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EFFECT OF ETHANOL ON DNA-POLYMERASE ACTIVITY IN SUBCELLULAR FRACTIONS OF THE LIVER OF ADULT AND OLD RATS

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One possible cause of the slowing of replication of nuclear and mitochondrial DNA during aging [7, 8, 10] may be an age-linked change in the multienzymic DNA replication complex. The enzymes of this complex which have been characterized most completely are the DNA-polymerases. Investigations have shown changes in the activity of these enzymes during aging [6], a decrease in the accuracy of the synthesis performed by them [9], and also an increase in the thermolability of their molecules [2].

However, the question whether sensitivity of DNA-polymerases to the specific inhibitors of their activity changes in old age and, if so, whether the age change in this sensitivity depends on the subcellular localization of the enzymes, has not yet been adequately studied.

In the present investigation ethanol was used as the inhibitor. In certain concentrations ethanol is known to inhibit activity of α- and β-DNA-polymerases (above 5% for α-DNA-polymerase [4] and above 20% for β-DNA-polymerase [3]). In this case, the concentration of ethanol chosen was 5%, in which it has no appreciable inhibitory action on DNA-polymerase activity in adult animals (according to data in the literature), but can inhibit (threshold concentration) α-DNA-polymerase. It was assumed that enzyme activity in old animals will be modified by ethanol in a concentration lower than the threshold level for adult animals.

The effect of 5% ethanol on DNA-polymerase activity of subcellular fractions (nuclei, mitochondria, microsomes, cytosol) of the intact and regenerating liver was studied in adult and old rats.

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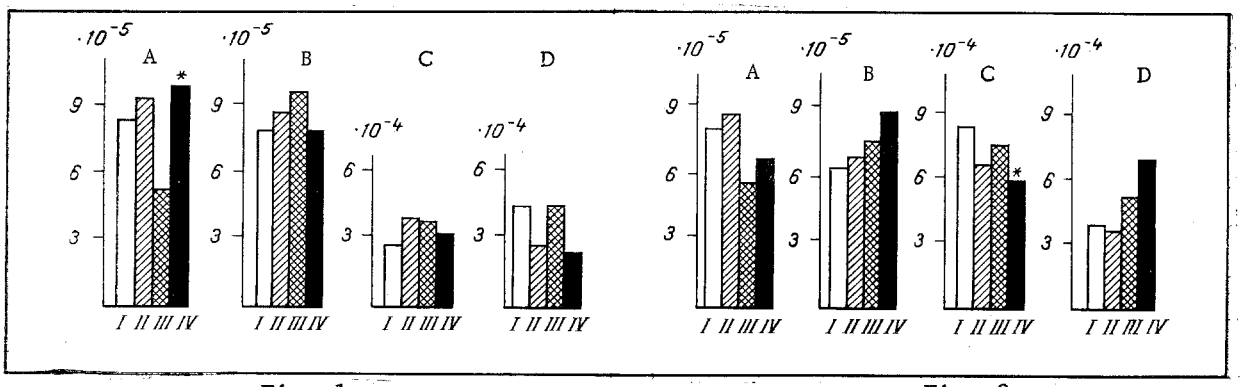

Fig. 1

Fig. 2

Fig. 1. Effect of 5% ethanol on DNA-polymerase activity in subcellular fractions of intact liver of rats aged 6 months (I, II) and 24 months (III, IV). A) Nuclei, B) mitochondria, C) microsomes, D) cytosol. I) Control (without ethanol), II) with ethanol, III) control, IV) experiment. *P < 0.05. Each group consisted of six animals.

Fig. 2. Effect of 5% ethanol on DNA-polymerase activity in subcellular fractions of regenerating liver of rats aged 6 and 24 months. Legend as to Fig. 1.

EXPERIMENTAL METHOD

Tissue from the intact and regenerating liver was taken from Wistar rats aged 6 months (adult) and weighing 200-250 g, and aged 24 months (old) and weighing 300-400 g. The nuclei and mitochondria were obtained under conditions preventing escape of enzymes from the organelles [12]. Microsomes and cytosol were obtained from the postmitochondrial supernatant (105,000 g, 1 h). The protein concentration was determined by Lowry's method [11]. DNA polymerase activity in nuclei, microsomes, and cytosol was determined by the method in [5] and mitochondrial DNA-polymerase activity by the method in [12]. The numerical results were subjected to statistical analysis by the Wilcoxon–Mann–Whitney nonparametric test [1].

EXPERIMENTAL RESULTS

The results of determination of the effect of 5% ethanol on DNA-polymerase activity of the subcellular fractions of adult and old rat liver are given in Figs. 1 and 2. In adult rats 5% ethanol had no significant effect on DNA-polymerase activity of any of the subcellular fractions in either the intact or the regenerating liver. In the liver of the old animals, however, under the influence of 5% ethanol, almost 100% stimulation of DNA-polymerase activity was observed in the nuclei of the intact liver, with a small decrease in its activity in the microsomes of the regenerating liver. On the basis of data in the literature, it can be postulated that in this case 5% ethanol evidently affected α-DNA-polymerase activity, since β-DNA-polymerase has been found to be sensitive to alcohol only in a concentration of over 20% [3].

Activity of DNA-polymerases in subcellular fractions of the adult rat liver thus do not change their activity under the influence of 5% ethanol, whereas during aging, changes in the activity of these enzymes are observed in the nuclear fraction under normal conditions and in the microsomal fraction during regeneration. These data may indicate that during aging changes may take place in the molecular properties of DNA-polymerases. However, the possibility cannot be ruled out that an age-linked change in the microenvironment may play a certain role in this situation, and may so influence the properties of the DNA-polymerases of old animals that they become sensitive to 5% ethanol.

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