quantity of nutrient medium. A similar activation system enabled BP to exhibit a mutagenic effect in a concentration of 5 μg per Petri dish in experiments on salmonellas [2]. It must also be borne in mind that, despite its fairly wide distribution in the environment, BP acts on man in relatively low concentrations. For example, the total dose of BP which can enter the human body during the lifetime of a person living in a zone polluted by waste products of organic synthesis [1] is measured in micrograms. According to the available data [4], the maximal allowable concentrations for BP in the air of industrial premises approved in the USSR is 15 μg/100 m^3. Accordingly the present investigations may be of definite importance for the detection of the mutagenic effect of BP in concentrations actually encountered in the environment.

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CHROMOSOMAL ABERRATIONS AND MOLECULAR WEIGHT OF SINGLE-STRANDED DNA FRAGMENTS IN EMBRYONIC FIBROBLASTS OF 101/H AND CBA MICE

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Attempts have recently been made to use mice of certain lines in order to create models of human diseases associated with chromosomal instability [6, 7]. Mouse line 101/H, characterized by a high spontaneous and induced level of chromosomal aberrations in the bone-marrow cells [4], and high susceptibility to induction of DNA injuries in embryonic fibroblasts under the influence of 4-nitroquinoline-1-oxide [1], is interesting from this point of view. The nature of the molecular events determining the increased mutability of the chromosomes in mice of line 101/H is not known.

The object of this investigation was to study the frequency of chromosomal aberrations, the molecular weight (mol. wt.) of DNA, and DNA-protein interaction in intact embryonic fibroblasts of 101/H mice. For comparison, mice of CBA line, relatively more resistant to mutagenic factors, were used [1, 4].

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TABLE 1. Spontaneous Level of Chromosomal Aberrations in Mouse Fibroblasts in Culture

Line of mice	Cultures tested	Cells tested	Cells with chromosomal injuries, %	Cells with structural injuries to chromosomes, %	No. of breaks per cell	No. of gaps per cell
CBA	13	700	9,8±1,3	3,0±0,7	0,04	0,08
101/H	11	750	18,5±2,2	9,0±1,3	0,15	0,16

Legend. Cells with structural chromosomal aberrations and (or) with gaps were taken to be injured.

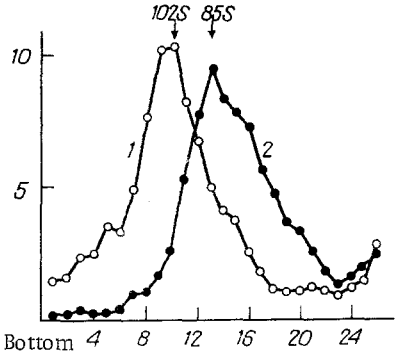

Fig. 1

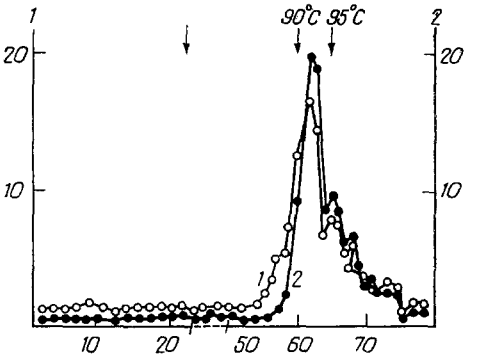

Fig. 2

Fig. 1. Sedimentation profiles of DNA of embryonic fibroblasts of CBA (1) and 101/H (2) mice. Abscissa, Nos. of fractions; ordinate, percentage of total radioactivity.

Fig. 2. NPC chromatography of DNA of embryonic fibroblasts of CBA (1) and 101/H (2) mice. Arrows indicate beginning and continuation of temperature gradients. Abscissa, Nos. of fractions; ordinate, left — number of cpm × 10^{-4} for ^{3}H, right — number of cpm × 10^{-3} for ^{14}C.

