Süleyman Demirel Üniversitesi
Eğirdir Su Ürünleri Fakültesi Dergisi
Cilt: 1, Sayı : 2 , (1-6) (2005)

Development of the liver and pancreas in the rainbow trout *(Oncorhynchus mykiss)*

KENAN ÇINAR NURGÜL ŞENOL

Department of Biology, Süleyman Demirel University, 32260 Isparta, Turkey

ABSTRACT

In this study, we aimed to determine the histochem ical structure and histological characters of development of the liver and pancreas. The development of the liver and pancreas was observed through different development periods in light microscopic. In total, 35 rainbow trouts (*Oncorhynchus mykiss*) were used. The lobules and bile ducts were studied in the liver in the early development periods. In addition, the dense PAS (+) characters and glycogen in hepatocytes were observed in the same periods. In the following periods, the weak PAS (+) and AB (+) chara cters were found in hepatocytes during the development of the lobules and bile duct. The reaction against PAS was also observed in the apical cells of the bile duct. During the early development periods, islets of langerhans were found in a few samples. Bu t, their number increased in the following periods. During all of the development periods, these cells in the exocrine part were proved to be of basophilic characters. Histochemical methods were applied on pancreas were in all periods.

Key words: Development, liver, pancreas, *Oncorhynchus mykiss*

Gökkuşağı alabalığında (*Oncorhynchus mykiss)* karaciğer ve pankreasın gelişimi

ÖZET

Bu çalışmada karaciğer ve pankresın histokimyasal yapısı ve histolojik karakterlerinin belirlenmesi amaçlandı. Kara ciğer ve pancreas farklı gelişim dönemlerinde ışık mikroskobik olarak gözlemlendi. Toplam 35 adet gökkuşağı alabalığı (*Oncorhynchus mykiss)* kullanıldı. Erken gelişim dönemlerinde karaciğerde, loplar ve safra kanalları belirlendi. Ek olarak aynı dönemlerde yoğun PAS (+) ve gilikojen içerikli hepatositler gözlendi. Takip eden dönemlerde lopların ve safra kanallarının gelişimi süresince hepatositlerde zayıf PAS (+) ve AB (+) karakter saptandı. Safra kanalının apikal hücrelerinde PAS' a karşı reaksiyonda gözle ndi. Erken gelişim dönemlerinde, langerhans adacıkları çok az örnekte bulundu. Fakat bu adacıkların sayısı takip eden dönemlerde artmaktadır. Tüm gelişim süresince ekzokrin bölümdeki hücreler bazofilik karakter göstermektedir. Histokimyasal metodlar pankre asa tüm dönemlerde uygulandı.

Anahtar kelimeler: Gelişim, karaciğer, pankreas, *Oncorhynchus mykiss*

INTRODUCTION

As it is the case in other vertebrates, the digestive system in fish consists of pancreas, liver, stomach and intestinal tract (Kjorsvik and Rhersen, 1992). The liver, which is one-lob in Salmonidae, and three-lobe in Scomberidae, is generally two-lobe in other fish species (Demir, 1992). Being covered with a serous membrane, it enables the liver lobes to merge by the elongation of this membrane together with the connective tissue into the parechyma of liver. Although this structure differs among different species of fish, it is not often distinctive. The most important function of the liver in the digestion is to secrete bile. This secretion is secreted by hepatic cells occupying intercellular bile duct. In the final periods the bile secreted into the hepatic duct is sent to duodenum. As for the stomachless fish, the bile duct opens into the anterior chamber of the intestines (Anderson and Mitchum, 1974).

As in other vertebrates, the pancreas in fish is an organ that consists of endocrine and exocrine parts (Pedersen and Petersen, 1992). Though endocrine and exocrine characterized pancreatic tissues are distinct from each other (Beccaria *et al.,* 1992) in some fish species, they have a similar compact structure as in high vertabrates (Demir, 1992; Anderson and Mitchum, 1974). This organ, however, largely develops around the digestive tract in most teleosts (Demir, 1992). In some fish species, pancreas grows together with the liver tissues, as a result of which the structure called hepato pancreas appears (Hibiya, 1982).

Enzyme and hormones secreted from exocrine and endocrine part of pancreas for digestion vary in some fish species. The enzymes such as amylase, maltase, lactase and lipase from exocrine part and the hormones such as glucagon, insuline, somatostatin, pancreatic polypeptide (PP), neuropeptide Y (NPY), peptide tyrosine tyrosine (PYY) are secreted from endocrine chamber (Demir, 1992; Anderson and Mitchum, 1974; Pan *et al.*, 2000; Al-Mahrouk and Youson, 1998; Youson *et al.*, 2001).

It is stated that this can be related to other ecological conditions as well as the nourishment and that the development of the digestive organs is triggered in the early periods along with the nourishment (Yoa, 1987).

