Influence of caffeine and cystein on the frequency of γ-ray-induced chromosomal aberrations in *Vicia faba*

Treatments	No. of cells analysed	Cells with breaks, bridges and micronuclei (per cent ± SE)	Modulation of index of aberration per cent
OR; Cystein	2856	—	—
OR; Caffeine	2937	—	—
200 R; Distilled water	2734	2.88 ± 0.32	—
Caffeine pre-treatment; 200 R	3096	11.53 ± 0.61	4.00
200 R; Caffeine post-treatment	3655	5.85 ± 0.40	2.03
Cystein pre-treatment; 200 R	4585	1.99 ± 0.21	0.69
200 R; Cystein post-treatment	4364	0.59 ± 0.12	0.20
(Caffeine + Cystein) pre-treatment; 200 R	3739	3.04 ± 0.29	1.06
200 R; (Caffeine + Cystein) post-treatment	3209	2.78 ± 0.29	0.97
Caffeine pre-200 R; Cystein post-treatment	2928	0.07 ± 0.05	0.02
Cystein pre-200 R; Caffeine post-treatment	3155	1.01 ± 0.18	0.35

Modulation index: defined as the ratio of the percent of cells affected by 200 R; distilled water to that irradiated with any pre- or post-treatment under comparison. When the value is about 1 = no modulation; when the value is significantly less than 1 = protection; when the value is significantly more than 1 = sensitization.

ments were allowed to recover for 16 h before fixation in a 1:3 acetic-alcohol. This allowed scoring of cells which were approximately in the G_1 stage of interphase[12] at the time of irradiation. 5 slides per treatment were prepared by Feulgen dyeing and from each of these 10 fields were analysed under high power. Cells with chromosomal aberrations at metaphase and anaphase and micronuclei at interphase were scored (Table). Appropriate non-irradiated controls were also studied but there were no significant effects.

Results and discussion. Our data show that, under conditions of experiment, cystein affords greater protection when applied immediately after irradiation. If radical scavenging is the major mechanism of radioprotection, cystein should be more potent when present during irradiation. As judged from our results, it appears that post-irradiatively added cystein might, in some manner, influence a pathway by which the radiation-induced initial lesions at G_1 are transformed into major chromosome breakage.

Caffeine administered before irradiation is more effective in potentiating the damage than when applied as a post-treatment. Furthermore, its radiosensitizing action gradually diminishes and finally disappears with increasing interval between irradiation and its post-treatment[4]. These observations seem to support the contention that caffeine blocks some of the rapid recovery steps involving either repair replication[9] or replicative synthesis[10,11]. The formation of certain complexes between caffeine and the radiation-induced lesions may, however, be the first step[13]. The observation of enhanced radioprotection following a sequential treatment with caffeine pre- and cystein post-irradiation can also be explained on the basis that caffeine first forms certain complexes with the radiation-induced lesions which are then more effectively removed by cystein. If cystein is not administered to the system immediately after irradiation, sensitization results. When the sequence of treatment is reversed (i.e.) cystein pre- and caffeine post-irradiation, the level of radioprotection is reduced by about 90%; however, it is still approximately 50% more than the cystein post-treatment alone. This may be due to a reduction of about 50% of the complexes formed if caffeine is not present in the system during irradiation.

When equimolar concentrations of both caffeine and cystein are applied, there is neither sensitization nor protection; it is not known whether one counteracts the effects of the other.

Since these results are largely confined to cells irradiated at the G_1 stage, there is need to investigate the modifying effects of caffeine and cystein on cells irradiated at their S and G_2 stages as well.

Résumé. Tandis que l'emploi de caféine avant l'irradiation tend à augmenter le dommage fait aux chromosomes, l'application de cystéine immédiatement après l'irradiation diminue ce dommage. Par conséquent, un traitement de caféine avant et de cystéine après l'irradiation offre le maximum de protection.

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Plastid Differentiation on 6-Azauracil Media

The antimetabolite 6-azauracil received attention in medicine because its cancerostatic activity[1] and its beneficial effect on cell cultures derived from patients afflicted by hereditary orotic aciduria[2]. The base is slowly, and its nucleoside is efficiently, metabolized by mammalian cells to 6-azauridine-5′-monophosphate but di- and triphosphates are apparently not formed[3,4] and the analog is not incorporated in substantial amounts into nucleic acids[5,6]. The primary biochemical effect of the drug is believed to be the inhibition of the activity of orotidylic

acid (05P) decarboxylase[7,8]. The resulting interference with de novo pyrimidine synthesis may deprive the carcinoma or leukemia cells from nucleic acid precursors. The mechanism of the beneficial effect of the drug on orotic aciduria is complicated[9,10]. Humans afflicted by this rare disease appear to be deficient either in both 05P-decarboxylase and 05P-pyrophosphorylase (Type I) or only in the first enzyme (Type II)[2]. Feeding to genetically defective cell cultures 6-azauridine, the level of these enzymes may be restored to near normal[9]. The drug causes accumulation of substrates of the pathway (e.g. orotic acid, dihydroorotic acid) and the latter may stabilize the defective enzymes or the inhibitory effect of 6-azauridylic acid may lead to derepression of the pathway by depletion of products[10].

