ENHANCEMENT OF DNA STRUCTURAL DAMAGE IN HUMAN LYMPHOCYTES DURING AGING

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An urgent problem in gerontology is the study of the strucutral and functional state of the genome during aging. Data in the literature on this question are contradictory [3, 9, 11]. The cause of the disagreements lies mainly in the low sensitivity and inadequacy of methods used to analyze lesions of the genome. The most sensitive method, which can be used to investigate native DNA molecules with mol. wt. of 10^7 kilodaltons or more, is elastoviscosimetry [7], whose physical significance is that it reflects the rate of relaxation during reversion of a disturbed DNA conformation into the initial conformation. A disturbed conformation arises due to the creation of a linear gradient in the solution located in the gap (1.5 mm) between the stationary stator and the moving rotor. The parameter of retardation time (τ_0), with appropriate extrapolations, characterizes on the whole the hydrodynamic volume of DNA in solution. The applicability of this method to the analysis of the state of the genome in trisomy for the 21st chromosome in human cells was demonstrated by the writers previously [5].

In this paper the results of an elastoviscosimetric study of the genome of peripheral blood lymphocytes from persons of different ages are described.

EXPERIMENTAL METHOD

Altogether 27 persons aged from 1 to 100 years were investigated. At the time of investigation this group of people was not receiving medication of any kind, and as regards their clinical status and results of laboratory tests, they conformed to the age norm. Blood was taken by venipuncture, with heparin used in a dose of 20 U/1 ml of blood as anticoagulant. Lymphocytes were isolated by the method in [6]. The cells were resuspended in physiological saline and lysed [10]. The use of lytic solutions containing 0.5 M EDTA, as was shown previously [1], completely rules out nuclease degradation of DNA. Measurements began 14-18 h after lysis. The design of the instruments and the method of recording the retardation time of DNA (τ_0) were described previously. To compare the elastoviscosimetric parameters of DNA, characterizing normal rates of aging and pathological rates characteristic of certain hereditary diseases, analogous investigations were carried out on cells of patients with trisomy for chromosome 21 (Down's syndrome). Altogether 16 patients aged from 1 to 30 years were investigated.

EXPERIMENTAL RESULTS

The method used to produce lysis of lymphocytes preserved DNA in the superhelical conformation. This conclusion is based on the study of the character of the change in retardation time depending on the ethidium bromide (EB) concentration in the solution (Fig. 1). EB, by intercalating into superhelical—standard DNA, induces its relaxation. After complete relaxation had been achieved, a subsequent increase in EB concentration leads to limited twisting of the double standard DNA helix to the opposite side. A further increase in EB concentration does not affect the degree of compactness of DNA, i.e., τ_0. The resulting curve (Fig. 1)

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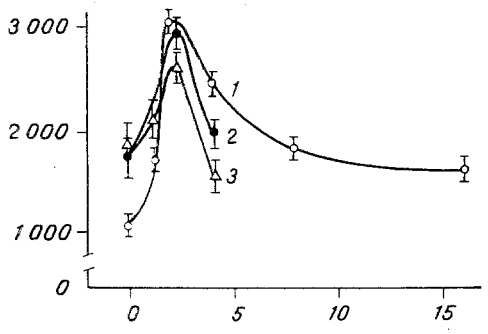

Fig. 1

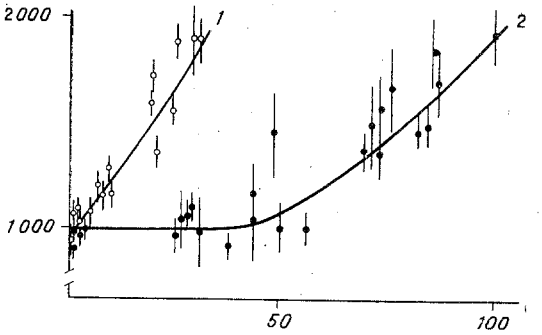

Fig. 2

Fig. 1. Changes in parameter of elastoviscosity in diploid and aberrant lymphocytes depending on ethidium bromide concentration. Abscissa, ethidium bromide concentration (in μg/ml) ordinate, parameters of elastoviscosity τ_0 (in sec). DNA of lymphocytes from: 1) subjects of control group, 2) elderly subjects, 3) patients with Down's syndrome.

Fig. 2. Changes in parameter of elastoviscosity depending on subjects' age. Abscissa, age (in years); ordinate, τ_0 (in sec). DNA of lymphocytes from: 1) patients with Down's syndrome, 2) subjects with normal karyotype.

is in full agreement with the transitions described above. Hence it follows that the parameter τ_0 of DNA, which we determined, corresponds in the absence of EB to the superhelical state of the chromosome.

