ACTIVITY OF ASPARTATE-CARBAMOYLTRANSFERASE, DNA-POLYMERASE, AND DEOXYRIBONUCLEASES IN THE HEMATOPOIETIC ORGANS OF RATS AFTER A SINGLE INJECTION OF DNA

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During the first day after injection of DNA from the calf thymus a twofold consecutive increase was observed in the activity of DNA-polymerase (after 1-3 h), of deoxyribonuclease DNase) I (after 1 h), and DNase II (after 1-6 to 24 h), and also of aspartate-carbamoyltransferase (after 24 h) in the bone marrow and thymus of rats.

The ability of exogenous DNA to stimulate hematopoiesis or to increase the magnitude of the immunological response to injection of antigens has been demonstrated experimentally [2, 5]. DNA fragments are considered to act as inducers of the deoxynucleoside-kinases, enzymes controlling DNA biosynthesis in the cell [5].

Since the mechanisms controlling DNA biosynthesis include not only the corresponding kinases but also several other enzymes [9], it was decided to study the effect of injection of DNA on the activity of some of these enzymes in the hematopoietic organs of intact rats, namely: aspartate-carbamoyltransferase – ACT (EC 2.1.3.2) and DNA-polymerase (EC 2.7.7.7). Activity of the deoxyribonucleases (EC3.1.4.5 and 3.1.4.6), protecting the cell genome against foreign DNA, also was investigated.

EXPERIMENTAL METHOD

Altogether 86 male rats (noninbred and Wistar) weighing 190-230 g were used in the experiments. Fifty of the rats received a subcutaneous injection of 4 mg high-polymer DNA from calf thymus in 4 ml of a citrate-salt solution (SSC = 0.15 M NaCl + 0.015 M Na citrate) or in 0.15 M NaCl, while the 22 control animals were injected with the corresponding solvents. The rats were decapitated after 1 h-17 days. Bone marrow, spleen, and thymus homogenates were centrifuged at 105,000 g (0-2°C, 1 h) and the activity of the following enzymes was determined in the supernatant: ACT [7], DNA-polymerase, using thymidyl C^{14}-nucleotides [4], and DNases I and II [1]. Carbamoylphosphate was synthesized by the method of Spector et al., [12] and the carbamoylaspartic acid in the incubation medium was determined by the method of Prescott and Jones [10]. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS AND DISCUSSION

The results in Table 1 show that from 3 h to 7 days after injection of SSC into rats the ACT activity in their bone marrow and spleen was reduced. Conversely, 24 h after injection of DNA the ACT activity in the bone marrow was 102.1% higher than in the corresponding control. The same effect was observed after injection of DNA solutions in 0.15 M NaCl, both in the bone marrow and in the thymus (by 114.3%; $P = 0.03$). DNA-polymerase activity in the bone marrow (Table 2) was increased 1 h and, more especially, 3 h after injection of DNA (by 2.3 times; $P < 0.05$), and also 5 days after the injection. Some tendency for the activity of this enzyme to be increased was observed in the thymus 1 h after injection. A significant increase in

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TABLE 1. ACT Activity in Organs of Rats after a Single Injection of DNA

Character of experiment (time after injection)	Bone marrow			Thymus			Spleen		
	1	2	P	1	2	P	1	2	P
Normal	0,90±0,07	—	—	0,61±0,15	—	—	0,76±0,04	—	—
3 h	0,44±0,11	0,39±0,05 (88,6±11,4)	0,70	0,58±0,07	0,74±0,07 (127,5±12,1)	0,15	0,54±0,06	0,51±0,11 (94,4±20,4)	0,85
1 day	0,47±0,06	0,95±0,15 (202,1±31,9)		0,81±0,16	1,03±0,10 (127,1±12,3)	0,27	0,65±0,02	0,65±0,02 (100,0±3,1)	No difference
		(168,0±32,0)*	0,02 0,09		(214,3±35,7)*	0,03		(85,7±14,2)*	0,50
7 days	0,36±0,14	0,38±0,11 (105,5±30,5)	0,92	0,81±0,16	0,47±0,12 (58,0±14,8)	0,13	0,55±0,04	0,67±0,04 (121,8±7,3)	0,08
15 days	1,35±0,42	1,07±0,25 (79,2±18,5)	0,57	0,66±0,16	0,75±0,12 (113,6±18,2)	0,70	0,76±0,10	0,99±0,24 (130,2±31,6)	0,45

Note: Enzyme activity expressed in micromoles carbamoylaspartate synthesized by 1 mg protein per hour. Column 1) injection of SSC, 2) of DNA. Figures in parentheses show percentage of changes due to injection of DNA and SSC solutions relative to corresponding control; results obtained (in percentages of corresponding control) after injection of DNA dissolved in 0.15 M sodium chloride solution marked by an asterisk.

