Step 2: hypoxanthine determination. A first spectrophotometric reading of the solutions obtained as described above was made at 293 nm to determine the initial absorbance. A 5-µl volume of 4 IU/ml XOD was then added and the conversion of hypoxanthine to uric acid was followed to completion at 293 nm. The reaction went to completion after 30 min at 25 °C. Hypoxanthine concentration was calculated from the optical density variation at 293 nm assuming a molar extinction coefficient of 12,000 for uric acid[10].

PRPP concentration was calculated as follows:

$$[PRPP] = 1.2 \times ([Hypoxanthine]_0 - [Hypoxanthine]_{PRPP}),$$

where $[Hypoxanthine]_0$ was the concentration of the purine base in the blank and $[Hypoxanthine]_{PRPP}$ was the concentration of the purine base in the samples at the end of the HGPRT catalyzed reaction.

Results and discussion. The calibration curve for the PRPP assay obtained by applying the procedure described under "Methods" on PRPP solutions of known concentration is reported in the figure. The relationship between PRPP concentration and the amount of hypoxanthine consumed is linear in the range studied. From the same series of experiments, a standard deviation of $\pm 8 \times 10^{-7}$ M was obtained for the PRPP assay.

Control experiments showed that PRPP at the concentrations employed, IMP and pyrophosphate (products of the HGPRT catalyzed reaction) added in equimolar amounts to PRPP (from 3.5×10^{-6} M to 5.8×10^{-5} M) do not influence the hypoxanthine assay.

The method for the determination of PRPP concentration described above is less time-consuming than the radiochemical procedures involving chromatographic or electrophoretic separation of nucleotides from the corresponding purine bases. The assay is sensitive and specific and responds linearly over the concentration range tested from 3.5×10^{-6} M to 5.8×10^{-5} M. Johnson et al.[8] have stated that no such linearity was found at PRPP concentration lower than 1×10^{-5} M using the alternate spectrophotometric method of Kornberg et al.[6,7].

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Genetic characterization of the new morphological and UV-sensitive mutants in *Coprinus cinereus*. I. A UV-sensitive mutation *rad* 1 associated with elevated frequencies of mitotic and meiotic recombination[1]

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Summary. Studies on the effect of an UV-sensitive mutation, *rad* 1, in meiotic and mitotic recombination in *Coprinus* indicated that, in homozygous condition, *rad* 1 increased the spontaneous meiotic recombination by 50% and UV-induced mitotic intergenic recombination by about 5-fold. The homozygous *rad* 1 diploid was shwon to be much more sensitive to the recombinogenic effects of polyfunctional than of mono- or non-functional alkylating agents.

The existence of relationships between radiation sensitivity, genetic recombination and repair was first suggested when UV-sensitive mutants of *Escherichia coli* were shown to be defective in recombination[3-5]. During the last decade, it has further been demonstrated that at least some of the enzymes responsible for the repair of induced DNA lesions are also involved in genetic recombination of eukaryotes such as *Ustilago*[6], *Neurospora*[7], *Aspergillus*[8], *Saccharomyces*[9-11] and *Drosophila*[12-14]. In *Coprinus*, 4 UV-sensitive mutants have previously been described[15] and tested for their effects on meiotic recombination. 2 were shown to be allelic (*uvs* 3-1 and *uvs* 3-2) and reduce the frequency of meoitic intergenic recombination, whereas the other 2 were mutations of other genes and did not affect meiotic recombination. None were tested for effects on mitotic recombination.

A UV-sensitive mutant (*rad* 1) in *Coprinus*, isolated from UV-irradiated oidial suspension produced from the morphological mutant *den*[2] (AJ1/65), was shown to increase by 50% the normal meiotic recombination frequency between the 2 morphological mutants *den*[2] and *zig* in linkage group III, either in repulsion or coupling (table).

The synthesis of diploid strains of *Coprinus* from 2 recessive morphological mutants in repulsion[16], carrying *rad* 1 mutation either in homozygous or heterozygous condition facilitated greatly the study of the effect of *rad* 1 on mitotic recombination. Thus a comparative study was made of the effect of UV-irradiation on oidial suspension from 3 diploid strains, all of which were heterozygous for *den*[2] and *zig* in repulsion; the strain AJZ4 was homozygous for *rad* 1, the strain AJZ1 heterozygous for *rad* 1 and the strain AJZ homozygous wild type for *rad* 1. The results of these studies were evaluated from the data of the dose-response curves together with the frequency of mitotic segregants expressed as a percentage of colonies on untreated controls. The same data were used to plot the frequency of UV-induced mitotic segregants against the surviving fraction (figure). As is apparent from these

