: zymogene granules.

all. Synthesis of these acids begins during the foetal stage both in domestic animals such as rat, rabbit, dog and sheep (Danielsson and Rutter, 1968; Jackson et al., 1971; Smallwood et al., 1972; Lester et al., 1973; Graham et al., 1979; Little et al., 1979; Suchy et al., 1987) and in man (Poley et al., 1964; Bongiovanni, 1965; Danielsson and Rutter, 1968; Subbiah and Hassan,1982). Their excretion has been observed in utero in rat (Little et al., 1979), dog (Jackson et al., 1971) and man (Poley et al., 1964, Bongiovanni, 1965). All the species studied secrete little bile at birth and during the first few days of life (Little et al., 1979, Hardy et al., 1980; Shaffer et al., 1985; Tavoloni et al., 1985; Suchy et al., 1987); at weaning, secretion intensifies and reaches levels similar to those of adults (Shaffer et al., 1985; Piccoli et al., 1986a, b; Suchy et al., 1987).

The differentiation of the exocrine cells of the pancreas is early in sea bass, sea bream and pike-perch. As in *Leiostomus xanthurus* (Govoni, 1980), *Scophthalmus maximus* (Cousin and Baudin-Laurencin, 1985) and *Coregonus fera* (Loewe and Eckmann, 1988), the first zymogen granules are synthesised during the endotrophic period. They become very abundant at mouth-opening. Their number and size decrease at the beginning of larval life in relation to zymogen excretion. Synthesis of zymogen granules is slight during the endo-exotrophic period and then intensifies.

Similar observations have been made during the development of mammals. Zymogen granules appeared at the end of gestation and filled the exocrine cells in rat at birth (Pictet et al., 1972; Ferraz de Carvalho et al., 1978; Uchiyama and Watanabe, 1984a). The first days following birth are characterized by a substantial fall in the number of zymogen granules and a decrease in their size in mouse (Munger, 1958) and rat (Uchiyama and Watanabe, 1984b; Ermak and Rothman, 1980). This neonatal period is also marked by a decrease in the pancreas enzyme content (Robberecht et al., 1971; Deschodt-Lanckman et al., 1974) and by the functional immaturity of pancreatic secretion (Grand et al., 1976; Lamers et al., 1985). The intensity of zymogen secretion does not resemble that of an adult until after the age of 3 weeks, at weaning (Ermak and Rothman, 1986; Uchiyama and Watanabe, 1984c).

The main stages of lipid absorption by fishes have been shown by ultrastructural studies performed on *Oncorhynchus mykiss* (Bergot and Fléchon, 1970a,b; Sire and Vernier, 1979,1981; Sire et al., 1981), on *Gadus morhua* (Kjorsvik et al., 1991) and on *Sparus aurata* (Calzada et al., 1998). Our results for sea bass (Deplano et al., 1989, 1991), sea bream (Diaz et al., 1997b) and pike-perch (Mani-Ponset et al., 1994) confirm the data. Lipid absorption is materialized by the formation of lipoprotein particles whose size leads to considering them as VLDL (20–70 nm) or chylomicrons (70–500 nm). They are synthesised in the endoplasmic reticulum and they then accumulate in the Golgi vesicles before transfer to the inter-enterocyte spaces.

The first signs of lipid absorption are seen in sea bass, sea bream and pike-perch during the endotrophic period and increase at mouth-opening before any feeding. Enterocyte lipoproteinogenesis continues but is weak and stops after 48 h if the larvae fast (unpublished results). When the periblast ceases to function, little lipoprotein is transferred to the inter-enterocyte spaces and is rare or absent in the bloodstream. The opposite situation is observed at the end

of the endo-exotrophic period when high levels of lipoproteinogenesis and lipoprotein transfer to the main blood stream are observed.

In fishes, the synthesis of lipoproteins by enterocytes before the start of feeding has been observed in the embryo of *Onchorhynchus mykiss* (Vernier and Sire, 1977a; Sire and Vernier, 1979). The same phenomenon is reported in *Sparus aurata* by Calzada et al. (1998) which strengthens our previous results from this species. It is also encountered in mammals such as rat (Mak and Trier, 1979) and man (Grand et al., 1976), whose foetal intestine has limited digestion and absorption capability (review by Thomson and Keelan, 1986). For example, Mak and Trier (1979) noted the presence of lipoproteins in the intestinal mucusae of rat foetus 3 d before birth and found that the quantity of these substances increased until the completion of gestation. The authors who reported this prenatal enterocyte lipoproteinogenesis consider that it might take place from substrates from cells discharged into the intestinal lumen or from biliary lipids. The latter hypothesis is supported by the fact that enterocyte lipoproteinogenesis continued in 18-d-old rats subjected to fasting for 48 h or 72 h, whereas it was very weak in subjects whose bile duct had been previously ligatured or sectioned.