TABLE 2. Activity of DNA-Polymerase and of DNases I and II in Organs of Rats under Normal Conditions and at Various Times after Injection of High-Polymer DNA from Calf Thymus

Enzyme	Organ	Normal	After injection of DNA					
			1 h	3 h	6 h	1 day	5 days	17 days
DNA-polymerase	Bone marrow	133±12	279±110	298±54*	99±19	166±45	249±32*	70±17*
	Thymus	611±75	777±14**	461±3	532±82	676±66	700±45	304±59*
	Spleen	40±7	35±1	26±5	42±3	26±5	46±15	48±5
DNase I	Bone marrow	0	0,5±0,3	0	0	0	1,6±1,0	1,0±0,8
	Thymus	1,3±0,6	3,9±0,6*	0,9±0,9	2,1±0,7	0,03±0,02	1,8±1,0	3,6±0,8*
	Spleen	0	0	0	0	0	0	0
DNase II	Bone marrow	3,0±1,1	5,2±1,3	5,6±2,0	7,4±1,2*	11,4±4,0*	3,5±0,4	2,7±1,1
	Thymus	6,4±1,2	11,6±1,1*	11,4±1,7*	9,9±0,9	6,5±0,6	10,1±0,5*	6,0±0,8
	Spleen	4,4±0,5	8,3±1,8*	3,9±0,4	6,4±0,8	2,3±0,5	5,8±0,6	13,4±2,1

*Differences significant $P < 0.05$.

†$P < 0.1$.

Note: DNA-polymerase activity expressed in pulses/min/mg protein per hour, of DNases as percentage of acid-soluble products formed from high-polymer DNA.

DNase I activity (Table 2) was observed in the bone marrow 1 h and 5-17 days, and in the thymus 1 h and 17 days, after injection of the DNA. Activity of DNase II also was increased in all the organs during the first few hours after injection of the DNA; the increase was particularly marked in the bone marrow (by 2.5-3.8 times at 6-24 h after injection), in agreement with data in the literature [5].

When injected, DNA can thus increase the activity of all the enzymes studied, and DNA-polymerase and the DNases were particularly sensitive. The changes observed are evidently the biochemical reflection of the proliferative stimulus induced in the cells by exogenous DNA and manifested as increased hematopoiesis and immunogenesis [4, 7]. Building material for proliferation during the first few hours could be exogenous DNA after its breakdown by the activated DNases I and II (Table 2). Degradation of DNA in vivo in fact takes place particularly intensively during the first 12 h after injection [11]. The increasing demands of proliferation must later be satisfied by endogenous sources, with the resulting stimulation of the enzyme ACT catalyzing the first reaction along the pathway of de novo biosynthesis of the pyrimidine precursors of nucleic acids [2]. The cause of inhibition of the activity of this enzyme is not clear. Perhaps as a result of the injection of citrate into the rats the phosphorylation of pyrimidine nucleotides to nucleoside triphosphates, which are inhibitors of ACT, takes place.

LITERATURE CITED

1. G. P. Benning, Uchen. Zapiski Kazan. Univ., 124, No. 1, 20 (1964).
2. S. A. Rogacheva, G. G. Rusanova, and É. G. Sharova, Tsitologiya, 7, No. 12, 912 (1970).
3. T. N. Rysina, R. E. Libinzon, and É. G. Sharova, Biokhimiya, 36, 532 (1971).
4. F. J. Bollum, J. Biol. Chem., 235, 2399 (1960).
5. W. Braun and W. Firshein, Bact. Rev., 31, 83 (1967).
6. J. C. Gerhart and A. B. Pardee, Fed. Proc., 23, 727 (1964).
7. S. Kim and P. P. Cohen, Arch. Biochem., 109, 421 (1965).
8. G. Crosbie, in: The Nucleic Acids, E. Chargaff and J. Davidson (editors), Academic Press.
9. K. G. Lark, in: Molecular Genetics [in Russian], Moscow (1964), p. 168.
10. L. M. Prescott and M. Jones, Analyt. Biochem., 32, 408 (1969).
11. P. G. Rubachev, T. A. Fedorova, V. K. Mazurik, et al., Studia Biophys. (Berlin), 29, 153 (1971).
12. L. Spector, M. Jones, and F. Lippman, Methods Enzymol., 3, 653 (1957).