Ultrastructure of Fish and Amphibian Liver during Catabolism of Degenerating Erythrocytes

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Similar catabolic features were found in macrophages of melano-macrophage centers of sturgeon liver and Kupffer cells of hibernating frogs and tadpoles underdoing metamorphosis. Catabolic process includes 4 stages: 1) intact phagocyted erythrocytes; 2) phagosomes with erythrocyte-like matrix density containing dense ferritin granules distributed between hypertrophied Golgi complex and smooth endoplasmic reticulum; 3) phagosomes with membrane structures and electron dense granules; 4) phagosomes with melanin granules. Products of erythrocyte catabolism are released through the bile duct. The origin of melanin granules is discussed.

Key Words: *erythrophagocytosis; pigment cells of the liver, melano-macrophage centers of fish liver*

Melano-macrophage centers (MMC) in fishes representing depots of catabolic products, in particular erythrocytes, were found in the spleen, kidneys, and liver [3]. Experiments with labeled tyrosine showed that Kupffer cells in amphibian liver can synthetize melanin granules [14], however, physiological mechanisms of the formation and origin of these granules in fish and amphibian liver remain unclear. We assume that in frogs melanin granules are normally formed during catabolism of phagocyted erythrocytes in Kupffer cells [1]. Here we studied the formation of melanin granules in fish MMC, compared our and published data on the formation of melanin granules in amphibian liver [14], and examined melanin granules in frog liver in spring, when the number of pigment cells decreases [11].

MATERIALS AND METHODS

We examined liver ultrastructure in adult sturgeons from the Volga basin, *Rana temporaria* tadpoles during their metamorphosis characterized by replacement of larval by mature erythrocytes, and adult frogs in winter and spring (May). Tissue was fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.2-7.4) and postfixed in 1% OsO_4 in the same buffer. Material was embedded in Epon, ultrathin sections were contrasted by the method of Rheinolds and examined under a JEM-100 C electron microscope.

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RESULTS

Whole phagocytosed erythrocytes were found in liver MMC macrophages of mature sturgeons. Numerous small round membrane-coated phagosomes of the same density as erythrocytes, but containing electron dense ferritin-like granules were observed in macrophage cytoplasm. These phagosomes were distributed among numerous vesicles of smooth endoplasmic reticulum, cisterns, and hypertrophied Golgi complex and few small mitochondria (Fig. 1, *a*). Local membrane thickenings (myelin figures) were seen at the periphery of these phagosomes. Close proximity of smooth endoplasmic reticulum and Golgi complex with fagosomes points to their role in phagosome transformation, which was shown histochemically at the electron microscopic level during erythrophagocytosis by Kupffer cells in rat embryonic liver [10]. Phagosomes of varying size and structure were found in macrophages: some of them contained melanin granules distributed in finely dispersed matrix, while others contained also smaller and less dense granules formed from the matrix (Fig. 1, *b*). Some of these granules contained lumens, while phagosomes contained membrane struc-

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tures similar to those observed in premelanosomes during the formation of melanin granules in amphibian liver after injection of labeled tyrosine [14]. No Golgi complex was found in these macrophages, while phagosomes at this stage could be refered as to heterolysosomes, because phagocyted material underwent enzymatic hydrolysis via contact with Golgi complex and smooth endoplasmic reticulum. The described changes should be regarded as successive stages of erythrocyte catabolism leading to the formation of melanin granules. Similar catabolism of phagocyted erythrocytes was observed in the liver of adult hybernating frogs and tadpoles undergoing metamorphosis [1]. We observed giant Kupffer cells containing whole phagocytosed erythrocytes with peripheral myelin-like structures, which reflects digestion of stroma lipids [2], or numerous phagosomes with electron dense ferritin-like granules distributed among the membranes of smooth endoplasmic reticulum and Golgi complex.

Three pigments melanin, hemosiderin, and lipofuscin were found in fish MMC [3]. Hemosiderin can be produced from destroyed hemoglobin of degenerating erythrocytes, lipofuscin from destroyed cell elements, while melanin appears after phagocytosis of melanin granules or their precursors in melanin-containing cells [3]. Our data suggest that melanin granules have similar origin and represent the product of erythrocyte catabolism. Pigment of amphibian liver is known to contain melanin, hemosiderin, and lipofuscin [12]. After dissociation of hemoglobin into hem and globin, hemic iron interacts with apoferritin with the formation of ferritin. Ferritin enriched with lipid and carbohydrate components is converted into hemosiderin. Globin-derived tyrosin is then converted into melanin by tyrosinase. Usually, macrophages excrete the excess of iron after erythrocytes phagocytosis. This excretion is completely blocked at 4°C [4]. In the presence of iron and tyrosin and at low temperature Kupffer cells become a depot of erythrocyte catabolites and a source of melanin granules. This can explain the increased content of pigment cells in amphibian liver during winter. The excess of iron is known to stimulate lipid peroxidation (LPO). Free radicals react with unsaturated fatty acids of membrane phospholipids [5,8]. Poikilothermal organisms, in particular fishes and amphibians require high tissue content of unsaturated lipids maintaining membrane fluidity and providing metabolism even at low temperatures. Unsaturated fatty acids are vulnerable to lipid peroxidation [3]. Being a potent antioxidant, melanin protects the liver of poikilotherms against free oxygen radicals performing the same function as superoxide dismutase in nonpigment tissues [9,13].

