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ERYTHROPHAGOCYTOSIS AND PIGMENTED CELLS OF THE AMPHIBIAN LIVER

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It is now established that pigment granules are not only accumulated, but are also synthesized in Kupffer cells [4, 5, 9]. Pigment granules have been shown to contain melanin, hemosiderin, and lipofuscin [2, 5], and the protein matrix of pigment granules possesses tyrosinase activity [10, 5], which is higher in winter and lower in summer. The melanin content reaches a maximum in the cold months of the year, and a minimum in the hot period [10, 5]. The question of the role of the pigmented cells of the liver and the causes of seasonal differences in melanin content remains unanswered. By studying the ultrastructure of the liver cells of adult frogs during hibernation and of tadpoles during metamorphosis, we also found changes in the Kupffer cells, and in our opinion these provide evidence relating to the causes of appearance of pigmented cells in the liver and their function.

EXPERIMENTAL METHOD

Kupffer cell ultrastructure was studied in the liver of adult frogs (*Rana temporaria*) in the winter period, the tadpole liver in the period of metamorphosis, when the tail is undergoing resorption, and the liver of frogs in the first year of life. Pieces of liver were fixed in 2.5% glutaraldehyde in phosphate buffer and postfixed with 1% OSO_4 in the same buffer. Material was embedded in Epon. Ultrathin sections were stained by Reynolds' method and examined in the IEM-100C electron microscope.

EXPERIMENTAL RESULTS

Kupffer cells of hibernating adult frogs and in the liver of tadpoles with resorbed tail are much larger than in the adult frog liver in the summer period. The nucleus is elongated and the heterochromatin is located juxtamurally in a wide border. The cytoplasm contains a few cisterns of the rough endoplasmic reticulum. The mitochondria are small, and oval or elongated in shape. In the hibernating frog the mitochondria have a denser matrix than normally, and a widened intercristal space, whereas in the liver of tadpoles during resorption of the tail the structure of the mitochondria is normal and indistinguishable from that In the adult frog liver in the summer period. Sometimes a whole erythrocyte can be seen in the cytoplasm of the Kupffer cells; the peripheral part of its cytoplasm appears to consist of membranes, arranged parallel to one another along the whole perimeter, and resembling myelin (Fig. 1). A distinguishing feature of the majority of Kupffer cells studied is the large number of phagosomes, the large Golgi complex, and a mass of small cross sections of the smooth

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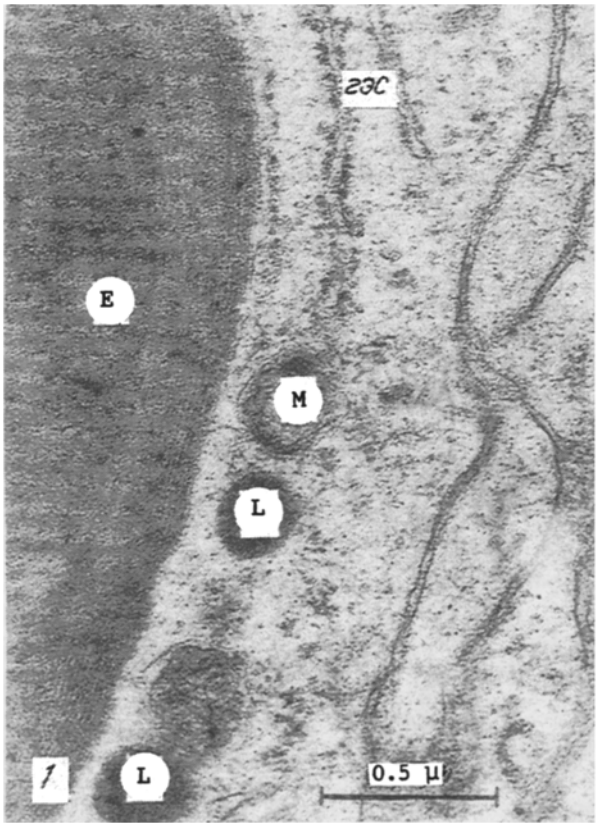

