INCORPORATION OF THYMIDINE-H^3 INTO DNP OF RAT TISSUES AFTER A SINGLE INJECTION OF DNA

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Injection of homologous DNA into rats caused increased incorporation of thymidine-H^3 into DNP of the bone marrow, spleen, thymus, and mucous membrane of the small intestine 24 h after the the injection. This effect was not found on the third day.

KEY WORDS: exogenous DNA; incorporation of thymidine-H^3.

Injection of high-polymer DNA into animals caused increased activity of some enzymes of DNA metabolism in the tissues [1, 3]. This phenomenon has been interpreted as a condition of stimulation of proliferation, as has been demonstrated in the hematopoietic organs of rats receiving an injection of DNA [2]. Intensified proliferation also assumes intensification of biosynthesis of homologous DNA in the recipient's own tissues, although direct proof of this statement can be obtained only in experiments with labeled precursors.

In the investigation described below the intensity of incorporation of thymidine-H^3 into DNP in the tissues of the bone marrow, spleen, thymus, and mucous membrane of the small intestine of rats was studied after a single injection of homologous DNA.

EXPERIMENTAL METHOD

Male Wistar rats weighing 160-170 g each received an intraperitoneal injection of 3 mg DNA (mol. wt. $3 \cdot 10^7$-$5 \cdot 10^7$ daltons), isolated from rat thymus by the detergent method [6]. The rats were decapitated 1 and 3 days after the DNA injection (the times of investigation were chosen to be at the peak and trough of mitotic activity in the cells of hematopoietic organs, based on observations by Rogacheva et al. [2]). The rats were given an intraperitoneal injection of thymidine-H^3 in a dose of 150 μCi/100 g body weight 30 min before sacrifice. Preparations of DNP were obtained in the cold by homogenization of the freshly obtained tissues in 0.14 M NaCl followed by repeated washing with the same solution by centrifugation. The DNA content was determined by the reaction with diphenylamine [4]. The radioactivity of thymidine-H^3 in acid hydrolyzates of DNP was measured by means of a liquid scintillation counter. The experiments on the intact animals were repeated seven times and those with injection of DNA four times. The experimental results were subjected to statistical analysis with the aid of Student's criterion.

EXPERIMENTAL RESULTS

As the results given in Table 1 show, the highest intensity of incorporation of thymidine-H^3 into DNP (specific radioactivity of DNP) took place in the mucous membrane of the small intestine. This observation agrees with the earlier observations of Gerber et al. [5] on the renewal time of DNA in rat tissues. They found correlation between the rate of renewal of DNA and the mitotic activity of the organs. The lowest specific radioactivity of DNP was found in the thymus; however, this fact was evidently more the result of dilution of the injected label on account of the particularly high endogenous pool of thymidine in that organ than a reflection of the state of the proliferative processes [7].

Injection of DNA into the rats (Fig. 1) caused no significant changes in the DNP concentration in the tissues of the organs studied; this index was reduced by 35% ($P = 0.015$) only in the spleen 24 h after the injection.

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TABLE 1. Concentration and Specific Radioactivity of DNA Present in the DNP Form in the Tissues of Organs of Normal Rats

Organ	Conc. (in mg/g tissue)	Specific radioactivity (in counts/mg DNA/min)
Bone marrow	11,78±0,67	73 000±13 000
Spleen	9,24±0,41	86 000±24 000
Thymus	17,60±0,90	12 200±1 800
Intestinal mucosa	5,00±0,37	241 000±61 000

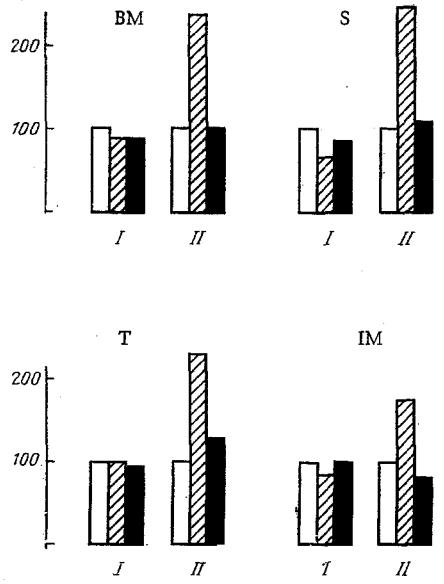

Fig. 1. DNP content in tissues of various organs of rats and incorporation of thymidine-H^3 into it after injection of DNA. BM) Bone marrow; S) spleen; T) thymus; IM) intestinal mucosa; I) DNP concentration (in % of normal); II) specific radioactivity of DNP (in % of normal). Unshaded columns represent intact control, obliquely shaded columns 24 h after injection of DNA, black columns 3 days after injection.

It must be emphasized that Rogacheva and co-workers [2], who injected a larger dose of DNA (5 mg) into rats, likewise observed not depopulation, but only a small and temporary decrease in the total number of cells in the hematopoetic organs, with considerable qualitative changes in the composition of the populations.

The specific radioactivity of DNP 24 h after injection of DNA was 2-2.5 times higher in all the tissues than in the intact animals; on the third day it returned to the normal level. This course of incorporation of the precursor into DNP corresponded to the dynamics of mitotic activity described for the cells of hematopoietic organs: an increase in the number of mitoses on the first day after injection of DNA and a decrease in their number in most cells capable of dividing on the third day [2]. During the first day, however, activation of certain enzymes of DNA synthesis has been observed in the hematopoietic organs under the influence of exogenous DNA [1]. It can accordingly be concluded that the increased incorporation of thymidine-H^3 into DNP reflected intensified premitotic synthesis of DNA in all the tissues studied. This process, however, did not lead to an increase in the DNP concentration in the tissues, such as may arise after changes in the cell populations which were found in the bone marrow, namely: the more rapid removal of mature cells and an increase in the number of young cells, against the background of some decrease in the total number of cells [2].

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