Table II. Chromosome aberrations induced in human 48 h leukocyte cultures by lead acetate, X-rays and the combination of both. Treatment time 48 h and 3 h before PHA addition

Treatment	Blood donor	No. of cells analyzed	Dicentrics % of cells	Dicentrics No. per cell	Rings % of cells	Rings No. per cell	Fragments % of cells	Fragments No. per cell
Control	FW♀	600	0.10	0.001	—	—	—	—
(without treatment)	MH♂	300	0.33	0.003	—	—	—	—
Lead acetate	FW♀	400	0.75	0.007	—	—	—	—
(10^{-5} *M*)	MH♂	200	1.00	0.010	—	—	—	—
100 R	FW♀	600	12.00	0.125	7.33	0.078	6.83	0.075
100 R Lead acetate (10^{-5} *M*)	FW♀	600	16.00	0.165	6.67	0.070	7.50	0.080
200 R	MH♂	200	26.00	0.340	15.00	0.160	8.00	0.085
200 R Lead acetate (10^{-5} *M*)	MH♂	300	30.67	0.340	13.00	0.140	7.67	0.083

culture initiation, with colcemid added $4^1/_2$ h before fixation. Table I shows the results of these experiments. Lead acetate induced achromatic lesions (AL), open chromatid breaks (B′), and isochromatid breaks (B″), in frequencies well over the baseline. Exchange type aberrations were completely absent. Chinon I induced the whole spectrum of chromatid aberrations, including exchange type aberrations. The combination experiments (lead acetate + Chinon I) revealed additive effects. Lead acetate has no influence on the induction of chromatid aberrations with the alkylating agent Chinon I.

Lead acetate and X-rays. Two cultures were prepared, each containing 10 ml of culture fluid (HAM'S F-10 medium, complemented with 10% fetal bovine serum, 2.0 mg dihydrostreptomycin, 200 IU penicillin), and 0.8 ml venous blood. To one of these cultures watery lead acetate was added to a final concentration of 10^{-5} *M*. The bottles were incubated for 3 h at 37 °C. After that, 0.3 ml PHA was added and the cultures were distributed in aliquots of 2.0 ml in disposable plastic syringes, 4 with lead acetate and 4 without. From each set of 4 syringes, 2 were irradiated with 100 R immediately after the addition of PHA (100 kV, 8 mA, 2.0 mm Al filter, 58 cm FD, dose rate per minute: 30 R) and 2 were left as controls. The cultures were incubated at 37 °C and chromosome preparations were made after 48 h, with colcemid added $4^1/_2$ h before fixation. In another set of experiments with 200 R we prepared 2 cultures with lead acetate and 1 culture without. The 100 R experiment was performed with female blood and the 200 R experiment with male blood.

As can be seen from Table II, lead acetate exhibits no influence on the X-ray induced chromosome aberrations. In the cells treated with lead acetate alone, some dicentric chromosomes were found. This aberration type was also found in lower frequencies in untreated controls of the blood donors used. This may be an outcome of the fact that both blood donors received high X-ray doses for therapeutical reasons some years before. In the lead acetate treated cultures, the rate of dicentric chromosomes is slightly enhanced. Possibly this is an outcome of the treatment. This is supported by the finding that in lead acetate treated 48 h cultures of another non-irradiated blood donor (BB♂), one dicentric chromosome was found among 500 analyzed cells, in 500 cells of untreated controls of the same donor this aberration type was absent (data not presented in the table). With all 3 blood samples mentioned, the rate of chromatid aberrations in the lead acetate treated 48 h cultures was not elevated over the baseline (data not reported in Table II).

The results of our experiments show that lead acetate has no influence on the rate of chromatid aberrations induced by an alkylating agent, and on the rate of chromosome aberrations induced by X-rays. Striking differences can be seen in cultures treated with lead acetate alone. In 72 h cultures a 24-h-treatment with lead acetate induced open chromatid type aberrations in frequencies well over the baseline. Irrespective of a much longer treatment time (48 h + 3 h), the frequencies of chromatid type aberrations are not elevated in 48 h cultures. In the latter culture system we found some indications of very few dicentric chromosomes being induced by lead acetate.

Zusammenfassung. Experimente mit menschlichen Leukocytenkulturen lassen vermuten, dass die Wirkung von Bleiacetat von der Kulturmethode abhängig ist. In 72-h-Kulturen wurde eine Erhöhung von achromatischen Läsionen, Chromatidbrüchen und Isochromatidbrüchen induziert, nicht jedoch in 48-h-Kulturen. Chemisch- und strahleninduzierte Aberrationshäufigkeiten wurden durch Bleiacetat nicht beeinflusst.

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Control of Tumour-Associated Peroxidases in a Genetic Tumour System in *Nicotiana*

Genetic tumours develop on individuals of specific genotypes. These have been extensively studied in the genus *Nicotiana*[1,2]. Tumours appear on hybrids between particular species of the genus *Nicotiana*, whereas the parental species involved in such hybrids are entirely non-tumourous. Näf[3] proposed that the species entering tumourous combinations may be divided into 2 groups: one arbitrarily designated the plus group, consisting of the species of the section Alatae; whereas the so-called minus group is comprized of species from the other sections of the genus *Nicotiana*. The contribution of these groups is envisaged as differing in some physiological or bio-

chemical manner so that products conducive to tumour growth are formed in the intergroup hybrids. Further characterization of the genetic tumour system in *Nicotiana* has led to a hypothesis[4] that species belonging to the section Alatae or the plus group of NÄF (such as *N. langsdorffii, N. longiflora*) carry a factor I, which is a simply inherited genetic component presumably controlling tumour initiation, whereas the species belonging to the minus group (such as *N. glauca, N. debneyi, N. tabacum*) contribute genetic components that govern different degrees of tumour expression and enhancement (ee). The interaction between these parental contributions (I/ee) in the interspecific hybrids results in tumour formation.