Our observations in sea bass, sea bream and pike-perch lead us to sharing the opinion of Mak and Trier (1979) and to favouring the hypothesis of the recovery of biliary lipids for the synthesis of the first enterocyte lipoproteins. The lipoprotein particles of pre-larval enterocytes doubtless mark the starting of the entero-hepatic flow of biliary lipids. The latter probably return to the liver as plasma lipoproteins present in the bloodstream during this period.

Like numerous teleost larvae (Iwai, 1968a,b; Stroband et al., 1979; Rombout et al., 1984; Watanabe and Sawada, 1985) and newly born mammals (Naito and Wisse, 1978; Grand et al., 1976), the larvae of sea bass, sea bream and pike-perch can absorb food lipids at their first feeding. This activity is slight at the beginning of trophic life and then increases at the end of the endo-exotrophic period. Our results are in agreement with the late appearance of lipolytic activities shown in larvae of *Acipenser fulvescens* (Buddington, 1985) and *Scophthalmus maximus* (Cousin et al., 1987) on day 16 and day 15, respectively. Fishes therefore react like the higher vertebrates, and especially man, as the work of Roy et al. (1975), Grand et al. (1976) and Aw and Grigor (1980) showed that the absorption of lipids is very slight in new-borns. This is probably the result of a low level of pancreatic lipase secretion (Lebenthal et al., 1981) or of the immaturity of the biliary function (Little and Lester, 1980; Watkins, 1981; Balistreri et al., 1983).

The decrease or stopping of the secretion of biliary lipids shortly after mouth-opening coincides with the decrease or stopping of intestinal absorption of lipids and of the flow of plasma lipoproteins. In conformity with the hypothesis put forward above to explain the early origin of enterocyte lipoproteinogenesis, this situation may indicate a decrease or interruption of the entero-hepatic flow of biliary lipids.

In parallel, changes and deterioration of the liver structure have been observed (Diaz et al., 1998). These changes characteristic of cholestasis are known in newly born mammals and are probably the result of imperfect entero-hepatic flow of biliary acids (reviews by Balistreri et al., 1983; Suchy et al., 1987; and Jacquemin, 1992). Like mammals, fish perhaps undergo ‘physiological cholestasis’ when they attain exotrophy. As in mammals at weaning (Balistreri et al., 1983), the mechanisms involved in entero-hepatic flows of biliary compounds may be perfected during the transition to strict exotrophy. This would explain why this developmental phase in sea bass, sea bream and pike-perch is marked by an increase in the intensity of biliary secretion, intestinal absorption of lipids and flow of plasma lipoproteins.

This discussion leads to two conclusions: (1) the fish post-embryonic lipid metabolism is divided into several phases marking the gaining of trophic autonomy; (2) this trophic autonomy is gained according to a pattern that displays great analogies between fishes and mammals.

(1) Four phases characteristic of early lipid metabolism are superimposed on the conventional endotrophic, endo-exotrophic and exotrophic periods (Fig. 5). The first, corresponding to the endotrophic period, begins at hatching and is completed just after mouth-opening. It is characterized (a) by an intense use of yolk reserves during which the periblast synthesises lipoproteins that are distributed by the bloodstream, (b) by organogenesis of the digestive tract and the establishment of activities for the use of food lipids (biliary secretion, pancreatic secretion and intestinal absorption). The second phase follows the first feeding and covers the very first days of larval life. It is marked by the exhaustion of yolk reserves, the stopping of periblast lipoprotein synthesis and the subsequent disappearance of lipoproteins from the bloodstream. It is also marked by weak hepatic and pancreatic activity closely correlated with weak intestinal absorption of lipids. The third phase is completed by the complete resorption of the yolk vesicle and consequently by the end of the endo-exotrophic period; it is characterised by a steady increase in hepatic synthesis (biliary lipids) and pancreatic synthesis (zymogen) while the intestinal absorption of lipids develops and lipoproteins are transferred into the larval bloodstream. The fourth phase corresponds to the strictly exotrophic period during which hepatic, pancreatic and intestinal activities display adult intensities.

