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Chromosome fragility and susceptibility of Bloom's syndrome fibroblasts to SV40 transformation[1]

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Summary. A comparison of the frequencies of chromosomal aberrations and the rates of SV40 transformation was made using fibroblasts obtained from 2 patients with Bloom's syndrome (BS) and from a normal individual. BS cells were found to be more susceptible to chromosome damage, in confirmation of earlier reports, but surprisingly, BS cells were distinctly less prone to transformation.

It has been suggested that cultured fibroblasts from patients with genetic disorders associated with chromosomal abnormalities, such as Fanconi's anaemia and Down's syndrome, which also have a high incidence of malignancy, are more susceptible than normal fibroblasts to in vitro transformation by Sendai virus (SV40)[3-6]. Bloom's syndrome (BS) is a rare autosomal recessive disorder characterized by growth retardation, light sensitivity, defective immune response, and predisposition to cancer[7,8]. In addition, BS cells show a high frequency of sister chromatid exchanges (SCE), chromosome breakage and symmetrical chromosome exchanges[7-9]. These particular cytological characteristics prompted us to investigate the relationship between the high risk fo cancer in BS and its sensitivity to transformation by SV40.

Materials and methods. Skin fibroblasts were cultivated in vitro from 1 normal individual and 2 patients with BS (GM 811 and GM 1493, obtained from the Institute for Medical Research, Camden, N.J.). Cultures were maintained in Eagle's minimum essential medium supplemented with 20% fetal calf serum. Fibroblasts during 7-12 passages were used. In order to differentiate sister chromatids, the cells were incubated in the dark in medium containing 10^{-5} M 5-bromodeoxyuridine (BrdU) for 2 cycles of DNA replication. Q-banding and BrdU-DAPI fluorescences were used for the staining of chromosomes[10,11]. The transformation procedure described by Todaro et al.[4] was followed.

Results and discussion. The results of the cytogenetic findings are presented in table 1. The frequency of spontaneous chromosome aberrations in BS fibroblasts was high, compared with that of the control. This result is consistent with earlier reports on BS lymphocytes and bone marrow cells in which the values for chromosomal aberrations were higher than those of normal cells[8,9,12,13].

As shown in table 1, the fibroblasts from a normal control showed a mean of 8.2 SCEs/cell. In contrast, the frequency of SCE in BS fibroblasts was about 10-fold greater than that of the normal control. A high incidence was observed in all metaphases after BrdU treatment, although SCE per BS cell varied (table 1). This is in agreement with the previous reports on lymphocytes and bone marrow cells from BS patients[8,9,12].

The percentages of BS cells surviving after infection with SV40 were comparable with those found for normal cells. Results of the transformation studies are shown in table 2. Only a slight difference was observed in the transformation frequency of cells (either normal or BS cells) between the 2 experiments. The transformation frequencies of cells from a normal individual are in good agreement with those reported by Todaro et al.[5]. The transformation rate was lower in cells from the 2 BS patients than in those from the control. This difference was even more pronounced in the repeated experiment in cells from 1 of the 2 BS patients (table 2). This clearly indicates that our fibroblasts from BS

Table 1. Chromosomal aberrations and sister chromatid exchanges in fibroblasts from 1 normal individual and from 1 patient (GM 1493) with Bloom's syndrome

Cell strain	Chromosomal aberrations						Sister chromatid exchanges		
	No. of cells examined	No. of abnormal cells (%)	No. of breaks per cell	No. of interchanges per cell	No. of dicentrics per cell	No. of fragments per cell	No. of cells examined	Mean	Range
Normal	35	2 (5.7)	0.08	0	0	0	32	8.2	3-16
GM 1493	70	24 (34.3)	0.37	0.09	0.13	0.16	20	81.7	55-125

patients had a low susceptibility to transformation with SV40.