Dabrowski (1986) states that digestive system takes an adult shape when Salmonidae starts to feed. The same researcher has stated that, as physiologically observed in postnatal periods of the mammals, some enzymatic differences occur depending upon the age. However, such process do not occur in cyprinid and salmonid' s larvae or juveniles.

Boglione *et al.*, (1992) and Kjorsvik *et al.*, (1991) affirm that digestive organs have peculiar physiological features in each period defined as embryonic, prelarval, larval, and juvenile. Moreover, Bonglione *et al.* (1992) state that the digestive tract and the glands completely differs from each other as far as the twelve-day larvae of *Chelon labrosus* species are concerned. In this study, we aimed to determine the histochemical structure and histological characters of development of the liver and pancreas.

MATERIALS and METHODS

In this study, thirty five rainbow trouts *(Oncorhynchus mykiss)* were used (Table 1). On the days illustrated in Table 1, rainbow trouts were fed with the fodders (Granule sizes 2, 3, 4, and Pellets 3 mm, 4 mm, 5 mm, 6 mm) three times a day. Water temperatures between the day 32 and 300 varied approximately from 7 to 16 ^{o}C, and the pH was 7 to 9.5.

Samples were taken from liver and pancreas tissues of the rainbow trout *(Oncorhynchus mykiss)* on the days 32, 59, 94, 120, 180, 210, 300. Pancreas tissue samples of 30 mm were fixed for 24 h in 10 % formaline and liver tissue samples of the same size in Carnoy' s. Fixed tissues were dehydrated in graded ethanol solutions, cleared in xylene, embedded in paraffin (vacuum embedded) and sectioned at 5-6 µm. sections were mounted on glass slides, deparaffinized stained by Hematoxylin-eosin (H&E) (Culling, 1963), Alcian blue (AB), Periodic acid-Schiff (PAS) (M.C. Manus, 1946), Best Carmine (Spicer and Mayer, 1960; Best, 1906).

Table 1: Measuring of rainbow trout *(Oncorhynchus mykiss)*

Day Post hatching	Total lenght (cm)	Weight (g)
32	2	1-1.5
59	3	3-3.5
94	6	8-11
120	10.5-12	20-25
180	17-17.5	60-65
210	19.5-21	76-83
300	22-23	255-270

RESULTS

On the day 32, the liver was proved to be in the anterio-ventral part of the stomach and the hepatocytes to contain numerous vacuoles. While there were a few numbers of sinusoids in liver tissues, no lobule structure was observed. Furthermore, no Langerhans islet was found in pancreatic tissues, which are densely distributed and observed among the pyloric sekas as well as the cells having intensive basophilic character in exocrine part. While liver hepatocytes react favorably to the both PAS and Best Carmine applications, it was revealed that pancreatic tissues did not respond to the histochemical applications.

On the day 59, vacuolization was found to exist in liver epithelium cells. Yet, no typical lobule was observed. Throughout this period, neither sinusoids nor central venas were not observed. In addition to the observation of a few langerhans islets, the cells in the exocrine part were proved to be of intensive basophilic character (Figure 1).

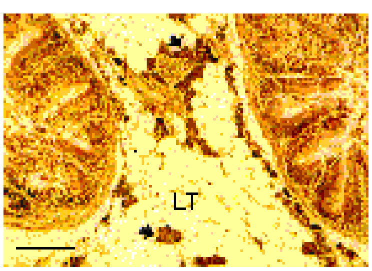

Figure 1: Pancreatic tissue of 59 days of *Oncorhynchus mykiss*. Lipoid tissue (LT) Bar:100 µm

As it was the case during the previous periods, hepatocytes reacted to the PAS and Best Carmine applications during this period, as well. As for pancreatic tissues, they haven't been dyed through histochemical methods being applied. As were in previous periods, vacuolization was determined to last in hepatocytes, too. Again, similar to the previous periods, numerous central venas turned out to exist, whereas no bile duct was observed during this period.

As in previous periods, many sinusoids and enlarged central venas were found in the livers of 120-day old fish. Liver cords were made up of two cell rows. Also, bile channels were determined to exist in fish during this period.

While no lobule was traced in 120, 180 and 210-day old fish, uneven lobules were traced to acquire a shape in 300-day old fish. Hepatic arter, vena and bile duct (duktus biliferus) were observed to form trio groups in connective tissue where lobules were interlocked. Fat tissues, which are among pancreatic tissues, were determined to increase more and more from the day 120 to 300. The Langerhans islets were found to have increased in number and the pancreatic channels in exocrine part to have been highly developed. Moreover, the cells in exocrine parts included intensive zymogene granule (Figure 2).

