CLASMATOSIS IN THE NORMAL AND PATHOLOGICAL LIVER

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The phenomenon of clasmatosis has been known for a long time. Pfuhl called it clasmatocytosis, considering that it was breaking of pieces of cytoplasm from histiocytes [9]. With the introduction of electron microscopy it could be seen how and what the cell gives off in each concrete case. The most familiar example of clasmatosis is separation of the territory of the cytoplasm of a megakaryocyte along a line of vesicles, followed by separation of platelets formed in this manner from it. There is little information in the literature about clasmatosis [1,3-6, 8, 9]. Nevertheless, this phenomenon deserves careful attention, for in certain cases the elucidation of the physiological importance of clasmatosis is essential.

During a study of the ultrastructure of liver cells from different animals under normal and pathological conditions, the writer discovered numerous examples of this phenomenon.

EXPERIMENTAL METHOD

The ultrastructure of liver cells was studied in the rat and chick during embryogenesis, in hungry fish (grass carp, silver carp), of year-old frogs (*Rana temporaria*), and of rats with disturbance of the hepato-intestinal circulation. Pieces of liver were fixed by Palade's method and in 2.5% glutaraldehyde solution in S-collidine buffer, pH 7.2-7.4, followed by post-fixation with osmium. The material was embedded in Epon and ultrathin sections were stained by Reynolds' method and with uranyl acetate, and examined in the JEM-100C electron microscope.

EXPERIMENTAL RESULTS

During a study of the ultrastructure of the liver cells of a chick embryo on the 7th day of incubation, when all blood coming from the yolk sac begins to pass through the liver and the liver takes part in the metabolism of these substances, outgrowths with the appearance of "tongues" begin to be formed on the sinusoidal surface of the cell membrane of the hepatocyte on the side of the sinosoids; rosettes and chains of ribosomes, and sometimes vesicles and granules of lysosomal type can be seen in these outgrowths (Fig. 1a). Similar fragments, but isolated from the cell, can be seen in the Disse's space and in the lumen of the sinusoids. In the writer's view, this is a distinct case of clasmatosis, which can be observed until the 13th day of incubation. Fragments of the cytoplasm separate either along a line of vesicles which stretches from one edge of the constricted part of the tongue to the other, or separating fragments of cytoplasm are located on a thin stalk, where their detachment from the cell takes place (Fig. 2).

On the 9th-10th day of incubation, when intensive liberation of bile products from the hepatocytes takes place, clasmatosis of some microvilli of the bile capillary is observed, and as a result of this, fragments of microvilli can be seen in its lumen, and the membrane of the bile capillary appears smooth in some places. Finally, on the 10th day of incubation, i.e., on the day after the beginning of rapid bile secretion, some mitochondria close to a bile capillary appear to be changed: destruction of the integrity of the outer membrane is observed for some distance with clasmatosis of individual fragments of mitochondria, as a result of which the mitochondria become irregular in outline (Fig. 1b), but after 2 days their ultrastructure is restored [3]. The same altered mitochondria close to bile capillaries could be seen in rat liver cells on the 19th-20th days of embryogenesis, when bile secretion