(2) Analysis of the results obtained in three teleost species makes it possible to stress the close correlation between the development of the biliary function, that of the exocrine function of the pancreas and that of the intestinal absorption of lipids. These activities established during the endotrophic period are effective at the first feeding. They are slight at the beginning of trophic life and then increase at the end of the endo-exotrophic period, reaching a level compatible with the satisfactory handling of food lipids when

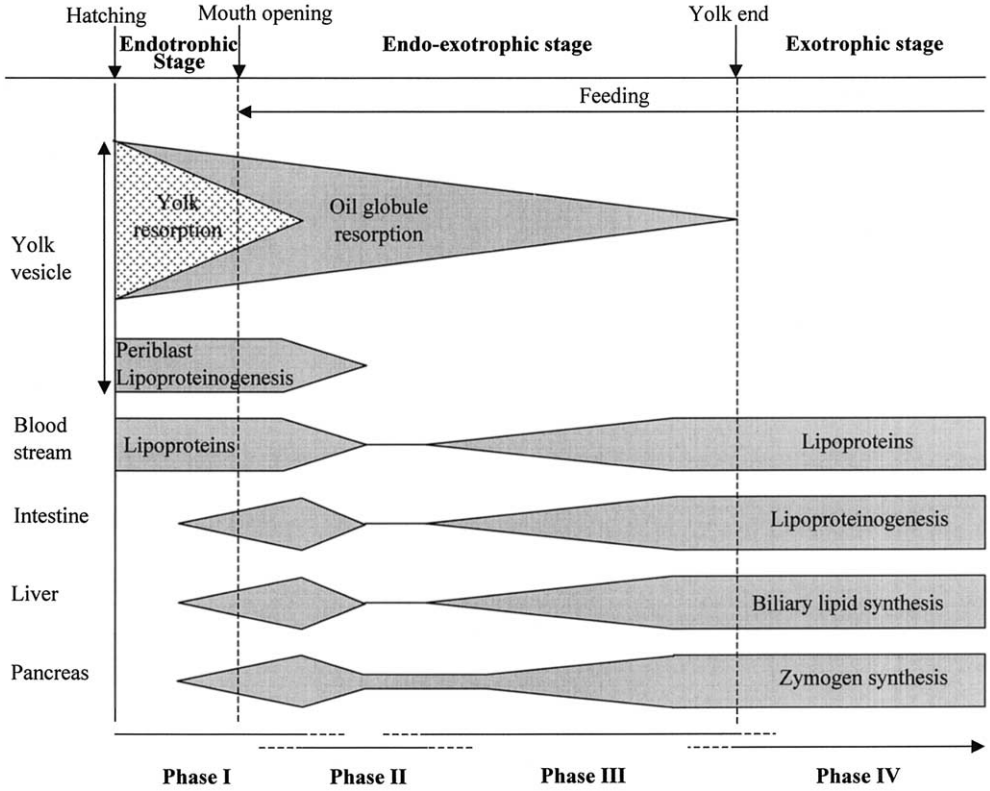

Fig. 5. Main physiological phases of lipid metabolism during early development in sea bass, *Dicentrarchus labrax*, sea bream, *Sparus aurata* and pike perch, *Stizostedion lucioperca*.

the strictly exotrophic stage is attained. Comparison with the activities observed in mammals makes it possible to put together the endotrophic or pre-larval period and the foetal period, the endo-exotrophic period and the suckling period, the strictly exotrophic period and the post-weaning period.

Acknowledgements

The authors thank Mr. T. Noëll and the staff of the Electron Microscopy Centre at the Université Montpellier II for their excellent technical collaboration.

References

Aw, T.Y., Grigor, M.R., 1980. Digestion and absorption of milk triacylglycerols in 1–14-day-old suckling rats. J. Nutr. 110, 2133–2140.

Babin, P.J., Bogerd, J., Kooiman, F.P., Van Marrewijk, W.J.A., Van der Horst, D.J., 1999. Apolipophorin II/I, apolipoprotein B, vitellogenin, and microsomal triglyceride transfer protein genes are derived from a common ancestor. J. Mol. Evol. 49, 150–160.