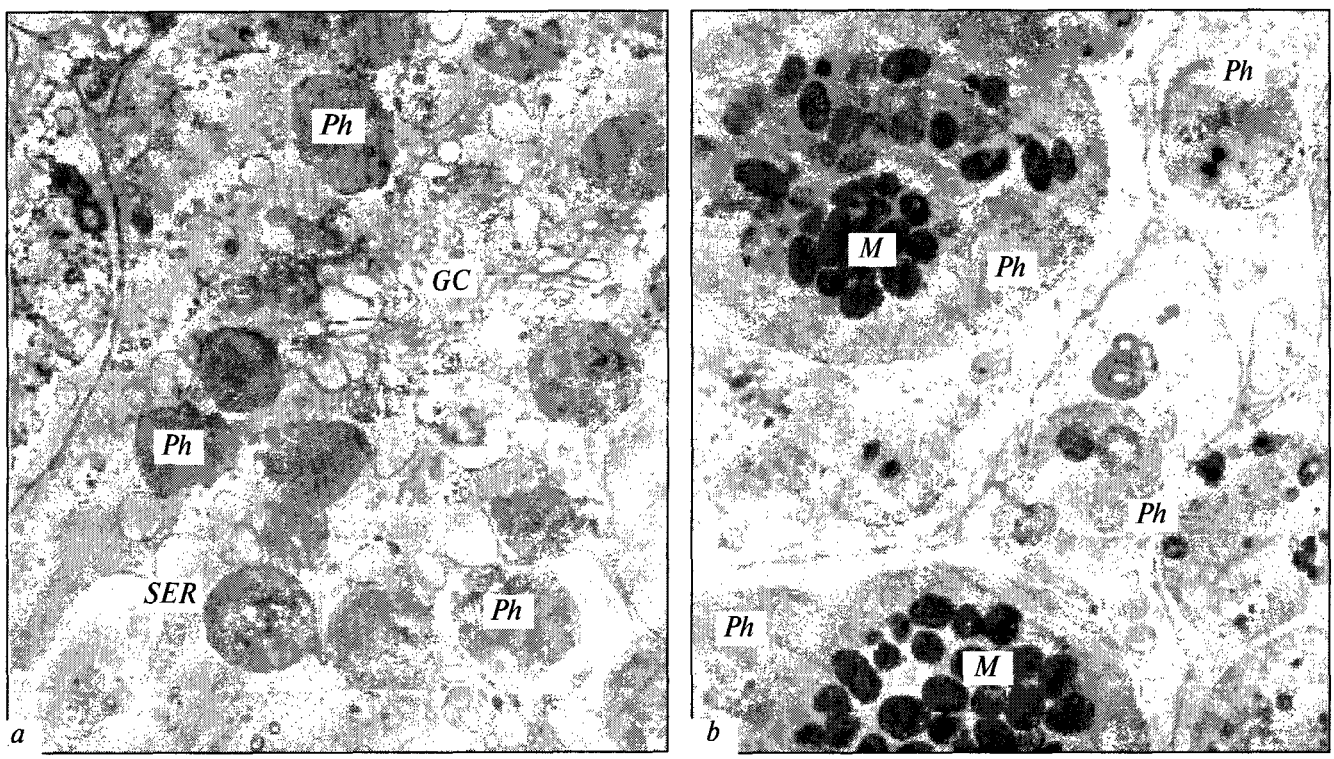

Fig. 1. Macrophages in sturgeon melano-macrophage center; *a*) phagosomes (*Ph*) with ferritin-like electron dense granules distributed among hypertrophied elements of Golgi complex (*GC*) and smooth endoplasmic reticulum (*SER*), ×10,500; *b*) melanin (*M*) and electron dense granules in macrophage phagosomes, ×10,850.

