Genotype-specific reduction in methyl nitrosourea (MNU) induced sister chromatid exchanges (SCE) in vivo during aging

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Summary. We studied mice from five strains (BALB/c, C3H/HeSnJ, C57BL/6J, Cs[b] and 129/ReJ) at two ages (young, 10 ± 1 weeks; and old, 67 ± 3 weeks) for the induction of sister chromatid exchanges (SCEs) in vivo by methyl nitrosourea (MNU). The SCE frequency is genotype-specific. The F_1 phenotype resembles the 'low' responding parent. SCE induction is significantly lower in the older animals of each strain than their younger counterparts, and the reduction of SCE/cell with old age is strain-specific. A general explanation for these results must include strain differences in relative mutagenic sensitivity, genotype-specific pattern of reduction in DNA repair and other such factors affecting SCE formation, with old age.
Key words. Methyl nitrosourea (MNU); sister chromatid exchange (SCE); aging; mutagenic sensitivity; background genotype.

Sister chromatid exchange (SCE) is one of the most sensitive and popular in vitro and in vivo eukaryotic assays for genetic toxicity, even though the mechanism of SCE formation and its biological significance remains speculative. SCE induction is influenced by a number of biological factors including some well-known mutations causing disease[1], cell types[2], developmental stage and species[3]. Further support for the role of gene(s) and genotype in SCE induction by a mutagen is provided by a number of studies on otherwise 'normal' individuals including different genetic strains of mice. In 1981, Dragani et al.[4] reported that mouse strains differed in SCE induction when challenged with cyclophosphamide (CP) in vivo, and Reimer and Singh[5] suggested that such differences could involve multiple loci that interact predominantly as non-additive factors. It is logical to argue that some of the genetic determinants that influence SCE induction may include normal genes of general metabolism and the role of such genetic determinants in SCE induction could be evaluated by the study of controlled genotypes during aging using the sensitive parameters of mutagenesis[6]. This may occur because most, if not all, of the genes that determine house-keeping metabolism appear to be subject to aging. In particular, the ability to repair induced DNA damage is known to decline with age for a number of species including mice[7], rats[8], and humans[9]. It may be pointed out that a number of genetic disorders (mutations) present symptoms which imitate a premature onset and an accelerated progression of normal biological aging[10] and most species have pre-determined and predictable life spans. The study of SCE induction on controlled genotypes, therefore, will permit assessment of the genetic determinants of relative sensitivity to mutagenic challenges that are prone to genotype dependent (strain-specific) aging and help assess the role of genetic determinants in SCE formation. Furthermore, such results are expected to be useful in the understanding of the genetic contributors of the phenomenon of aging.

In this study we present data on methyl nitrosourea (MNU, a direct acting alkylating agent and effective inducer of SCE both in vitro and in vivo[11]) induced SCEs in male mice at two ages (10 ± 1 weeks and 67 ± 3 weeks) in vivo. The two ages represent approximately 10 and 70% of the life span, respectively, of the five genetic strains studied under our management practices. The strains included in this study (BALB/c, Cs[b], C3H/HeSnJ, C57BL/6J and 129/ReJ) represent divergent gene pools (background genotype) in homozygous conditions. The data obtained were used to determine the role of background genotype on relative sensitivity to MNU particularly during aging in otherwise normal individuals. The observed genetic differences are attributed to the factors involved in protection against genetic damage and repair efficiency of the DNA following such damage.

Material and methods. Breeding pairs for the mice used in this experiment were obtained as follows; BALB/c, Charles River, Quebec; C57BL/6J, 129/ReJ and C3H/HeSnJ, Jackson Lab, Bar Harbor, Maine; and Cs[b], Dr T. W. Clarkson, University of Rochester, New York. Animals were housed in 28.5 cm × 11.5 cm × 112.5 cm polycarbonate cages with sawdust bedding, and fed Mouse Chow (Purina Canada Inc) and water ad libitum. They were kept in a thermostatically controlled room (23 °C) with a 14:10 h light-dark photoperiod in the animal care facilities of the University of Western Ontario, London, Canada.