Balistreri, W.F., Heubi, J.E., Suchy, F.J., 1983. Immaturity of the enterohepatic circulation in early life: factors predisposing to "physiologic" maldigestion and cholestasis. J. Pediatr. Gastroenterol. Nutr. 2, 346–353.

Beccaria, C., Diaz, J.P., Connes, R., Chatain, B., 1991. Organogenesis of the exocrine pancreas in the sea bass. *Dicentrarchus labrax* L., reared extensively and intensively. Aquaculture 99, 339–354.

Bergot, P., Fléchon, J.E., 1970a. Forme et voie d'absorption intestinale des acides gras à chaine longue chez la truite arc-en-ciel (*Salmo gairdneri* Rich). I. Lipides en particules. Ann. Biol. Anim. Biochim. Biophys. 10, 459–472.

Bergot, P., Fléchon, J.E., 1970b. Forme et voie d'absorption intestinale des acides gras à chaîne longue chez la Truite arc-en-ciel (*Salmo gairdnerii* Rich.). II. Lipides "étalés". Ann. Biol. Anim. Biochim. Biophys. 10, 473–480.

Bongiovanni, A.M., 1965. Bile acid content of gall bladder of infants, children and adults. J. Clin. Endocrinol. Metabol. 25, 678–685.

Buddington, R.K., 1985. Digestive secretions of lake sturgeon, *Acipenser fulvescens*, during early development. J. Fish Biol. 26, 715–723.

Calzada, A., Medina, A., de Canales, M.L.G., Medina, R.P., 1998. A fine structure of the intestinal development in cultured sea bream larvae. J. Fish Biol. 53, 340–365.

Cousin, J.C.B., Baudin-Laurencin, F., 1985. Morphogenèse de l'appareil digestif et de la vessie gazeuse du Turbot, *Scophthalmus maximus*. L. Aquaculture 47, 305–319.

Cousin, J.C.B., Baudin-Laurencin, F., Gabaudan, J., 1987. Ontogeny of enzymatic activities in fed and fasting turbot, *Scophtalmus maximus* L. J. Fish Biol. 30, 15–33.

Crawford, J.M., Berken, C.A., Gollan, J.L., 1988. Role of the heptocyte microtubular system in the excretion of bile salts and biliary lipid: implications for intracellular vesicular transport. J. Lipid Res. 29, 144–156.

Crawford, J.M., Vinter, D.W., Gollan, J.L., 1991. Taurocholate induces pericanalicular localization of C6-NBD-ceramide in isolated hepatocyte couplets. Am. J. Physiol. Endocrinol. Metabol. 260, G119–G132.

Danielsson, H., Rutter, W.J., 1968. The metabolism of bile acids in the developing rat liver. Biochemistry 7, 346–352.

Deplano, M., Connes, R., Diaz, J.P., Paris, J., 1989. Intestinal steatosis in the farm-reared sea bass *Dicentrarchus labrax*. Dis. Aquat. Org. 6, 121–130.

Deplano, M., Diaz, J.P., Connes, R., Kentouri-Divanach, M., Cavalier, F., 1991. Appearance of lipid absorption capacities in larvae of the sea bass *Dicentrarchus labrax* L., during transition to the exotrophic phase. Mar. Biol. 108, 361–371.

Deschodt-Lanckman, M., Robberecht, P., Camus, J., Baya, C., Christophe, J., 1974. Hormonal and dietary adaptation of rat pancreatic hydrolases before and after weaning. Am. J. Physiol. 226, 39–44.

Diaz, J.P., Carmona-Sepulveda, M., Connes, R., 1992. L'organogenèse du pancréas exocrine chez la daurade *Sparus aurata*. Cah. Agric. 1, 101–108.

Diaz, J.P., Connes, R., 1991. Development of the liver of the sea bass, *Dicentrachus labrax* L (Teleost, Fish): II, Hepatocyte differentiation. Biol. Struct. Morphog. 3, 57–65.

Diaz, J.P., Mani-Ponset, L., Guyot, E., Connes, R., 1997a. Biliary lipid secretion during early post-embryonic development in three fishes of aquacultural interest: sea bass, *Dicentrarchus labrax* L, sea bream *Sparus aurata* L., and pike perch *Stizostedion lucioperca* (L). J. Exp. Zool. 277, 365–370.

Diaz, J.P., Guyot, E., Vigier, S., Connes, R., 1997b. First events in lipid absorption during post-embryonic development of the anterior intestine in gilt-head sea-bream. J. Fish Biol. 51, 180–192.