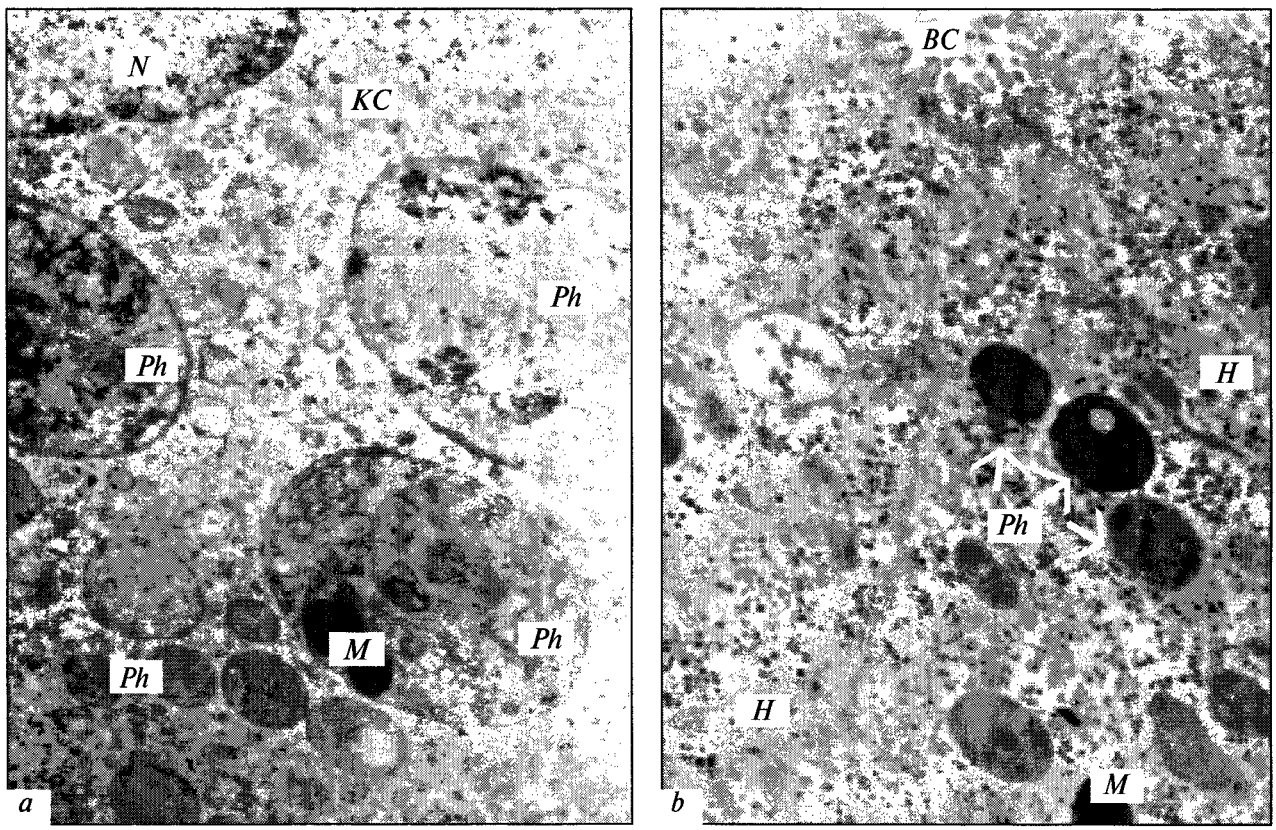

Fig. 2. Liver of adult frog fixed in May. *a*) various phagosomes (*Ph*) containing electron dense granules, membrane structures, and few melanin (*M*) granules in Kupffer cell (*KC*), ×15,000; *b*) various phagosomes and melanin granules near bile capillary (*BC*) of hepatocyte (*H*), ×15,000; nucleus (*N*).

The presence of numerous pigment cells in amphibian liver in winter and their low number in summer [11] attracted our attention to ultrastructure of hepatocytes fixed in May. We found some Kupffer cells containing pigment granules with destroyed outer membrane, which was also noted by other authors [6]. We also found Kupffer cells containing large phagosomes with pigment granules surrounded by small phagosomes with ferritin-like granules. Other Kupffer cells included phagosomes with ferritin-like granules, membrane structures, and few melanin granules (Fig. 2, *a*). In these animals, the pericapillary zone of hepatocytes contained all types of phagosomes found in Kupffer cells and few pigment granules (Fig. 2, *b*). This points to penetration of phagosomes from Kupffer cells to hepatocytes and their release in bile through bile ducts. Thus, amphibian liver excretes pigment granules and phagosomes with ferritin and hemosiderin. It was reported that the number of MMC in fish liver increased with age [7]. This probably means that, in contrast to frogs, pigment-saturated macrophages are not removed from fish liver.

Our findings and published data [3,4] show that pigment granules are located inside phagosomes. However, some authors suggest that pigment granules undergo lysis in phagosomes [14]. Our data indicate that pigment granules are formed in these phagosomes, which represent fragments of degenerating erythrocytes.

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