PHYSIOLOGICAL AND REPARATIVE REGENERATION OF MITOCHONDRIA

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The ultrastructure of the mitochondria of hepatocytes was studied under normal and pathological conditions. Restoration of the ultrastructure of swollen mitochondria with a translucent matrix was shown to be completed within 24 h in rat and chick hepatocytes during embryogenesis. One method of intramural regeneration of the mitochondria in hepatocytes of chick embryos and of mice poisoned with CCl_4 twice a week for five months whas shown to be clasmatosis of the damaged fragments of mitochondria, their removal through the partially destroyed outer membrane of the mitochondrion, and subsequent restoration of the integrity of the outer membrane. The process of regeneration of the mitochondrion after clasmatosis of its fragments takes two days in chick embryonic hepatocytes.

KEY WORDS: ultrastructure of mitochondria; intramural regeneration of organoids; clasmatosis.

There are several aspects to the problem of restoration of the ultrastructure of mitochondria [1, 4, 7, 9, 10, 12]: 1) regeneration of the mitochondrial membranes, 2) reproductive processes in their matrix, 3) the limits within which changes in mitochondria are reversible, 4) methods of reproduction of mitochondria, 5) the velocity of repair processes in mitochondria, and 6) hypertrophy of mitochondria.

The object of this investigation was to study the methods of repair processes in mitocondria of liver cells under normal and pathological conditions.

EXPERIMENTAL METHOD

Hepatocyte ultrastructure was studied in rats during embryonic (from the 13th to the 21st days inclusive) and postnatal development (1st, 4th, 14th, and 30th days after birth), in chick embryos from the sixth day of incubation until hatching, and in mice after poisoning with CCl_4 twice a week for five months. The liver tissue was fixed by Palade's method at pH 7.2-7.4 and embedded in Araldite. Ultrathin sections were stained by Reynolds' method and examined in the JEM-7A electron microscope.

EXPERIMENTAL RESULTS

During the study of the embryonic development of the rat liver, the times of recovery of the ultrastructure of the mitochondrial matrix were established. Diurnal rhythmic changes were found in the ultrastructure of the mitochondria and cisterns of the granular endoplasmic reticulum (GER). For instance, whereas during the first day the mitochondria in the cytoplasm of the hepatocytes were enlarged, swollen, and had a translucent matrix frequently with disoriented cristae, and the cisterns of the GER were dilated (Fig. 1), next day the mitochondria were smaller, their matrix became dense, the arrangement of the cristae became regular, and the cisterns of the GER once more became flat (Fig. 2). This diurnal alternation was observed throughout the period of embryogenesis and affected most cells. In the writer's view, these morphological pictures reflect a wave-like course of protein synthesis in the cells and, consequently, in the organ as a whole. Hepatocytes with swollen mitochondria and dilated cisterns of the GER evidently reflect a phase of intensive synthesis for export and, consequently, a phase of self-utilization, whereas hepatocytes with mitochondria with a dense matrix and with flattened cisterns of the GER represent the phase of physiological regeneration. Since the ultrastructure of the mitochondria during embryonic development changed every

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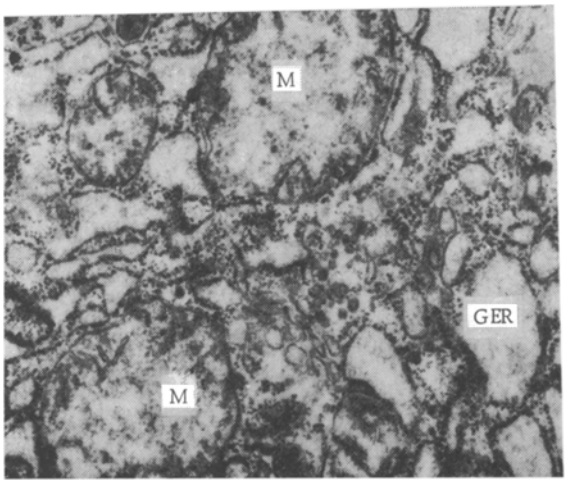

Fig. 1

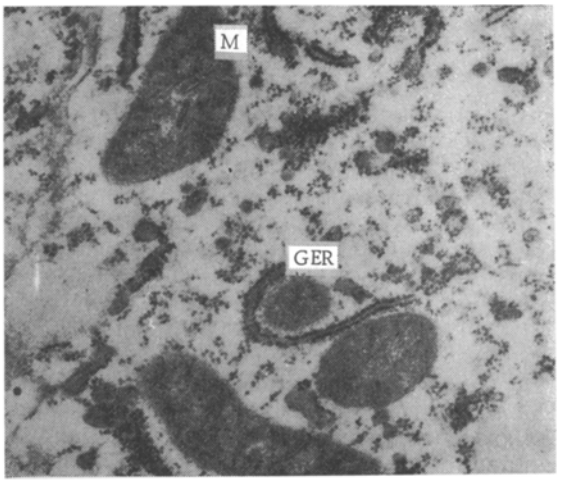

Fig. 2

Fig. 1. Swollen mitochondria (M) and dilated cisterns of GER in cytoplasm of hepatocyte of rat embryo on 18th day of development (25,000×).