In *im* mutants (Requests for seed should be addressed to G. P. RÉDEI) of a chromosomal gene locus of the angiosperm *Arabidopsis* when grown under conditions severely interfering with leaf pigment production (aseptic culture under high intensity continuous illumination) 6-azauracil, 6-azauridine and 6-azacytidine partially restore the green color of the plants[11,12] (Figure 1). The response of the plants to feeding the analog is a gradual process. In a period of 2 month culture the pigment content may reach over 60% of that of the wild type plants and may display 10-fold increase relative to the control (Figure 2). In contrast to the utilization of the analog in animal cells[3], in these plants, on molar basis, the azanucleoside is less effective than the base. The metabolism of 6-azauracil to its monophosphate is efficient[13] through the de novo synthetic pathway but the operation of the salvage path appears to be of minor importance. In *Arabidopsis*[13], like in the cocklebur[14], a small amount of radioactivity is incorporated into nucleic acid from ^{14}C-labelled 6-azauracil. The mutant plant tissues also display anomaly in pyrimidine synthesis but in contrast to human orotic aciduria where enzyme activity is reduced, in these plants, grown on minimal media, the activities of both 05P-pyrophosphorylase and decarboxylase are elevated[13]. Furthermore, unlike in hereditary orotic aciduria Type I, where 6-azauridine supply restores the activity of both enzyme[9], in the *Arabidopsis* tissues the analog reduces the amount of the active phosphoribosyltransferase and increases the level of the decarboxylase[13]. The block in the pyrimidine path, the reduction of the level of 05P-pyrophosphorylase, and the inhibition of the activity of the 05P-decarboxylase, results in an accumulation of orotic acid[13].

Dietary orotic acid supply to rats involves the accumulation of lipids in the liver presumably through an imbalance between pyrimidines and purines[15–17]. In the case of abundance of orotic acid the pyrimidine pathway competes apparently more successfully than the purine path for the limited phosophoribosyl pool[18]. Blocking pyrimidine synthesis in *Arabidopsis* distal to orotic acid restores the development of the internal membranes of the plastids (consisting largely of lipids) and it makes possible pigment production under otherwise unfavor-

Fig. 1. *Arabidopsis* mutant *im*^{1} *gi*^{2} grown on minimal (left) and $1.5 \times 10^{-5}M$ 6-azauracil media (right) under continuous illumination for 2 months. The gene *gi*^{2} was used only to delay flowering and to obtain larger plants.

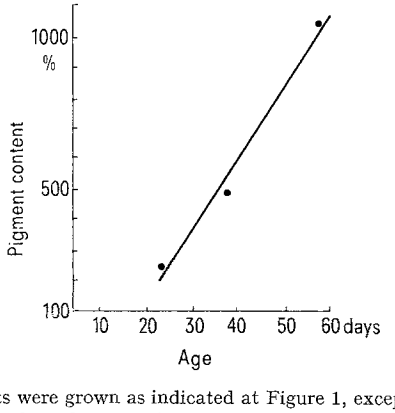

Fig. 2. Plants were grown as indicated at Figure 1, except that they were harvested at the ages given on the abscissa. Chlorophyll was extracted in 85% acetone and absorption was determined and the quantities were calculated on the basis of the formulae given by RÖBBELEN[20]. The pigment content of the azauracil treated plants is expressed in percent of that of the control.

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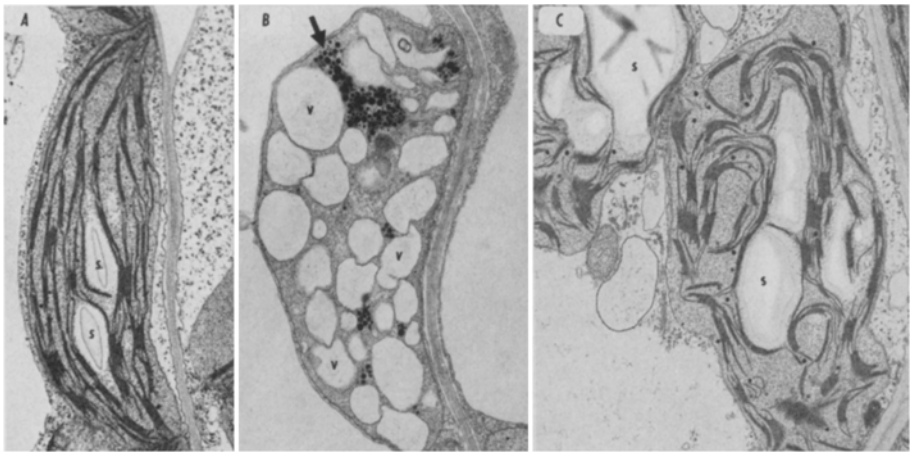

