AUTORADIOGRAPHIC STUDY OF THE RELATIONSHIP BETWEEN THE REPARATIVE REGENERATION OF THE LIVER AND THE RHYTHM OF PATHOGENIC ACTION

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UDC 616-003.93-073.916

The dynamics of DNA synthesis in the liver tissue after injection of CCl_4 at different frequencies was studied by autoradiography. The frequency of increased synthetic activity of the hepatocytes was found to correspond to the frequency of action of the hepatotropic poison. This shows that adaptation of the body to a changing rhythm of exposure to a pathogenic stimulus is achieved through corresponding fluctuations in the intensity of regeneration.

Preliminary investigations showed that the intensity of intracellular regenerative processes varies with the intensity of the functional load and also with the strength of action of the pathogenic stimulus [1-3]. It was therefore decided to continue the study of the adaptive change in the rhythm of regeneration under the influence of changing external environmental conditions.

In the investigation described below the course of reparative regeneration of the liver tissue was studied during administration of CCl_4 to animals at different frequencies.

EXPERIMENTAL METHOD

Experiments were carried out on three groups of noninbred mice: the animals of group 1 received CCl_4 once a week, those of group 2, twice a week, and those of group 3, daily. The dose of CCl_4 per injection was the same in all experiments: 0.2 ml of a 40% solution in peach oil (subcutaneously). From the 14th to the 22nd day after the first injection of CCl_4 the number of hepatocytes synthesizing DNA intensively was determined daily for 30 days. For this purpose two experimental mice of each group and one control mouse received and intraperitoneal injection of thymidine-H^3 (specific activity 4.6 Ci/mmole) in a dose of 1.3 μCi/g daily at 8 AM. The animals were sacrified 70 min later and the liver was fixed in Carnoy's fluid. Paraffin sections were coated with type M (VNIIKhimFoto) emulsion, exposed for 30-40 days, developed, and stained with hematoxylin-eosin. To determine the index of labeled cells (ILC) 10,000 hepatocytes from each animal were studied.

EXPERIMENTAL RESULTS

When CCl_4 was injected once a week, on the second day after each injection an increase in ILC was observed, and during the next two to four days, although falling sharply, it remained above the control level. The degree of increase in DNA synthesis after each injection of CCl_4 was well marked, but as the number of injections increased the increase in ILC became smaller (Fig. 1). On the day after the injection, marked degenerative changes and areas of necrosis were found in the liver tissue. On the subsequent days, as the result of reparative regeneration, these changes disappeared almost completely, and foci of infiltration remained only in the periportal zones. Each successive increase in synthetic activity of the liver tissue thus

A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. A. Vishnevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 5, pp. 113-115, May, 1972. Original article submitted November 24, 1971.

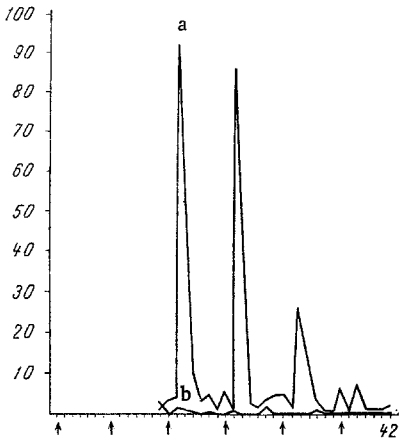

Fig. 1

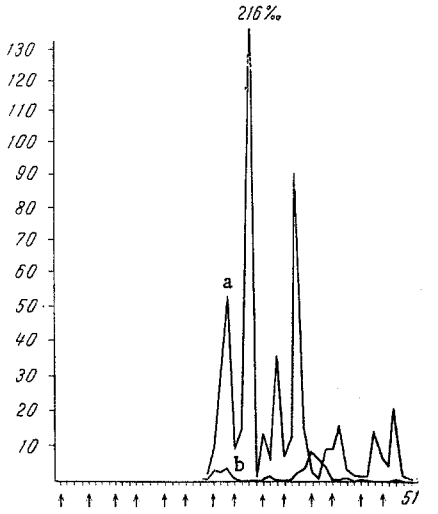

Fig. 2

Fig. 1. Dynamics of DNA synthesis by hepatocytes during injection of CCl_4 once a week: a) experiment, b) control. Abscissa, days of experiment. Arrows mark days of CCl_4 injection. Ordinate, ILC (in percent).