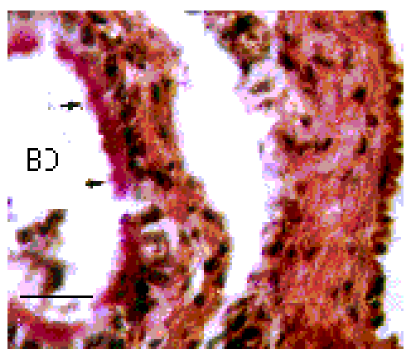

Figure 2 : Liver of 180 days of *Oncorhynchus mykiss*. Bile duct (BD), Bar: 50 µm.

DISCUSSION

The liver in newly hatched Atlantic cod (*Gadus morhua*) individuals have been reported to acquire a primitive shape and many liver hepatocytes to involve basophilic character as well as numerous vacuols (Pedersen and

Petersen, 1992). As for *Hippoglossus hippoglossus* species, it was determined that the liver had developed by making a posterior protrusion from the digestive tract to the heart on the first day after the hatching. Then, it established itself in ventral (Kjorsvik and Rhersen, 1992). Similarly, it was observed to acquire a shape in the form of a bud in digestive tract of the primordial liver during the prelarval periods of Gilt-Head Sea Bream (*Sparus aurata)* species. As for the development of hepatocytes, it began to form after the day 9 and had no bile ducts (Guyot *et al.*, 1995). In this study, the liver of rainbow trouts *(Oncorhynchus mykiss)*, which are in the early states of their development, was located in anterio-ventral of the stomach and their hepatocytes were mostly of basophilic character.

In accordance with the information obtained regarding *Gadus morhua* (Pedersen and Petersen, 1992), it was observed that some hepatocytes had vacuolization. Besides, bile ducts of Gilt-Head Sea Bream (*Sparus aurata)* acquired their shapes in the early periods of development (Guyot *et al.*, 1995), whereas bile ducts were first observed 120-day of rainbow trouts *(Oncorhynchus mykiss)* in the study.

Boulhic and Gabaurden (1992) determined that liver of *Solea solea* had no glycogen storing feature after 30 days from the hatching. However, glycogen storing process took place intensively in the following periods of their development.

Moreover, Santos and Vinagre (1991) stated that the amount of glycogen in the liver was high and even increased through the following periods of the development. In another study (Hyvaeriner *et al.*, 1985), it was suggested that the liver glycogen varied in amount according to the age and seasonal changes. Glycogen was reported to be observed in 15-day *Gadus morhua* (Kjorsvik *et al.*, 1991) larvae and in the hepatocytes of Acipencer (Hung *et al.*, 1990) juveniles. In this study, a great amount of glycogen was determined in the hepatocytes of fish almost in the same periods.

Also, the liver responded to the PAS applications positively in all groups, which supports the data obtained by Pedersen and Petersen (1992) and Hibiya (1982). In accordance with data obtained with respect to *Salmo aurata* (Guyot *et al.*, 1995) species, the liver glycogen was determined to decrease in the following periods of the development. Similar to data obtained with regard to *Hippoglossus hippoglossus* (Kjorsvik and Rhersen, 1992), sinusoids, which were less in the beginning of the development, were found to increase in the following periods. As stated by Guyot *et al.* (1995) and Yao (1987), liver courts were proved to be composed of two cell layers and to make anostomosis with each other.

The pancreas was observed to be located in the posterior part of the intestines during the early stages of the development in some fish species (Kjorsvik and Rhersen, 1992), whereas it was in the liver paranchima in some other species (Cataldi *et al.*, 1987). Kjorsvik and Rhersen (1992) stated that pancreatic tissues started to form for the first time in the posterior part of the liver after the day 20 from the hatching. While Hibiya (1982) states that endocrine and exocrine tissues are of identical origin and that these structures develop during the embryonic period. Boulhic and Gabaudan (1992) reported that the liver of *Solea solea*, gall bladder and pancreas became distinctive on the day 30 after the brood.

In this study, as stated for *Gadus morhua* (Pedersen and Petersen, 1992), the pancreas was determined to take place immensely among the pyloric caeca. As for the cells forming this organ, it was determined that they were of asidophilic and basophilic character. In accordance with the data obtained in the study of *Hippoglossus hipp*oglossus (Kjorsvik and Rhersen, 1992), zymogen granules were determined in asidophilic cells. As for *C. fera* (Loeve and Eckmann, 1988) species, a few, small Langerhans islets were reported to exist in the pancreas 10 days after the nourishment. In addition, we determined that Langerhans islets acquire their shape by the day 59 and their number increase in the next periods of the development. Since the liver glycogen cannot perform its absorption-related duties of the intestines efficiently in the early stages of the development, it was interpreted that the liver consists of intensive glycogen so as to meet the energy gap that is likely to occur.

