TRADESCANTIA-MICRONUCLEUS TEST ON POTASSIUM DICHROMATE

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ABSTRACT

Cuttings with inflorescences of Tradescantia clone 4430 were allowed to absorb several different concentrations of potassium dichromate during 6 hours and analysed 30 hours later. The frequencies of micronuclei (MCNs) in tetrads were more than twice those of the control. The relationship concentration-frequency was asymptotic.

RESUMEN

Se permitió que cortes de Tradescantia clone 4430 con inflorescencias absorbieran diversas concentraciones de dicromato de potasio durante seis horas y fueran analizadas 30 horas después. Las frecuencias de los micronúcleos (MCNs) observados en las tétradas rebasaron el doble del registro en los testigos, la relación concentración-respuesta fue asintótica.

INTRODUCCION

Nowadays and specially due to industrial development, heavy metals have obtained an important place among environmental pollutants and represent a potential health risk due to their acute toxicity.

A common pollutant is chromium, which is considered an essential micronutrient, involved in the normal metabolism of glucose (Mertz 1969, Freund et al. 1979), and as a cofactor in the increased activity of some enzymes (Mertz 1969). It provides additional structural configuration and stabilization to nucleic acids and it also serves in the maintenance of protein structure (Wacker and Vallee 1959).

Chromium is never found in the uncombined state. Its is found in several oxidation states, the most common are hexavalent (VI) and trivalent (III) forms.

Although in nature plants contain small amounts of metals (Gruskho 1948,
Gilbert 1957, Starich and Blincoe 1983), the role they play in their metabolism is not known (Gilbert 1957). In alfalfa, an organic chromium complex of about 2600 daltons was found. In other plants this complex has a different molecular weight (Starich and Blincoe 1983).

The extent of contamination depends mainly on soil condition, plant type (Davis 1982) and availability to the plant (Davis 1956).

Some of the effects produced by an excess of chromium in plants are: the prevention of development of oats (Hunter and Vergnano 1953), beans (Walace et al. 1976), Rhodesian grass (Soane and Saunder 1959), sugar beets (Pesek and Kolsky 1967), barley (Skeffington et al. 1976), and rice (Kamada and Doki 1974); the inhibition of inflorescence development and delay of stem growth in tobacco (Soane and Saunder 1959); the inhibition of seed germination in Picea abies (Supuka 1974) and in beans (Mukherji and Kumar 1978); and chlorosis in tomatoes, sugar beets, potatoes (Hewitt 1953), tobacco (Soane and Saundor 1959), oats (Hunter and Vergnano 1953) and rice (Verfaillie 1974).

The heavy metal effects involve several plant tissues. As an example the gametes which develop in the inflorescences have been widely used in genetics toxicology as the mother cell chromosomes of pollen grains are sensitive to physical (Sparrow 1951, Ma et al. 1980, 1982, Villalobos-Pietrini and Balderas 1982) and chemical agents (Ma et al. 1978, 1983, 1984, Ma 1979, 1981b, Villalobos-Pietrini and Balderas 1982). The sensitivity of the gametic cells to the mutagens is related to the state of the microsporogenic cycle in which the treatment is applied. There is agreement in the studies made on different plants treated with several agents that the most sensitive state is prophase I (Sparrow 1951, Sparrow et al. 1952, Ochlewsa 1964, Ma et al. 1978, 1980, Ma 1979, 1981a, b, Villalobos-Pietrini and Balderas 1982).

One of the most used plants to detect the damage produced by pollutants in germ cells is Tradescantia, a member of the Commelinaceae, the microsporogenic cycle of which is well known, so for T. reflexa it takes 15 days (Sax and Edmonds 1933) and for T. paludosa it lasts 17 days (Taylor 1950). Among Tradescantia the clone 4430 has been commonly used because of its high sensitivity to chemical mutagens (Van’t Hof and Schairer 1982, Villalobos-Pietrini et al. 1986). In order to evaluate the damage produced to Tradescantia, probably the easiest and most efficient method is the microunuclei (MCNs) in tetrads, at the end of the meiosis (Ma et al. 1978).

Some responses obtained after exposures are acentric fragments (Sparrow and Singleton 1953, Read 1959, Schmid 1976, Ma 1979, 1981a, b), chromosomes with inactivated centromere and isochromosomes (Gómez-Arroyo and Villalobos-Pietrini 1983, Gómez-Arroyo et al. 1985) that are excluded from the daughter nuclei at the end of the cell division and remain in the cytoplasm of the tetrads as MCNs (Ma 1979, 1981a). This test system has also been used to monitor polluted location in situ (Ma et al. 1980, 1984).