Fig. 2. Dynamics of DNA synthesis by hepatocytes during administration of CCl_4 twice a week (legend as in Fig. 1).

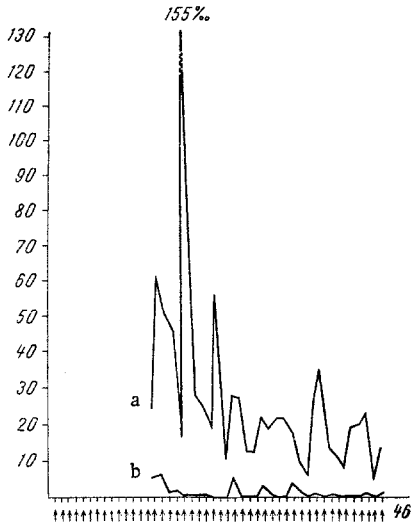

Fig. 3. Dynamics of DNA synthesis by hepatocytes during daily injection of CCl_4 (legend as in Fig. 1).

coincided the periods of most marked degenerative changes. It must also be emphasized that necrotic changes were observed in the liver tissue only after the first five injections, and at subsequent injections were not accompanied by necrosis but produced merely moderate degenerative changes. The increase in DNA synthesis was correspondingly reduced. This phenomenon must be regarded as due to adaptation of the liver tissue to the repeated action of the toxic agent.

If CCl_4 was injected more frequently (twice a week) the same high increase in ILC was observed, although in the animals of group 2 these increases were more frequent than in those of group 1, to correspond to the more frequent rhythm of pathogenic action (Fig. 2). Just as in group 1, the highest peak of increase of ILC appeared consistently on the seventh day after each successive injection. In this series of experiments, as Fig. 2 shows, intervals of two days between successive injections alternated with intervals of three days. After intervals of two days the increase in ILC was greater than after intervals of three days.

Despite the fact that the animals of group 2 received CCl_4 more often and, consequently, the total dose of the poison received by the animals was twice as large, the necrotic changes after the successive injections were much less severe in these animals or were absent altogether (except after the first two-three injections).

A particularly marked increase in DNA synthesis by the hepatocytes was observed in the mice of group 3 after daily injections of CCl_4. With this frequency of action of pathogenic stimulus, the increase in DNA synthesis remained wave-like, but in this case no connection could be found between successive injections and successive increases in ILC, as was found in groups 1 and 2. Another characteristic feature of experiments of group 3 was that the minimal level of DNA synthesis was higher than in groups 1 and 2 (Fig. 3). The total quantity of DNA synthesized in the animals of group 3 was thus considerably larger.

No definite connection could be found between successive injections of CCl_4 and the severity of the necrotic and degenerative changes in the animals of group 3, by contrast with those of groups 1 and 2: de-

generative changes grew stronger and weaker with a period of five to six days, although the injections were given daily.

These results of these experiments showed that the increase in the number of cells synthesizing DNA and providing for reparative regeneration of the liver tissue directly depends on the frequency of action of the pathogenic stimulus: more frequent injections of CCl_4 correspond to more frequent increases in synthetic activity of the liver tissue. Consequently, the frequency of action of the pathogenic stimulus determines and, apparently, induces the corresponding rhythm of increase in ILC. This connection must be regarded as an adaptive response of the liver tissue aimed at restoring the disturbed structure and function. This conclusion is confirmed by the following fact: when the toxic substance was injected twice a week necrotic changes were found less frequently and they were less severe than when it was injected once a week, which can only be explained by the higher intensification of reparative regeneration in the first case. The increase in DNA synthesis under these conditions provides for two principal processes: an increase in the intensity of cell division and activation of intracellular reparative regeneration. The fact that increased DNA production does not go entirely toward an increase in mitotic activity, but also helps to provide for intracellular compensatory processes is not entirely spent on an increase in mitotic activity, but also provides for intracellular compensatory processes is shown by the greater increase in ILC than in the mitotic index, which does not conform to the relationships established [2]. During CCl_4 administration marked polyploidization and hypertrophy of the hepatocytes are observed, whereas few mitoses are found [4, 5].

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