EXPERIMENTAL METHODS

Experiments were carried out on primary cultures of embryonic fibroblasts from mice of lines 101/H and CBA. The cells were cultured in Eagle's medium with the addition of glutamine, antibiotics, and 20% bovine serum. Colchicine was added in a final concentration of 2 μg/ml 2 h before the end of culture. Preparations of metaphase plates for cytogenic analysis were obtained by the standard method [7]. To investigate mol. wt. of the DNA the cells were labeled with ^{3}H-thymidine (5 μCI/ml) or ^{14}C-thymidine (2 μCi/ml) for 24 h. Molecular weight of single-stranded DNA fragments was determined by a modified method [8]. Successive layers of 0.1 ml of lytic mixture (1 N NaCl, 0.1 M EDTA), 0.1 ml of cell suspension ($5 \cdot 10^{4}$ cells in Hanks' solution), and 0.2 ml of lytic mixture were poured above the peak of a 5-20% linear alkaline sucrose gradient (0.7 N NaOH, 0.3 N NaCl, 0.01 M EDTA), in a volume of 4.8 ml. Lysis continued for 2 h at 20°C. The samples were centrifuged in SW-65 and AH-650 rotors on the Beckman L-2-65 centrifuge at 35,000 rpm for 90 min at 20°C. The gradient was fractionated from below. The sedimentation coefficient was determined from a nomogram. Molecular weight was calculated by the method described in [8]. Stability of the DNA-protein bond was determined by nucleoprotein-celite chromatography (NPC chromatography) [2]. Statistical analysis was carried out by the use of Student's t-test.

EXPERIMENTAL RESULTS

As Table 1 shows, the percentage of cells with structural injuries to the chromosomes and the number of breaks per cell were significantly higher in the 101/H mice than in the CBA mice ($P < 0.01$). Thus both the level of chromosomal aberrations and the degree of injury

to the cells were higher in intact cultured mouse fibroblasts of the 101/H line than in CBA mice. All observed aberrations were of chromatid type, but the spectra of the chromosomal injuries differed in the different lines. In CBA fibroblasts the chromosomal aberrations consisted of single fragments only, whereas in 101/H mice 12% of the aberrations were exchanges (dicentrics and rings). The results are evidence that the high spontaneous mutability of chromosomes characteristic of the cells of 101/H mice *in vivo* also is preserved during culture of embryonic fibroblasts. This may perhaps be due to insufficiency of intracellular mechanisms maintaining the stability of the genetic material.

Various events in the cell such as changes in the DNA bases, modification, single and double breaks, or changes in the character of the DNA-protein bond [3] may lead to the appearance of chromosomal aberrations. Investigation of mol. wt. of single-stranded DNA fragments showed that the mean mol. wt. of fibroblast DNA of 101/H mice was $(9.32 \pm 0.37) \cdot 10^7$ daltons, whereas in CBA mice it was $(1.37 \pm 0.04) \cdot 10^8$ daltons. The number of cultures tested was 15 and 11 respectively. Typical sedimentation profiles of DNA of embryonic fibroblasts are illustrated in Fig. 1. The mol. wt. of DNA from 101/H mouse cells was thus considerably less than CBA mica ($P < 0.001$).

The low mol. wt. of DNA of the 101/H mouse fibroblasts may be due to true DNA breaks — both single- and double-stranded, and also to the presence of alkaline-labile regions, in the form of apurinic and apyrimidinic zones, modified bases, and esterified phosphates [5]. In the present experiments it was impossible to identify which injuries were responsible for the decrease in mol. wt. of fibroblast DNA in the 101/H mice.

Changes in mol. wt. of DNA may be accompanied by disturbances of DNA-protein interaction, as is observed following exposure of cells to irradiation or chemical mutagens [3]. In the present investigation an attempt was made to compare the stability of DNA-protein interaction in chromatin of embryonic fibroblasts of mice of the 101/H and CBA lines by means of NPC chromatography, which enables the stability and resistance to dissociation of nucleic acid-protein interactions to be determined. In this method of investigation proteins of nucleoproteins are irreversibly adsorbed on celite, whereas nucleic acids of different types are successively liberated from their bonds with proteins and are fractionated in accordance with the strength of these bonds, i.e., their resistance to dissociating forces. It will be clear from Fig. 2 that the chromatographic position of DNA of the fibroblasts coincided practically exactly in the two lines of mice.

The cause of the increased spontaneous mutability of the chromosomes of 101/H mouse cells was thus evidently potential injuries to DNA, in the form of alkaline-labile regions or breaks in DNA. The results, together with those showing increased spontaneous and induced mutability of chromosomes *in vivo*, may point to defective repair of DNA injuries in 101/H mice.

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