Differences between the area of the lamelloplasm and area of clearing are evidently explained as follows. The transformed cells often formed long processes. The receptors were cleared only from the surface of small lamellae located at the end of these processes. The surface of the remainder of the processes, which had stable edges, was not cleared although their cytoplasm contained no granules and, for that reason, during quantitative measurements they were regarded as lamelloplasm. The reduction in the area of the lamelloplasm of the transformed cells had the result that the zone of clearance reached its maximal size during short-term incubation of these cells with CA. In normal cells, however, the zone of clearance reached its maximum during long-term incubation with CA. A further increase in the incubation time to 2 h caused no change in the dimensions of the zone of clearance.

It was thus shown that the zone of clearance from conglutinated receptors corresponded to the area of lamellar cytoplasm in normal NMF and NRK cells, but was a little smaller than the area of the lamelloplasm in transformed L and Ki-MSV cells.

Reduction of the clearance zone was characteristic of the transformed cells studied. Further investigations are necessary to determine to what extent this property is common to other types of transformed cells.

The mechanism lying at the basis of differences between the lamelloplasm and other parts of the cytoplasm remains unexplained. It can be tentatively suggested that an important role in the movement of receptors is played by microfilaments located in the cortical layer of the lamelloplasm, and that the character of interaction between the microfilaments and receptors differs in different parts of the cell cytoplasm [2, 3].

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ELECTRON-MICROSCOPIC AND HISTOCHEMICAL CHARACTERISTICS OF HEPATOMAS ARISING AFTER PROLONGED ADMINISTRATION OF CARBON TETRACHLORIDE

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During prolonged injection of CCl_4 into animals, against the background of degenerative and regenerative changes in the liver, in 100% of cases hepatomas appear and gradually increase in size, turning into large tumor nodules. The ultrastructural data on the structure of these hepatomas are not numerous, and it was accordingly decided to investigate this problem. Experiments were carried out on 40 noninbred mice receiving injections of 0.2 ml of a 40% solution of CCl_4 in peach oil twice a week for 13 months. The animals were killed 7, 8, 10, 11, 12, and 13 months after the beginning of CCl_4 administration. The following methods of light microscopy were used: staining with hematoxylin and eosin, with picrofuchsine, impregnation with silver by Gomori's method, staining for fat with Sudan III, Brachet's reaction with ribonuclease control, PAS reaction with amylase.

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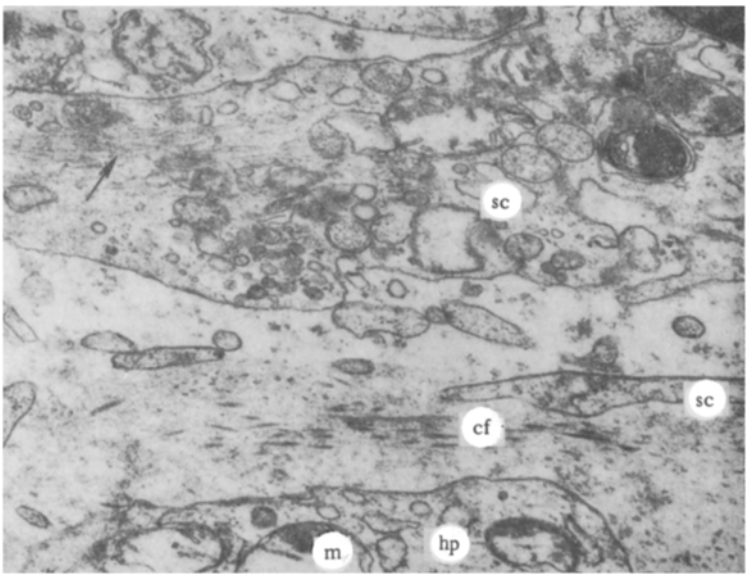

Fig. 1. Very thin fibrils in cytoplasm of sinusoidal cell (sc) 1 month after beginning of CCl_4 injections. hp) Hepatocyte; m) mitochondrion; cf) collagen fibers, 30,000×.

