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# SISTER CHROMATID EXCHANGES INDUCED BY CADMIUM IN *Vicia faba*

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ABSTRACT

The cytogenetic damage induced in *Vicia faba* by two cadmium compounds, nitrate ($Cd(NO_3)_2$) and chloride ($CdCl_2$) in concentrations of 0.001, 0.01, 0.1 and 0.5 ppm was analysed by means of the sister chromatid exchanges (SCE) test. The results were obtained with differential stain techniques including 5-bromodeoxyuridine incorporation and the Feulgen reagent. The data showed that chloride did not increase the frequencies of SCE with increasing concentrations, in contrast to the nitrates.

RESUMEN

Se analizó el daño citogenético provocado en *Vicia faba* por dos compuestos de cadmio, el nitrato ($Cd(NO_3)_2$) y el cloruro ($CdCl_2$) en concentraciones de 0.001, 0.01, 0.1 y 0.5 ppm, por medio de la prueba de intercambio de cromátidas hermanas (ICH) empleando la técnica de tinción diferencial que incluye la incorporación de 5-bromodesoxiuridina y el reactivo de Feulgen. Los resultados mostraron que el cloruro no elevó significativamente las frecuencias de ICH al incrementarse las concentraciones, mientras que el nitrato sí lo hizo.

## INTRODUCTION

It has been shown that the alterations in the chromosomes of higher plants give an adequate criterion to evaluate the effects produced by environmental pollutants (De Serres and Shelby 1978, Ma 1982).

*Vicia faba* has been used in numerous studies to detect mutagens through chromosomal aberrations (Gläss 1956, Kihlman 1966, Michaelis and Rieger 1968, Gómez-Arroyo and Villalobos-Pietrini 1983, Gómez-Arroyo *et al.* 1985, 1986) and sister chromatid exchanges (Kihlman 1975, Gómez-Arroyo and Castillo-Ruiz 1985, Gómez-Arroyo *et al.* 1988a, b).

Among the agents able to induce genetic alterations are heavy metals in airborne particles or disolved in water or soil (Hutton 1983). Heavy metals can bind to nitrogen bases, phosphate groups and sugars in DNA nucleotides (Rossman 1981). Linked to the DNA polymerase, heavy metals produce mishapes in DNA replication (Sirover and Loeb 1976, Miyaki *et al.* 1977, Rossman 1981). Cadmium reacts with the polynucleotides in electron donor sites, then the metabolism or the synthesis of nucleic acids can be affected directly (Degraeve 1981). This alteration significantly inhibits *E. coli* and human DNA polymerase (Miyaki *et al.* 1977).

Cadmium can increase or suppress RNA synthesis in microorganisms, plants and mammalian cells (Hoffman and Niyogi 1977, Enger *et al.* 1979, Kazantzis 1981). It also causes breakage of the single-strand in DNA (Mitra and Bernstein 1977, 1978), degradation of the repair system (Klein *et al.* 1976), decrease in the fidelity of DNA synthesis and induce base misparing (Sirover and Loeb 1976, Hoffman and Niyogi 1977, Loeb *et al.* 1977).

The incorporation of tritiated thymidine and uridine is avoided by cadmium applied to mice, rats and rabbits (Lunan *et al.* 1975, Cihak and Inoue 1979). But, when low concentrations of cadmium are used, then the incorporation of tritiated thymidine is increased and doubles the amino acid rate of RNAt (Lishko *et al.* 1985, Hellman 1986, Nicholls *et al.* 1986). In microorganisms, such as *Bacillus subtilis* strains H17 and M45, the effects of cadmium chloride and sulfate are positive in the rec assay, whereas nitrate produces less evident results. In *Escherichia coli* (try$^-$) compounds of cadmium are inactive (Venitt and Levy 1974, Nishioka 1975). The histidine reversion of *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537 and TA1538) also gives negative results with and without metabolic activation (Nishioka 1975, Heddle and Bruce 1977, Kalinina and Polukhina 1977, Polukhina *et al.* 1977, Bruce and Heddle 1979). But in the host mediated assay system in mice, cadmium increases the reversion frequency in *Salmonella typhimurium* (Kalinina *et al.* 1977).

In higher plants,namely *Allium cepa*, *Beta vulgaris*, *Pisum abyssinicum*, *Nigella damascena*, *Crepis capillaris*, *Vicia sativa*, *Hordeum sativum* and *Vicia faba*, cadmium induces chromosomal aberrations (Oehlkers 1953, von Rosen 1953, 1954, Gläss 1955, 1956, Moutschen-Dahmen *et al.* 1965, Degraeve 1971, Ruposhev 1976, Ruposhev and Garina 1976). In rice, chlorophilic mutations are produced by this heavy metals (Reddy and Vaidyanath 1978).

Negative results were observed in *Drosophila melanogaster* treated with $CdCl_2$ when recessive lethal mutations linked to X-chromosome were scored and sterile accumulated in the 2nd chromosome (Ramel and Friberg 1974, Inoue and Watanabe 1978). However, a positive response in dominant lethal mutations are induced in males in the media containing $CdCl_2$ (Vasudev and Krishnamurthy 1979). Chromosomal aberrations and

tetraploidy were observed in the grasshopper *Poecilocerus pictus* (Kumaraswamy and Raja Sekarasetty 1977).

