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

animals was processed according to Schmid[26], with the only modification being the suspension of the cells in 1 ml of bovine calf serum, instead of 5 ml. Special centrifuge tubes were used for this purpose.

Micronucleated polychromatic erythrocytes were scored from 1000 polychromatic erythrocytes counted for each animal, cell counts were made on coded slides.

The significance tests of the linear regression coefficients for the mean micronucleated polychromatic erythrocytes counts of individual mice against the dose logarithms were performed by the usual method of t-computation.

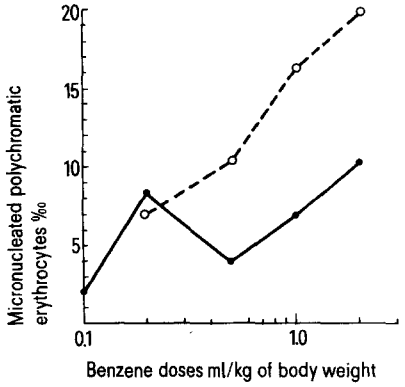
Results and discussion. The s.c. injection of 2 ml/kg of pure benzene in oil to mice was followed by acute symptoms, including nervous system excitation, convulsion, dyspnea and asphyxia. From our scores, including from 9 to 13 animals per dose, it was concluded that the LD_{50} is around 2.5 ml/kg and the LD_5 about 0.8 ml/kg.

Regarding the influence of benzene on polychromatic erythrocytes, a linear dose effect relationship was observed for the percentage of micronucleated erythrocytes against the benzene-dose logarithms. Mean score for each dose and the linear regression coefficients are shown in the table, for 2 experiments, 30- and 54-h-long. The corresponding graphic plottings are shown in the figure.

Micronucleated polychromatic erythrocytes after benzene treatment in the mouse

Benzene doses ml/kg	Micronuclei/1000 erythrocytes					
	6 h from the last injection			30 h from the last injection		
	No. of animals	$\bar{x}$	s	No. of animals	$\bar{x}$	s
Control	3	2.00	0.000	3	6.00	2.646
0.1×2	3	2.00	2.000	-	-	-
0.2×2	3	8.33	5.859	3	7.00	1.000
0.5×2	3	4.00	2.000	3	10.33	3.215
1.0×2	3	7.00	2.646	3	16.33	3.786
2.0×2	3	10.33	1.527	2	20.00	8.485
b	b = 4.431			b = 1.345		
t_{n-2}	$t_{13} = 2.252$ ($p < 0.05$)			$t_9 = 4.151$ ($p < 0.01$)		

$\bar{x}$ = mean micronuclei scores for the animals injected with each dose. s = standard deviation. b = regression coefficient. t = Student's t-test coefficient computed for regression significance.



Dose effect curves for benzene induced polychromatic erythrocytes. Each point is the mean value of 3 animals injected twice with each benzene dose. Animals were sacrificed 6 (——O——) or 30 (- - - - O - - - -) h after the last injection.

In both experiments, a significant dose effect correlation was found, but the results were more striking in the 54-h-long experiment than in the 30-h one. The control range varied from 0.1 to 0.6% micronucleated polychromatic erythrocytes, a result which parallels those reported by other authors[25,26].

Micronucleated normochromatic erythrocytes were within the range 0 to 0.1% in the animals tested in our laboratory.

These data indicate a clastogenic activity of benzene on erythroblastic chromosomes within the toxic dose range.

Thus, benzene should be classified as a weak mutagen in relation to micronuclei induction, its potency being comparable to that of ethylmethane-sulfonate[25]. Lyon[15] reports significant results in the micronuclei test performed on rats at doses from 0.1 to 0.5 ml/kg given in 2 i.p. injections of half the total dose each, at 30 and 6 h before killing the animals. The mean scores of micronucleated polychromatic erythrocytes observed for these 2 doses were 5.0 and 6.8‰, and the difference from the control is significant to the level $p = 0.01$. Such values are in the range of our own results for low doses in mice under similar conditions. The clastogenic action of benzene after acute treatment in rats was studied previously by other authors, by scoring chromosome breaks and gaps in bone marrow metaphase[11,15,16]. Their results are concordant with ours, in showing benzene as a clastogen under acute in vivo treatments. The data reported by Dean[11] are comparable to ours even in the observed levels of chromosome damage, but those reported by Philip and Jensen[16] show a much higher frequency of chromosome breaks. Lyon[15] also reported high levels of chromatid and chromosome deletions in the bone marrow of rats 24 h after the i.p. injection of 0.5 ml/kg of benzene.