Diaz, J.P., Mani-Ponset, L., Guyot, E., Connes, R., 1998. Hepatic cholestasis during the post-embryonic development of fish larvae. J. Exp. Zool. 280, 277–287.

Ermak, T.H., Rothman, S.S., 1980. Large decrease in zymogen granule size in the postnatal rat pancreas. J. Ultrastruct. Res. 70, 242–256.

Ermak, T.H., Rothman, S.S., 1986. Zymogen granule size in pancreas of nursing rats. J. Morphol. 187, 289–299.

Ferraz de Carvalho, C.A., Laurindo, F.R.M., Taga, R., Sesso, A., 1978. Ultrastructural morphometric study on developing acinar cells of the rat pancreas and parotid gland. Acta Anat. 101, 234–244.

Gallagher, M.L., Luczkovich, J.J., Stellwag, E.J., 2001. Characterization of the ultrastucture of the gastrointestinal tract mucosa, stomach contents and liver enzyme activity of the pinfish during development. J. Fish Biol. 58, 1704–1713.

Govoni, J.J., 1980. Morphological, histological, and functional aspects of alimentary canal and associated organ development in larval *Leiostomus xanthurus*. Rev. Can. Biol. 39, 69–80.

Graham, T.O., Van Thiel, D.H., Little, J.M., Lester, R., 1979. Synthesis of taurocholate by rat liver in organ culture: effects of cortisol in vitro. J. Am. Physiol. 237, E177–E184.

Grand, R.J., Watkins, J.B., Torti, F.M., 1976. Progress in gastroenterology: Development of the human gastrointestinal tract: a review. Gastroenterology 70, 790–810.

Guyot, E., Connes, R., Diaz, J.P., 1993. Résorption des réserves vitellines et passage de l'endotrophie à l'exotrophie chez la larve de daurade (*Sparus aurata*) nourrie et à jeun. In: Barnabé, G., Kestemont, P. (Eds.), Production, Environment and Quality. Bordeaux Aquaculture 1992. Eur. Aquac. Soc. Spec, 18. Ghent, Belgium, pp. 213–226.

Guyot, E., Diaz, J.P., Connes, R., 1995. Organogenesis of liver in sea bream *Sparus aurata*. J. Fish Biol. 47, 427–437.

Hardy, K.J., Hoffman, N.E., Mihaly, G., Sewell, R.B., Smallwood, R.A., 1980. Bile acid metabolism in fetal perinatal changes in the bile acid pool. J. Physiol. 309, 1–11.

Iwai, T., 1968a. The comparative study of the digestive tract of teleost larvae. V. Fat absorption in the gut epithelium of goldfish larvae. Bull. Jpn. Soc. Sci. Fish. 34, 973–978.

Iwai, T., 1968b. Fine structure and absorption patterns of intestinal epithelial cells in rainbow trout alevins. Z. Zellforsch. 91, 366–379.

Jackson, B.T., Smallwood, R.A., Piasecki, G.J., Brown, A.S., Rauschecker, H.F.J., Lester, R., 1971. Fetal bile salt metabolism.I. The metabolism of sodium cholate -14C in the fetal dog. J. Clin. Invest. 50, 1286–1294.

Jacquemin, E., 1992. Développement et physiopathologie de la sécrétion biliaire. Arch. Fr. Pediatr. 49, 741–748.

Kjorsvik, E., Van Der Meeren, T., Kryvi, H., Arnfinnson, J., Kvenseth, P.G., 1991. Early development of digestive tract of cod larvae, *Gadus morhua* L., during start-feeding and starvation. J. Fish Biol. 38, 1–15.

Lambson, R.O., 1970. An electron microscopic study of the entodermal cells of the yolk sac of the chick during incubation and after hatching. Am. J. Anat. 129, 1–20.

Lamers, W.H., Mooren, P.G., Charles, R., 1985. Perinatal development of the small intestine and pancreas in rat and spiny mouse. Its relation to atricial and precocial timing of birth. Biol. Neonate 47, 153–162.

Lebenthal, E., Choi, T.S., Lee, P.C., 1981. The development of pancreatic function in premature infants after milk based and soy based formulas. Pediatr. Res. 15, 1240–1244.

Lester, R., Little, J.M., Greco, R., Piasecki, G.J., Jackson, B.T., 1973. Fetal bile salt formation. Pediatr. Res. 6, 375.