Methyl nitrosourea (MNU) was prepared by acidifying sodium nitrite (Sigma Chemical Co., St. Louis MO, USA) and N-methylurea (Aldrich Chemical Co.) following Venitt and Perry[12]. Colchicine and bromodeoxyuridine (BUdR) were also obtained from the Sigma Co., St. Louis. Animals were anesthetized with a 12.5% solution of avertim-tribromoethanol prior to surgical implantation of the BUdR tablet.

An i.p. injection of MNU dissolved in 10 mM sodium citrate pH 6.0 at a dose of 0, 2.5, 25, or 50 mg/kg b.wt was given at the time of BUdR implant. Two animals of a given age and genotype (replicates) were used for each treatment. After 21 h, each animal was given a single injection of colchicine (4 mg/kg b.wt, i.p.). Animals were sacrificed by cervical dislocation 24 h after the BUdR tablet implantation. Femurs were removed, and the collected bone marrow cells were processed for SCE studies. Twenty well-spread metaphase cells were evaluated for sister chromatid exchanges for each animal. The data so obtained were analyzed using parametric statistical methods to evaluate the effect of MNU doses, strains (genotype) and age on sister chromatid exchange induction. Significant differences were established using analysis of variance (ANOVA) and the Student's t-test.

Results and discussion. The data consist of SCEs/cell (fig. 1) for male mice from five genetic strains in response to increasing doses (0, 2.5, 25 and 50 mg/kg) of MNU in vivo. SCE counts are based on 20 bone marrow cells per animal and two animals were studied for each strain-dose combination. Such a data set was obtained for animals of two age categories (young, 10 ± 1 weeks and old, 67 ± 3 weeks). An analysis of variance on the complete data suggests that increasing doses of MNU cause significant increases in the SCEs/cell ($F = 9723$, $p < 0.001$). Also, the SCE induction following MNU treatments is significantly different among strains ($F = 104$, $p < 0.001$), and the animals of the two age groups differ significantly in their rate of SCE induction from each other ($F = 807$, $p < 0.001$), but there are no significant differences between the two animals (replications) evaluated for a given treatment-strain-age combination ($F = 0.0059$, $p < 0.999$). Table 1 shows the mean (± SEM)

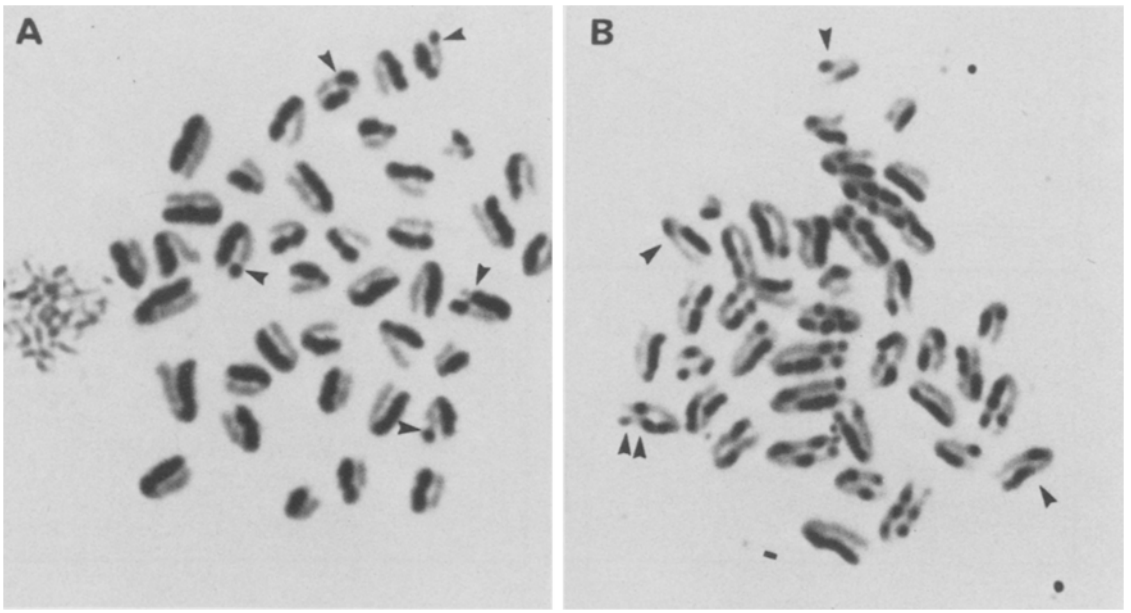

Figure 1. Chromosome spreads from two cells showing (*A*) low and (*B*) high number of sister chromatid exchanges.

