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EFFECT OF ANTIHEPATOCYTOTOXIC SERUM ON DNA SYNTHESIS IN THE RAT

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Incorporation of thymidine-^{3}H into parenchymatous and reticulo-endothelial cells of the liver was studied autoradiographically in adult female rats treated with small doses (0.06 μg/100 g body weight per injection) of antihepatocytotoxic serum (AHTS), the γ-globulin isolated from it (γAHTS), and the γ-globulin fraction of normal rabbit serum (γNRS) to intact animals and to rats with liver damage caused by carbon tetrachloride (CCl_4). Following injection of γAHTS and, to a lesser degree, of AHTS into intact animals the index of labeled nuclei of both the parenchymatous and the reticulo-endothelial cells was increased. When given after preliminary CCl_4 administration, γAHTS stimulated reparative regeneration. The action of γAHTS took place in phases: A period of increase in the index of labeled nuclei was followed by a period of decrease, and this again was followed by a fresh period of stimulation of proliferative processes.

KEY WORDS: liver; antihepatocytotoxic serum; carbon tetrachloride; index of labeled nuclei.

A definite role in the activity of organs under normal and pathological conditions is ascribed to antitissue autoantibodies. The possibility of their participation in the growth of organs has been discussed [5]. The problem of their harmful and protective action is in process of solution [7, 10, 13, 15]. The use of heterogeneic antibodies may help to solve this problem.

Small doses of antihepatocytotoxic serum (AHTS) have been shown to have a normalizing action on the functions of the liver and its metabolism, when disturbed by carbon tetrachloride (CCl_4) or by exogenous bile acids [1-3, 8].

The protective action of antibodies is largely ascribed to neutralization of the antigens formed during tissue destruction [6, 7]. However, a stimulating action of antibodies on the organ may also be expected. There are two possible pathways for this to occur: Either antibodies stimulate metabolic processes only in the cytoplasm of the cell and enhance its functional capacity, or, under the influence of antibodies, DNA replication and cell proliferation take place. It has been shown that AHTS increases the mitotic index in the liver of intact rats [4, 14].

The object of this investigation was to study incorporation of thymidine-^{3}H into liver cells (parenchymatous and reticulo-endothelial) of rats following injection of small doses of AHTS, of the γ-globulin fraction isolated from it (γAHTS), and the γ-globulin fraction of normal rabbit serum (γNRS) into intact animals and to animals with liver damage caused by CCl_4.

EXPERIMENTAL METHOD

Experiments were carried out on female Wistar rats weighing 170-200 g. The AHTS for rats was obtained by immunizing rabbits with a saline extract of rat liver. The titer of the serum in the complement fixation test was 1:320. γ-Globulin was isolated from the AHTS and NRS by Kendall's method [12].

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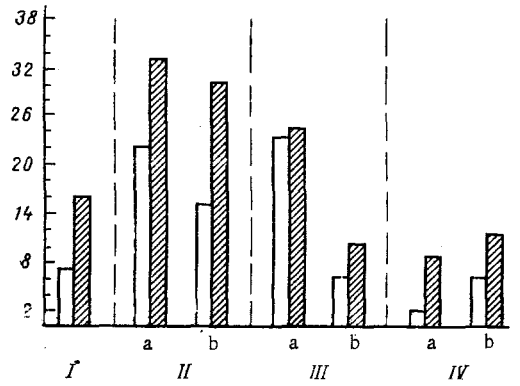

Fig. 1. Changes in index of labeled nuclei in rat liver after administration of γAHTS, AHTS, and γNRS. Ordinate, index of labeled nuclei (in ‰). I) Control, II) γAHTS, III) AHTS, IV) γNRS. a) 1st Day, b) 4th day. Unshaded columns represent parenchymatous, shaded columns reticulo-endothelial cells.

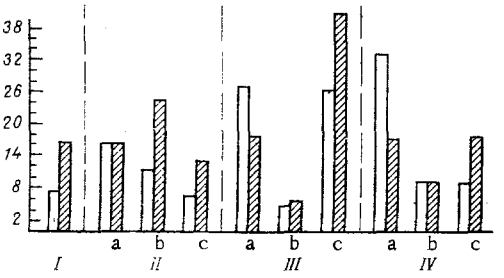

Fig. 2. Changes in index of labeled nuclei in rat liver after injection of γAHTS and γNRS following liver damage caused by CCl_4. I) Control, II) CCl_4, III) CCl_4 + γAHTS, IV) CCl_4 + γNRS. a) 7th Day, b) 10th day, c) 20th day. Remainder of legend as in Fig. 1.