The results of chromosome studies in mammalian and human cells, both *in vitro* and *in vivo* have given contradictory data. In cultured human leucocytes, Shiriashi *et al.* (1972) and Röhr and Bauchinger (1976) have described chromatid and isochromatid breaks and translocations. In contrast, cadmium applied to cultures of human lymphocytes and fibroblasts, of mammary carcinoma cells of mice and in primary cultures of rat embryos did not increase the frequency of SCE and chromosomal aberrations (Zasukhina *et al.* 1977, Voroshilin *et al.* 1978, Umeda and Nishimura 1979, Deaven and Campbell 1980).

The response of mammals in most of the cases of Cd exposure *in vivo* is negative (Ramaya and Pomerantzeva 1977, Bruce and Heddle 1979, Deknutd and Gerber 1979, Watanabe *et al.* 1979). But, $CdCl_2$ has been reported to produce numerical chromosomal aberrations in 8 % of the oocytes of female mice (Shimada *et al.* 1976). In leucocytes of lambs, Cd produces hypodiploidy (Doyle *et al.* 1974) and in mice, chromosomal aberrations in bone marrow cells and spermatocytes (Felten 1978).

In order to verify cadmium as an inductor of SCE in meristematic root cells of *Vicia faba*, several concentrations of cadmium chloride and nitrate were tested.

## MATERIAL AND METHODS

*Vicia faba* (var. minor) seeds were germinated between 2 cotton layers wetted with tap water. The primary roots that reached 2-3 cm were introduced into a solution containing 100 $\mu$M 5-bromo-2'deoxyuridine (BrdUrd), 0.1 $\mu$M 5-fluorodeoxyuridine (FdUrd) and 5 $\mu$M of uridine (Urd) for 20 h. After this, they were treated for 1 h with cadmium chloride and cadmium nitrate, separately, in concentrations of 0.001, 0.01, 0.1 and 0.5 ppm.

Fresh solutions of BrdUrd, FdUrd and Urd were applied for a second replicate cycle (20 h). The treatments were carried out in the dark at 19 °C. Controls were exposed to distilled water and underwent the same procedure.

After this treatment, the meristems were cut and treated with 0.5 % colchicine for 3 h and stained using the Feulgen differential technique described by Tempelaar *et al.* (1982) with the following modification: cuttings were fixed with glacial acetic acid for 1 h, then put into ethanol-acetic acid (3:1) for 2 days at -20 °C and later in 70 % ethanol for 15 min and after hydrolysis in the 5N HCl for 80 min at 28 °C. They were then washed 3 times with distilled water and stained with the Schiff reagent (Feulgen staining) for 10 min in the dark. Then the cuttings were treated with 2 % pectinase dissolved in 0.01 M citrate buffer (pH 4.7) for 15 min at 28 °C, followed by 45 % acetic acid for 10 min and then they were finally transferred to cold 70 % ethanol for 30 min.

Squashing was carried out using 45 % acetic acid and the slides were made permanent using the dry-ice technique (Conger and Fairchild 1953), dehydrated by means of two changes of absolute butanol and then mounted in Canada balsam.

The scoring of the SCE was made in 25 metaphase cells for each experiment and its replicate. Statistical analysis was applied by means of the Student "t" test.

Slides were scored in code so that it was not known to which group they belonged.

## RESULTS AND DISCUSSION

Since the earliest experiments, *Vicia faba* has been very sensitive in showing the genetic damage produced by heavy metals (Gläss 1956, Moutschen-Dahmen *et al.* 1965, Degraeve 1971).

In this investigation it has been observed that in spite of the SCE frequency increasing when the dose of cadmium chloride was increased, there were not significant differences from the controls (Table I). However, 0.01 and 0.1 ppm of cadmium nitrate (Table II) produced SCE frequencies significantly different and with the latter the frequency double the control values were obtained. Cell damage was produced with 0.5 ppm cadmium nitrate.

The basel value for the number of SCE/metaphase in *Vicia faba* is 20 in chromosomes with TT/TB constitution (when cells persist the first cycle in the presence

TABLE I. SISTER CHROMATID EXCHANGES INDUCED BY CADMIUM CHLORIDE IN *Vicia faba*

| *Treatment (ppm)* | *SCEs/metaphase* $\bar{X} \pm S.E.$ | *Value "t"* |
|---|---|---|
| Control | 30.00 ± 0.89 | – |
| 0.001 | 31.02 ± 0.92 | 0.564 N.S. |
| 0.010 | 35.76 ± 1.13 | 2.851 N.S. |
| 0.100 | 36.41 ± 0.88 | 3.621 N.S. |
| 0.500 | 36.81 ± 1.55 | 2.791 N.S. |

N.S. not-significant

of BrdUrd and the second one in thymidine) and 29 in chromosomes TB/BB (when cells remained in both cycles in BrdUrd) (Kihlman and Andersson 1984). This number was in agreement with that obtained in this investigation, where controls, maintained during both replication cycles in BrdUrd, showed frequencies of 30 and 32 SCE/metaphase, respectively (Tables I and II).

