biarmed chromosomes, those with 1 biarmed and 1 acrocentric chromosome, and those with 2 acrocentric chromosomes. In 3 cases, a fourth type was noted in which there were 2 biarmed chromosomes but one (a subtelocentric) had a distinctly shorter second arm than the other (a submetacentric). The chromosome with the shorter second arm apparently arose from a deletion, since the missing heterochromatin was not found translocated to any other chromosome of the complement.

G-bands revealed distinct homologies between the 2 largest autosomes in every case. The only regions not matching band-for-band were those regions that had stained positive for constitutive heterochromatin. Figure b illustrates the G-band patterns of the sex chromosomes and the 4 configurations of the polymorphic pair that were observed by us.

We interpret the C-band and G-band patterns of the heteromorphic autosomal pair as clear indications that the large submetacentric and the large acrocentric chromosomes are homologous except that one has sustained a pericentric inversion in the region containing constitutive heterochromatin. G-bands and C-bands themselves give no indication as to which form gave rise to the other but certain bits of circumstantial evidence indicate that the ancestral form is the submetacentric form. First, the fact that in *Peromyscus, Mesocricetus, Dipodomys*[5,10] totally heterochromatic short arms are abundant without any evidence for pericentric inversions suggests that the majority of events involving heterochromatin are additions/deletions or possibly translocations. Second, one of us (JWW) has examined over 400 *N. micropus* karyologically and found that the number of individuals homozygous for the submetacentric form of the polymorphic chromosome was greater (by over 20%) than the number of heterozygous individuals and more than 3 times as great as the number of individuals homozygous for the acrocentric form. This indicates that the acrocentric form may be less desirable and would be expected, if the placement of heterochromatin next to genes in the euchromatin has induced adverse position effects. Third, *N. micropus* and *N. floridana* appear to be very closely related (morphologically and karyologically) and probably have had a common ancestor[3,11,12]. Since the karyotypes of *N. floridana* and individuals of *N. micropus* with the two large submetacentric autosomes are identical, it seems logical to infer that the submetacentric is the ancestral form and that the subtelocentric and acrocentric forms are recent derivatives of it.

*Résumé.* Les bandes C et G des fivroblastes en culture de *Neotoma micropus*, Baird (le Plains Woodrat), indiquent que la base de la variation chromosomique de cette espèce est une inversion péricentrique comprenant un bloc d'hétérochromatine constitutive. On trouve aussi chez cette espèce une rature encore non-décrite affectant une partie de ce même bloc.

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[10] C. J. Bostock, D. M. Prescott and F. T. Hatch, Expl. Cell Res. *74*, 487 (1972).
[11] W. H. Burt, Misc. Publs Mus. Zool., Univ. Mich. *113* (1960).
[12] E. R. Hall and K. R. Kelson, *The Mammals of North America* (Ronald Press, New York 1959).
[13] Predoctoral fellow in biology supported by National Institutes of Health training grant No. T01 CA 5047 awarded by the National Cancer Institute.
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## A Sequential Caffeine-Cystein Treatment and Enhanced Radioprotection of *Vicia faba* Chromosomes

Recent studies reveal that, in addition to radical scavenging, cystein has a more important biochemical component to its pathway of radioprotection[1-3]. Caffeine (1.3,7 trimethylxanthine) is currently gaining importance as a radiosensitizer of $\gamma$- and x-ray-induced damage in a wide range of actively metabolizing test systems[4,5]; however, in dry barley seeds Kesavan et al.[6] have recently found that it effectively decreases the magnitude of post-irradiation oxygen-dependent damage. There are also a few reports to the effect that caffeine has neither a sensitizing nor a protective action[7,8]. The radiosensitizing action of caffeine has been interpreted as due to its inhibition of either the repair replication[9] or a repair process confined to the replicative synthesis[10,11].

Since metabolic effects of a radioprotector may be expected to be antithetic to that of a radiosensitizer, we initiated a series of studies to investigate the mode and magnitude of modulation of gamma ray-induced chromosomal aberrations in *Vicia faba* pre- and post-treated with cystein and caffeine separately, sequentially or both simultaneously. The results presented here show that a treatment with caffeine applied before irradiation, and cystein immediately afterwards, gives greater protection than cystein alone to chromosomes irradiated at the $G_1$ stage of interphase.

*Materials and methods.* Solutions ($5 \times 10^{-3} M$) of caffeine and cystein separately, as well as a mixture of these in equimolar concentration, were used for treating the actively growing secondary roots of *Vicia faba* at 25 °C for 2 h. The roots were subjected to a total dose of 200 R $\gamma$-rays (3680 Ci $^{60}$Co) at a dose-rate of 38.5 R/sec. The pre-treatment solution were removed immediately after irradiation and the roots were washed in running water before placing them in the respective post-treatment solutions for 2 h. The time elapsed between irradiation and post-treatment was less than 10 min. Roots following all treat-

[1] Zs. Nagy, F. Hernadi, P. Kovacs and T. Valyi-Nagy, Radiation Res. *35*, 652 (1968).
[2] D. Billen, Biochim. biophys. Acta *72*, 608 (1963).
[3] P. Kovacs, Cs. Kari, Zs. Nagy and F. Hernadi, Radiation Res. *36*, 217 (1968).
[4] G. Ahnström and A. T. Natarajan, Int. J. Radiat. Biol. *19*, 433 (1971).
[5] K. Yamamoto and H. Yamaguchi, Mutation Res. *8*, 428 (1969).
[6] P. C. Kesavan, S. Trasi and A. Ahmad, Int. J. Radiat. Biol., *24*, 581 (1973).
[7] D. Gaudin, R. S. Gregg and K. L. Yielding, Proc. Soc. exp. Biol. Med. *141*, 543 (1972).
[8] S. Wolff and D. Scott, Expl. Cell Res. *55*, 9 (1969).
[9] H. E. Bendigkeit and P. C. Hanawalt, Bact., Proc. *103*, 36 (1968).
[10] F. Fabre, Molec. gen. Genet. *117*, 153 (1972).
[11] J. E. Cleaver and G. H. Thomas, Biochim. biophys. Acta *36*, 203 (1969).

Influence of caffeine and cystein on the frequency of $\gamma$-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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*16 April 1973.*

[12] H. J. EVANS and D. SCOTT, Genetics *49*, 17 (1964).
[13] M. DOMON, B. BARTON, A. PORTE and A. M. RAUTH, Int. J. Radiat. Biol. *17*, 395 (1970).
[14] One of us (R.B.) is grateful to the Council of Scientific and Industrial Research, India, for the award of a Junior Research Fellowship during the tenure of this study. We wish to thank Col S. K. MAZUMDAR and Mr. T. KOSHY of Institute of Nuclear Medicine and Allied Sciences, Ministry of Defense for $\gamma$-ray irradiation facility.

## 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