ELECTRON-AUTORADIOGRAPHIC INVESTIGATION OF DNA SYNTHESIS IN HEPATOCYTES AFTER CARBON TETRACHLORIDE ADMINISTRATION

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A sharp increase in the intensity of DNA synthesis was found in the hepatocytes regularly after injection of carbon tetrachloride. The increased intensity of DNA synthesis in the cell was analyzed quantitatively by electron-autoradiography, and the relationship between the silver grains and the cell membranes, especially the nuclear membranes, was investigated.

Investigations have shown that after administration of CCl_4 to experimental animals the intensity of degeneration and regeneration in the liver tissue varies depending on the frequency of injection of the compound [1-3]. For example, if CCl_4 was injected twice a week, more frequent and higher increases in the level of DNA synthesis were observed in the nuclei of the liver cells compared with those observed after weekly injections [4].

It was decided to conduct a fine structural analysis of the increased intensity of synthesis in nuclei of the hepatocytes. This is an important subject, first, as a prelude to the further study of the dynamics of formation of substances (RNA) responsible for intracellular regeneration after the action of a pathogenic factor, and for their transportation from nucleus to cytoplasm. Second, it is an interesting problem in connection with the further improvment in the technique of quantitative analysis of the increased intensity of DNA synthesis. The method of electron-autoradiography was selected for this purpose.

EXPERIMENTAL METHOD

Twice a week noninbred mice weighing 25 g received a subcutaneous injection of 0.2 ml of a 40% solution of CCl_4 in peach oil. After the sixth injection of CCl_4, two experimental and two intact control mice received two intraperitoneal injections of thymidine-H^3 (specific activity 4.6 Ci/mmole) in a dose of 10 μCi/g at an interval of 1 h. The animals were sacrificed 70 min after the last injection of thymidine. The liver was fixed with 1% OsO_4 solution, dehydrated in alcohol and acetone, and embedded in Araldite. Ultrathin sections were obtained on the LKB ultratome, stained with lead citrate, and coated with a thin (50Å) layer of carbon.

To obtain autoradiographs, a monolayer of type M emulsion (State Photographic Chemical Research Project) was applied to the sections. After exposure for 2 months the autoradiographs were developed in metol-hydroquinone developer and examined in the UÉM-100V microscope. The photographs were taken on high-contrast production plates.

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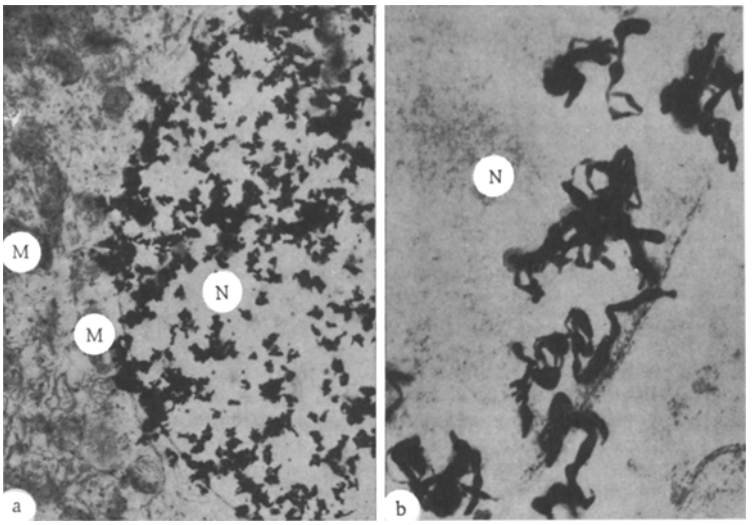

Fig. 1. Electron-autoradiographs of hepatocyte nuclei from a mouse on the 2nd day after injection of CCl_4: a) many silver grains in hepatocyte nucleus, 15,000×; b) the silver grains are shaped like hooks, commas, and coils, 42,000×; N) nucleus; M) mitochondrion.

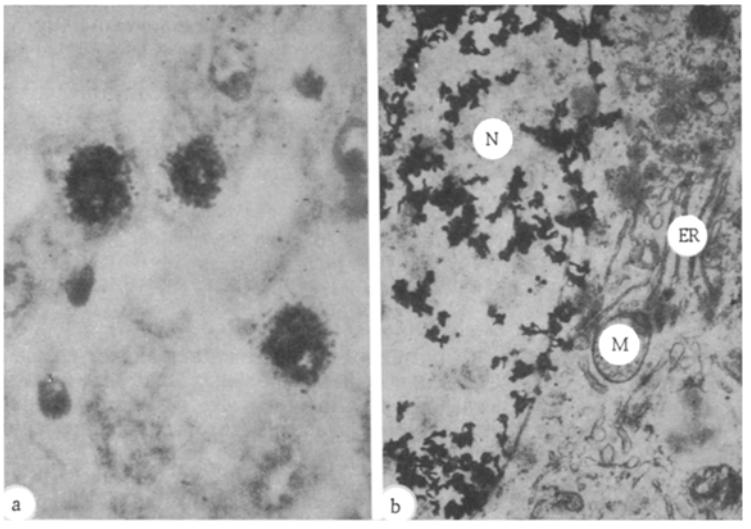

Fig. 2. Comparative picture of intensive DNA synthesis by hepatocytes as shown by light (a) and electron (b) autoradiography: a) many silver grains massed together; nucleus appears as a black spot, 1200×; b) electron-autoradiograph clearly shows separate grains of silver, 25,000×; N) nucleus; M) mitochondrion; ER) endoplasmic reticulum.

EXPERIMENTAL RESULTS

On the second day after injection of CCl_4 many grains of silver were found in many nuclei of the hepatocytes. This agrees with the results obtained by light microscopy, showing that the index of labeled cells can rise by 100-200 times after injection of CCl_4 [4]. Whereas there were either no labeled cells or only solitary grains of silver, the significance of which it is difficult to estimate, in the liver sections of the control animals when examined under the electron microscope, in every section from the liver of the experimental mice there were several nuclei containing many grains of silver. Because of the very large number of silver grains these nuclei appeared as dark round or oval structures under the low power of the electron microscope (Fig. 1a). On electron-micrographs taken under high power the grains of silver appeared as black bands, curved into hooks or comma-shaped structures, or twisted into coils of curious shapes (Fig. 1b). These grains of silver were located above the nuclei and were absent in the cytoplasm, corresponding to the localization of DNA in the cell. The relationship between the silver grains and membranous structures of the cell, especially the nuclear membrane, could be demonstrated by electron-autoradiography. The reason for the presence of single grains of silver in the cytoplasm around the nucleus must be that radiation from the tritium atoms inducing the formation of these grains was directed tangentially to the surface of the section and emulsion.

The technique of electron-autoradiography is a much improved method of quantitative analysis of fluctuations in the intensity of nucleic acid synthesis in single cells. This is particularly true if the increase in intensity of synthesis is marked. Under the light microscope in such cases the nuclei appear as almost confluent black stains (with exposures required for the formation of a clear autograph in the control series), in which it is very difficult to count the grains because of their very large number (Fig. 2a). On electron-autoradiographs in these cases quantitative analysis can be carried out by counting the silver grains, taking each isolated deposit of silver in the shape of a comma or coil etc., as a separate grain (Fig. 2b). An important advantage of quantitative analysis performed on electron-autoradiographs is that all the grains lie in the same plane, whereas if grains are counted in the light autodiograph errors may arise because of the omission or duplication of the granules counted in different planes.

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