The non-radioactive method of DSBs estimation had an important limitation: it was not reliable in the case of asynchronous cell cultures, as DNA release from S-phase cells diminished considerably compared to interphase cells [8]. We omitted this problem by using human lymphocytes, 20-24 h after stimulation with phytohemagglutinin A. At that time, the cells made a synchronous population in the G_1 phase of the cell cycle. Figure 4 presents dose-response curves for DSBs induction in human lymphocytes by X-irradiation. The relationship was linear within the whole range of the doses applied. The slopes of the linear regression curves were very similar for the lymphocytes obtained from all the three donors, with the sensitivity of the assay below 0.5 Gy. These parameters confirm that the method is suitable for estimation of DSBs in stimulated human lymphocytes exposed to low doses of ionising radiation.

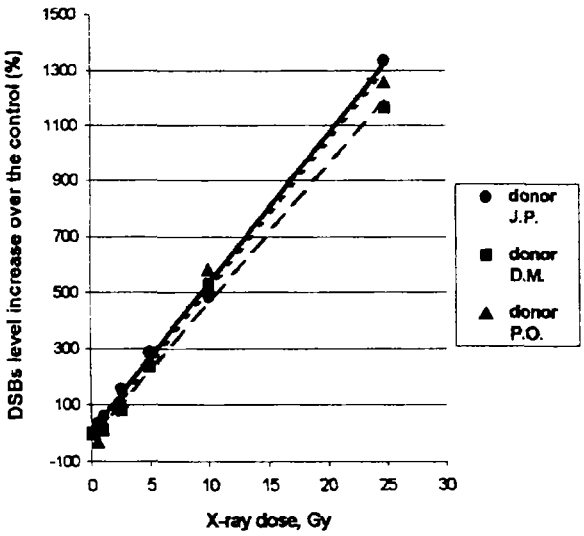

Fig.4. Dose-response curves for DSBs induction in lymphocytes from three donors. The slopes of the linear regression curves were: 52.6%·Gy^{-1} (for J.P.), 47.8%·Gy^{-1} (for D.M.) and 51.8%·Gy^{-1} (for P.O.).

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REPAIR OF DNA DOUBLE STRAND BREAKS IN ATAXIA TELANGIECTASIA HOMO- AND HETEROZYGOTE CELLS

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Ataxia telangiectasia (AT) is an autosomal recessive disorder characterized by progressive neurological impairment, immunodeficiencies and high cancer frequency. In vitro cells from AT patients are more sensitive that normal cells to killing by ionizing radiation and also show a greater incidence of chromosomal aberrations. Even AT heterozygotes (approximately 1% of the population) show increased incidence of some cancers, and cells from heterozygotes show a sensitivity to radiation that is intermediate between that of AT homozygotes and normal cells. The gene responsible for the AT defect is ATM (for AT mutated).

The following lymphoid cell lines were used to study the initial DNA damage and repair after X-irradiation (5 Gy): two human ataxia-telangiectasia (AT) Epstein-Barr virus-tranformed; GM00736A and GM00717C, heterozygous and homozygous for the ATM gene, respectively, and, as control, two apparently normal cell lines GM03798A and GM01953C. We measured the DNA lesions using the alkaline version of the comet assay [1]. To determine the initial DNA damage, cells were irradiated on ice. For the DNA repair experiments cells were irradiated at room temperature and, after medium change, were incubated at 37°C for 3 and 24 h. We have shown (Fig.) that the AT cells, both heterozygous and homozygous (GM00736A and GM00717C) repair radiation-induced DNA damage as efficiently as normal cells (GM03798A and GM01953C). The initial DNA damage in GM00717C (homozygous) after X-irradiation with 5 Gy was the highest but the difference was not statistically significant. Irradiated AT cells were shown to exhibit various in vitro abnormalities, such

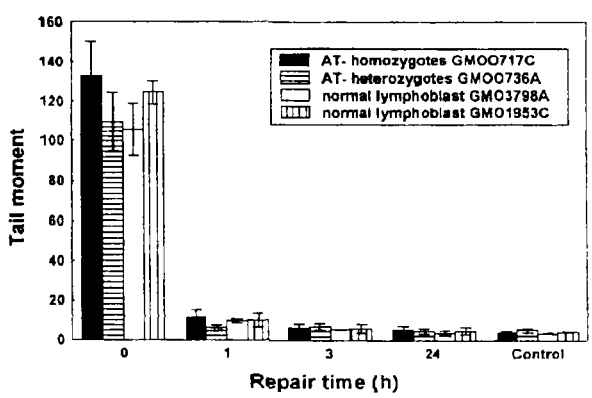

Fig. DNA damage and repair kinetics measured by the alkaline comet assay in human lymphoid cell lines irradiated with 5 Gy X-rays. The bars represent the mean tail moment values ±SD of 3 independent experiments (100 comets were measured per experimental point).

as elevated frequencies of chromosome aberrations [2] and micronuclei induction [3] and defective mitogen and cell-cycle responses to radiation [4]. Sikpi et al. proposed that AT cells rather than possessing an actual DNA repair defect are defective in regulating radiation-induced DNA repair and less-than-normal rejoining fidelity [5].