REFERENCES

Anderson B.G., Mitchum D.L., 1974. Atlas of Trout Histology, Wyoming Game Fish Department Bulletin, 13: U.S.A

Al-Mahrouki, A.A., Yousan, J.H., 1998. Immunohistochemical studies of the endocrine cells within the gastro-entero-pancreatic system of *Osteoglosso morpha*, an ancient teleostean group, Gen. Comp. Endcrinol., 110 (2):125-39 pp.

Beccaria, C., Diaz, J.P., Connes, R., 1992. Effects of dietary conditions of the exocrine pancreas of the sea bass, *Dicentrarchus labrax* L., Aquacult., 101:163-176 pp.

Best, F., 1906. Unber karmin ferbung des gkykogens und der kerne, Z. Wiss. Mikr., 30:53-60 pp.

Boglione, B., Bertolini, B., Russiello, M., Cataduella, S., 1992. Embryonic and larval development of the thick-lipped mullet (*Chelon lobrosus*) under controlled reproduction conditions, Aquaculture, 101: 349-359 pp.

Boulhic, M., Gabaudan, J., 1992. Histological study of the organogenesis of the digestive system and swim bladder of the docer solea, *Solea solea* (L., 1758).Aquaculture, 102:373-396 pp.

Cataldi, E., Cataudella, S., Monaco, G., Rossi, A., Tancioni., 1987. A study of the histology and morphology of the digestive tract of the sea-bream, *Sparus aurata*, Journal of Fish Biolgy, 30:1,35-145 pp.

Culling, C.F.A., 1963. Handbook of Histological Techniques (Including Museum Techniques) Second Edition, London, Butterworhts, 553 p.

Dabrowski, K.R., 1986. Ontogenetical aspects of nutritional requirements in fish, Comp., Biochem. Physiol., 85:639-655 pp.

Demir, N., 1992. İhtiyoloji, İ. Ü. Yayın., Sayı: 3668 , İstanbul.

Guyot, E., Diaz, J.P., Connes, R., 1995. Organogenesis of the liver in sea bream, J. of Fish Biol., 47:427-437 pp.

Hibiya, T., 1982. An Atlas of Fish Histology Normal and Pathological Features, College of Agriculture and Veterinary Medicine, Nihon Univ., 73-93 pp., Tokyo, Japan.

Hung, S.S.O., Groff, J.M., Lutes, P.B., Alkins, F.K.F., 1990. Hepatic and intestinal histology of juvenile white sturgean fed different carbonhydrates, Aquaculture, 87:349-360 pp.

Hyvaeriner, H., Holopainen, I.J., Piironon, J., 1985. Anaerobic wintering of crucian carp (*Carassius carassius* L.) 1. annual dynamics of glycogen reserves in nature, Comp. Biochem. Physiol., 82:797-803 pp.

Kjorsvik, E., Rehersen, A.L., 1992. Histomorphology of the early yolk-sac larva of the atlantic aalibut (*Hippoglossus hippoglossus* L.) an indication of the timing of functionality, J. of Fish Biol., 41:1-19 pp.

Kjorsvik, E., Van Der Meeren, T., Kryvı, H., Ainfinnos, J., Kuenseth, P.G., 1991. Early development of digestive tract of cod larvae, *Gadus morhua* L., during start-feeding and starvation, J. of Fish Biol., 38:1-15 pp.

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

Manus, M.C., 1946. Histologic demonstration of mucin after periodic acid, Nature, 158-202 pp.

Pan, Q.S., Fang, Z.P., Huang, F.J., 2000. Identification, localization and morphology of APUD cells in gastroenteropancreatic system of stomach containing teleosts, Journal Gastroenterol, 6(6):842-847 pp.

Pedersen, T., Petersen, I.B., 1992. Morphological changes during metamorphosis in rod (*Gadus morhua* L.), with particular reference to the stomach and pyloric caeca, J. of Fish Biology, 41:449-461 pp.

Santos, E.A., Vinagre, A.S., 1991. Carbonhydrate metabolizm during embryonic and larval development of *Odonthostes humensis* (DeBuer,1953) (Pisces-Atherinidae), J. of Fish Biol., 39:239-344 pp.

Spicer, S.S., Mayer, D.B., 1960. Histochemical different section of acid mucology saccharides by means of combined aldehyde fuchsin-alcian blue staining, Techn. Bull. Regist. Med. Fechn., 30:53-60 pp.

Yao, C., 1987. A study of the histology and morphology of the liver and pancreas of the chinese paddle fish *Psephrus gladius* (Martens), Acta-Hydrobiol., 11:21-26 pp.

Yousan, J.H., Al-Mahrouki A.A., Naumovski, D., Conlon, J.M., 2001. The endocrine cells in the gastroentoropancreatic system of the bowfin *A. calva* L, an immunohistochemical, ultrastructural and immunocyto chemical analysis, J. of Morpho., 250(3):208-24 pp..