AUTORADIOGRAPHIC STUDY OF DNA AND RNA SYNTHESIS IN LIVER CELLS OF MICE WITH CHRONIC CARBON TETRACHLORIDE POISONING

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A long course (3-4 months) of CCl_4 injections leads to a marked increase in the intensity of DNA synthesis in the liver tissue. The rhythm of the increase in thymidine-labeled cells corresponded to the rhythm of CCl_4 administration. Incorporation of radioactive RNA precursor (orotic acid) in animals with chronic CCl_4 poisoning was less than in control animals, indicating a decrease in synthesis or a marked increase in the rate of breakdown of RNA under the influence of CCl_4.

A previous investigation [1] showed that administration of CCl_4 to mice stimulates DNA synthesis in the liver and that the frequency of peaks of intensity of DNA synthesis depends on the frequency of administration of CCl_4 to the animals (once or twice a week).

Since the response of cells to pathogenic agents is determined by nucleocytoplasmic relations, it was decided to study the character of synthesis not only of DNA, but also of RNA, by the liver cells. For this purpose a comparative analysis of DNA and RNA synthesis was carried out on animals receiving a long course of CCl_4 injections.

EXPERIMENTAL METHOD

Adult noninbred mice received subcutaneous injections of 0.2 ml of 40% CCl_4 solution in peach oil twice a week. DNA synthesis was investigated from the 98th to the 117th day of the experiment. For this purpose two mice of the experimental group and one intact (control) mouse received daily intraperitoneal injections of thymidine-H^3 (specific activity 4.6 Ci/mmole) in a dose of 1.3 μCi/g. The animals were sacrificed 70 min after injection of thymidine and the liver was fixed in Carnoy's fluid. To determine the RNA synthesis, from the 119th to the 125th day of the experiment two mice of the experimental group and one control mouse received daily intraperitoneal injections of the RNA precursor orotic acid-5-H^3 (Radiochemical Center, Amersham, England, specific activity 20 Ci/mole) in a dose of 8 μCi/g. The animals were sacrificed 4 h after the injection, and the liver was fixed in a formalin–alcohol–acetic acid mixture (30:10:3) and embedded in paraffin wax. To investigate DNA synthesis, sections were coated with type M liquid emulsion and exposed for 30 days, while to investigate RNA synthesis they were coated with type R emulsion and exposed for 14 days. The sections were developed and then stained with hematoxylin–eosin and methyl green–pyronine. To determine the index of thymidine-labeled cells (ILC) 3500 hepatocytes from each animal were studied. The quantity of orotic acid incorporated into RNA was determined from the number of silver grains counted separately above the nucleus and cytoplasm of the hepatocytes. Grains were counted in 100 hepatocytes from each animal.

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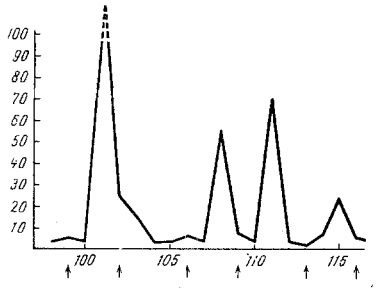

