HEPATOCYTE ULTRASTRUCTURE OF THE SEVAN TROUT (*Salmo ishchan gegarkuni* KESSEL) DURING ONTOGENY

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KEY WORDS: hepatocyte ultrastructure; ontogeny of fishes.

Data on morphology of the liver during ontogeny of fish and, in particular, of trout, are extremely scanty [3, 6, 7]. The aim of this investigation was to study the role of the liver in the early development of fish against the background of a change of feeding methods (endogenous, mixed, exogenous), which is interesting from both theoretical and practical aspects.

EXPERIMENTAL METHOD

The test object was the liver of larvae and fry of the Sevan trout (*Salmo ishchan gegarkuni* Kessel), reared at the Karchakhpyur Fish Farm. Material was fixed for light microscopy in Bouin's fluid and for electron microscopy in a 2.5% solution of glutaraldehyde in phosphate buffer followed by postfixation with osmium tetroxide in the same buffer, and embedded in Epon. Ultrathin sections were stained by Reynold's method and studied in the JEM-100C electron microscope. The liver of the larvae was fixed immediately after hatching, when the fish were on endogenous feeding with yolk sac products, and also 5 and 10 days after the beginning of mixed feeding; the liver of the fry (exogenous feeding) also was fixed. Yolk was used for supplementary feeding. The relative volumes of the organelles were calculated by means of a random step grid [2].

EXPERIMENTAL RESULTS

The light-optical investigation showed that in the Sevan trout larva at the time of hatching from the membrane the liver lies on the dorsal surface of the yolk, is partially submerged in it, and is separated from it by a layer of peritoneal epithelium. The parenchyme of the liver is penetrated by many vascular lacunae, filled with blood. The hepatocytes are joined together into tubules with a biliary capillary in the center. Droplets of fat of different sizes can be seen in the cytoplasm of the hepatocytes. In the period of mixed feeding the liver is located on the residual part of the yolk sac. No vascular lacunae can be found, but sinusoids are distinctly visible. Droplets of fat in the hepatocytes are reduced in number. The liver of the fry is clearly demarcated from the adjacent organs, and no fat is present in the hepatocytes.

Electron microscopy of liver cells of the larvae immediately after hatching out showed that the hepatocytes are small in size, with high nucleo-cytoplasmic ratios. The nucleus contains one or two large, wide-looped nucleoli, but little heterochromatin (Fig. 1, I). The nucleus is irregular in shape due to deep invaginations of the nuclear membrane inside the karyoplasm. Mitochondria are numerous, polymorphic, with a moderately dense matrix, and with numerous cristae. Organelles up to 10 μm in length can be found. Cisterns of the rough endoplasmic reticulum (RER) are numerous, long, somewhat dilated, and contains material of low electron density in their lumen; they are arranged in parallel rows or they form a network and are in close typographic contact with the mitochondria. Many free ribosomes are present in the cytoplasm. The Golgi complex occupies a large zone of cytoplasm between the nucleus and the biliary capillary, and is formed by several dictyosomes, each of which consists of two or three long, flat cisterns, arranged parallel to one another, with small vesicles and large vacuoles. It will be clear from Fig. 1 that small vesicles with contents of average electron density detach themselves from cisterns with similar con-

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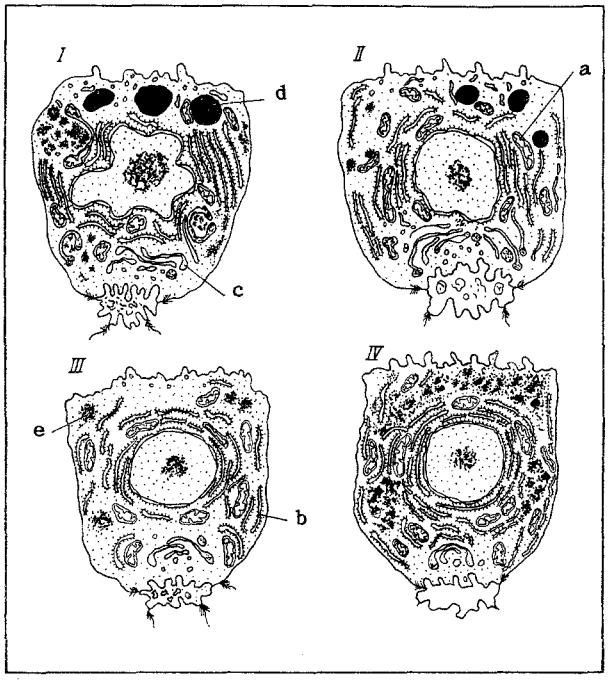