It has been shown that chromium (VI) induces mutations in Salmonella typhimurium (Petrilli and De Flora 1977, 1982, Bennicelli et al. 1983), in Escherichia coli (Venitt and Levy 1974, Nakamura et al. 1978, Petrilli and De Flora 1982) and in Schizosaccharomyces pombe (Bonatti et al. 1976); it also produces lethality, mitotic gene conversion and reverse mutations in Saccharomyces cerevisiae (Kharab and Singh 1985); causes lesions to chicken embryo hepatocytes DNA (Tsapakos et al. 1983); induces chromosomal aberrations in bone marrow cells of rats.
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(Bigaliev et al. 1976), embryo cells of hamsters (Tsuda and Kato 1977) and in mouse cells in culture (Umeda and Nishimura 1979); inhibits DNA synthesis and decreases cell survival in hamster fibroblasts (Levis et al. 1977, 1978). It also produces chromosomal aberrations, sister chromatid exchanges (SCE) and mitotic delay in ovary embryos of hamsters (CHO) (Majone and Levis 1979); SCE in human lymphocytes in vitro (Gómez-Arroyo et al. 1981) and in rodent cell cultures, and transformation of hamster cells in vitro (Bianchi et al. 1983); C-mitosis in meristematic cells of Allium cepa (Levan 1945) and Vicia faba root tips (Gómez-Arroyo and Vilalobos-Pietrini 1983); chromosomal aberrations (Gómez-Arroyo and Vilalobos-Pietrini 1983) and SCE in Vicia faba (Gómez-Arroyo et al. in preparation).

Due to the fact that hexavalent forms of the chromium compounds, are the most toxic to living organisms it is worth knowing the effects of potassium dichromate on gametic cells of Tradescantia clone 4430 using the micronuclei in tetrads as a test system.

MATERIAL AND METHODS

The biological material used was Tradescantia clone 4430 which originated as a result of the interspecific cross between T. hirsutiflora and T. subacaulis (Sparow and Sparrow 1976).

The plants were reproduced vegetatively and maintained in plastic trays with a mixture of leaf and moldy sand (2:1) in the greenhouse of the Centro de Ciencias de la Atmósfera, UNAM. They were irrigated twice a week.

210 plants were selected with vigorous stems and young inflorescences. They were cut 6 cm from the top and formed 7 groups.

Cuttings were allowed to absorb through its normal vascular system the potassium dichromate solutions of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0% contained in glasses. The control was exposed to distilled water. All groups were treated for 6 hours.

Later, cuttings were washed with tap water and placed in glasses containing tap water and constant aeration for 30 hours. The inflorescences were fixed in ethanol-acetic acid (3:1) and after 24 hours transferred to 70% ethanol (Ma 1981a).

By means of a dissecting microscope furnished with a micrometric objective, buds of 2.0 — 3.5 cm were selected because in previous observations, tetrads were found in buds of these lengths. Buds were dissected on slides and the anthers were placed in 0.5% aceticarmine and pressed in order to let the pollen mother cells free and to verify the presence of tetrads. The debris were removed before application of the coverglass. Then the slide was heated over an alcohol flame avoiding boiling. Gentle pressure was applied with the palm of the hand over the coverglass and some layers of absorbent paper. Permanent slides were made following Conger and Fairchild technique (1953). In order to avoid bias, slides were handled with a code. For each concentration, 1500 to 3000 tetrads were observed, scoring normal tetrads and tetrads with different numbers of MCNs, expressed at last as number of MCNs per 100 tetrads.

To compare the mean values obtained in the treated and control groups, the difference of proportion test (Spiegel 1961) was applied. The data are the results of one experiment and its replica.
RESULTS

The *Tradescantia* cuttings exposed to the lower concentrations (0.1, 0.2 and 0.4%) of potassium dichromate did not show physiological damage, but up to 0.6%, necrotic areas in the stem and leaves were observed to reach fading in the cuttings treated with 1.0%. In all cases the inflorescences were not affected. The damage observed at the end of the treatment increased after 30 hours.

Table I shows means and standard deviations of MCNs per 100 tetrads for each group. There was not a concentration-response relationship, but by statistical analysis the frequencies of MCNs were found significantly different from the controls (P < 0.001). The frequency of MCNs per 100 tetrads in the groups treated exceeded double that of the control. The response was asymptotic to concentrations (Table I).