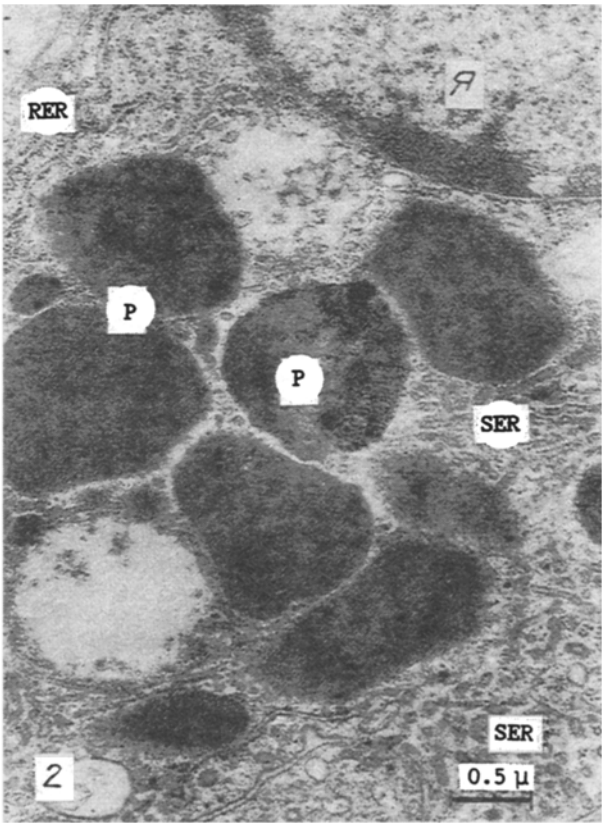

Fig. 1 Fig. 2

Fig. 1. Kupffer cell in liver of hibernating frog containing erythrocyte in its cytoplasm.

Fig. 2. Multiple phagosomes and profiles of smooth endoplasmic reticulum in cytoplasm of Kupffer cell of hibernating frog.

endoplasmic reticulum (SER). For instance, in Kupffer cells of the liver of hibernating frogs numerous phagosomes measuring 0.05-0.15 μm and similar in density to erythrocytes, but containing granules that are even more electron-dense in their matrix, and resembling ferritin (Fig. 2), can be seen. These phagosomes are located along a large mass of vesiculiform and vermiform cross sections of SER, many of which establish direct topographic contact with phagosomes. The Golgi complex is large.

Polymorphism of the phagosomes is more clearly visible in Kupffer cells of the tadpole liver during resorption of the tail The phagosomes vary in size from 0.03 to 0.7 μm. Round and oval granules are most frequently seen, and against the background of their homogeneous contents, electron-dense granules resembling ferritin can be distinguished. Very large complex phagosomes measuring 0.25-0.5 μm can be seen, and contain either granules similar to those described above (Fig. 3a) or membrane profiles of varied configuration, beneath their membrane; myelin figures resembling fingerprints or the myelin sheath of a nerve can be distinguished (Fig. 3b). Incidentally, the phagosomes described above are very similar in their ultrastructure to those present in macrophages ingesting erythrocytes in the melanomacrophagal centers of the spleen and liver in fish [13]. We sometimes observed pictures suggesting that the outer membrane of Kupffer cells, packed with phagosomes, ruptures and their contents escape into the lumen. All phagosomes, just as in the Kupffer cells of the hibernating frog, lie among a large number of tiny circular, oval, and vesiculiform profiles of the SER, and of cisterns of the Golgi complex, which is large in size (Fig. 3b). The topographic closeness of the cisterns of SER and elements of the Golgi complex to phagosomes points to the involvement of all these organelles in the transformation of phagosomes, which has been demonstrated by histochemical methods at the electron microscopic level during the description of erythrophagocytosis by Kupffer cells in the rat embryonic liver [8].

The Kupffer cells in the liver of frogs in the 1st year of life, after metamorphosis, become smaller in size, but resorbed erythrocytes are still found in their cytoplasm as before. There is evidence that erythrophagocytosis in the period of metamorphosis is connected with the fact that the larval erythrocytes are replaced by the adult type [3]. Erythrophago-