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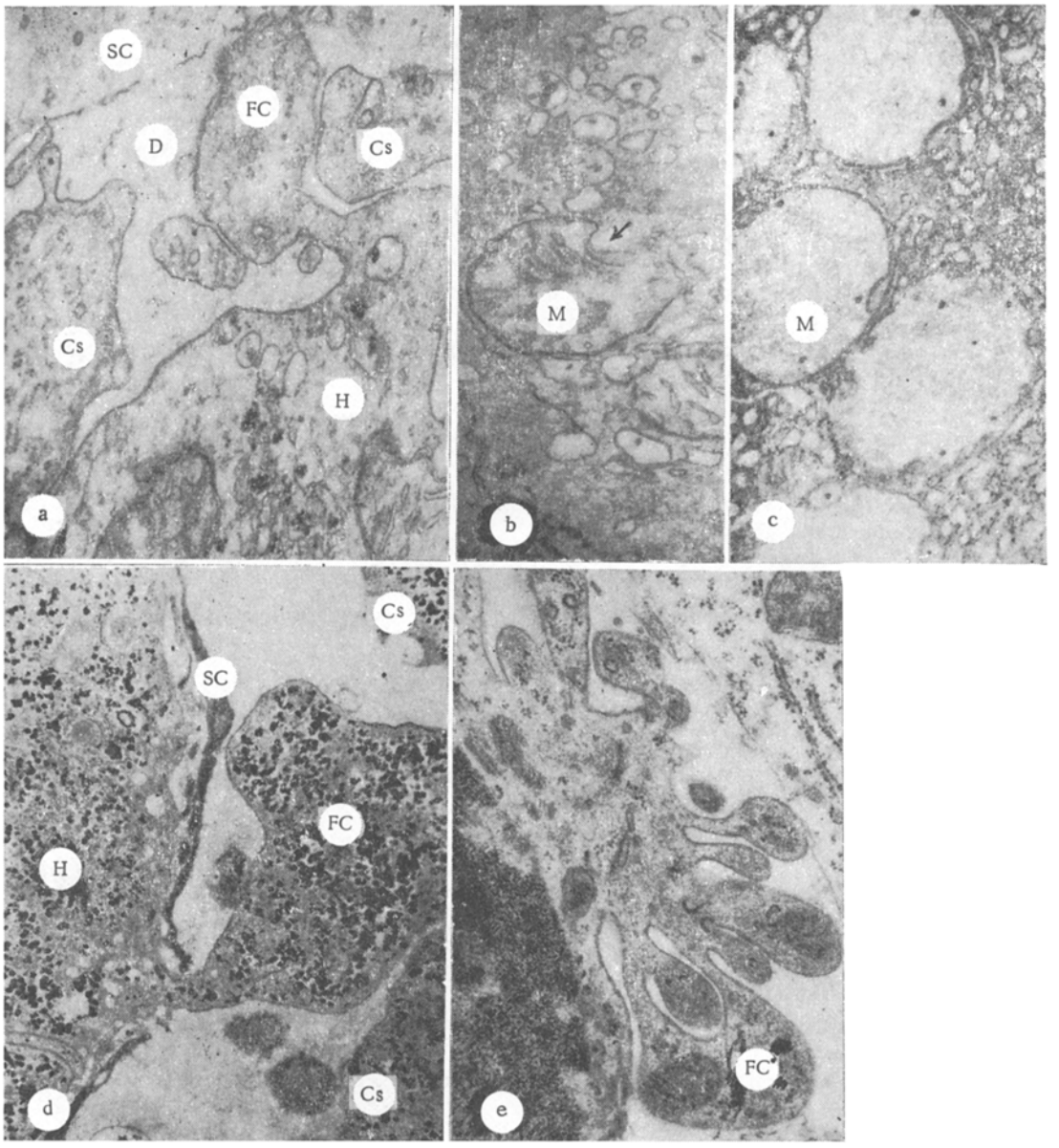

Fig. 1. Clasmatosis of fragments of cytoplasm of chick embryonic hepatocyte. a) On 9th day of incubation (17,250 ×); b) stage after clasmatosis of fragments of mitochondrion (arrow) in chick embryonic hepatocyte on 10th day of incubation (34,650 ×); c) initial state of clasmatosis in hepatocyte of year-old frog (21,000 ×), d) hepatocyte of grass carp (11,220 ×); e) erythroblast in rat liver on 21st day of embryonic development (28,050 ×). [see Fig. 2 for explanations of symbols.]

is beginning. Similar pictures of contact of cristae with the inner membrane of mitochondria, partial destruction of the outer membrane of the mitochondria, followed by clasmatosis of mitochondrial fragments (Fig. 2), described by the writer previously [3, 4], could be observed in liver cells of a year-old frog, i.e., at a stage when the animal is growing rapidly and feeding actively. Mitochondria of this kind were found in hepatocytes in which no morphological evidence of destruction of other organelles could be seen. Numerous cisterns and vesicles of the rough endoplasmic reticulum and many mitochondria, some of them with an unusual structure, could be seen in the cytoplasm of these hepatocytes. Incidentally, the density of the matrix in these mitochondria of year-old frogs varies. In some hepatocytes the density of the matrix of the whole mitochondrion and of its parts bounded by the crista, in contact with the inner membrane of the mitochondrion, is uniform. In other hepatocytes the density of the matrix in small fragments undergoing clasmatosis is much higher than the density of the matrix of the mitochondrion as a whole (Fig. 1c). As we know [2], mitochondria with a dense matrix are more highly charged energetically. In the writer's view, the separation of an

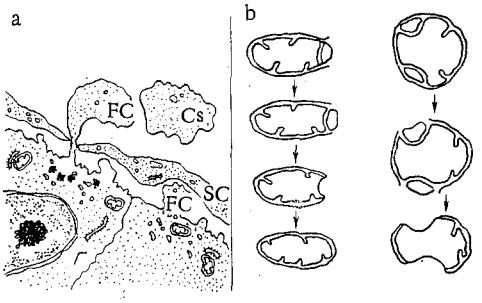

Fig. 2. Schematic drawing of clasmatosis of cytoplasmic fragments (a) and mitochondrial fragments (b).