In the experiments of series I intact rats were given three injections of γAHTS at intervals of 2 days. In series II intact animals were given whole AHTS by the same scheme. In series III intact rats were given injections of γNRS. The tests in these series were carried out on the 1st and 4th days after the last injection of the sera. In the next series of experiments γAHTS and γNRS were injected into rats with liver damage caused by CCl_4. In series IV the rats received CCl_4 only, by three subcutaneous injections at intervals of 2 days in a dose of 0.5 ml/100 g body weight, the compound being diluted 1:1 with sunflower oil. In series V and VI, γAHTS and γNRS (respectively) were injected into rats on the day after each injection of CCl_4. In all series γAHTS and γNRS were injected intravenously into rats in a dose of 0.06 μg protein/100 g body weight per injection. Whole AHTS was injected in a dose containing the same quantity of protein in its γ-globulin fraction.

At all periods of the investigation, thymidine-^3H (specific activity 12 Ci/mmole) was injected intraperitoneally into the rats in a dose of 1 μCi/g body weight 1 h before sacrifice. From 3 to 5 animals were used at each time of testing. The control group consisted of 8 intact rats, into which radioactive thymidine was injected 1 h before sacrifice. All tests were carried out at the same time of day.

Histological sections were cut from the liver, stained with hematoxylin, coated with type M emulsion (Photographic Chemical Research Institute project), and exposed for 28 days. After development, the sections were stained with eosin and the index of labeled nuclei determined as the number of labeled cells per 1000 parenchymatous and per 1000 reticuloendothelial cells. The latter group included Kupffer cells, lymphocytes,

histiocytes, and cells of the walls of the blood vessels and bile ducts. Two to four sections from each animal were analyzed. The results were subjected to statistical analysis by means of the Wilcoxon–Mann–Whitney nonparametric criterion (U) [9].

EXPERIMENTAL RESULTS

Changes in the index of labeled nuclei under the influence of γAHTS, AHTS, and γNRS are shown in Fig. 1. After administration of γAHTS the number of labeled parenchymatous and reticulo-endothelial cells increased. Thymidine-^{3}H was incorporated more frequently into mononuclear parenchymatous cells, but sometimes into binuclear cells also. On the first day the number of labeled parenchymatous cells was 3 times greater than the control, and the number of labeled reticulo-endothelial cells was doubled. The number of labeled cells remained high on the 4th day ($P = 0.05$ for the parenchymatous cells, $P < 0.05$ for reticulo-endothelial cells). Injection of whole AHTS also led to an increase in the index of labeled parenchymatous cells on the first day after the last injection ($P < 0.05$), but on the 4th day the index of labeled parenchymatous and reticulo-endothelial cells was indistinguishable from normal. The action of whole AHTS was thus less prolonged than the action of its γ-globulin fractions. Following injection of γNRS, unlike γAHTS and AHTS, the number of labeled parenchymatous cells decreased during the 1st day after the last injection ($P < 0.05$), but on the 4th day the number of cells incorporating radioactive thymidine was indistinguishable from normal.

Changes in the index of labeled nuclei in the liver of the rats receiving γAHTS and γNRS after damage to the liver produced by CCl_4 are shown in Fig. 2. In animals receiving CCl_4 only, an increased number of labeled parenchymatous cells was found on the 7th day after the last dose of CCl_4 in the liver ($P = 0.05$). Later in the course of the investigation (10th and 20th days) their number returned to normal. In the group of animals receiving γAHTS after CCl_4 poisoning, the index of labeled parenchymatous cells on the 7th day after the last injection of CCl_4 was higher than at the same time in animals receiving CCl_4 only ($P = 0.05$). At the time of the next investigation (10th day) the number of labeled reticulo-endothelial cells was reduced, to a level lower than that in the intact animals ($P = 0.05$), whereas the index of labeled parenchymatous cells showed only a tendency to decrease ($P > 0.05$). On the 20th day the number of labeled cells again rose sharply and exceeded the control level (for both types of cells $P < 0.05$). Injection of γNRS after previous liver damage by CCl_4, like γAHTS, increased the index of labeled parenchymatous cells at the first time of testing (the 7th day after the last injection of CCl_4) ($P = 0.05$). At subsequent times (10th and 20th days) the number of labeled cells gradually returned to normal. After injection of γNRS, unlike γAHTS, no decrease in the number of labeled cells on the 10th day or increase on the 20th day was observed.