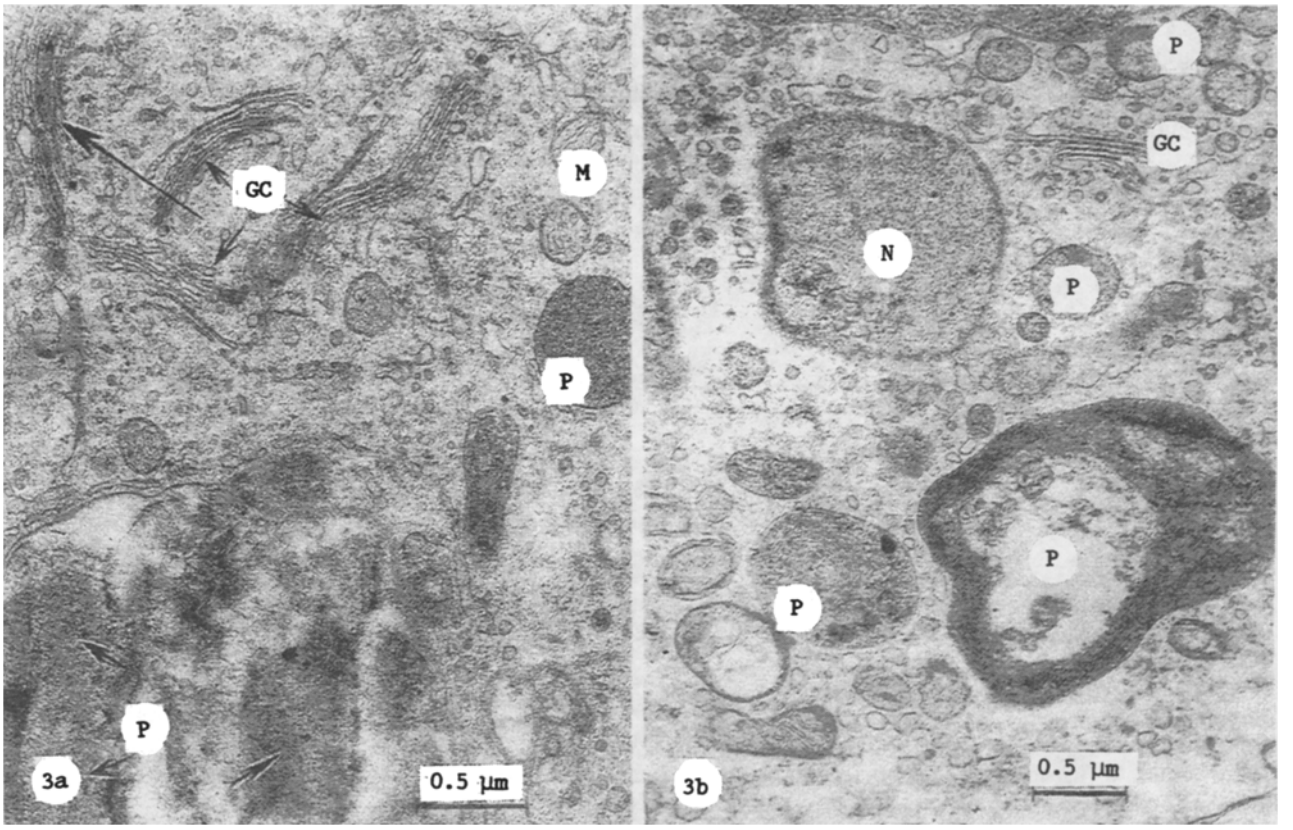

Fig. 3. Areas of cytoplasm of Kupffer cells of a tadpole during metamorphosis with large Golgi complex and with complex phagosomes, containing (a) granules (arrows), and (b) membrane profiles. E) Erythrocyte, M) mitochondrion; RER) rough endoplasmic reticulum; SER) smooth endoplasmic reticulum, L) lysosome, N) nucleus, GC) Golgi complex, P) phagosome.

cytosis in the liver of adult hibernating frogs is evidently connected with physiological death of a considerable number of erythrocytes.

If our morphological data are compared with information in the literature on synthesis and distribution of melanin granules in the Kupffer cells, we can conclude that erythrophagocytosis is the source of this process. The presence of hemosiderin, arising from ferritin and a component of hemoglobin breakdown products, in the pigment granules besides melanin is further proof that at least one source of formation of the pigment granules is resorbed erythrocytes. The role of the pigmented cells that are beginning to appear, as a depot for the products of catabolism, thus becomes evident. Usually macrophages, when carrying out phagocytosis of erythrocytes, secrete the excess of iron. There is evidence that at 4°C this secretion stops almost completely [1]. This may perhaps explain the increase in the melanin content in the liver of frogs in winter, for metabolism of the phagocytosed erythrocytes during this period is evidently aimed at melanin formation. There is another good reason for this, which is that melanin is a powerful antioxidant, it protects cells against free oxygen radicals, and it acts in a similar way to superoxide dismutase — the enzyme which performs this function in nonpigmented tissues [6, 7, 11, 12]. Thus melanin, an end product of catabolism, performs a very important protective function.

Unfortunately we could find no reference to the melanin content in frogs in the 1st year of life and in tadpoles during metamorphosis. Data in the literature indicating a fall in the melanin content in the liver of adult frogs in summer suggest that the pigment is somehow eliminated by the frog. Otherwise it would accumulate from year to year in the frog's liver, which would impair Kupffer cell function.

At this point we must mention the melanomacrophagal centers in the spleen, kidneys, and liver of fish, and which are known to be formed from macrophages of sinusoids phagocytosing foreign material and remnants of dying erythrocytes and other cells, after which they migrate into hematopoietic tissue [13]. Their function, namely elimination of endogenous metabolites, degenerating cells, and ingested foreign material [13] – is analogous to the function of the pigmented cells in the amphibian liver.

Thus whereas in fish melanin is formed during metabolism of erythrocytes and concentrated in melanomacrophagal centers, in amphibians phagocytosed erythrocytes are transformed into pigment granules, which are concentrated in Kupffer cells; the pigment of both amphibians and fishes performs an antioxidant function, in mammals this function is performed by specific enzymes (superoxide dismutase, catalase, etc.), and the metabolism of dying erythrocytes takes place in the spleen and liver, but in this case without the formation of melanin granules, possibly in connection with the fact that mammals are warm blooded.

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