Fig. 3. Electron micrographs of normal wild type chloroplast (A), of *im* mutant (B) as illustrated on the left of Figure 1 and that of the same mutant grown on $1.5 \times 10^{-5} M$ azauracil medium (C). The technique of electronmicroscopic manipulations was given earlier[21]. *s* starch; — osmiophilic globuli; v vacuoles.

able conditions. In the control white cells of the plants, plastid differentiation is arrested at an early stage. Though the outer membrane of this organelle as well as other membranes (nuclear, mitochondrial) appear normal in this condition, the plastids in the white cells display only osmiophilic globuli and vacuoles (Figure 3B). At low concentrations of 6-azauracil the majority of the green cells produced exhibit chloroplasts undistinguishable from the normal ones (Figure 3A). At higher concentrations many of the mutant cells have green and functional chloroplasts as indicated by the large amount of starch accumulated yet the shape of the thylakoids is characteristically curved (Figure 3C). Such abnormal chloroplasts can be seen only rarely in the cells of the wild type plants grown under identical conditions, and even then, the expression of this altered form is much less conspicuous[19].

In the higher plant *Arabidopsis*, mutants at a chromosomal gene locus *im* fail to differentiate normal chloroplasts under high intensity continuous illumination when grown on mineral-glucose-agar aseptic medium. Under these conditions the activity of de novo synthesis of pyrimidines is accelerated. Feeding 6-azauracil to the plants partially restores pigment production and chloroplast differentiation. On $1.5 \times 10^{-5} M$ 6-azauracil media the functional chloroplasts of the mutant cells, exhibit curved thylakoids, however. An important metabolite of the analog, 6-azauridylic acid, selectively inhibits the activity of orotidylic acid decarboxylase and reduces the level of orotidylic acid pyrophosphorylase, and thus increases the orotic acid pool. It is suggested that the increased orotic acid supply may be one factor conducive to plastid differentiation in the mutants where normally, in the absence of the analog orotic acid is rapidly converted into nucleotides and RNA, and the pyrimidine-purine ratio deviates from normal.

Résumé. Les mutants au locus *im* de l'*Arabidopsis* ne réussissent pas à former de chloroplastes normaux dans certaines conditions et montrent des activités plus hautes en synthèse de pyrimidines que le témoin. La nutrition aseptique par 6-azauracile, inhibiteur spécifique de cette voie métabolique, restaure la différenciation de structure lamellaire dans la plupart de ces organites, mais aux concentrations élevées, apparaissent des chloroplastes morphologiquement déviants, quoique de fonction efficace.

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Heterochromatin Localization in the Chromosomes of *Lycosa malitiosa* (Arachnida)

This is a preliminary report on a cytogenetic study of *Lycosa malitiosa*. The main purpose of this work is to study the meiotic process and the heterochromatin localization.

Six males and 8 females from Marindia (Uruguay) were employed. The females were injected with 0.1 ml., 0.04% colchicine solution. After 20 h they were sacrificed and the hemolymphe was extracted from the dorsal vessel and legs and placed in isotonic saline solution (ISS). They were pretreated with ISS and distilled water 1:1 for 25 min and then fixed in metanol-acetic 3/1. The testicular material was dilacerate and treated with 0.025% trypsin solution and all the preparations were stained with Giemsa. The heterochromatin stain procedure was made according to ARRIGHI and HSU's[1] technique with slight modifications.

In the 20 metaphases studied we found identical chromosome complements with diploid number, $2n = 20 + X_1X_2O$ in the male and $2n = 20 + X_1X_1X_2X_2$ in the female, all chromosomes being telocentric (Figure 1).

During the meiotic prophase, the sex chromosomes were observed as strongly condensed in relation to the autosomes. The *X* elements were clearly recognized in the meiotic metaphase. Almost always they were present in an eccentric position in relation to the spindle, quite separate from the autosomes, and identified as 2 long rods lying parallel to each other. During diplonema each bivalent had 1 distal quiasma in the majority of cases and occasionally 2 proximal-distal chiasmata. In the somatic

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