The Ultrastructure of Liver Cells of Some Herbivorous Animals

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It is shown that hepatocytes of grass carp and silver carp contain much glycogen, but have very few cisternae of agranular endoplasmic reticulum. The cytoplasm of chicken and pigeon hepatocytes contains glycogen, lipid droplets, as well as a fair amount of granular and agranular endoplasmic reticulum. Agranular endoplasmic reticulum predominates in hepatocytes of herbivorous mammals (sea cow, muskrat, musk deer, bison, and elk) and the cells contain various kinds of inclusions as well.

Key Words: *hepatocyte; ultrastructure; herbivores*

There are few reports dealing with the ultrastructure of liver cells in herbivorous animals [1,2,9,11-13].

The aim of the present investigation was to study the ultrastructure of liver cells of some herbivores and to identify features of similarity and difference among them.

MATERIALS AND METHODS

The ultrastructure of liver cells was studied in fed and fasting mature grass carp, silver carp, chicken, pigeon, sea cow, muskrat, musk deer, elk, and bison. Fixation was performed with 2.5% glutaraldehyde on S-collidine buffer at pH 7.2-7.4 followed by additional fixation with osmium tetroxide on the same buffer. The samples were embedded in Epon. Ultrathin sections were stained after Reynolds and examined with a JEM-100C electron microscope.

RESULTS

Hepatocytes in algivorous mature grass carp and silver carp contain predominately one nucleus with a nucleolus and heterochromatin situated close to the karyolemma and partially around the nucleolus. Cisternae of granular endoplasmic reticulum (GER) in the cytoplasm embrace the nucleus as well as each mitochondrion. The Golgi apparatus is of moderate size and consists of several flattened cisternae and vesicles which do not contain particles of very low density lipids (VLDL). Golgi apparatus is situated close to the bile capillary, which is formed by the membrane of one hepatocyte in these fishes. The bile ducts are lined with bile duct epithelial cells and are therefore autonomic [2]. A huge amount of glycogen fills a major part of the hepatocyte cytoplasm in these fishes. Among the glycogen profiles of agranular endoplasmic reticulum (AGER) are rather seldom encountered. It should be noted that glycogen is often found in intercellular gaps, as well as in the cytoplasm of Ito cells and sinusoidal cells, as other authorities have reported [11]. Widening of intercellular gaps and clasmatosis of cytoplasmic fragments with glycogen into the sinusoidal lumen was noted in the liver of fasting fishes. The cytoplasm of hepatocytes in grass and silver carp has inclusions which are granules consisting of thin fibrils and electron-dense granulation (Fig. 1, *a*). The ratio of these two components is different in different granules. The impression is gained that

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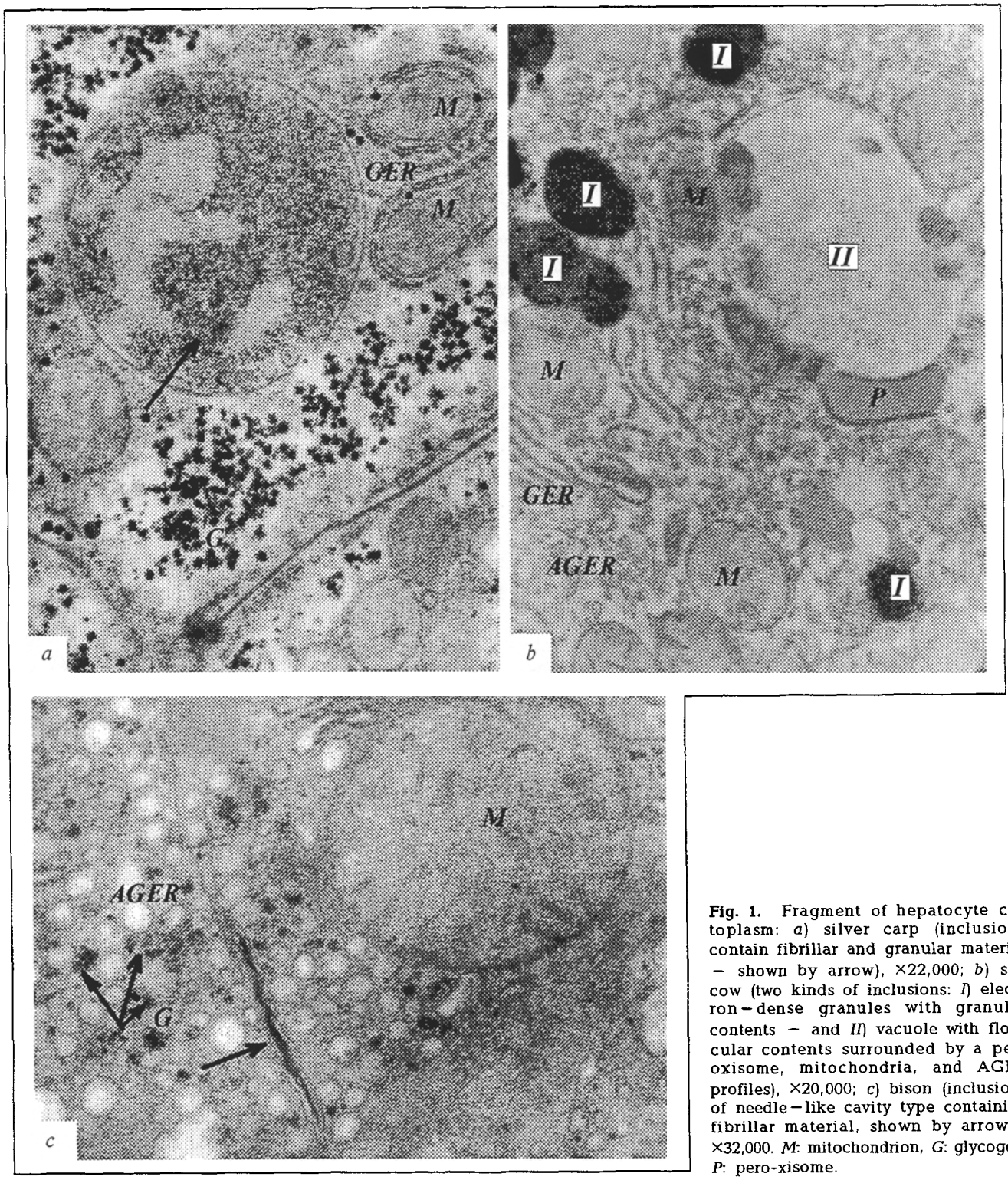