The effect of UV-sensitive mutation *rad*1 on meiotic recombination between the 2 morphological mutants *den*^{2} and *zig*, either in coupling or in repulsion

Cross	Number of replicas	Total progeny N	Heterogeneity between replicas χ^2	P	% Recombination $\bar{p} \pm 2SE(\bar{p})$	Replica data Recombinants/sample size
$\times \frac{den^2\ zig}{+\ +}\ \frac{+}{+}$	5	1027	8.887	0.1–0.05	18.3±3.65[b]	28/121 35/158 50/239 30/204 45/305
$\times \frac{den^2\ +}{+\ zig}\ \frac{+}{+}$	4	2829	0.962	0.9–0.8	20.0±1.50[a]	150/752 121/573 151/794 144/710
$\times \frac{den^2\ zig}{+\ +}\ \frac{+}{rad1}$	5	1396	3.023	0.6–0.5	20.3±2.15[a]	50/229 71/401 79/362 42/189 42/215
$\times \frac{den^2\ +}{+\ zig}\ \frac{rad1}{+}$	3	2079	3.248	0.2–0.1	19.5±2.38[b]	62/301 120/553 223/1225
$\times \frac{den^2\ zig}{+\ +}\ \frac{rad1}{rad1}$	5	1246	4.859	0.4–0.3	31.4±3.38[b]	152/433 40/146 39/137 22/69 138/461
$\times \frac{den^2\ +}{+\ zig}\ \frac{rad1}{rad1}$	4	681	4.281	0.3–0.2	30.5±4.22[b]	38/129 73/258 59/159 38/135

[a] $SE=[\bar{p}\ (1-\bar{p})\ /N]^{1/2}$. [b] SE according to Snedecor and Cochran 26, p. 241.

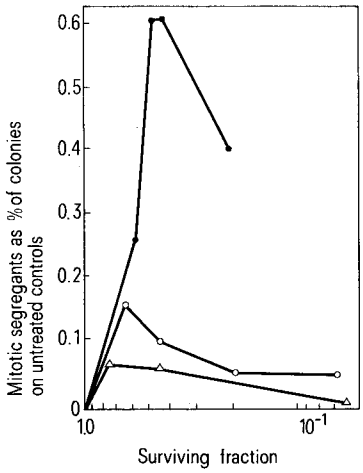

Fig. 1. The effect of UV-irradiation on mitotic segregation at different survival levels, in 3 diploid strains, to demonstrate their relative sensitivity towards the recombinogenic effect of UV-light. Oidia were treated under a 6-W Philips TUV germicidal lamp at the distance of 12 cm with an energy output of 500 erg cm^{-2} sec^{-1}. The dose was adjusted by altering the time of treatment. All 3 strains had the constitution A_6/A_6 B_5/B_6 *den*^{2} +/+ *zig*, but differed with respect to *rad*1:
● *rad*1/*rad*1 (AJZ4), ○ *rad*1/+ (AJZ1), △ +/+ (AJZ).

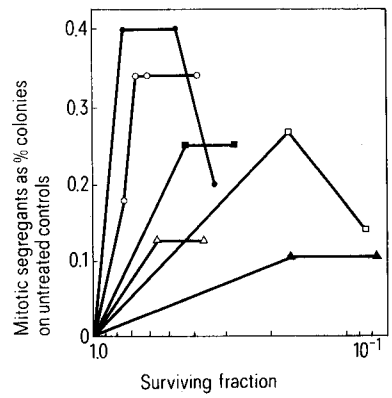

Fig. 2. Comparative effects of mono- or polyfunctional alkylating agents and nitrous acid on mitotic segregation in the strain AJZ4, homozygous for *rad*1, at different survival levels.
●, Tepa; ○, thiotepa; ■, methylmethanesulfonate; ▲, compound AI3-50991; △, AI3-50990 (mono- and difunctional alkylating compound, respectively); □, nitrous acid.

curves, the diploid AJZ4, homozygous for *rad*1, exhibited about 5-fold higher frequency of mitotic segregation than the strains AJZ1 or AJZ.