BS and Fanconi's anaemia are associated with an increase in chromosomal anomalies and also have a high incidence of cancer[6-8]. BS cells show predominately homologous interchanges and an unusually high frequency of SCE whereas Fanconi's anaemia cells show a relatively high proportion of chromosomal breaks and a normal level of SCE[9,12-14]. However, the decreased susceptibility of BS cells to SV40 transformation is contrary to the situation for Fanconi's anaemia where the cells are unusually susceptible to SV40 transformation[6,7]. Our results suggest that chromosomal aberrations are not directly related to the high cancer risk, but may provide a predispository background. Possibly, a defective immune response in BS may play an important role in the incidence of malignancy, which is a hypothesis proposed by Miller and Todaro[3]. It is also known that fibroblasts from immunodeficient patients, who also had a high risk of developing malignancies, only had a low or regular susceptibility to SV40 transformation[15].

Table 2. Rates of transformation by SV40, of cells in human fibroblast cultures from 1 normal individual and from 2 Bloom's syndrome patients (GM1493 and GM811)

Cell strain	Transformation rate* Experiment 1	Experiment 2
Normal	2.2± 1.3	2.9± 1.5
GM1493	0.9± 1.0	1.1±0.9
GM811	-	0.4±0.6

* The number of transformed colonies/2×10^4 cells seeded out 24 h after SV40 infection with $0.5\times10^{8.5}$ plaque forming units/dish. A total of 14 replicate dishes were set up for each experiment.

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Studies on benzene mutagenesis. I. The micronucleus test

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Summary. The mutagenic action of benzene was studied by means of the micronucleus test performed on mice. A linear dose effect relationship was found for the percentage of micronucleated erythrocytes, against the benzene-dose logarithms. A significant dose effect correlation was found either after the standard 30-h experiments or after a prolonged 54-h one. A higher effect was found in the prolonged experiments, suggesting the induction of a delay in the cell cycle by benzene.

Epidemiological human data show a significant relationship between occupational benzene exposure and an increased incidence of leukemia[1-4]. In a recent study[5] concerning the industrial hazards from carcinogen exposure, benzene ranked third in a list of the most hazardous chemicals. Several cytogenetics studies of workers exposed to benzene[6-10], have shown a high incidence of chromosome aberrations in peripheral blood lymphocytes as well as in bone marrow cells.

Leukemogenic action of benzene has not been demonstrated in experimental animals, but several reports have shown a clastogenic action of benzene on mammalian cells in vivo[11-16] and in vitro[17-19].

Inhibition of H^3-thymidine incorporation into mammalian cells exposed to benzene has been reported after autorradiographic studies in vivo[20-22] and in vitro[17,23].

The repair of breaks induced by gamma rays on human lymphocytes in vitro is inhibited by benzene at several concentrations in the culture medium as reported by Morimoto[22].

We considered it convenient to begin our studies on benzene mutagenesis by testing its potency with the micronucleus test, a recently developed, well standarized method for mutagen screening[25,26]. In the present paper we report the results obtained after benzene injection in the micronucleus test performed on mice.

Materials and methods. Analytical grade benzene (Carlo Erba), mixed with olive oil in different proportions was injected s.c. to hybrid F_1 male mice from the cross CSW × CS No. 1 (obtained from the National Atomic Energy Commission of the Argentine Republic).

3 animals were injected per dose. The different doses used, from 0.1 to 2.0 ml/kg of b.w., are listed in the table.

Animals weight ranged from 25 to 31 g. The total value of the benzene oil mixture injected per animal was 0.10–0.12 ml.

A first control group of 3 animals was injected with olive oil alone. A second control group of 3 mice received injections of 10 mg/kg of cyclophosphamide. Cyclophosphamide (Endoxan) was obtained as the commercial preparation for medical use and diluted in physiological saline. Each animal received 2 injections under the abdominal skin, separated by a 24-h interval. The animals were sacrificed by neck extension, either 6 or 30 h after the last injection according to 2 alternative protocols.

The bone marrow from both femurs of the sacrificed