Little, J.M., Lester, R., 1980. Ontogenesis of intestinal bile salt absorption in the neonatal rat. Am. J. Physiol. 239, G319–G323.

Little, J.M., Richey, J.E., Van Thiel, D.H., Lester, R., 1979. Taurocholate pool size and distribution in the fetal rat. J. Clin. Invest. 63, 1042–1049.

Loewe, H., Eckmann, R., 1988. The ontogeny of the alimentary tract of coregonid larvae: normal development. J. Fish Biol. 33, 841–850.

Mak, K.M., Trier, J.S., 1979. Lipoprotein particles in the jejunal mucosa of postnatal developing rats. Anat. Rec. 194, 491–506.

Mani-Ponset, L., Diaz, J.P., Schlumberger, O., Connes, R., 1994. Development of yolk complex, liver and anterior intestine in pike-perch larvae, *Stizostedion lucioperca* (Percidae), according to the first diet during rearing. Aquat. Living Resour. 7, 191–202.

Mani-Ponset, L., Guyot, E., Diaz, J.P., Connes, R., 1996. Utilization of yolk reserves during post-embryonic development in three teleostean species: the sea bream *Sparus aurata*, the sea bass *Dicentrarchus labrax*, and the pike-perch *Stizostedion lucioperca*. Mar. Biol. 126, 539–547.

Munger, B.L., 1958. A phase and electron microscopic study of cellular differentiation in pancreatic acinar cells of the mouse. Am. J. Anat. 103, 1–33.

Naito, M., Wisse, E., 1978. Filtration effect of endothelial fenestrations on chylomicron transport in neonatal rat liver sinusoids. Cell. Tissue Res. 190, 371–382.

Noble, R.C., Cocchi, M., 1990. Lipid metabolism and the neonatal chicken. Progr. Lipid Res. 29, 107–140.

Piccoli, D.A., Muller, E.S., Vanderslice, R.R., Watkins, J.B., 1986a. Bile salt secretion in the developing rat: implications for cholestasis. Gastroenterology 90, 1801.

Piccoli, D.A., Muller, E.S., Vanderslice, R.R., Watkins, J.B., 1986b. Maturation of biliary steady uptake and secretion capacity in the perfused rat liver. Hepatology 6, 1185.

Pictet, R.L., Clark, W.R., Williams, R.H., Rutter, W.J., 1972. An ultrastructural analysis of the developing embryonic pancreas. Develop. Biol. 29, 436–467.

Plonné, D., Winkler, L., Franke, H., Dargel, R., 1992. The visceral yolk sac: an important site of synthesis and secretion of apolipoprotein B containing lipoproteins in the feto-placental unit of the rat. Biochim. Biophys. Acta 1127, 174–185.

Poley, J.R., Dower, J.C., Owen, C.A., Stickler, G.B., 1964. Bile acids in infants and children. J. Clin. Invest. 63, 838–846.

Poupard, G., André, M., Durliat, M., Ballagny, C., Bœuf, G., Babin, P.J., 2000. Apolipoprotein E gene expression correlates with endogenous lipid nutrition and yolk syncytial layer lipoprotein synthesis during fish development. Cell Tissue Res. 300, 251–261.

Robberecht, P., Deschodt-Lanckman, M., Camus, J., Bruylands, J., Christophe, J., 1971. Rat pancreatic hydrolases from birth to weaning and dietary adaptation after weaning. Am. J. Anat. 221, 376–381.

Rombout, J.H.W.M., Stroband, H.W.J., Taverne-Thiele, J.J., 1984. Proliferation and differentiation of intestinal cells during development of *Barbus conchonius* (Teleostei, Cyprinidae). Cell. Tissue Res. 236, 207–216.

Roy, C.C., Ste-Marie, M., Chartrand, L., Weber, A., Bard, H., Doray, B., 1975. Correction of the malabsorption of the preterm infant with a medium chain triglyceride formula. Pediatrics 86, 446–450.

Seligman, A.M., Wasserkrug, H.L., Hanker, J.S., 1966. A new staining method (OTO) for enhancing contrast of lipid-containing membranes and droplets in osmium tetroxide-fixed tissue with osmiophilic thiocarbohydrazide (TCH). J. Cell Biol. 30, 424–432.

Shaffer, E.A., Zahavi, I., Gall, D.G., 1985. Postnatal development of hepatic bile formation in the rabbit. Digest. Dis. Sci. 30, 558–563.