To test further the sensitivity of the diploid strain AJZ4 towards the recombinogenic effects of other mutagens, known mutagens such as methylmethanesulphonate, nitrous acid, mono- and poly-functional alkylating aziridinyl phosphine oxides were used for treating diploid oidia obtained from the strain AJZ4. The data from the dose-survival curves and the frequency of chemically-induced mitotic segregation at the same dose levels were used to plot the frequency of chemically-induced mitotic segregants against surviving fraction (figure 2). A common feature of the effect of all 6 mutagens is that, with the increase in dose of mutagen or decrease in surviving fraction, there is a gradual increase in mitotic segregation up to peak level, which is characteristic for each compound tested. Thus the tri-functional alkylating compounds Tepa and Thiotepa are presented by their highest peaks in comparison with their chemically-related mono- or difunctional compounds (figure 2). Although at this stage it seems premature to draw a conclusion as to the mode of action of an alkylating compound on mitotic recombination, the results are consistent with the findings of others that monofunctional agents are usually less efficient in inducing chromosomal aberrations than their polyfunctional analogues[17]. The cross-linking agents, such as polyfunctional compounds, have been shown to increase the frequency of genetic exchanges in fungi[18,19] and homologous non-sister exchanges in human leukocytes and fibroblasts[20,21].

The increased sensitivity of the homozygous *rad*1 diploid (AJZ4) as compared with the +/+ and +/*rad*1 strains, towards the recombinogenic effects of UV and chemical mutagens may be assignable to its decreased capacity to repair single strand nicks. As demonstrated in *Coprinus*, the recombination frequency is controlled by the rate of nicking and the time in which unrepaird nicks match and ultimately crossover[22]. UV-sensitive mutants have also been

reported in *Ustilago*[23], *Aspergillus*[24] and *Saccharomyces*[10,25], which enhance mitotic inter- and intragenic recombination in diploid strains, but the possibility that some of the mitotic segregants may have been due to chromosomal abnormalities has not been ruled out.

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Differential lethality in developmental stages of *Drosophila* following X-irradiation

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Summary. A *Drosophila melanogaster* line has been treated with ionizing radiations. The dose-response relationship has been studied upon separate treatment of male and female gametes. The results show that while the total survival is similar, at different developmental stages differences can be observed between progenies from treated male and female gametes. It is suggested that developmental patterns may affect the expression of induced mutations.

Induced mutation frequencies result from a number of factors both internal and external to the cell[1,2]. Treatments with mutagens have shown different responses due to age, developmental stage of treated individuals, stage of cellular division, environmental conditions, sex and so on.

The different sensitivity to X-rays treatment of male and female germ cells, already reported[3] as a greater induced mutation frequency in the males, is thought to be a process of cell selection, which could be more effective in virgin females, considering the different stage of germ cells maturation in the adults of the 2 sexes[4]. On the other hand, the mechanisms effective at the molecular level in modifying the response to mutagenic treatments which may be visualized by the differential damage produced, involve enzymatic activities which are crucial for cellular metabolism[5].

Gatti et al.[6] also explain the induced mutation frequency on the 2 sexes by differences in the efficiency of the induced damage recovery mechanisms, linked to particular enzymatic complexes, which are more or less active in the 2 sexes. These enzymatic activities could be the same involved in the recombination process. On the other hand, genetic mechanisms are needed satisfactorily to explain the genetic control of the mutagenic action, since differences in mutagenic responses are often found to be under genetic control. In a previous experiment[7], different mutagenic responses were obtained by irradiation of different selection lines genetically related; on the basis of the experimental design used, it was argued that the different mutation frequencies observed could result from some kind of control of gene activity.

Since differential gene activity could be expressed at various developmental stages, the work reported in the present paper was aimed at investigating the mutagenic effect of ionizing radiation in successive developmental stages of genetically related progenies.

Material and methods. Flies from a line *(K)* of *Drosophila melanogaster* were treated with several doses of X-rays (1.5, 3.0, 4.5, 6.0 Kr; filter 4 mm. Al.; 250 kV; 8 mA).

The *K* line has the following characteristics: 1. it was obtained by selecting for short wing heterozygous flies vg^+/vg (vg = vestigial on the 2nd chromosome); 2. it is still maintained under selection; matings are allowed only be-

't'-values for the comparison between the regression coefficients of A and B crosses within each line

	K b±SE	t	*Canton* b±SE	t
L/E				
Cross A	−5.56±0.359	4.51**	−6.03±0.804	1.98
Cross B	−9.36±0.762		−8.33±0.838	
1-(L-F)/E				
Cross A	−2.17±0.447	6.43**	−1.98±1.699	1.99
Cross B	1.86±0.379		1.54±0.518	
F/E				
Cross A	−7.27±0.564	0.91	−8.86±1.952	0.56
Cross B	−8.07±0.657		−7.62±1.078	

* = $p < 0.05$; ** = $p < 0.01$.