of nectar robbing, 7% and 76% fruit set was recorded among perforated and nonperforated flowers respectively. Thus nectar robbing appears detrimental to the pollination and subsequent fruit set in *V. negundo*.

Rhynchium and *Ropalidia* wasps can be classified as 'nectar-foraging-perforating robbers'[4] or as 'primary robbers'[8]. Other foragers obtain nectar through the holes that have bitten by the primary robbers but do not bite themselves are known as 'secondary robbers'[9].

Generally corolla perforation behaviour can be expected in cases of insects having shorter tongue lengths or flowers with narrow corolla tubes. From the literature, it was proved that perforation behaviour is not necessarily restricted to circumstances where tongue lengths are insufficient to reach nectar[6,10]. Nectar robbing is not always detrimental; some nectar robbers steel the nectar through the perforations, but also collect pollen, thus resulting in pollination[6,7].

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Micronucleus induction in a mammalian cell line subjected to X-radiation and hyperthermia as studied by cytokinesis block method

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The micronuclei induction in a human tumour cell line 4197 *in vitro* was studied after treatment with 4.0 Gy of X-rays, 43°C for 1 h, or X-rays followed by heat, using the cytokinesis block (CB) method with cytochalasin B (CyB). Irradiation produced significantly higher number of binucleate cells (BNC) with micronuclei than hyperthermia. Hyperthermia after radiation reduced the number of cells entering division and further cell progression and also markedly increased the number of BNC bearing more than two micronuclei. This test appears to be a good method to study the relative sensitivity of the mitotically active cells to different treatments.

THE micronucleus technique is a viable alternative to chromosome aberration analysis for evaluating the cytogenetic damage in cells induced by chemical and physical agents. Micronuclei are formed from acentric chromosome fragments[1] or whole chromosomes[2], lagging behind during the anaphase separation and hence excluded from the daughter nuclei. Therefore, at least one cell division is needed before the micronuclei can be produced. However, the conventional micronucleus technique does not give accurate information, since the cells that have divided only once cannot be distinguished from the cells that have undergone more than one division or no division at all. Therefore, counting the micronuclei per nucleus or per 100 nuclei, as is generally done, will not reveal the actual chromatin injury due to treatments. To overcome this problem in human lymphocytes, Fenech and Morley[3] developed a method to block cytokinesis using cytochalasin which does not hinder karyokinesis, so that the once-divided cells can be identified as binucleated cells and the micronuclei score in these cells can be done without ambiguity. *In vitro* studies demonstrated that the cytochalasin block method is more sensitive than the conventional micronuclei assay[4,5]. Ramalho *et al.*[6] have confirmed that the frequency of micronuclei detected by this method is a suitable indicator of the frequency of chromosomal aberrations induced by ionizing radiations. Fenech *et al.*[7] also concluded that the cytokinesis block (CB) micronucleus assay may have the potential to complement metaphase analysis of chromosomes for estimating chromosome damage in human lymphocytes following *in vitro* irradiation. We have used the CB method to study the *in vitro* sensitivity of a human tumour cell line to radiation and hyperthermia.

The tumour cell line 4197 originally derived from a human oral carcinoma was established *in vitro* at the Institute for Medical Radiobiology, University Clinics Essen, Germany. The cells were grown in minimum essential medium (MEM, Gibco) with 20% foetal calf serum. Twentyfour-hour-old cultures were used for the experiment. In a preliminary test, six samples containing 2×10^5 cells each were incubated in 5 ml MEM in 35-mm culture dishes. Cytochalasin B (Sigma, Germany) in DMSO was added at 1 $\mu g\ ml^{-1}$ to each 24-h-old culture and incubated again. Two samples were removed at 24 h, 48 h and 72 h and processed for micronuclei assay by the method described by Shibamoto *et al.*[8] Briefly, the cells were fixed in 1% glutaraldehyde in phosphate buffer after aspirating the medium, treated

with 5 N HCl for 20 min and stained with Schiff's reagent for 60 min in the dark. The cells were then washed in 0.05% N HCl with 0.5% $K_2S_2O_5$. The binucleated cells were scored under the oil immersion of a light microscope (Zeiss, Germany) using a phase-contrast objective. The experiment was repeated once and the pooled data are presented (Figure 1). From this study 48 h was selected for studying the effect of irradiation and hyperthermia.