Sire, M.F., Lutton, C., Vernier, J.M., 1981. New views on intestinal absorption of lipids in Teleostean fishes: an ultrastructural and biochemical study in the rainbow trout. J. Lipid Res. 22, 81–94.

Sire, M.F., Vernier, J.M., 1979. Formation de VLDL intestinales endogènes. Etude ultrastructurale sur un nouveau modèle, l'embryon et l'adulte à jeun, de truite. Biol. Cell 35, 271–280.

Sire, M.F., Vernier, J.M., 1980. Lipid staining on semithin sections with sudan black B or nile blue sulphate. Application to intestinal fat absorption. Acta Histochem. Cytochem. 13, 193–201.

Sire, M.F., Vernier, J.M., 1981. Etude ultrastructurale de la synthèse de chylomicrons au cours de l'absorption intestinale des lipides chez la truite. Influence de la nature des acides gras ingérés. Biol. Cell 40, 47–62.

Smallwood, R.A., Lester, R., Piasecki, G.J., Klein, P.D., Greco, R., Jackson, B.T., 1972. Fetal bile salt metabolism II. Hepatic excretion of endogenous bile salt and of a taurocholate load. J. Clin. Invest. 51, 1388–1397.

Stroband, H.W.J., Meer, H.V.D., Timmermans, L.P.M., 1979. Regional functional differentiation in the gut of the grass carp, *Ctenopharyngodon idella* (Val). Histochemistry 64, 235–249.

Subbiah, M.R., Hassan, A.S., 1982. Development of bile acid biogenesis and its significance in cholesterol homeostasis. Adv. Lipid Res. 19, 137–161.

Suchy, F.J., Bucavalas, J.C., Novak, D.A., 1987. Determinants of bile formation during development: ontogeny of hepatic bile acid metabolism and transport. Seminars in liver disease 7, 77–84.

Tavoloni, N., Jones, M.J.T., Berk, P.D., 1985. Postnatal development of bile secretory physiology in the dog. J. Pediatr. Gastroenter. Nutr. 4, 256–267.

Thomson, A.B.R., Keelan, M., 1986. The development of the small intestine. Can. J. Physiol. Pharmacol. 64, 13–29.

Uchiyama, Y., Watanabe, M., 1984a. A morphometric study of developing pancreatic acinar cells of rats during prenatal life. Cell Tissue Res. 237, 117–122.

Uchiyama, Y., Watanabe, M., 1984b. Morphometric and fine structural studies of rat pancreatic acinar cells during early postnatal life. Cell Tissue Res. 237, 123–128.

Uchiyama, Y., Watanabe, M., 1984c. A morphometric study of 24 hours variations in subcellular structures of rat pancreatic acinar cells during the periweaning period. Cell Tissue Res. 237, 131–138.

Vernier, J.M., Sire, M.F., 1977a. Lipoprotéines de très basse densité et glycogène dans le syncytium vitellin, l'épithélium intestinal et le foie, aux stades précoces du développement embryonnaire chez la truite arc-en-ciel. Biol. Cell 29, 45–54.

Vernier, J.M., Sire, M.F., 1977b. Plaquettes vitellines et activité hydrolasique acide au cours du développement embryonnaire de la truite arc-en-ciel. Etude ultrastructurale et biochimique. Biol. Cell 29, 99–112.

Walzer, C., Schönenberger, N., 1979a. Ultrastructure and cytochemistry study of the yolk syncytial layer in the alevin of trout (*Salmo fario trutta* L. and *Salmo gairdneri* R.) after hatching. I - The vitellolysis zone. Cell. Tissue Res. 196, 59–73.

Walzer, C., Schönenberger, N., 1979b. Ultrastructure and cytochemistry of the yolk syncytial layer in the alevin of trout (*Salmo fario trutta* L. and *Salmo gairdneri* R.) after hatching. II - The cytoplasmic zone. Cell Tissue Res. 196, 75–93.

Watanabe, Y., Sawada, N., 1985. Larval development of digestive organs and intestinal absorptive functions in the freshwater goby *Chaenogobius annularis*. Bull. Tohoku Reg. Fish. Res. Lab. 47, 1–10.

Watkins, J.B., 1981. Role of bile acids in the development of the enterohepatic circulation. In: Lebenthal, E. (Ed.), Gastroenterology and Nutrition in Infancy. Raven Press, New York, pp. 167–176.