Fig. 2. Mitochondria (M) with dense matrix and flattened cisterns of GER in cytoplasm of hepatocyte of rat embryo on 19th day of development (25,500×).

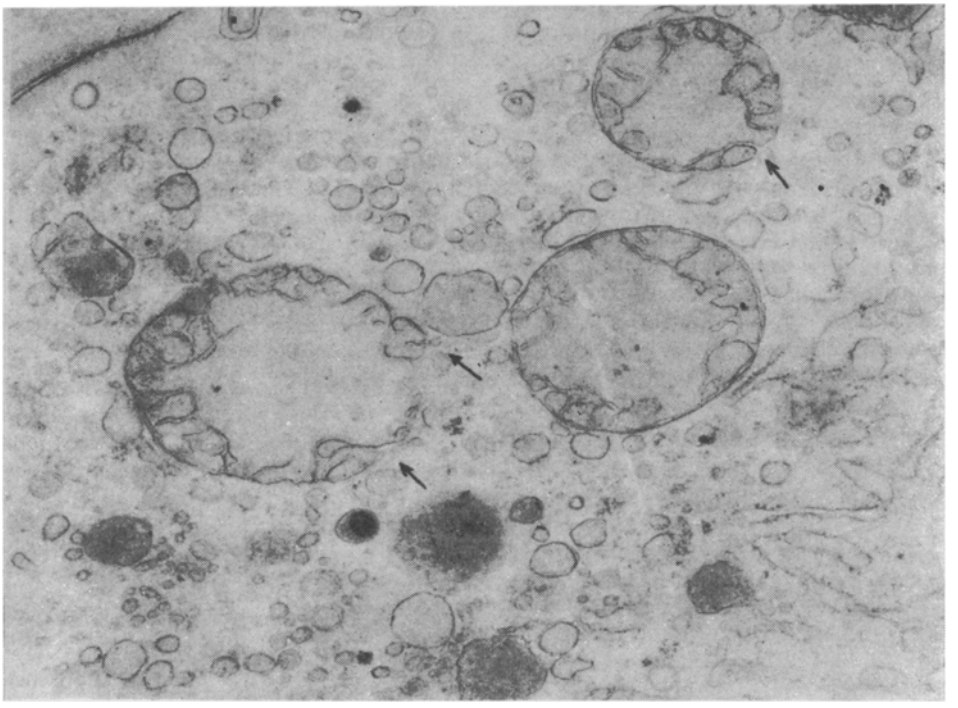

Fig. 3. Clasmatosis of fragments of mitochondria (arrow) in hepatocyte after prolonged (5 months) administration of CCl_4 twice a week to mice (26,400×).

day (from swollen to flat), in the writer's view the time taken for normalization of mitochondria with a swollen matrix and with disoriented cristae is one day.

The sharpest changes in the mitochondria were observed in hepatocytes of developing chick embryos on the day after the beginning (ninth day) of intensive secretion of bile into the bile capillary. All the mitochondria of the hepatocytes had a clear matrix, disoriented cristae, and a wavy outline of their outer and inner membranes; the integrity of the outer membrane of many mitochondria was disturbed and clasmatosis of isolated fragments was observed. For a certain distance the outer membrane of the mitochondria was absent, and alongside the

mitochondrion lay a fragment possessing one membrane, similar in shape and size to the region of the mitochondrion removed by clasmatosis. Daily study of the ultrastructure of chick embryonic hepatocytes showed that the density of the matrix and the orderly arrangement of the cristae were restored after one day, just as during embryonic development in rats, whereas normalization of the outline and restoration of the integrity of the outer mitochondrial membrane occurred only after two days, for these changes were still taking place after 1 day.

The mechanism of clasmatosis could be studied in more detail only by examination of the ultrastructure of the mitochondria under pathological conditions: by the study of mictochondrial ultrastructure in the hepatocytes of mice during prolonged (five months) poisoning twice a week with CCl_4. Under pathological conditions the process of elimination of dying fragments from the mitochondrion evidently takes place more slowly, and as a result it can be studied more clearly.