Six cultures were set as before and two cultures were treated at 24 h with either 2.0 Gy, 4.0 Gy or 6.0 Gy of X-rays (250 kVp, 15 mA, 0.5 mm Cu filter) at a dose rate of 1.0 Gy min^{-1} and incubated with cytochalasin B (CyB) for 48 h. The cells were then processed for micronuclei scoring as before. Duplicate samples were studied from each culture. There was a dose-dependent increase in the number of binucleate cells (BNC) with micronuclei and the BNC with more than two micronuclei showed a significant increase from 4.0 Gy to 6.0 Gy (data not shown). On the basis of this study 4.0 Gy was selected for the experiment with radiation and hyperthermia.

Twelve cultures were set up in 35-mm culture flasks with 10^6 cells per flask. Twentyfour hours after incubation at 37°C they were divided into four groups of three dishes each. One group was exposed to 4.0 Gy of X-rays (RT), the second subjected to 43°C for 60 min in a circulating water bath (HT), and the third group was given radiation followed immediately by HT. The remaining group of three dishes was sham-treated and served as control. Immediately after the treatment all the cells were trypsinized and two subcultures of 2×10^5 cells were set from each flask in 35-mm-culture dishes containing 5 ml medium. CyB at 1 $\mu g\ ml^{-1}$ was added to each dish and incubated for 48 h. The cells were then fixed *in situ* in glutaraldehyde and processed as before for micronuclei scoring in binucleate cells. Six hundred cells were scored from each sample and the percentage of BNC, BNC with one micronucleus, two micronuclei and more than two micronuclei, and cells with more than two nuclei (multinucleates) were scored. The experiment was repeated twice with duplicate samples. Statistical analysis was done by the Student's *t* test.

BNC in normal culture increased from 24 to 48 h and then decreased with a corresponding increase in tri- and tetra-nucleated cells (multinucleates), indicating further progression in cell cycle (Figure 1).

Hyperthermia for 1 h at 43°C did not produce any marked change in the number of cells undergoing first division after treatment (Table 1), but total number of cells entering division (binucleates + multinucleates) was slightly lesser than in control. After irradiation, though there was no change in the number of binucleate cells (BNC), cell progression after the first division was delayed, as indicated by the reduction in the multinucleate cells compared to the control (Table 1). Radiation was more effective than HT in inducing micronuclei and resulted in a significantly higher number of cells with 1 and 2 and more than two micronuclei than in the latter (Table 1). Irradiation followed by hyperthermia resulted in a reduction in the cells which underwent division and a smaller number of cells had progressed to second division at 48 h after treatment. The number of BNC with 1 and two micronuclei was comparable to that in the 4.0 Gy alone group, but there was a significant increase in the BNC with more than two micronuclei, indicating the presence of more unrepaired DNA lesions (dsb) after the combined treatment (Table 1).

Radiation has been reported to induce micronuclei in dividing cells *in vitro* and *in vivo* in a dose-dependent

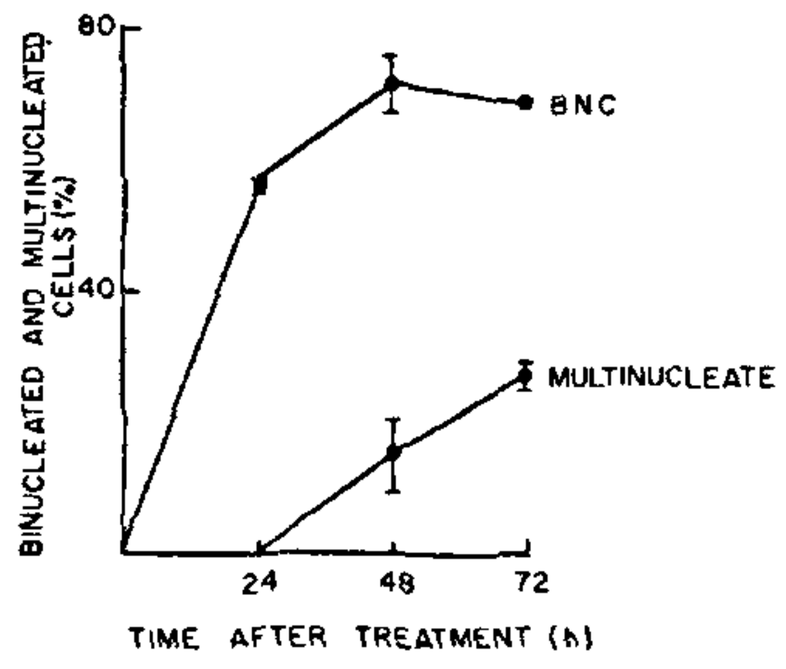

Figure 1. Change in the frequency of binucleate and multinucleate cells with time after cytochalasin B treatment.