Fig. 1. Fragment of hepatocyte cytoplasm: *a*) silver carp (inclusions contain fibrillar and granular material – shown by arrow), ×22,000; *b*) sea cow (two kinds of inclusions: *I*) electron-dense granules with granular contents – and *II*) vacuole with floccular contents surrounded by a peroxisome, mitochondria, and AGER profiles), ×20,000; *c*) bison (inclusions of needle-like cavity type containing fibrillar material, shown by arrows), ×32,000. *M*: mitochondrion, *G*: glycogen, *P*: pero-xisome.

granular material is converted to thin fibrillar material. Ito cells contain large swollen mitochondria with a light matrix.

The grain-feeding chicken and pigeon have hepatocytes with the usual ultrastructure. Their cytoplasm abounds in oval or elongated mitochondria containing a moderate number of cristae, GER and AGER, lipid droplets, and glycogen which is situated among the AGER profiles. The bile capillaries are formed by membranes of contacting hepatocytes, but in the pigeon the bile capillary is sometimes constructed by a membrane of one hepatocyte, as other authorities have also noted [12].

The sea cow, whose consumption of algae is four times as great as its body weight [10], has hepatocytes containing 1-2 nuclei, numerous mitochondria with a small number of cristae, several GER cisternae, peroxisomes without crystalloid, a moderate-sized Golgi apparatus without VLDL particles in its cavities, and diverse profiles of AGER which predominate in the cytoplasm. It should be noted that some hepatocytes contain much more GER. There are two kinds of inclusions in the cytoplasm. The first type consists of electron-dense granules which, according to numerous electron micrographs, are formed by small electron-dense particles coming from the sinusoids; they agglomerate, move into the zone of the bile capillaries, and discharge into their lumen (Fig. 1, *b*). The second type of inclusions comprises large vacuoles sometimes the size of the nucleus, with light floccular contents. They are surrounded by mitochondria, peroxisomes, and AGER profiles. Myelin structures may be found in vacuoles in zones of contact between mitochondria and vacuoles. Glycogen is extremely rarely found, and in a very small amount, and lipid droplets are absent in the hepatocyte cytoplasm. Sinusoidal cells are large, and besides organelles they contain numerous phagosomes and sometimes phagocytized erythrocytes.

The muskrat, which feeds mainly on succulent plants and sometimes small invertebrates, has hepatocytes with an ultrastructure similar to the fine structure of sea cow hepatocytes. They also contain 1-2 nuclei, a large nucleolus, a large number of mitochondria, which differ in size with a small number of cristae, peroxisomes without crystalloid, little GER (around the nucleus and mitochondria), an enormous number of AGER profiles, a moderate-sized Golgi apparatus without VLDL particles in its cavities, and two structurally similar kinds of inclusions. Glycogen and lipid droplets are not found. Sinusoidal cells contain numerous inclusions similar to those of the first type.

