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CHANGES IN THE PRIMARY STRUCTURE OF DNA IN SOME RAT ORGANS DURING AGING

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Aging is essentially the summation of changes arising at different levels of organization, including the biochemical level. It is generally accepted that the metabolically most stable component of the cell is DNA. However, even in nondividing cells, extracopying of DNA fragments from individual regions of the genome can take place [4]. In ontogeny the level of DNA methylation changes [2] and the DNA undergoes injuries, which are removed in the course of reparative synthesis [12], the effectiveness of which diminishes with age [15]; this evidently leads to the accumulation of mistakes in the genetic apparatus [8]. An important role in this situation is played by free-radical processes [7], the inhibition of which lengthens the duration of life [6].

The object of this investigation was to study the organ specificity of age changes in the primary structure of DNA.

EXPERIMENTAL METHOD

Noninbred albino rats aged 1, 12, and 30 months were used. The animals were decapitated, the organs quickly removed, the nuclei isolated in the presence of EDTA [10], and DNA was isolated from the nuclei [11, 14]. The DNA was hydrolyzed to pyrimidine fragments of different lengths — blocks or isopliths [5]. The isopliths were separated according to length and composition by thin-layer chromatography [9]. For subsequent quantitative analysis, thin-layer disks were scanned on a chromatogram spectrophotometer (Opton, West Germany) at 270 nm, i.e., at the isobestic point of pyrimidines, determined by analysis of UV reflection spectra. The composition of the DNA bases also was determined by thin-layer chromatography. The relative percentages of the different bases were calculated by multiplying the areas of peaks on the densitograms by coefficients determined empirically for each base, and on the basis of direct correlation between the quantity of material in the spot and the area of the corresponding peak on the densitogram, provided that the quantity of material in the spot did not exceed 1 μg. The chromatograms were scanned in reflected UV light using a slit measuring 12 × 0.2 mm, the velocity of the disks relative to the beam was 10 mm/min and the tape winding speed of the automatic writer was 30 mm/min. The coefficients for bases dispersed in an alkaline solvent [3] were as follows: adenine (A) — 1.00, guanine (G) — 1.56, cytosine (C) — 1.96, thymine (T) — 1.51, 5-methylcytosine (5-MC) — 1.37. Scanning was carried out at 260 nm to analyze the ordinary bases and at 290 nm to determine the 5-methylcytosine content.

EXPERIMENTAL RESULTS

As Table 1 shows, a general tendency was observed in all investigations for the size of the pyrimidine blocks to decrease with age, as shown by an increase in the number of mono-

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TABLE 1. Frequency of Finding Pyrimidine Isopliths in Nuclear DNA from Some Rat Organs

Age of animals, months	Source of nuclear DNA	Content of pyrimidine isopliths, moles %						
		mono	di	tri	tetra	penta	hexa	higher
1	Liver	11,5	9,7	8,5	6,6	4,5	3,0	6,2
	Spleen	11,3	9,5	8,7	6,0	4,1	2,8	7,6
	Kidney	11,9	10,2	8,0	6,2	4,2	3,2	6,3
	Heart	11,6	9,9	8,3	6,5	4,4	3,1	6,2
	Brain	11,9	10,2	8,4	6,7	4,3	3,0	5,5
12	Liver	12,3	10,7	8,6	5,8	4,0	2,9	5,7
	Spleen	12,5	10,4	9,0	5,2	3,8	2,3	6,8
	Kidney	12,4	10,8	7,9	5,8	4,0	3,3	5,8
	Heart	11,9	10,3	8,1	6,2	4,2	3,0	6,3
	Brain	13,0	11,5	7,8	5,4	3,8	3,2	5,3
30	Liver	12,8	11,4	8,6	5,5	4,0	2,6	5,1
	Spleen	13,0	11,0	9,1	5,0	3,8	2,4	5,7
	Kidney	13,5	12,0	7,5	5,1	3,7	3,0	5,2
	Heart	13,1	11,4	8,2	5,4	4,0	2,7	5,2
	Brain	12,7	12,5	7,9	5,3	3,6	2,9	5,1
	Chaotic distribution	12,75	12,38	9,38	6,19	3,83	2,27	3,28

Legend. Number of determinations for each case was three. Scatter of data, expressed as standard deviation within limits of 5%.