number of SCEs/cell and the induced (treatment-control or MNU = 0 mg/kg) SCEs/cell following MNU challenge in bone marrow cells of young (10 ± 1 weeks) mice from the five strains. A similar data set for corresponding old animals (67 ± 3 weeks) is given in table 2. It is apparent from these tables that the increasing doses of MNU cause an increase in SCEs/cell ($p < 0.001$) for all mouse strains at both ages. The increase in mean SCEs/cell is also associated with an increasing variance around the mean for all strains. This variance is significantly different among the strains for young mice ($F = 20.40$, $p < 0.001$). The SCE results for each dose of MNU, however, are not uniform for all animals. It depends on the strain (genotype) and age of the mice. Mice from some strains are relatively more sensitive at a young age (BALB/c, table 1) while others are more sensitive at an old age (C3H/HeSnJ, table 2) as compared to the other strains studied. It is interesting to note that the relative sensitivity among strains to SCE induction is MNU dose-dependent. For example, the induced SCEs in the strain 129/ReJ is over seven times that seen in C3H/HeSnJ at 2.5 mg/kg, more than two times at 25 mg/kg and similar at 50 mg/kg of MNU (table 1). This suggests that these strains may vary in their relative sensitivity at different thresholds of MNU challenge. Figure 2 summarizes the mean dose response to MNU (represented by SCEs/cell) for the two age groups in the five strains. Here the older animals generally have lower SCEs/cell at most MNU challenges in all strains. The overall dose response is significantly different between the two age groups in three strains (129/ReJ, Cs^b and C57BL/6J). Also, the difference between the two age groups is significantly different when

Table 1. Observed SCEs/cell (mean ± SEM) and induced (treatment-control) SCEs/cell by different doses of MNU in vivo in young mice (10 ± 1 weeks) from five genetic strains. Induced (dose-control) values are given in brackets.

Dose (MNU)	Strains BALB/c	C3H/HeSnJ	C57BL/6J	Cs^b	129/ReJ
0	8.28 ± 0.49	10.35 ± 0.48	10.90 ± 0.5	5.97 ± 0.26	9.75 ± 0.43
2.5	12.75 ± 0.78	11.63 ± 0.76	14.95 ± 0.73	15.48 ± 0.48	19.03 ± 0.76
	(4.47)	(1.28)	(4.05)	(9.51)	(9.28)
25	32.75 ± 0.72	21.25 ± 0.90	30.28 ± 0.81	18.05 ± 0.98	33.08 ± 1.11
	(24.47)	(10.9)	(19.38)	(12.08)	(23.33)
50	57.98 ± 1.18	47.45 ± 1.34	40.48 ± 0.97	38.85 ± 0.91	45.20 ± 1.21
	(49.70)	(32.1)	(29.58)	(32.88)	(35.45)

Table 2. Observed SCEs/cell (mean ± SEM) and induced (treatment-control) SCEs/cell by different doses of MNU in vivo in old mice (67 ± 3 weeks) from five genetic strains. Induced (dose-control) values are given in brackets.

Dose (MNU)	Strains BALB/c	C3H/HeSnJ	C57BL/6J	Cs^b	129/ReJ
0	7.15 ± 0.32	8.25 ± 0.52	8.08 ± 0.46	5.78 ± 0.37	4.27 ± 0.28
2.5	10.97 ± 0.48	11.33 ± 0.81	11.63 ± 0.76	12.2 ± 0.61	9.88 ± 0.78
	(3.82)	(3.08)	(3.55)	(6.42)	(5.61)
25	28.08 ± 0.79	25.5 ± 0.83	21.28 ± 0.60	15.25 ± 0.63	17.13 ± 0.86
	(20.93)	(17.25)	(13.20)	(9.47)	(12.86)
50	36.75 ± 0.97	50.30 ± 1.34	36.15 ± 1.12	37.80 ± 0.70	36.90 ± 0.97
	(29.60)	(42.05)	(28.07)	(32.02)	(32.63)