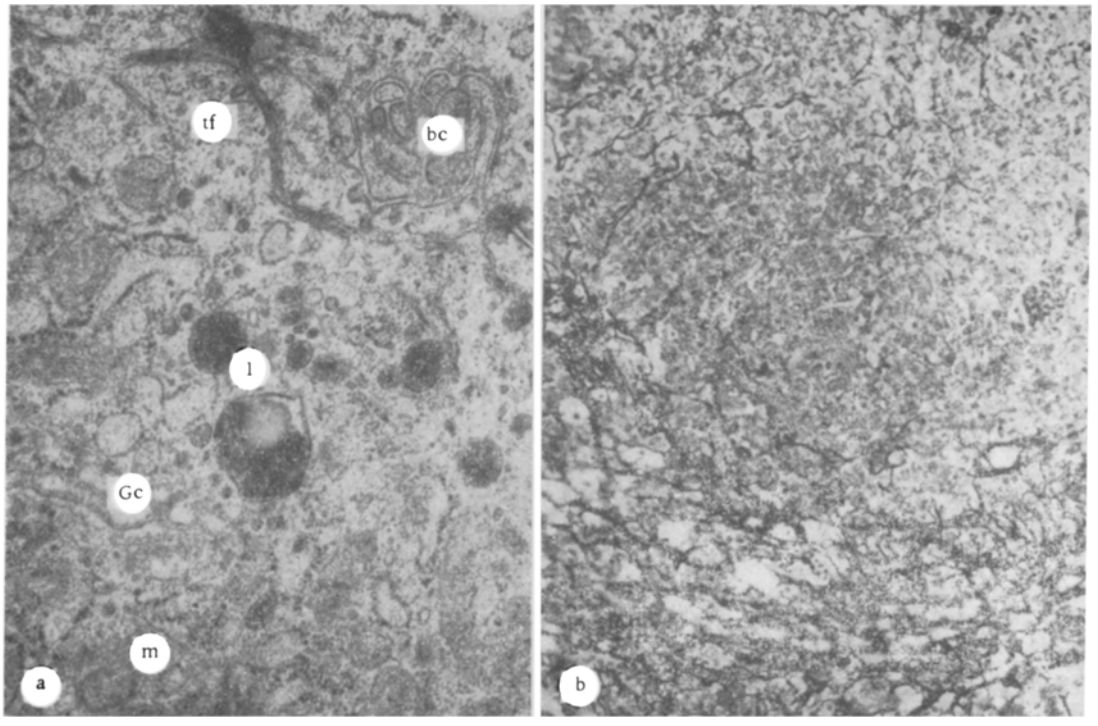

Fig. 2. Weakening of reticular skeleton and associated changes in ultrastructure of hepatocytes. a) Tonofibrils (tf) around bile capillary (bc) in cytoplasm of hepatocyte from hepatoma 10 months after beginning of CCl_4 injections. Gc) Golgi complex; m) mitochondrion; 1) lysosome; 33,000×. b) Weakening of reticular skeleton in hepatoma. Dense network of reticular fibers at periphery of nodule and single reticular fibers in center of hepatoma. Silver impregnation by Foot's method, 120×.

Activity of the following oxidoreductases and hydrolases was determined in frozen sections: NAD- and NADP-diaphorases, and succinate, lactate, and α-glycerophosphate dehydrogenases. Material for electron-microscopic study was fixed by Palade's method and embedded in Araldite; ultrathin sections were examined in the IEM7A electron microscope.

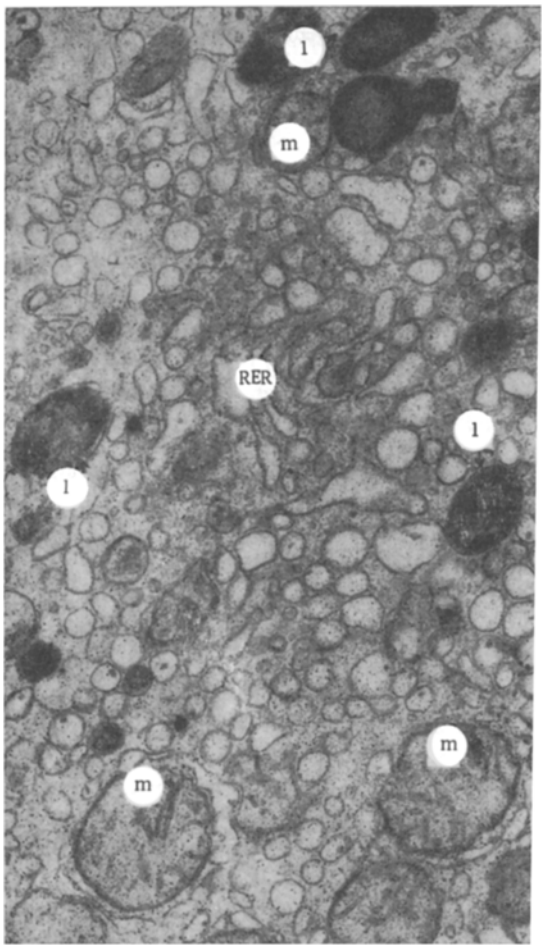

Fig. 3. Type 4 cells in hepatoma 13 months after beginning of CCl_4 injections. m) Mitochondrion; RER) rough endoplasmic reticulum; 1) lysosomes, 66,000×.

EXPERIMENTAL RESULTS

Administration of CCl_4 causes the formation of fibrosis in the liver and the onset of cirrhosis and hepatomas [3].

Three types of cells were found in hepatomas arising 7-10 months after the beginning of the injections. In cells of type 1, the predominant features were of fatty and vacuolar degeneration. Histochemical investigation showed that their cytoplasm contains little ribonucleoprotein (RNP) and glycogen, and that weak activity of oxidative and hydrolytic enzymes is present. Electron-microscopic investigation revealed many lipid drops of different sizes in the nucleus, as well as in the cytoplasm. Vacuoles with electron-optically transluscent contents, often displacing the nucleus toward the cell membrane, were found in the cytoplasm. Cisterns of the rough endoplasmic reticulum (RER) were greatly widened, few ribosomes were present on their membranes, and the matrix of the mitochondria was clear.