The increase in the number of cells synthesizing DNA could be evidence of stimulation of proliferative processes [11], which together with polyploidization are responsible for regeneration in the liver of adult rats. The results showed that AHTS increases the number of parenchymatous and reticulo-endothelial cells synthesizing DNA in both the intact and the damaged liver, and consequently, it stimulates physiological and reparative regeneration. The effect of AHTS is due mainly to antiliver antibodies, for it was stronger during the action of the γ-globulin fraction and much stronger than the action of nonimmune γAHTS on DNA synthesis in the liver when damaged by CCl_4 exhibit phases. A similar series of phases, with times comparable to those in the present investigation, also was observed when the effect of AHTS on bile formation was studied [1]. These data indicate that reparative regeneration may be the basis for restoration of the disturbed functions of the liver by the action of AHTS.

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LATE CHANGES IN THE COURSE OF MITOSIS IN A SYNCHRONIZED CHINESE HAMSTER CELL CULTURE AFTER INHIBITION OF RNA AND PROTEIN SYNTHESIS

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The effect of inhibition of synthesis of various types of RNA and proteins at different periods of interphase on the course of late mitosis was studied in a synchronized culture of Chinese hamster cells. Analysis of the mitotic index and forms of pathology of division showed that the action of different doses of actinomycin D and puromycin in the first half of interphase produces an identical effect: C-mitoses in the immediate and late waves of cell division. Suppression of synthesis of total cell RNA and proteins in the second half of interphase was accompanied by delay of the cells in metaphase, with scattering of the chromosomes, evidence of a disturbance of the synthesis of the components of the division spindle. It is suggested that proteins (tubulins) and RNA participate as a reserve pool in the organization of the division spindle of late mitosis.

KEY WORDS: interphase; late mitosis; actinomycin D; puromycin; pathology of mitosis.

In continuously proliferating cell populations after division of the maternal cell the daughter cells, when commencing a new mitotic cycle, contain reserves of several structural proteins and enzymes, and also a set of different types of RNA synthesized in the preceding interphase, which provide for the passage of the cell through the initial and also, possibly, the late stages of the cycle. It was accordingly decided to use inhibitors of RNA and protein synthesis in an attempt to discover the relationship between synthetic processes in interphase and the course of the second mitosis after synchronization and to determine any functional relations between them.

EXPERIMENTAL METHOD

Experiments were carried out on a synchronized culture of Chinese hamster cells of strain B11du FAF-28, clone 237. The cells were synchronized by mitotic selection after preliminary treatment of the cells with colcemid [3, 6]. The harvested population of metaphase cells was seeded on penicillin flasks containing 2 ml culture medium at the rate of 100,000 cells to 1 ml. To inhibit rRNA synthesis actinomycin D was added to the cells in a dose of 0.1 μg/ml, and to inhibit the transcription of the total nuclear RNA (xRNA) the actinomycin D concentration was increased to 1 μg/ml. Puromycin (10 μg/ml) was used as inhibitor of protein synthesis. Incubation of the cells with the antibiotics began immediately after their emergence from the colcemid block. The inhibitors were added to the cells in the first half of interphase for 2 h (beginning of the G_1-period), for 4 h (G_1 and beginning of the S-period), for 6 h (G and first half of the S-period), and also in the second half of interphase for 6 h (second half of the S-period and G_2-period), for 4 h (end of the S-period and G_2-period), and for 2 h (principally the G_2-period). After incubation with the inhibitors the cells were thoroughly rinsed in Hanks' solution and transferred to fresh nutrient medium, in which incubation continued at 37°C until the cycle of the second wave of mitoses. The cells were fixed (alcohol : acetic acid 3 : 1) in the period of entry of the maximal number of synchronized cells into the second mitosis (23-24 h after emergence of the cells from

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