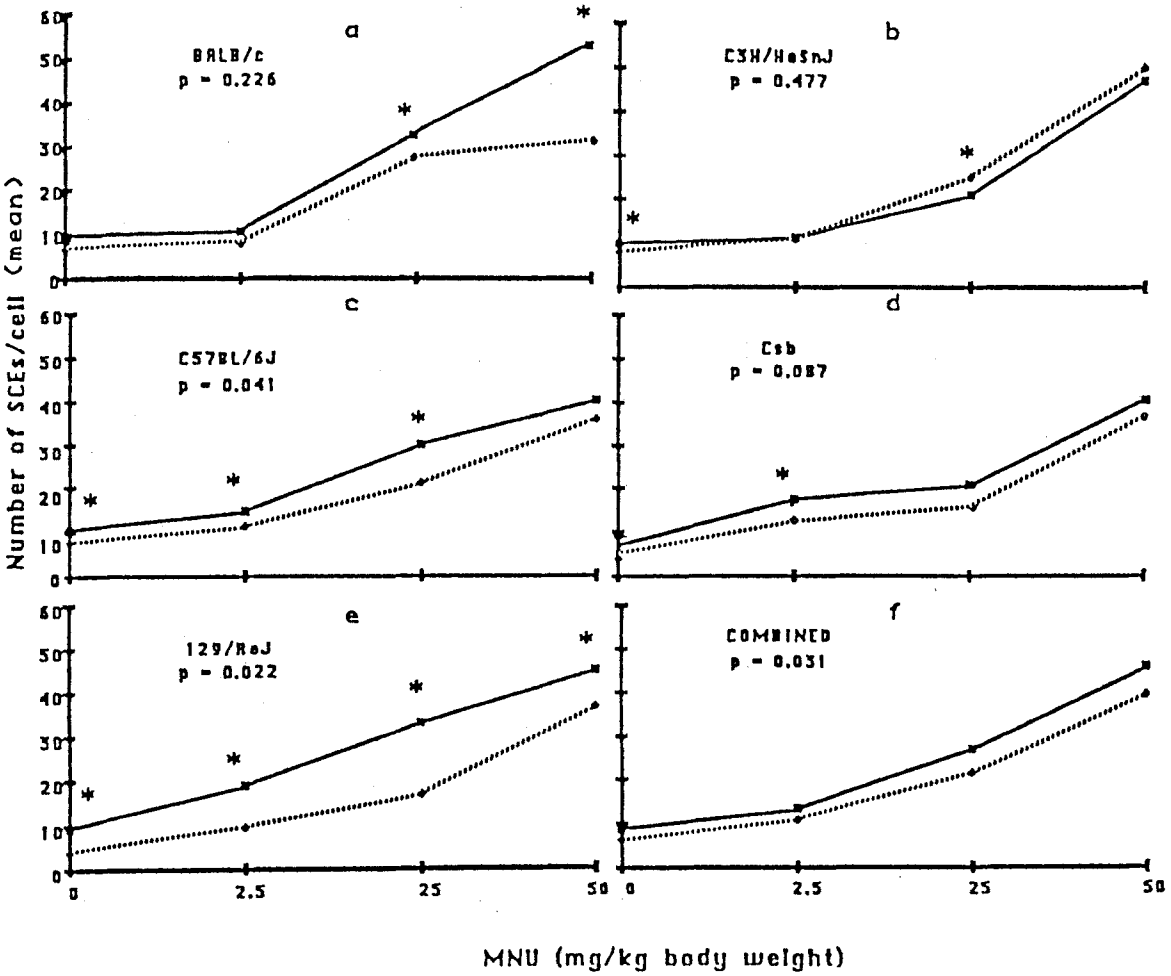

Figure 2. Methyl nitrosourea (MNU) dose response in young (10 ± 1 week, solid lines) and old (67 ± 3 weeks, broken lines) mice for five genetic strains (panels a–e). Panel f represents average of all strains. P values given in each panel show significance of the difference between the two ages over all MNU doses. * represent significant differences ($p < 0.05$) between young and old mice at a given dose.

Table 3. Aging index [(young – old)/young × 100 %] for SCEs/cell in response to different methyl nitrosourea (MNU) doses in five genetic strains of mice.