Changes in the parameter τ_0 of DNA from peripheral blood lymphocytes of subjects of different ages are shown in Fig. 2. It was found that over 60 years of age there is a significant increase in τ_0, whereas in the other age groups (down to 1 year) τ_0 was constant. Similar changes in τ_0, incidentally, are characteristic of DNA of lymphocytes from patients with Down's syndrome (Fig. 2), but the rates of increase in the value of τ_0 in this pathology are much greater. By the end of the first 10 days of life an increase in elastoviscosity of DNA was observed, and toward the age of 20 years this parameter corresponded to values characteristic of healthy subjects aged 70-80 years. Down's syndrome, as we know, is one of the most widespread hereditary diseases, characterized by accelerated rates of aging [2]. Statistical errors of individual determinations also are shown in Fig. 2. Despite the scatter of the values obtained, definite correlation can be seen between the parameter of elastoviscosity and the donors' age, and also with the presence of pathology accompanied by accelerated aging.

The most probable cause of the increase in τ_0 is partial relaxation of superhelical DNA as a result of the presence of spontaneous injuries in it. The role of DNA injuries in relaxation of the superhelix was studied by the writers in detail previously [1]. It must be noted that the decrease in the number of turns of the DNA superhelix was found also in late passages in cell culture [4].

During aging, changes in the subpopulation composition of the lymphocytes (a fall in the number of T-cells) is observed in the peripheral blood, and an increased number of undifferentiated lymphocytes is released into the blood stream [8]. However, these changes in the subpopulation composition of the lymphocytes cannot be the cause of the differences observed in the values of τ_0. This conclusion is based on the normal values of the hydrodynamic volume of DNA in patients with Down's syndrome during the first years of life, whereas immunologic disturbances take place in these patients actually from the time of birth [12].

There is one other control which confirms the validity of this explanation. On complete despiralization of DNA with EB, the elastoviscosimetric parameters of DNA ought to be the same for all specimens irrespective of the donors' age. As can be seen in Fig. 1, despite differences in the retardation time with zero concentration of EB the maximal values of τ_0 were virtually identical. The differences we observed in the hydrodynamic volume of DNA of peripheral blood lymphocytes of persons over 60 years of age and of patients with Down's syndrome are thus connected with relaxation of some of the superhelical regions of DNA as a result of an increase in the frequency of spontaneous injuries.

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STRENGTHENING OF THE DNA-PROTEIN COMPLEX DURING STATIONARY PHASE AGING OF CELL CULTURES

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The writers previously postulated that limitation of proliferation of cells of the body is the main cause of accumulation of injuries to genetic material with age. It was accordingly suggested that stationary cell cultures (i.e., cultures consisting of undividing cells) be used to model processes of aging taking place at the molecular level [6-9]. Experiments showed that changes similar to those taking place in cells aging *in vivo* over a period of many years also take place in the cells of such cultures, but within a short time (not more than 2-3 weeks). In particular, accumulation of alkali-labile regions in DNA [9] and an increase in the number of spontaneous sister chromatid exchanges [6, 7] were found.

In the investigation described below the possibility of accumulation of cross-linkages in the DNA-protein complex was studied during stationary phase aging of cells in culture. Investigation of injuries of this type to genetic material was indicated for the following reasons. The theory according to which the primary cause of aging is progressive accumulation of cross-linkages between proteins, nucleic acids, and other macromolecules, was formulated by Bjorksten as long ago as in 1941-42 [11, 12] and was developed in his subsequent researches [13, 14] on the basis both of his own data and of results obtained by other investigators. Several papers have recently been published [1-3, 10, 15] in support of this concept. In particular, it is an interesting fact that cells of patients with progeria (a syndrome of premature aging) are unable to repair induced cross-linkages of the DNA–protein complex [10], although no other defect of the DNA repair system could be found in them [4].

EXPERIMENTAL METHOD

Experiments were carried out on cultures of Chinese hamster cells (line B 11 dii-FAF 28), normal human diploid embryonic fibroblasts (strain E2), and fibroblasts from a patient with xeroderma pigmentosum (strain IMG-667). The Chinese hamster cells were grown on Eagle's medium with glutamine, containing 10% bovine serum, the human fibroblasts on medium consisting of 80-85% of Eagle's medium with glutamine, 10-15% bovine serum, and 5% of human umbilical serum. Cells removed from the glass with trypsin solution were reseeded in several Carrell's flasks (1:6 in the case of Chinese hamster cells, 1:2 in the case of fibroblasts from the patient

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