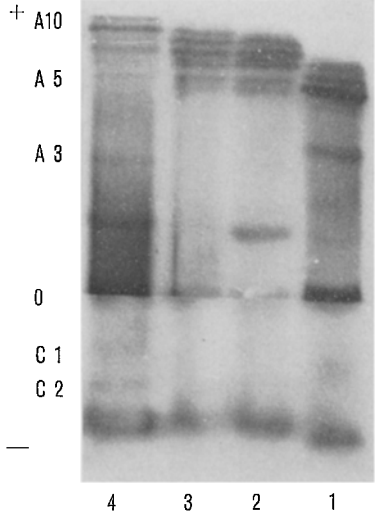
Tumour-prone hybrid plants grow normally under optimal conditions until the flowering stage, suggesting that the action of genetic components (I/ee) remains repressed at the juvenile stage of plant development. Tumours generally appear during and after the flowering phase of the hybrid plants, suggesting that a shift in the hormonal relationship, or some other mechanism, accompanies the change from vegetative (elongation) phase to flowering (senescent) phase, thereby activating the 'tumour genes'. Stresses such as wounding, crowding, chemical treatments, irradiations, etc. can initiate and enhance tumour formation at any stage of the hybrid plant[2,4].

The role of auxin, indole 3 acetic acid (IAA), has been recognized in the process of tumour formation[5–8]. Further peroxidases have been implicated in the oxidation metabolism of IAA[9]; conversely the fact that IAA alters the activity of peroxidase in plant tissues[10,11] suggests that mutual interaction between the auxin and the enzyme may be important in the regulation of growth.

The present investigation was undertaken to study the peroxidase isozymes and their role, if any, in tumour formation in a genetic tumour system in *Nicotiana*.

Leaf tissues from the normal parental species, *N. longiflora, N. debneyi-tabacum*, and their tumour-prone line, in which the *longiflora* chromosome fragment is integrated into the genome of *debneyi-tabacum*[4,12], were used for the present analysis. Tumour tissue was also included in this study.

Electrophoretic techniques for the peroxidases were employed according to the following methodology[13].



Zymogram showing the isozyme pattern of peroxidases in *N. longiflora* (1), *N. debneyi-tabacum* (2), tumour-prone plant (3), and tumour (4). Band A3 is present in *N. longiflora* and the tumour, but it is absent in the tumour-prone plant.

Equal amount (500 mg) of tissue from each type were taken, washed twice with distilled water and ground in chilled mortar. Electrophoresis was conducted in a discontinuous buffer system at pH 8.4, under an applied gradient of 8–10 V/cm for 4–5 h or until the borate front zone had migrated to about 7 cm from the sample slot. During the run, the gel was kept in a refrigerator maintained at 4 °C. After the run, the gel was sliced horizontally into two parts. The presence of peroxidases was demonstrated by incubating the gel strips in a mixture of benzedine solution (1 g benzedine, 10 ml acetic acid and 39 ml water) and equal amount of 3% hydrogen peroxide. The zymogram pattern, which appeared within a few sec, was photographed.

The zymogram of peroxidases is shown in the Figure. The zymogram shows maximally 14 visible isozymes. Since stem tissues did not exhibit any appreciable peroxidase activity, they were not included in the present study. There are 3 distinct patterns of isoperoxidases in the leaves of 3 strains examined. *Longiflora* shows just 9 isoperoxidases as it lacks bands A 8–10, C1 and C4, but it has the isoperoxidase A3; while *debneyi-tabacum* leaf shows 13 isoperoxidases as it lacks A3 band and the intensity of bands A2, A4, and C2 is very light. The tumour-prone plant showed practically the same pattern of major bands as that of *debneyi-tabacum*. The band A3 is absent in the pre-tumourous plant, while this isoperoxidase is present in the tumourand the intensity of bands A2, A4, C1 and C2 is more in the tumour. This indicates that the isoperoxidase A3 is not activated in the pre-tumourous plant until tumour formation. This observation implies that the gene for the A3 transcribes a tumour-specific isoperoxidase which is inherited from the *longiflora* parent, while the genes for A4 and C1, in addition to the genetic background, are contributed from *debneyi-tabacum*. It has been shown[14] recently in the present tumour test system that pre-tumourous plant tissues contain relatively higher levels of IAA compared with their normal parents, and it may be presumed that high IAA levels repress the action of A3 locus in the hybrid before tumour formation.

Zusammenfassung. Isoperoxide-Muster von Nicotianaarten und ihren tumorbildenden Hybriden sind charakteristisch verschieden. Das Tumorgewebe zeigt spezifische Isoenzyme der Peroxidase, die im gesunden Gewebe derselben Pflanze nicht vorkommen.

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