Fig. 1. Trend of changes in ultrastructure of Sevan trout hepatocytes during ontogeny. I) Endogenous feeding (after hatching); II, III) mixed feeding (5 and 10 days after switching to exogenous feeding); IV) exogenous feeding (fingerling); a) mitochondrion; b) rough endoplasmic reticulum; c) Golgi complex; d) fat; e) glycogen.

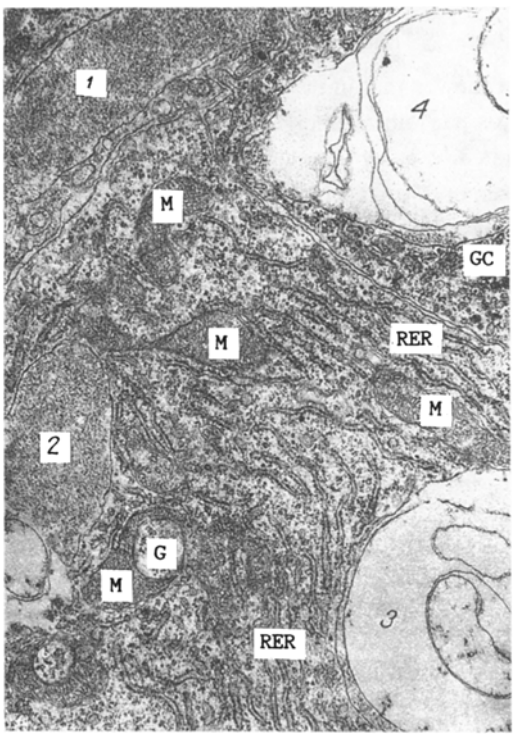

Fig. 2. Different types of vacuoles (1, 2, 3, 4) with mainly lipid contents, undergoing transformation, in cytoplasm of hepatocyte of trout larva immediately after hatching (endogenous feeding). 15,000×. M) Mitochondrion; RER) rough endoplasmic reticulum; GC) Golgi complex; G) glycogen.

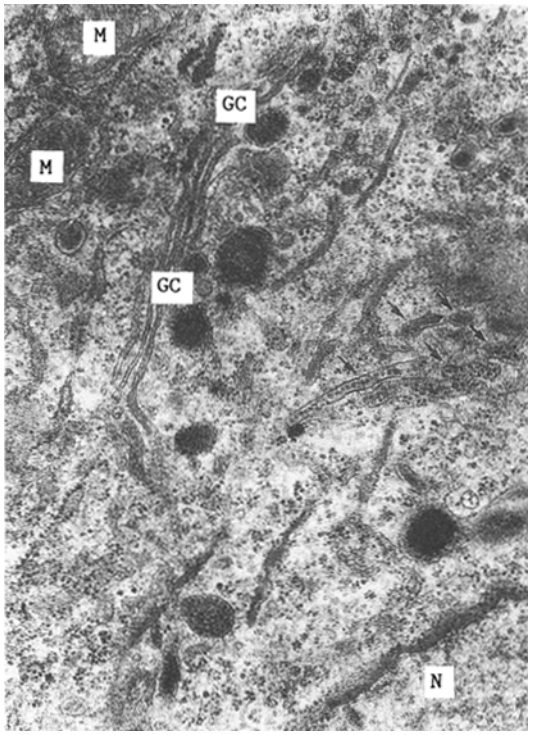

Fig. 3. Chains of particles of VLDL type in cisterns of SER (arrows) in zone of large Golgi complex 5 days after beginning of feeding (mixed feeding). 20,000×. N) nucleus; remainder of legend as to Fig. 2.