Dose (MNU) (mg/kg)	Strains BALB/C	C3H/HeSnJ	C57BL/6J	Cs[b]	129/ReJ
0	13.6	20.3	2.59	3.20	56.2
2.5	13.9	2.6	22.2	19.3	48.1
25	14.3	− 20.0	29.7	12.7	48.2
50	36.6	6.0	10.7	2.70	12.4
AVG ± SEM	19.6 ± 11.3	8.9 ± 10.0	16.3 ± 12.0	9.5 ± 8.0	41.2 ± 19.7

viewed over all strains (panel f, fig. 2). The percent decrease/increase (aging index) in SCEs/cell can also be examined for each strain, at different levels of MNU challenge (table 3). These estimates are used to evaluate differential aging of these strains. We wish to point out the two main features of the aging index for these strains; the average index (over all MNU doses) is highly variable among strains (i.e. 8.9 ± 10.0 for C3H/HeSnJ and 41.2 ± 19.7 for 129/ReJ) and the maximum reduction in SCEs/cell in the old animals is realized at different levels of MNU challenge in different strains (i.e. 50 mg/kg MNU for BALB/c and 25 mg/kg for C57BL/6J). Such observations suggest that the strains included in this study differ in relative sensitivity to MNU and that there is a strain-specific rate of loss of ability to deal with mutagenic challenge (i.e. DNA repair) during aging.

Evaluation of the genetic contributor(s) to SCE induction was attempted using F_1 analysis of SCEs/cell for selected strains; BALB/c (high inducer) and C57BL/6J (low inducer). Figure 3 shows the distribution of SCEs/cell in these two strains and their F_1 hybrid (BALB/c ♀ × C57BL/6J ♂) in response to increasing doses of MNU. It shows that the F_1 hybrid exhibits an SCE distribution similar to the C57BL/6J strain. Also, the mean SCEs/cell at various doses of MNU (given in the insert of fig. 3) shows that the F_1 hybrid is similar to the strain C57BL/6J ($p < 0.05$). This finding is in agreement with another study which found that the F_1 hybrid, when exposed to CP, followed the SCE mean and distribution pattern of the parent with lower induction [5]. These results indicate that there must be genetic factors involved in the induction of SCEs.