### TABLE I. MICRONUCLEI (MCNs) IN TETRADS OF *TRADESCANTIA* INDUCED BY SEVERAL CONCENTRATIONS OF POTASSIUM DICHROMATE

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>MCNs/100 tetrads</th>
<th>X ± S. D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>3.38 ± 0.47</td>
</tr>
<tr>
<td>0.1</td>
<td></td>
<td>7.15 ± 0.68 *</td>
</tr>
<tr>
<td>0.2</td>
<td></td>
<td>7.93 ± 0.47 *</td>
</tr>
<tr>
<td>0.4</td>
<td></td>
<td>6.89 ± 0.40 *</td>
</tr>
<tr>
<td>0.6</td>
<td></td>
<td>7.83 ± 0.33 *</td>
</tr>
<tr>
<td>0.8</td>
<td></td>
<td>7.14 ± 1.04 *</td>
</tr>
<tr>
<td>1.0</td>
<td></td>
<td>7.36 ± 1.00 *</td>
</tr>
</tbody>
</table>

* P < 0.001

DISCUSSION

As noted in Table I, potassium dichromate can affect the chromosomes of the pollen mother cells in prophase I. The plants showed the damage 30 hours later as MCNs which are the consequences of the production of acentric fragments (Read 1959), chromosomes with inactivated centromere (Gómez-Arroyo y Villalobos-Pietrini 1983, Gómez-Arroyo *et al.* 1986) and isochromosomes (Schmid 1976, Gómez-Arroyo y Villalobos-Pietrini 1983, Gómez-Arroyo *et al.* 1986) that are excluded from the nuclei at the end of the meiotic division (Ma 1979, 1981a). The results agree with those of Gómez-Arroyo and Villalobos-Pietrini (1983) who found that the same compound induced MCNs in the meristematic root tips cells of *Vicia faba* and that there was not a concentration-response relationship. However, Ma *et al.* (1984) treating *Tradescantia paludosa* Sax clone 03 with potassium chromate, obtained negative results in the induction of MCNs.

Plants take up chromium from the soil, accumulate it mainly in roots (90%) and of the remainder only 2% is translocated to leaf structures (Huffman and Allaway 1973). In spite of the fact that the cuttings treated in this work do not have roots, the whole amount of chromium probably did not reach the inflorescences, because they did not show physiologic alterations even with the highest concentrations.

Chromium (VI), the form contained by potassium dichromate, has an increased biological activity because it was able to cross the cell membranes (Mertz 1969, Taylor and Parr 1978), using the same transport mechanisms to enter the cells as other oxyanions like phosphate and sulphate (Jennette 1981). In the cytoplasm it is reduced by the enzymes composing the electron-transport cytochrome P-450 and by NADPH (nicotinamide and adenine reduced dinucleotide phosphate) to form Cr III (Jennette 1981) which binds nucleic acids and nucleoproteins (Herrmann and Speck 1954, Levis et al. 1977) and thus can lead to mutagenic and carcinogenic activity (Tsapakos and Wetterhan 1983).

When Cr (VI) comes into contact with micromosomal fraction before crossing the Salmonella cell membrane it is reduced to Cr (III) and thus mutagenic activity is decreased (Löfroth 1978, Bennicelli et al. 1983. Chelators also reduced Cr (VI) to Cr (III) preventing entrance into the cell and decreasing or eliminating its mutagenicity (Gentile et al. 1981). Chromium is one of the carcinogenic heavy metals which is consistently detected as being mutagenic (Babich et al. 1985).

In rats injected intravenously with potassium dichromate, the reduction of Cr (VI) to Cr (III) is made in the blood (Cavalleri et al. 1985) and the only way the latter penetrated liver cells was by binding to a low-molecular-weight substance (Yamamoto et al. 1981). Ormos and Mányai (1977) found that higher chromate concentrations than normal inhibited chromate uptake in the red blood cells.

Tsapakos and Wetterhahn (1983) found little Cr (III) bound to DNA in the absence of the complete microsomal reducing system. Meanwhile the binding of Cr (VI) to DNA in the presence of microsomes and NADPH was caused by a labile intermediate chromium oxidation state, Cr (V) was produced and required for the maximum binding (Jennette 1982).

Within the cell Cr (VI) also damages the lysosomes, leaving free among other enzymes, lipases which hydrolyse tissue lipids producing epoxyaldehydes that have been related to cancer (Schoental 1975).

As can be seen in Table I, the frequency of MCNs in all the groups treated with chromium goes beyond double the control value and is significantly different (P < 0.001). These data point out that potassium dichromate is a good inducer of MCNs, although there was not a concentration-frequency relationship. Instead the response was asymptotic to concentration. This was probably due to inhibition of entry of chromium into the cell when high concentrations were reached (Ormos and Mányai 1977), some extracellular reduction of Cr (VI) occurred (Jennette
1981), low translocation of Cr occurred in the upper part of the plants (Huffman and Allaway 1973), or there was a saturation of sensitive sites of the chromosomes.

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REFERENCES