The discrepancy between the results of these authors can probably be explained by differences in the chromosome breaks scoring criteria and/or differences in strain susceptibility to the mutagen.

Bone marrow metaphase damage has been studied also after chronic treatment of rats[12,14] and rabbits[13] with benzene. High levels of damage were reported after such treatment, which points to cumulative effects of benzene on bone marrow cells.

The differences in the results between the 30- and 54-h experiments can be interpreted as the consequence of a cell cycle delay induced by benzene. Other authors have reported that benzene induces an inhibition of DNA synthesis in erythroid precursor cells in vivo in rats[20] and rabbits[21,22] and in human lymphocytes and HeLa cells in vitro[6,23]. These results have been related to a prolonging of the S-phase[20,23] and, therefore, of the cell cycle, in agreement with our findings and interpretation.

The low number of micronuclei observed in normochromatic erythrocytes implies that there is no significant contribution from nondysjunction to micronuclei scores after benzene treatment.

In conclusion: by using the micronuclei test for detection of chromosome damage, we have found a clastogenic effect of benzene acute in vivo treatments within the toxic dose range. In addition to this activity, it seems reasonable to assume that benzene can induce a delay in the cell cycle. Both effects are supported also by evidence in previous papers by other authors, suggesting that cell cycle delay occurs at the S-phase.

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Flavonol glycosides and seed coat structure in certain species of *Epilobium* – A correlation?[1]

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Summary. In a survey of 239 populations of *Epilobium* representing 17 taxa the following flavonol glycosides were found: myricetin 3-O-arabinoside; 3-O-glucoside; 3-O-rhamnoside; quercetin 3-O-arabinoside; 3-O-glucoside; 3-O-diglucoside; 3-O-rhamnoside; kaempferol 3-O-glucoside; and 3-O-rhamnoside. A correlation appears to exist between seed coat sculpturing as determined in a previous study using SEM techniques, and the flavonoid profiles.

The genus *Epilobium* is quite cosmopolitan in distribution, with several circumboreal species[2]. In many respects it appears to be ecologically nonspecific, but at the same time exhibiting a wide range of morphological and cytological variation. This is shown to its greatest degree in western North America[3]. The present day distribution of the genus indicating a possible centre of origin in this region[4].

With this in mind a survey of 17 taxa representative of the genus in northwestern America was carried out to determine if a parallel complexity occurred with respect to leaf flavonoid glycosides.

In the Onagraceae, in general, studies have been restricted mainly to the genera Oenothera[5], Circaea[6], with little being carried out on other taxa. Studies have shown that the commonly distributed flavonol glycosides[5] are present, and rarely more complicated flavonoids such as methylated derivatives and glyco-flavones[6].

In the present study the leaves of 239 populations were sampled representing 17 taxa (table) previously studied for their seed coat structures[7]. A minimum of 6 populations (10 g dry wt) were examined in any taxon. Isolation of flavonoid glycosides, hydrolytic procedures, and spectral analyses were carried out using standard methods[8], with modifications when discontinuities in distribution of compounds was found. In such situations flavonoids were exhaustively extracted using 70% methanol reduced to dryness and loaded in a minimum volume of water onto a sephadex LH 20 column. Gradient elution of glycoside mixtures was then carried out using water-methanol-acetone gradients. Flavonoids were then re-isolated and characterized using normal analytical procedures.

Flavonol glycosides in *Epilobium*

Taxon	Samples	m 3 glc	m 3 rh	m 3 arab	q 3 glc	q 3 rh	q 3 arab	q 3 diglc	k 3 glc	k 3 rh
E. latifolium	66	+	+	+	+	+	+	+	+	+
E. angustifolium	52	+	+	+	+	+	+		+	48
E. glandulosum	6	+	+	+	+	+	+			+
E. paniculatum	8	+	+	6	+	+	7			7
E. hirsutum	8	+	+	+	+	+	+			7
E. anagallidifolium	8	+	+	7	+	+	+			+
E. platyphyllum	6	+	+		+	+	+			+
E. hornemanii	12	+	+		+	+	10			+
E. clavatum	6	+	5		+	+	5			+
E. lactiflorum	6	+	+		+	+	+			
E. alpinum	17	+	+		+	+	+			
E. luteum	6	+	+		+	+	4			
E. davuricum	6	+			+	+				
E. leptophyllum	6	+			+	+				
E. palustre	14	+			+	+				
E. palustre var. *grammadophyllum*	12	+			+	+				
E. palustre var. *monticolor*	10	+			+	+				
Total	239									

m, myricetin; glc, glucose; q, quercetin; arab, arabinose; k, kaempferol; rh, rhamnose.