Legend (Figs. 1 and 2): H) hepatocyte, D) Disse's space, SC) sinusoidal cell, Cs) clasmasome, FC) fragment of cytoplasm of hepatocyte undergoing clasmatosis, M) mitochondria.

energetically more charged fragment from the mitochondrion is due to the need to supply energy quickly for certain synthetic processes in the cell. Hence it follows that during rapid utilization of energy-yielding products mitochondria not only undergo partial utilization, but also destroy themselves.

The results of a study of the ultrastructure of liver cells of starving fish revealed separation of areas of cytoplasm packed with glycogen (Fig.1d). These fragments moved into the Disse's space, and from it into the sinusoid, they were club shaped, and connected with the hepatocyte only by a thin stalk of cytoplasm which, on stretching, evidently breaks away from the cell, since similar fragments of cytoplasm, no longer connected to the hepatocyte, can be seen in the lumen of the sinusoid.

During a study of erythrocyte maturation in the rat embryonic liver the writer found that before enucleation, organelles are removed from the erythroblast by clasmatosis of individual fragments of cytoplasm together with mitochondria and ribosomes (Fig. 1b).

The study of the ultrastructure of the liver cells during disturbance of the hepato-intestinal circulation in rats [5] revealed two types of clasmatosis at different stages of the investigation, both in sinusoids, and differing in the content of the clasmasomes. On the first day after disturbance of the hepato-intestinal circulation, when the hepatocytes were under severe functional strain, the clasmasomes contained numerous small profiles of the smooth endoplasmic reticulum, whereas on the 5th day, they contained small vesicles and free ribosomes.

Clasmatosis of mitochondrial fragments during long-term administration of CCl_4 has been described previously [3]. Thus under normal physiological conditions and in pathology clasmatosis occurs at two levels: at the cell level, when clasmatosis of fragments of cytoplasm takes place, and at the organelle level, when clasmatosis of fragments of mitochondria takes place, but could be seen only by electron microscopy.

Detachment of areas of cytoplasm into the bile capillaries, in the writer's view, is passive clasmatosis, connected with enforced detachment of microvilli because of the rapid separation of vesicles containing bile products from the cell. Similar phenomena, in the writers view, take place during intensive apocrine secretion in various glands. Separation of fragments of cytoplasm into Disse's space, which the writer observed in the chick liver during embryogenesis, in the liver of starving fish, and in rats with disturbance of the hepato-intestinal circulation, is, however, an active process for the cell, connected with release of certain substances necessary for the body into the blood stream, and which in each concrete case are contained in liver cells. Whereas in the chick liver during embryogenesis these substances are most probably of protein nature, in starving fish they consist of glycogen, an essential source of energy for the fish. The glycogen is released into the bloodstream, evidently, for rapid metabolism by blood enzyme, for we know that hepatocytes of fishes contain only small quantities of enzymes of glycogenolysis, and as a result, even during starvation, the liver glycogen of fishes is utilized very slowly [7].

Clasmatosis of mitochondrial fragments, which in each of the cases described above under normal conditions is also observed during very intensive utilization of energy by the cell, is very interesting.

Thus clasmatosis of their fragments in the cell and its organelles takes place whenever it is necessary to supply the organism or the cell quickly with certain metabolic products or to remove something from the cell quickly, as in the case of the erythroblast; in other words, it is one of the mechanisms of adaptation of the cell and its organelles to changing environmental conditions.

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TIME COURSE OF CHANGES IN RAT HEPATOCYTE ULTRASTRUCTURE AFTER HEPATIC ISCHEMIA

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Temporary exclusion of the liver from the circulation during operations is attended by the risk of irreversible changes in the hepatocytes. There is as yet no general agreement on the optimal time for which the organ can be excluded from the circulation [1, 2, 4, 5] while preserving its function. Hepatic ischemia, however, (caused total extirpation of the organ before transplantation, compression of the main vessels during operations on the "dry" liver, embolism, thrombosis, atherosclerosis, and so on), leads to structural changes in the organ [1, 2, 4, 5]. In this connection it is useful to have a clear idea of the character of the changes, especially subcellular, taking place in cells of the liver after its exclusion from the circulation.

The aim of this investigation was to study the subcellular organization of rat hepatocytes during and at various times after ischemia of the liver.

EXPERIMENTAL METHOD

Forty male Wistar rats weighing 150-180 g were used. For 12-14 h before the experiments the rats were deprived of food but had free access to water. Intact animals (group 1) served as the control. The operation consisted of compression of the hepato-duodenal ligament after preliminary isolation of the bile duct under intraperitoneal pentobarbital anesthesia (40

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