TABLE 1. Relative Volumes of Organelles and Inclusions in Sevan Trout Liver at Different Stages of Ontogeny

Test object	Mitochondria	RER	SER	Golgi complex	Lysomes	Peroxisomes	Glycogen	Fat
Larva after hatching (endogenous feeding)	10,45±0,09	12,8±0,19	1,05±0,03	6,95±0,16	1,25±0,07	—	12,85±0,02	1,65±0,06
Larva 5 days after switching to exogenous feeding (mixed feeding)	10,6±0,24	15,0±0,24	1,27±0,14	8,17±0,22	1,25±0,11	—	3,17±0,08	—
Fingerling (exogenous feeding)	12,0±0,38	23,8±0,28	2,3±0,12	5±0,28	1,6±0,19	—	18±1,1	—

tents. Since products synthesized by the hepatocyte accumulate in the vacuoles and vesicles, we regard them as primary lysosomes. Frequently, the primary lysosomes lie very close to the hepatocyte membrane at the sinusoidal pole. In this region outlines of the smooth endoplasmic reticulum (SER) with material of average electron density in their lumen can be found. Glycogen granules are quite numerous and diffusely distributed in the cytoplasm, but sometimes they form large concentrations at the periphery of the cell. Around them are concentrated large mitochondria and cisterns of the RER, which in the zone of contact with glycogen, lose their ribosomes. Vacuoles up to 4 μm in diameter with mainly lipid contents, some of which was lost during dehydration with alcohols, can be seen at the sinusoidal pole of the hepatocyte. By their morphological appearance it can be concluded that transformation of the contents takes place in them. For instance, some vacuoles were seen with only finely granular contents, others in which there were myelin figures as well as finely granular contents, and a 3rd group containing myelin figures alone (Fig. 2). The fact that such finely granular material is observed in the wide intercellular spaces and in Disse's space suggests that the contents of the vacuoles are products brought in by the blood and are undergoing transformation in the hepatocyte. Mitochondria, cisterns of the RER and, frequently, a Golgi complex are arranged around the vacuoles. Biliary capillaries are formed by membranes of four to eight hepatocytes, at the meeting points of which tight junctions and desmo-

somes are formed. Microvilli, large and small vacuoles, with vesicles of different sizes and shapes, and structures resembling myelin figures can be seen in the lumen of the biliary capillaries.

In larvae at the mixed feeding stage (5 days after the beginning of feeding) the hepatocytes are larger but the nucleocytoplasmic ratios smaller. Many nuclei are round in shape, but some are lobular. The nucleoli are large. Changes in ultrastructure of the hepatocytes relate mainly to the Golgi complex: the degree of saturation of the cisterns, vacuoles, and vesicles with electron-dense granules of the very low density lipid (VLDL) type is appreciably increased [9]. Flat cisterns (evidently of the SER) are located in the zone of the Golgi complex, and contain chains of granules of VLDL type in their lumen (Fig. 3); often, however, these cisterns have direct topographic contact with granules containing electron-dense material. Glycogen disappears almost completely (Table 1).

In liver cells of larvae 10 days after the beginning of feeding (mixed feeding) no significant changes can be observed. The only difference is that the numerous primary lysosomes, which were distributed in the zone of the Golgi complex near the biliary capillaries, have disappeared. The process of secretion of bile products into the biliary capillaries was evidently intensified.