Concerning the effect of cadmium on organisms there are still controversies about its mutagenic activity, because the results reported by different laboratories are contradictory, even though the same test system was used (Felten 1978, Bruce and Heddle 1979).

In the cells of mammals, the results depended upon the anion involved. Cadmium sulfide induced chromatid and isochromatid breakages and translocations in human lymphocytes in culture (Shiriashi *et al.* 1972, Röhr and Bauchinger 1976), whereas in other cell lines, the effects were observed but only in toxic dose levels because the non toxic doses were ineffective (Deaven and Campbell 1980, Mukherjee *et al.* 1984). Although, cadmium chloride was not clastogenic in mammalian systems *in vitro* (Sharma and Talukder 1987), cadmium acetate was a very weak clastogen (Gasiorek and Bauchinger 1981).

In most cases with the different evaluation systems for mammals *in vitro*, the results with $CdCl_2$ were negative (Epstein *et al.* 1972, Leonard *et al.* 1975, Bruce and Heddle 1979, Deknudt and Gerber 1979, Sutuo *et al.* 1980). However, $CdCl_2$ injected subcutaneously in female mice, induced numerical aberrations in meiosis (Shimada *et al.* 1976, Watanabe *et al.* 1979). Also acute exposure to this salt induced aberrations in bone

TABLE II. SISTER CHROMATID EXCHANGES INDUCED BY CADMIUM NITRATE IN *Vicia faba*

| *Treatment (ppm)* | *SCEs/metaphase* $\bar{X} \pm S.E.$ | *Value "t"* | |
|---|---|---|---|
| Control | 32.04 ± 0.75 | – | |
| 0.001 | 32.98 ± 0.90 | 0.570 | N.S. |
| 0.010 | 53.63 ± 2.04 | 7.783 | * |
| 0.100 | 73.02 ± 1.85 | 13.158 | * |
| 0.500 | cellular damage | – | |

N.S. not-significant; * $p < 0.001$

marrow cells of mice (Muramatsu *et al.* 1980) and spindle disturbances (Mukherjee *et al.* 1984). On the other hand, cadmium chloride produced chromosomal aberrations as well as spindle disturbaces in plants, namely *Allium cepa*, *Beta vulgaris* (von Rosen 1954), *Hordeum vulgare*, *Nigella damascena* (Degraeve 1971, Moutschen-Dahmen *et al.* 1956), *Crepis capillaris* (Ruposhev 1976, Ruposhev and Garina 1976), *Allium sativum* (Mukherjee *et al.* 1984), and *Eichhornia crassipes* (Rosas *et al.* 1984).

Although, it has been considered that for plants the pH of the solution (6 to 8) and the kind of anion used (chloride, bromide, iodine, nitrate, sulfate and acetate) did not influence the results (von Rosen 1954, Degraeve 1971), in this investigation the type of anion was important because the chloride did not induce SCE whereas the nitrate did. The ingress of nitrate into the cell occurred generally by a mechanism of active transfer, adjusted to the ATPase, but in the case of chloride, transfer stimulated by ATPase has not been found with exception of plants living in strongly saline environments (Clarkson 1984).

In relation to the molecular mechanism involved in the genetic damage induction, cadmium is known to induce DNA single-strand scissions. The incomplete reunion of the altered DNA could be due to the reaction of the metal with the enzymes related to its repair which has been described. However, it has not been possible to produce the same effect in anaerobic conditions, that requires oxygen to induce the mutagenic action because the oxygen-active species produced DNA alteration after exposure of the cells to cadmium (Ochi *et al.* 1983 a, b, Ochi and Ohsawa 1985).

The oxygen-active species superoxide, a hydroxil free radical and hydrogen peroxide were formed as intermediate reactives when oxygen was reduced in order to produce water (Vuillaume 1987).

The divalent metals capable of redox reactions, shared in the enzymatic and autoxidative peroxidation of polyunsaturated fatty acids. Cadmium at higher concentrations than $5x10^{-5}$ M induced the establishment of lipid peroxides in isolated hepatocytes (Stacey *et al.* 1980). Malondialdehyde, secondary product of lipid hydroperoxide, reacted with the DNA inducing mutagenic and carcinogenic effects (Brooks and Klamerth 1968, Schamberger *et al.* 1974, Mukai and Goldstein 1976).

In V79 of Chinese hamster cells treated in culture with cadmium chloride, antioxidants and scavengers of oxygen-active species as catalase, manitol and butylated hydroxytoluene, suppressed partially or completely the induction of the chromosomal aberrations (Ochi and Ohsawa 1985). The fact that aminotriazol (catalase inhibitor) increased the clastogenicity of cadmium, furnished the evidence that hydrogen peroxide was formed by the action of this metal (Ochi and Ohsawa 1985) and that the former was an active builder of fatty acid peroxides. These effects have been mentioned above (Cerutti 1985, Vuillaume 1987).

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