The ultrastructure of hepatocytes in musk deer, which feeds mainly on lichens [6], is much the same as in the sea cow and muskrat. The difference lies in the presence of inclusions of the second type only in the cytoplasm of musk deer hepatocytes. Glycogen is absent and lipid droplets are found not only in the hepatocyte cytoplasm, but in sinusoidal cells as well. Particles of the VLDL type are situated in the Golgi apparatus cavities.

The ultrastructure of hepatocytes in elk and bison, which feed on tender branches [7], is similar, but it differs from that of hepatocytes of the above-mentioned animals in the presence of glycogen and needle-like inclusions. AGER with randomly scattered rosettes of glycogen predominates in the majority of cells. Hepatocytes in which GER prevails occur more rarely. The hepatocytes contain rather numerous mitochondria with few cristae. The Golgi apparatus is moderate-sized and does not contain VLDL particles. Needle-like cavities with fibrillar contents frequently occur among the AGER (Fig. 1, *c*). Inclusions of the granular type with electron-dense particles inside resembling compactly arranged glycogen are found in the sinusoidal cells.

Comparing the ultrastructure of hepatocytes in herbivorous fishes and in mammals, one may note the abundant glycogen in fishes, its moderate amount in bison and in elk, a minimum amount in sea cows and a total absence in muskrat and musk deer. The cytoplasm of hepatocytes in grass and silver carp contains a minimum of AGER profiles whereas in mammals AGER packs the entire space which is free of other organelles. In fasting herbivorous and carnivorous animals clasmatosis of cytoplasm fragments with glycogen into the sinusoid may be an adaptive device [3] which serves for the rapid digestion of carbohydrates by blood enzymes, because it is known that the liver in fishes has few glycogenolysis enzymes and glycogen is expended slowly even under fasting conditions [4].

An abundance of AGER and inclusions of different types is common for hepatocytes of all herbivorous mammals. Although the majority of hepatocytes showed a predominance of AGER, individual hepatocytes with predominant GER were found in all animals, which probably "handle" the protein supply of the organism. Identical inclusions of two kinds were found in the sea cow and muskrat. The content of inclusions of the first type in the sea cow and muskrat liver and their situation on the sinusoidal and biliary poles, set us thinking that substances of high electron density, probably metals (iron and others) which are contained in aquatic plants, issue from the blood into the cell. The accumulation of these inclusions in the bile capillary zone testifies that they are excreted from the cell with the bile. The second type of inclusions were found in the sea cow, muskrat, and musk deer. These animals feed on plant food rich in carbohydrates, which supply starch and sugar to the organism. On the basis of published data we consider the vacuole contents to be sucrose and products of its hydrolysis, namely glucose and fructose, because these substances were found in similar vacuoles in hepatocytes of rats which were in-

jected with a concentrated solution of sucrose [14,15]. The noted close topographic contact of mitochondria, peroxisomes, and AGER with these vacuoles as well as the appearance of myelin figures in them testify to the involvement of hepatocyte organelles in the metabolism of the vacuole contents.

The minimum amount of glycogen in sea cow hepatocytes and its total absence in muskrat and musk deer hepatocytes is surprising. An explanation emerges from experiments on rats [8,15] injected with concentrated solutions of sucrose. It was established that hydrolysis of sucrose and further metabolism of fructose yield an enormous accumulation of fructose-1-phosphate; the ATP content drops, as does the content of uridine triphosphate and uridine diphosphoglucose, resulting in the impossibility of glycogen synthesis.

The cytoplasm of hepatocytes in herbivorous mammals contains an enormous number of AGER cisternae. In rat experiments when a concentrated sucrose solution was administered the formation of "spirals" of AGER cisternae that were direct extension of GER [15] was also noted, but in a lesser amount than in the studied animals. Such an abundance of AGER may be interpreted as follows. It is known that essentially all plants contain an array of substances (alkaloids, tannins, glycosides, and estrogens) which are therapeutic in low doses, but when absorbed in high doses become toxic [5]. In addition, many plants accumulate pesticides. Since animals consume large amount of plant food, a system of detoxication has to exist. This is represented in hepatocytes by an abundance of AGER, in which processes of detoxication of medicinal and harmful substances go on in addition to the metabolism of carbohydrates and lipids.

Thus, animals consuming abundant plant food have adapted to metabolize sugar, which they "pack" into vacuoles. When rats, which are not used to consuming such an amount of sugar were experimentally administered it, they used the same adaptations as herbivorous animals do naturally. In bison and elk, which feed on protein rich tender branches [7], glycogen is found in the cytoplasm of hepatocytes, but inclusions comprise needle-like cavities containing a fibrillar substance, presumably protein.

This study in our view readily demonstrates the correlation between the ultrastructure of hepatocytes and the food an animal consumes.

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