In cells classed as type 2, besides degenerative features, signs of intracellular regeneration could be observed. For instance, in many cells the nuclei were larger and the nucleoli more numerous, and their DNA content was increased considerably. The cytoplasm of these cells contained a moderate amount of RNP and glycogen. Activity of oxidative enzymes in these cells was higher than in the type 1 cells. Electron-microscopic investigation showed that the nuclear membrane forms numerous projections and invaginations. The same organoids in the cytoplasm of the same cell could be in different states. For instance, the RER could be observed as greatly dilated cisterns or as flattened cisterns, breaking up into vesicles. Swollen mitochondria with a clear matrix were seen together with other mitochondria with a dense matrix and an increased number of cristae, probable evidence of compensatory processes taking place in these organoids. One of the mani-

festations of regeneration in response to fatty infiltration is a sharp increase in the number of microparticles in the cytoplasm of most hepatocytes [5]. The Golgi lamellar complex in hepatocytes is relatively large and contains many lysosomes. Another manifestation of the regeneration reaction was the presence of binuclear cells and also of giant cells containing a large nucleus, with many invaginations of the nuclear membrane and many organoids in the cytoplasm.

Sometimes large hepatocytes, classed as type 3, were seen. Histochemical investigation revealed much RNP and glycogen in the cytoplasm of these cells, high activity of oxidative enzymes, and moderate hydrolase activity. Electron-microscopic investigation showed that the nuclei of these cells contain several nucleoli and their cytoplasm an enormous number of cisterns of the RER, forming dense parallel rows. These cells evidently reflect the intensive course of intracellular regeneration. The relative numbers of the different types of cells in different nodules in the liver of the same animal differed. Soon after the beginning of CCl_4 injections collagen fibers, normally not present, began to appear in the Desse's spaces and between the hepatocytes. Fibroblastic sinusoidal cells increased in size; their cytoplasm contained many phagosomes and very thin fibrils, the initial stage of formation of collagen fibrils (Fig. 1).

Between 11 and 13 months after the beginning of CCl_4 injections numerous large nodules developed in the liver of the mice, and by contrast with the early period, they grew irreversibly despite discontinuation of CCl_4 injection, evidence of malignant change in the hepatomas. The hepatoma cells were characterized by polymorphism and different variants of the cells of types 2 and 3 were represented, although features not observed previously were noted in the cytoplasm of many cells. For instance, whereas the predominant form of RER in the hepatomas after 7-10 months was variously dilated cisterns, after 11-13 months the RER quite often showed a lacelike pattern. Often, well-developed desmosomes were located around the bile capillaries and gave off bundles of tonofibrils into the depths of the cytoplasm; these tonofibrils have a supporting function, and normally they are never found (Fig. 2a). Impregnation with silver by Gomori's method showed that the quantity of reticular stroma in the nodule was much less and it was less uniformly distributed than in the surrounding parenchyma (Fig. 2b). One of the characteristic features of hepatomas at this period was the greatly increased folding of the cell membrane of the hepatocytes, enabling them to increase considerably in size in case of necessity. Another distinguishing feature of the hepatomas was the appearance, besides of cells of the three types described above, of hepatocytes differing noticeably from those normally found. Their nuclei contained large nucleoli. Much RNP was present in their cytoplasm, activity of oxidative enzymes was depressed, but hydrolase activity was increased. High aerobic glycolysis is a characteristic feature of the metabolism of tumor cells. Electron-microscopic examination revealed a decrease in the number of cisterns of the RER in the cytoplasm of these cells; the RER consisted of short, flat tubules, weaving around separate mitochondria, or it had the appearance of vesicles with very few ribosomes on them. Small mitochondria and many free ribosomes, microparticles, and cytosegresomes were distributed in the cytoplasm (Fig. 3). Besides marked evidence of collagen formation in the liver at this time of investigation, a picture of resorption of collagen fibers could be observed, evidently taking place with the aid of hepatocytes and sinusoidal cells [1].

With an increase in the period of administration of CCl_4, a steady increase was found in the number of cells less sensitive to its action, a feature noted also by Kostyrev [2]. It is quite possible that resistance to the action of the harmful factor is formed in some cells through adaptive changes in their metabolism [4].

Hepatomas arising in mice as a result of prolonged administration of CCl_4 thus consist of cells in which signs of degeneration and regeneration are represented in different proportions. The appearance of cells similar in ultrastructure to the cells of malignant hepatomas in these hepatomas at the late stages of development, and also the appearance of signs of resorption of collagen fibers, histochemical differences betwen the hepatoma cells, and the fact that development of the tumor is not reversible on discontinuation of CCl_4 administration are evidence that the hepatomas have a tendency toward malignancy.

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