In the liver of the fingerling (exogenous feeding) the hepatocyte ultrastructure has come to resemble that observed in the adult. This period of the investigation is characterized by a clear arrangement of organelles: cisterns of the RER and mitochondria are arranged in a circle around the nucleus, and also near the biliary capillary and sinusoid, with large complexes of glycogen between them. The relative volume of the Golgi complexes is reduced, whereas those of the mitochondria and cisterns of RER are increased (Table 1), although the mitochondria are reduced in size and their polymorphism has disappeared. The relative volume of glycogen again is increased (Table 1), as other workers also have found in the liver of the coho [11]. Thus, hepatocytes in the early stages of ontogeny are already in a state of high functional activity. The abundance of free ribosomes indicates protein synthesis for the needs of the cell itself. The large nucleoli, lobular nuclei, numerous cisterns of the RER and mitochondria are evidence of active protein synthesis "for export." The large Golgi complex, the abundance of primary lysosomes, and the localization of the Golgi complex in the zone of the biliary capillary all indicate that large quantities of products to be excreted from the cell and, in particular, bile products, accumulate in it. The wide biliary capillaries and the various structures arranged in their lumen are evidence of secretion of bile products into the biliary capillaries [1]. The arrangement of structures of primary lysosome type at the sinusoidal pole close to Disse's space indicates that the hepatocyte is preparing the synthesized products for secretion into the bloodstream. All the facts described above are evidence that the larval liver in the period of endogenous feeding undertakes a gigantic task, synthesizing protein and bile products, laying down fat and glycogen in the cytoplasm, and secreting synthesized products into the sinusoid. There is no doubt that the original material for such a gigantic task consists of yolk sac products transformed by syncytial cells of the yolk sac [13] and transported by the bloodstream to the liver. This is in agreement with our discovery that the liver is situated immediately on the yolk sac, from which it is separated only by a layer of peritoneal epithelium, which also has been observed in the brook trout [12]. The presence of glycogen in the hepatocytes which we noted immediately after hatching, and the sharp decrease in its level during switching to mixed feeding confirm data obtained by other workers [12], who also noted that carbohydrates are utilized first, followed by proteins and fats. Further evidence of glycogenolysis is given by the close topographic contacts between mitochondria and glycogen. The decrease in size of the lipid drops and their complete disappearance are due to utilization of this material in the hepatocytes during VLDL synthesis for export into the bile and blood, which takes place in RER and SER and is completed in the Golgi complex, from which large vacuoles with particles of VLDL become detached [5, 8, 9]. Some of them become components of the lipid complex of the bile and also are secreted into the blood at the sinusoidal pole of the hepatocyte. We know from the literature that in the steelhead and striped bass the utilization of glycogen and polar lipids is increased before the switch to active external feeding, and as a result, the content of phospholipids increases [4]. On the complete transition to active feeding, the relative volume of the organelles responsible for protein synthesis "for export" is increased and the hepatocytes also become a depot for glycogen, depositing it in large amounts in the cytoplasm. The decrease in size of the Golgi complex is evidently connected with the fact that the liver becomes significantly enlarged and that the functional load on each hepatocyte becomes smaller. At this time the hepatocytes acquire the typical appearance of the adult fish.

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CONTROL OF HUMORAL TRANSPORT IN TISSUES OF THE EYE

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Anatomical and physiological data on communication of the drainage channels of the eye with the lymphatic system and on the existence of prelymphatic formations in the eye [7-9, 12-14] have provided a basis for using the results of clinical lymphology, a new and developing trend in Soviet medicine [2, 3], in order to study regional differences in the control of humoral transport in the eye as well as differences between different structures and tissues of the eye in this respect. The first positive experimental [7, 8] and clinical results on the use of lymphotropic agents, stimulating the hydrodynamics and the lymphatic drainage channels of the eye have now been obtained. However, we could find no information in the accessible literature on the possibility of exercising isolated control of humoral transport in tissue formations of the eye. It can be tentatively suggested that a detailed analysis of this problem could reveal new aspects in ophthalmology, where the problem of the causes of selective tissue damage frequently arises, and where selective and specific action of this kind must be carried out at the tissue level rather than that of the eye itself.

The aim of this investigation was accordingly to study the possibility of exercising isolated control of humoral transport in individual eye tissues, using lymphotropic agents differing in their mechanism of action.

EXPERIMENTAL METHOD

There were three series of experiments on 65 White Giant rabbits. The agents used in the experiments of series I were dalargin [1], in a solution of 0.1% concentration and in a dose of 0.04-0.05 ml/kg body weight (30 eyes), in series II terrilytin was used in a concentration of 25,000 U in 0.1 ml physiological saline, in a dose of 0.1-0.2 ml/kg body weight, injected subconjunctivally or by electrophoresis (30 eyes), and in series III a 10% solution of mannitol was injected subconjunctivally in a dose of 0.2-0.4 ml (20 eyes). Thirty minutes after paracentesis and escape of the aqueous from the anterior chamber, an India ink—gelatin mass (India ink jelly) or Gerota's mass was injected into it or into the vitreous body (VB) in a volume equal to that of the fluid removed. The animals were decapitated 3 h later, the eyes were enucleated, and a detailed macroscopic study of the eye was undertaken with the MBS-2 microscope, and film preparations obtained from different tissues of the eye were studied under the light microscope, and the state of the optic nerve and the internal membranes of the eye also was studied. Paraffin and celloidin sections were stained with hematoxylin-eosin and by Van Gieson's method. Control experiments were carried out on 25 rabbits

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