10. A. L. Shapiro, E. Viñuela, and J. V. Maizel, Biochem. Biophys. Res. Commun., 28, 5 (1968).

EFFECT OF EXOGENOUS DNA ON CONTENT AND BIOSYNTHESIS OF ENDOGENOUS DNA IN RAT BONE MARROW

V. B. Shorokhova

UDC 612.419.015.3:612.398.145.1

The effect of exogenous DNA on the content and rate of biosynthesis of endogenous DNA in rat bone marrow was studied. After injection of high-polymer homologous DNA into intact rats the endogenous DNA content per gram bone marrow was found to be reduced for the first 3 days (the decrease was most marked after 3 days, namely by 36%) and it returned to normal by the 6th day. The rate of DNA biosynthesis in rat bone marrow was increased after 18 h (when it was twice as high as in the control) and after 6 days (by 58%), and it was close to normal again 1-3 days after injection of DNA.

KEY WORDS: *Exogenous DNA; bone marrow; rate of DNA biosynthesis.*

Data showing that homologous high-polymer exogenous DNA, if injected parenterally into rats, causes an increase in proliferative activity of the bone marrow blast cells [3] and differentiation of hematopoietic stem cells [1]. Since the molecular basis of these processes is nucleic acid metabolism, in order to understand the action of exogenous DNA information is required on its effect on endogenous DNA metabolism.

The object of this investigation was to study the content and rate of biosynthesis of DNA in rat bone marrow at various times after administration of exogenous DNA.

EXPERIMENTAL METHOD

Male Wistar rats weighing 170-180 g were used. Thymidine-^{3}H was used as the label. The experimental animals were given DNA, obtained by a modified Kay's method [7], by intraperitoneal injection in a dose of 5 mg per rat in SSC solution in a concentration of 2-2.25 mg/ml. The molecular weight of the DNA was 20-25 million daltons, protein impurities did not exceed 0.5%, and RNA 1%. Control rats received the corresponding volume of solvent; thymidine-^{3}H was injected in a dose of 200 μCi/100 g body weight 1 h before sacrifice. The rats were decapitated 18 h and 1, 3, and 6 days after injection of DNA. Bone marrow for investigation was taken from the femur and tibia. The DNA content in the bone marrow was determined by the method of Schmidt and Thannhauser [8] followed by spectrophotometry by Spirin's method [6]. Radioactivity in a neutralized aliquot of DNA digest was determined in a liquid scintillation counter. The specific radioactivity of DNA (in counts/min/mg DNA) was calculated.

The experimental data were subjected to statistical analysis with calculation of the arithmetic mean values and the errors of the means. The significance of differences was determined by Student's criterion [4].

EXPERIMENTAL RESULTS

The results in Table 1 show that the DNA content per gram bone marrow of the experimental rats fell during the 3 days after injection of DNA by comparison with its level in the control. The decrease in the DNA content was greater after 3 days (by 36% com-

(Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 8, pp. 952-953, August, 1976. Original Article submitted April 19, 1975.

TABLE 1. Content of Rate of Biosynthesis of DNA in Rat Bone Marrow after Injection of DNA (M±m)

Time of investigation	No. of animals	DNA content		Specific radioactivity of 1 mg DNA	
		in mg/g tissue	in % of control	in counts/min	in % of control
Control	10	14,0±0,41	100	116 600±1 200	100
18 h	5	10,2±0,38*	73	230 000±7 400*	197
1 day	5	10,8±0,18*	77	119 000±7 560	102
3 days	5	8,9±1,53*	64	119 900±17 500	103
6 days	5	15,3±1,16	109	184 000±8 900*	158

*Differences between control and experimental groups significant — $P \leq 0.05$.

pared with the control). By the 6th day after injection of the compound the DNA content per gram bone marrow of the experimental rats increased to the control level.

The rate of DNA biosynthesis, which was determined from the specific radioactivity of this biopolymer in the rat bone marrow, was twice as high as in the control 18 h after injection of the DNA. It was close to normal again 1-3 days after injection of the compound. By the 6th day the specific radioactivity was again significantly higher than in the control (by 58%).

The rate of biosynthesis of endogenous DNA in the bone marrow of the rats was thus increased 18 h after injection of exogenous DNA. This led to increased proliferation of hematopoietic cells. As Rogacheva [3] found, DNA when injected into rats stimulated medullary hematopoiesis and increased the mitotic activity of the hematopoietic cells of the bone marrow in the early period after injection of the biopolymer. At the same time, the number of the youngest hematopoietic cells was reduced, evidently on account of their more rapid differentiation. This could perhaps be connected with the decrease in the DNA content in the bone marrow found in the present experiments during the first 3 days after DNA injection. In fact, proliferating bone marrow cells in the greater part of the mitotic cycle contained DNA in an amount greater than that which corresponds to a diploid set of chromosomes. A decrease in the number of these components of the cell population ought therefore to be accompanied by a decrease in the total DNA concentration. By the sixth day, when the number of blast cells in the myelogram was increased, the DNA concentration in the bone marrow also was restored to normal.

The increase in DNA synthesis 18 h after injection of DNA can be considered to be due to the stimulating action of exogenous DNA, assimilated by the young bone marrow cells. After intraperitoneal injection of labeled DNA it can be found as early as after 6 h in the high-molecular-weight fraction of bone marrow, but most of it is eliminated from the bone marrow relatively quickly, within a space of time comparable with the transit time of these cells [5]. Exogenous DNA evidently stimulates the proliferation and differentiation of stem cells, as a result of which a second increase in DNA biosynthesis is observed 6 days after administration of the compound. Luzanov [1] showed that stimulation of stem cells is possible in principle. As regards the molecular mechanisms of activation of DNA biosynthesis in bone marrow, one possibility is that fragments of exogenous DNA and its low-molecular-weight breakdown products induce activation of the corresponding enzymes of DNA synthesis [2].

LITERATURE CITED

1. V. M. Luzanov, Byull. Éksp. Biol. Med., No. 10, 94 (1974).
2. V. K. Mazurik and E. Yu. Moskaleva, Byull. Éksp. Biol. Med., No. 2, 32 (1974).
3. S. A. Rogacheva, G. G. Rusinova, and É. G. Sharova, Tsitologiya, No. 7, 912 (1970).
4. P. F. Rokitskii, Fundamentals of Statistics for Biologists [in Russian], Minsk (1961).
5. G. G. Rusinova, Biokhimiya, No. 5, 889 (1971).
6. A. S. Spirin, Biokhimiya, No. 5, 656 (1958).
7. E. R. M. Kay, Nature, 191, 387 (1961).
8. G. Schmidt and S. J. Thannhauser, J. Biol. Chem., 161, 83 (1945).