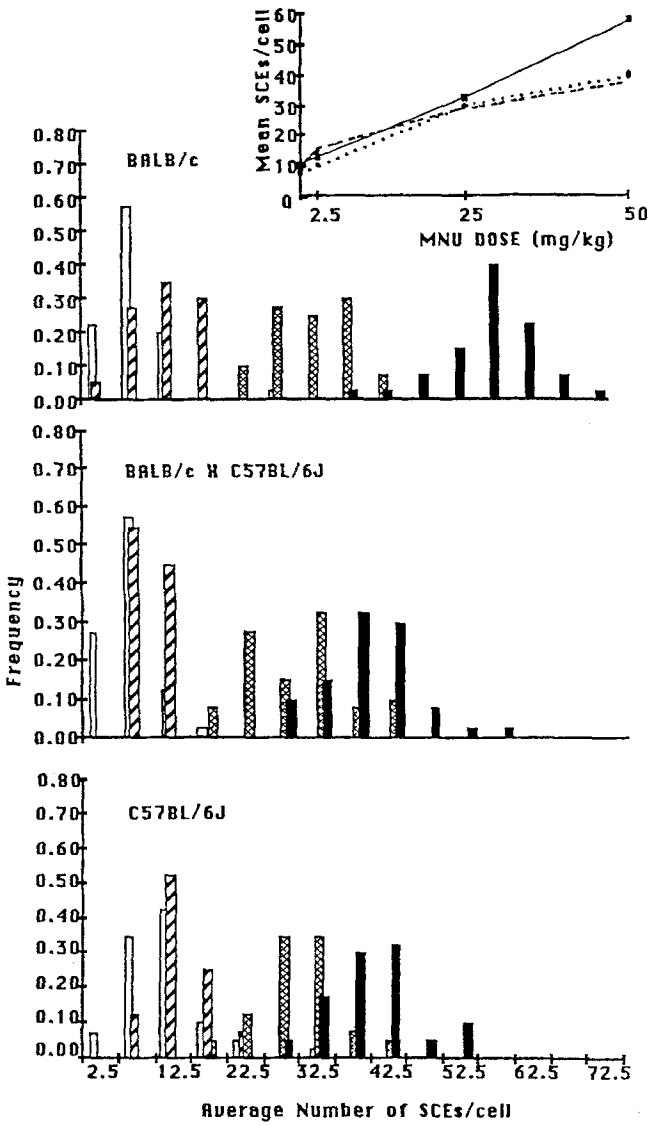

Figure 3. Distribution of SCEs/cell in BALB/c, C57BL/6J and their F_1 hybrid (BALB/c ♀ × C57BL/6J ♂) at four doses of MNU (□ 0 mg/kg; ▨ 2.5 mg/kg; ▩ 25 mg/kg; ■ 50 mg/kg) in young mice (10 ± 1 weeks). Insert shows SCEs/cell (mean ± SEM) as a function of MNU dose. — BALB/c; ··· BALB/c × C57BL/6J; - - - C57BL/6J.

It may be pointed out that the observed heterogeneity probably represents the interaction of a number of biological and non-biological factors. The known biological components may have genetic predispositions and include the ability to protect the DNA from damage directly or through detoxification of the mutagen, and the ability to repair any induced damage, among others. It is difficult, if not impossible, to identify all of the biological components of this heterogeneity. However, in general, these results follow earlier reports that older individuals are generally more sensitive to chromosomal damage by physical and chemical agents [13, 14] and less able to repair any induced damage [7–9, 14]. The data set presented here points to a complex pattern of causations for in vivo SCE induction, in particular, and relative mutagenic sensitivity in general. Although it is not possible to evaluate the actual genetic determinants involved in relative sensitivity through the data set presented, these conclusions have implications in two areas. First, towards the understanding of the process of SCE formation, it suggests involvement of a number of biological components that are prone to genotype-dependent aging (i.e. DNA repair). Second, towards the use of SCEs in mutagenesis testing, it offers reason for caution and selecting animals of comparable age and genetic background to be used.

1 German, J., in: DNA Repair Mechanisms, pp. 625–631. Eds Hanawalt et al. Academic Press, New York 1978.
2 Mitchel, G. A., Meher-Homji, K. M., and Baker, R. S. U., Envir. Mutagen. *4* (1982) 267.
3 Barnett, R. I., and Wallace, J., Experientia *38* (1982) 542.
4 Dragani, T. A., Zunino, A., and Sozzi, G., Carcinogenesis *2* (1981) 219.
5 Reimer, D. L., and Singh, S. M., Genetics *105* (1983) 169.
6 Singh, S. M., Toles, J. F., and Reaume, J., Can. J. Genet. Cytol. *28* (1986) 286.
7 Licastro, F., and Walford, R., Mech. Ageing Dev. *35* (1986) 123.
8 Kennah, H. E., Coetzie, M. L., and Ove, P., Mech. Ageing Dev. *29* (1985) 283.
9 Kovacs, E., Weber, W., and Muller, H., Mutat. Res. *131* (1984) 231.
10 Schneider, E., Genetics of Aging. Plenum Press, New York 1978.
11 Kaina, B., Mutat. Res. *142* (1975) 49.
12 Venitt, S., and Perry, J. M., Mutagenicity testing: A practical approach. IRL Press, Washington, D.C. 1984.
13 Hedner, K., Hogstedt, B., Kotnig, A. M., Mark Vendel, E., Strombeck, B., and Mitedman, F., Human Genet. *62* (1981) 305.
14 Reimer, D. L., and Singh, S. M., Mech. Ageing Dev. *21* (1983) 59.

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Chromosome aberrations in cattle raised on bracken fern pasture

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Summary. Thirteen cows maintained on natural bracken fern (*Pteridium aquilinum*) were analyzed cytogenetically. The frequency of structural chromosome aberrations detected in peripheral blood cells was significantly higher when compared to that detected in animals raised on pasture containing no bracken fern. We discuss the clastogenic action of fern and its synergistic action with infection by type 2 and 4 papilloma virus in the same animals.
Key words. Pteridium aquilinum; bovine papilloma virus; chronic enzootic hematuria; alimentary cattle cancer; chromosome aberrations; clastogenicity.

The occurrence of bladder and digestive tract tumors in cattle seems to be related to the presence of the fern *Pteridium aquilinum* in the pasture and to infection with bovine papilloma virus. The radiomimetic and carcinogenic action of fern