Table 1. Micronuclei (MN) frequency in binucleate cells (BNC) at 48 h after irradiation and hyperthermia

Treatment	BNC (%)	BNC with 1 MN (%)	BNC with 2 MN (%)	BNC with >2 MN (%)	Multinucleates (%)
Control	70.10 ± 1.00	8.78 ± 0.57	2.67 ± 0.40	0.46 ± 0.46	17.77 ± 2.15
HT (43 C, 60 min)	70.60 ± 1.75	15.07 ± 2.50	6.83 ± 1.60	5.82 ± 1.22	9.16 ± 1.01
4.0 Gy	79.16 ± 0.51^{a2}	28.95 ± 1.40^{a2}	16.17 ± 0.49^{a3}	11.68 ± 1.68^{a1}	9.88 ± 0.87
4.0 Gy + HT	67.88 ± 0.53^{b3}	24.45 ± 3.40^{a1}	19.37 ± 0.49^{a2,b2}	32.20 ± 5.61^{a2,b1}	5.83 ± 0.84^{a1,b1}

a, Compared with HT group; *b* Compared with 4.0 Gy group. 1, $P < 0.05$; 2, $P < 0.01$; 3, $P < 0.001$.
600 cells were scored for each group.
All values in the treated groups, except BNC (%) in HT, are significantly different from control at $P < 0.05$ to < 0.001

manner[6,9,10]. Shibamoto *et al.*[8] observed in different tumours that, results from *in vivo* and *in vitro* experiments did not differ significantly. A synergistic increase in the BNC with many micronuclei and also a decreased cell progression are evident in the cells treated with HT immediately after 4.0 Gy. This indicates the presence of multiple lesions and unrepaired residual lesions remaining after cell division. Thus hyperthermia immediately after irradiation appears to enhance the cytogenetic damage by inhibiting repair of radiation-induced lesions, which may also convert some of the potentially lethal damage to lethal damage. This could explain the high increase in the cells with many micronuclei. *In vivo* studies on mouse bone marrow after whole body irradiation and local hyperthermia have shown that moderate post irradiation hyperthermia significantly increased the micronuclei frequency and also produced more cells with >1 micronucleus than in the animals receiving only radiation[11].

Even though the micronuclei frequency may not be directly correlated with the number of the chromosome aberrations, it can possibly be used as an index of the response of the mitotically active cells like the proliferating cancer cells and the normal cell renewal systems to combined modality treatment in cancer therapy. This is very important in that many cells which have received lesions will not be able to undergo more than one division after which they will be eliminated from the division pool and hence will not be available for studies at a later stage. Such an elimination has been observed in mouse bone marrow where there was a drastic reduction in cells bearing chromosome aberrations at 48 h after irradiation from the peak value of chromosome lesions observed at 24 h (ref 12).

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Photoinduced DNA strand scission by 1,8-naphthalimide-derived intercalators

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DNA cleavage by non-enzymatic chemical reagents has both basic and applied value. We report here photo-induced strand scission properties of novel DNA intercalators, 4-nitro(*N*-hexylamine)1,8-naphthalimide and 4-amino(*N*-hexylamine)1,8-naphthalimide. The results indicate that the cleavage takes place via a free-radical mechanism and the efficiency of cleavage is unaltered over a long range of pH and ionic strength. These intercalators, therefore, provide a probe for studying DNA conformation, conformational micro-heterogeneity, DNA–ligand interactions and have potential use in genome targeting.

CHEMICAL modification of DNA strand has facilitated DNA sequencing[1], conformational studies[2] and understanding of interaction of small molecules to DNA[3]. Novel mechanistic approaches have been used for DNA cleavage[4], which are also important for their application in chemotherapy[5], gene regulation[6] and genome targeting[7]. A variety of synthetic[8] and natural products[9] have been used in these studies. 1,8-Naphthalimide-derived synthetic DNA intercalators, 4-nitro-(*N*-hexylamine)1,8-naphthalimide **a** and 4-amino(*N*-hexylamine)1,8-naphthalimide **b** (Figure 1) have strong fluorescent properties and have been shown to intercalate with the double helical DNA (unpublished results). Also, a substantial increase in the life-time of the excited state is observed upon intercalation. This increased life-time of the excited state and binding of **a** and **b** to DNA, prompted us to investigate if these intercalators have DNA-cleavage property. We report here that compounds **a** and **b** indeed cause DNA cleavage upon irradiation with visible light.

The DNA-cleavage analysis was carried out by comparing the reduction in intensity of the supercoiled DNA and reciprocal increase in relaxed DNA (and also aggregated DNA under stronger cleavage conditions). At a fixed DNA/intercalator ratio, irradiation time was varied and increased cleavage was observed with

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