In a partially destroyed mitochondrion the distal end of a crista comes into contact with the mitochondrial membrane and an area of the outer mitochondrial membrane in this zone is destroyed, and a small fragment of the mitochondrion is separated from it along the arch-shaped crista; this is evidently followed by growth of the outer membrane of the mitochondrion (Fig. 3). As a result, mitochondria with an irregular outline — "moth-eaten" mitochondria — in which the outer membrane is absent along part of the mitochondrion, can be seen in the cytoplasm of the hepatocyte. In the writer's view, clasmatosis of fragments of a mitochondrion followed by restoration of the ultrastructure of the organelle is a method of intramural regeneration of the mitochondrion that has not been described in the accessible literature.

Normalization and intensification of the working activity of the cell, during both normal development and pathology, take place through the normalization of the ultrastructure of injured mitochondria or through an increase in the number of mitochondria produced by division of existing ones along the cristae or by a sharp increase in size of mitochondria present in the cells, with an increase in the number of cristae in them and in the density of their matrix. Combinations of the various methods are also possible.

There are differences of opinion on the biogenesis of mitochondria [6]. In the present writer's view, the number of mitochondria in the cells of an organ which has started to function increases on account of their division along the cristae. The process of division of mitochondria along the cristae, when the crista grows out as far as the opposite wall of the mitochondrion, was observed during a study of the ultrastructure of hepatocytes in early rat and chick embryos, and during regeneration after partial hepatectomy [2] and after prolonged administration of CCl_4 to mice. A sharp increase in the size of mitochondria accompanied by an increase in the number of cristae and in the density of the matrix was observed during postnatal development in rats, when the functional load on the liver was sharply increased as a result of a change of diet [3]; during the fetal period of embryonic development in the chick, when the functional load on the liver is increased because the fetus begins to assimilate amniotic fluid together with protein; and under pathological conditions — during irradiation [2]. In any form of intramural regeneration of the mitochondrion (repair of the partially destroyed membrane, normalization of the density of the matrix, hyperplasia of the mitochondrion and its matrix and cristae), processes of synthesis take place. The question of how the ultrastructure of the mitochondria is restored so quickly to normal naturally arises. We now know that the mitochondria possess their own DNA and protein-synthesizing system [6, 13]. There is also evidence that mitochondrial proteins are synthesized both in the mitochondrion itself and in the cytoplasm also, from which they enter the mitochondrion [5], and that the rate of renewal of proteins of the outer mitochondrial membrane is higher than that of the inner [11], and that the half-renewal time of the mitochondrial proteins varies from 1 h to several days [8, 14]. All the biochemical data given above confirm the morphological observations indicating rapid intramural regeneration of the mitochondria.

In conclusion, many morphological changes in the mitochondria are similar under normal and pathological conditions and their ultrastructure is restored in the same way; however, the times taken for normalization evidently differ.

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EFFECT OF REPEATED COOLING ON THE STATE OF THE ADRENALS AND PROLIFERATION OF THE CORNEAL EPITHELIUM IN ALBINO RATS

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The effect of repeated cooling on the state of the adrenals and on the mitotic cycle and number of DNA-synthesizing nuclei in the corneal epithelium was studied in albino rats. The animals were cooled by a contact method to a body temperature of 28°C and exposed at that temperature 1 h daily for 5 days. Marked activation of the adrenals was observed: The weight of the glands was doubled, their cholesterol concentration reduced by two-thirds, their blood 11-hydroxycorticosteroid level increased fourfold, and their adrenalin excretion stimulated. The mean number of mitoses in the cornea was reduced by half. The depression was not connected with any change in the rate of mitosis but was due to delay in interphases. There was no change in the level of pathological mitoses. Chronic exposure to stress was not accompanied by any change in the number of DNA-synthesizing nuclei or the intensity of DNA synthesis.

KEY WORDS: mitotic activity; stress; hypothermia; DNA synthesis; cornea.

In previous investigations the writers found that chronic exposure to stress (intravenous injection of pyrogenal daily for 5 days) causes prolonged depression of mitotic activity without changing the index of labeled nuclei [10].

The object of the present investigation was to test if this rule applies also to the effect of a different stressor, namely moderate hypothermia. The study of this problem is of applied importance: moderate hypothermia is widely used in clinical practice. However, the basic research into the effects of low temperatures on cell proliferation has been undertaken on tissue cultures [2, 8, 13]. The few studies of the action of cold on cell division which have been undertaken on homeothermic animals have given contradictory results and were carried out on specialized tissues which play an active part in adaptation to low temperature [3, 12].

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