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VALIDATION OF A NEW MODIFICATION OF THE NEUTRAL COMET ASSAY

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Comet assay under neutral conditions allows the detection of DNA double-strand breaks, considered to be the biologically relevant radiation-induced lesion. In this report we describe modifications of the neutral comet method, which simplifies and facilitates its use for estimation of DNA double strand breaks in X-irradiated mammalian cells in culture. The analysis carried out according to this protocol takes less time than those most often applied. Also, the use of lysis at 50°C is avoided; this is important in view of the presence of heat-labile sites in the chromatin of irradiated cells, recently reported by Rydberg [1]. The comets have well defined, sharp limits, suitable for image analysis. The specificity of the assay for DSB was confirmed by measuring the repair rate of X-ray-induced lesions in DSB repair-competent Chinese hamster ovary cells, CHO-K1 cells (wild type) and in the DSB repair-defective xrs-6 mutant. As shown in Fig.1, the mutant cells do not repair efficiently the DNA damage revealed by the neutral comet assay, in agreement with the defect in non-homologous rejoining of double strand breaks.

To further verify the specificity of the method we measured the DNA lesions that should be "invisible" under neutral, but not under alkaline conditions. Such criteria are fulfilled by DNA damage induced by UV-C (254 nm) (Fig.2) or H_2O_2 (Fig.3). For the alkaline assay the cells were processed as described by Kruszewski et al. [2].

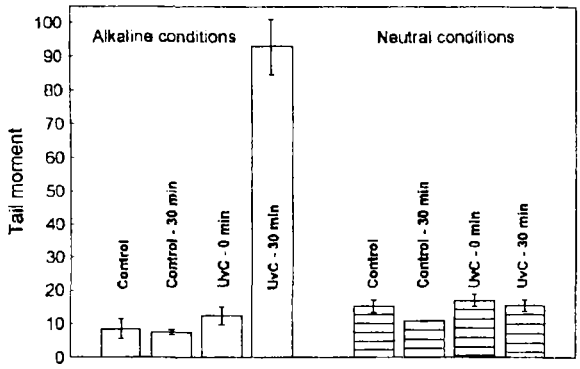

Fig.1. Repair of the DNA damage in CHO-K1 and xrs-6 cells after X-irradiation (10 Gy) at time zero or repair intervals (0.5-24 h). The mean tail moment values were measured by the neutral comet assay. Bars represent mean tail moment values ±SD of 3 independent experiments (100 comets were measured per experimental point).

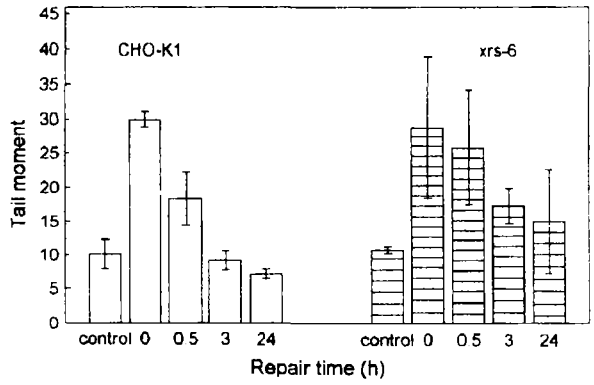

Fig.2. The DNA damage representing incomplete excision repair sites after exposure to UV-C (fluence of 7 J/m^2) and 30 min incubation at 37°C estimated in CHO-K1 cells by the alkaline or neutral comet assay. Bars represent mean tail moment values ±SD of 3 independent experiments (100 comets were measured per experimental point).

The results point to a satisfactory sensitivity of the modified neutral comet assay and its specificity for DSB.

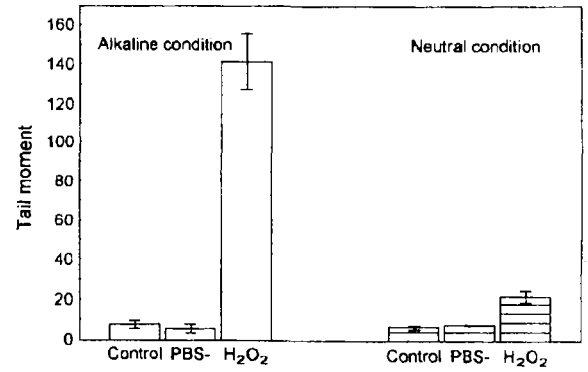

Fig.3. DNA damage induced in CHO-K1 cells by 15 min treatment with 10 μM H_2O_2 measured by the alkaline or neutral comet assay. Bars represent mean tail moment values ±SD of 3 independent experiments (100 comets were measured per experimental point).

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