TABLE 2. Content of Pyrimidine Oligonucleotides of Different Length and Composition in Liver DNA from Rats Aged 1 Month (A) and 30 Months (B)

Isopliths	Composition of oligonucleotides	Quantity of nucleotides, moles %	
		A	Б
I	Cp_2	4,7	4,9
	Tp_2	6,8	7,9
II	C_2p_3	1,9	2,1
	CTp_3	5,3	6,0
	T_2p_3	2,5	3,3
III	C_3p_4	0,8	0,9
	C_2Tp_4	3,2	3,0
	CT_2p_4	3,1	3,1
	T_3p_4	1,4	1,6
IV	C_4p_5	0,4	0,4
	C_3Tp_5	1,4	1,5
	$C_2T_2p_5$	2,4	1,9
	CT_3p_5	1,6	1,1
	T_4p_5	0,8	0,6
V	C_5p_6	0,3	0,3
	C_4Tp_6	0,6	0,6
	$C_3T_2p_6$	1,0	0,9
	$C_2T_3p_6$	1,2	1,1
	CT_4p_6	1,0	0,8
	T_5p_6	0,4	0,3
>V	C } Total	3,5	3,3
	T } quantity	5,7	4,4

Legend. Number of determinations was five. Scatter of data reflected in standard deviation not more than 6%.

and dipyrimidine sequences with age and a decrease in the number of sequences of more than six pyrimidines. The course of the general redistribution of the pyrimidine block by size was close to chaotic [13], in agreement with views on the randomized free-radical injury to DNA in the course of vital activity [7]. The decrease in size of the pyrimidine blocks with age took place mainly on account of a decrease in size of blocks of thymidine-rich sequences,

TABLE 3. Content (moles %) of G + C + MC and 5-MC in DNA from Rats of Different Ages

Source of nuclear DNA	Age of animals, months								
	1			12			30		
	n	GC	MC	*n*	GC	MC	*n*	GC	MC
Liver	27	43,2±0,13	0,99±0,02	11	43,3±0,15	1,02±0,01	31	43,3±0,07	0,96±0,03
Spleen	21	42,9±0,11	1,07±0,01	15	43,2±0,13	0,97±0,02	25	43,3±0,12	0,91±0,02
Kidney	19	43,3±0,09	0,85±0,04	9	43,4±0,16	0,90±0,01	26	43,4±0,08	1,11±0,01
Heart	25	43,2±0,15	1,05±0,01	13	43,2±0,09	0,98±0,02	22	43,3±0,04	0,96±0,05
Brain	24	43,1±0,05	1,09±0,02	12	43,3±0,11	0,97±0,01	29	43,5±0,09	0,91±0,03

Legend. Error expressed as standard deviation. n) Number of determinations.

most probably on account of the greater lability of AT pairs, formed by means of two hydrogen bonds, by contrast with the more stable GC pairs, which contained three hydrogen bonds (Table 2).

A shift in the nucleotide composition of DNA toward enrichment with GC pairs also was found in all the organs, and this was not random in character because, with the appearance of transversions and transitions relative to GC- and AT-pairs the formation of a stable GC pair was a more likely event (Table 3).

One other cause of a shift in the composition of DNA bases toward enrichment with GC pairs in ontogeny may be a change in the level of DNA methylation. According to one opinion, demethylation of animal DNA can take place through excision of 5-MC [1]. Mistakes in repair of regions injured in this way may be a component of age changes in the primary structure of DNA revealed by this investigation. These considerations are in agreement with the fact that the greater increase in the GC content in DNA from the spleen and brain compared with DNA from other organs (Table 2) corresponds to a higher degree of demethylation of the DNA of these organs, and also, possibly, to the more frequent change in level of their DNA during ontogeny.

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