Fig. 1

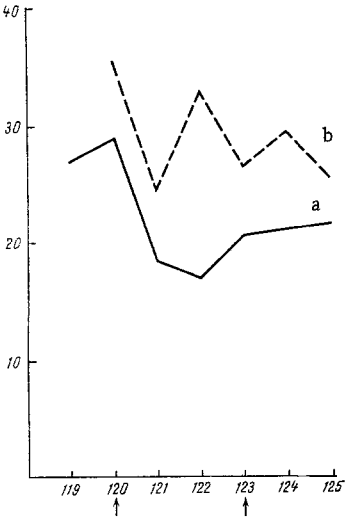

Fig. 2

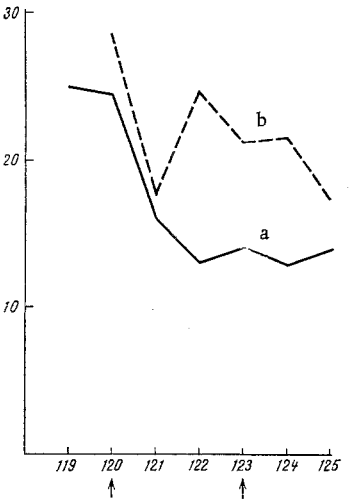

Fig. 3

Fig. 1. Dynamics of DNA synthesis by liver tissue during prolonged administration of CCl_4 twice a week. Abscissa, time (in days), arrows denote CCl_4 injections; ordinate ILC in %. Scale chosen so that level of DNA synthesis in control animals coincides with abscissa.

Fig. 2. Intensity of incorporation of orotic acid into liver cell nuclei during prolonged administration of CCl_4 twice a week. Abscissa, time (in days), arrows mark injections of CCl_4; ordinate, mean number of grains of silver above nucleus; a) experiment, b) control.

Fig. 3. Concentration of orotic acid in cytoplasm of liver cells during prolonged administration of CCl_4 twice a week. Ordinate, mean number of silver grains above cytoplasm of hepatocyte; remainder of legend as in Fig. 2.

EXPERIMENTAL RESULTS

Degenerative changes developed in the liver of the experimental group of animals and were most severe during the first and second days after successive CCl_4 injections. Throughout the period of observation ILC was significantly higher than the control level and it rose sharply after successive CCl_4 injections (Fig. 1).

Orotic acid was incorporated into nearly all cells except in small foci of necrosis. In both experimental and control mice, the cell nuclei were most intensively labeled. Since all or nearly all the RNA of the cell is synthesized in the nucleus [2, 3, 4], the number of silver grains above the nucleus reflects the intensity of DNA synthesis, while the number of grains above the cytoplasm corresponds to the amount of RNA passing from the nucleus into the cytoplasm. As is clear from Figs. 2 and 3, in most animals of the experimental group the number of silver grains above the nucleus and cytoplasm was less than in the controls. Statistical analysis of the experimental results was carried out by comparing the number of silver grains above the nuclei and above the cytoplasm separately in the control and experimental animals. Analysis of the results using the Kolmogorov–Smirnov criterion showed that the difference between the experiment and the control corresponds to the 95% level of probability accepted in biological research (for comparing groups by the number of grains above the nucleus $\lambda^2 = 2.43$, for comparing groups by the number of grains above the cytoplasm $\lambda^2 = 1.97$; $\lambda^2_{05} = 1.84$, $\lambda^2_{01} = 2.65$).

The experiments showed that injection of CCl_4 into animals is followed by a marked increase in DNA synthesis and a decrease in the number of grains of silver reflecting incorporation of the radioactive RNA precursor above both nucleus and cytoplasm of the hepatocytes. These opposite changes in the indices of DNA and RNA synthesis can be explained, first, by the fact that intensification of DNA synthesis does not always lead to intensification of RNA synthesis; some workers [5, 6] associate increased DNA synthesis entirely with increased mitotic activity. However, it is now known [7] that during pathological processes in the liver tissue an increase in DNA synthesis may be associated not only with activation of the mitotic activity of the hepatocytes, but also with their polyploidization, which must be accompanied by increased RNA synthesis in the cells concerned. Evidence of intensification of RNA synthesis is given by the increase in

size and number of the nucleoli in the hepatocytes observed constantly in the present investigation under the influence of prolonged CCl_4 injections. However, despite these observations, not only were no signs of an increase in RNA synthesis observed, but the number of silver grains above the nucleus and cytoplasm of the cells was actually smaller in the experimental animals than in the controls. It can accordingly be postulated that in the experimental animals an increase in the rate of RNA synthesis was accompanied by an even greater increase in the rate of its breakdown.

Further investigations will help to shed light on the cause of the lower incorporation of RNA precursor into hepatocytes under the influence of CCl_4.

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