CYTOTOXIC AND CLASTOGENIC EFFECTS OF SOLUBLE CHROMIUM COMPOUNDS ON MAMMALIAN CELL CULTURES

A. G. LEVIS AND F. MAJONE

From the Institute of Animal Biology, University of Padua, Italy

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Summary.—The inhibition of cell growth, the reduction of cell survival and the induction of chromosome aberrations and of sister chromatid exchange (SCE) have been determined in cultured hamster cell lines (BHK and CHO) treated with 11 water-soluble compounds of hexavalent and trivalent chromium.

All Cr$^{6+}$ compounds inhibit growth of BHK cells and reduce survival of CHO cells to levels comparable to those obtained only after exposure to 100–1000 times higher Cr$^{3+}$ concentrations. The cytotoxicity curves obtained with the different Cr$^{6+}$ compounds are almost overlapping, whereas marked differences of activity are noticeable among Cr$^{3+}$ compounds. Giant cells are obtained after exposure to Cr$^{6+}$ and Cr$^{3+}$ compounds, as shown by the rise of DNA and RNA per cell, and are due to the blockage of the cell cycle without sudden inhibition of macromolecular syntheses.

Both Cr$^{6+}$ and Cr$^{3+}$ compounds are able to induce chromosome aberrations, whereas Cr$^{3+}$ is absolutely incapable of inducing SCE, only Cr$^{6+}$ being active. The frequency of chromosome aberrations is increased about 10-fold after exposure to 1.0 $\mu$g/ml Cr$^{6+}$, whereas it is only doubled after treatment with up to 150 $\mu$g/ml Cr$^{3+}$. On the other hand, in spite of the sensitivity of CHO cells to the induction of SCE by mitomycin C, the frequency of SCE hardly doubles after exposure to Cr$^{6+}$ compounds.

The present data confirm that Cr$^{6+}$ compounds are characterized by a marked cytotoxicity and clastogenic action on mammalian cell cultures and show that Cr$^{3+}$ compounds, though cytotoxic only at extremely high concentrations and not increasing the frequency of SCE, are not completely without cytogenetic effect, as they are able to induce chromosome aberrations.

A COMPARISON of the cytotoxic and cytogenetic effects of Cr$^{6+}$ (as potassium dichromate) and Cr$^{3+}$ (as chromium chloride) on mammalian cell cultures was carried out in our laboratory, and showed quite different mechanisms of action, on the basis of their effects on the physico-chemical properties of nucleic acids (Tamino, 1977), the uptake of nucleosides and nucleic acid synthesis (Levis et al., 1978a, b; Bianchi et al., 1979), the mitotic cycle (Majone, 1977) and the frequencies of chromosome aberrations and sister chromatid exchanges (Majone & Levis, 1979; Majone & Rensi, 1979). The study of the cytotoxic and clastogenic action of chromium on mammalian cells grown in vitro has been extended here to 11 water-soluble compounds of Cr$^{6+}$ and Cr$^{3+}$.

MATERIALS AND METHODS

Cells.—Cultures of the established pseudodiploid BHK21 Syrian hamster fibroblast line, and of the established pseudodiploid CHO Chinese hamster ovary fibroblast line were grown at 37°C as monolayers, in Eagle's minimal essential medium supplemented with 10% calf serum. Cultures in glass Petri dishes were maintained in a humidified 5% CO$_2$ atmosphere. 0.25% trypsin (Difco 1:250, Detroit, Mich., U.S.A.) was routinely used for subculturing. The cultures were periodically tested for the presence of Mycoplasma by Dr L. Conventi (Institute of Microbiology, University of Padua) with standard selective culture media (Barile, 1973).

Cell growth and labelling.—To determine the cytotoxic action of Cr on cell growth, 10$^5$ BHK cells from log-phase cultures were seeded in 60mm Petri dishes and treated as
specified in the Results section. Cell growth was estimated on the basis of the nucleic-acid content (RNA + DNA) of each culture, determined 5 days after seeding, when controls became confluent. To this purpose nucleotides and nucleic acids were sequentially extracted by differential hydrolysates with perchloric acid (PCA): soluble nucleotides of the intracellular pool were extracted with 5% PCA at 4°C for 30 min, RNA was hydrolysed with 10% PCA at 37°C for 1 h, and DNA was hydrolysed with 10% PCA at 70°C for 2 h. Soluble nucleotides and RNA were measured by UV absorption at 260 nm, DNA was measured at 268 nm, with a Hitachi 200-20 double-beam spectrophotometer. In order to label nucleic acids, the cultures were incubated with tritiated nucleosides (Radiochemical Centre, Amersham, England): thymidine-6-3H ([3H]-Tdr; 2 Ci/mm) and uridine-5-H3 ([3H]-UR; 2-5 Ci/mm) were used at the concentration of 2 μCi/ml. After labelling, nucleotides and nucleic acids were extracted and determined as above, and the radioactivity of liquid samples (0-5 ml) of the different fractions was counted in a Packard Tri-Carb 2425 scintillation counter, using 10 ml of Bray’s solution. As stated elsewhere (Levis et al., 1978a, b) the extraction procedure with PCA allows both quantitative determinations and radioactivity counting of different fractions (soluble nucleotides, RNA and DNA) obtained from the same culture.

Cell survival.—To determine the effects of Cr on cell survival, CHO cells were harvested from log-phase cultures, and samples of single-cell suspensions, properly diluted with growth medium, were plated in 60mm Petri dishes directly in the presence of Cr. The medium was not changed until the 8th day, when the plates were stained with acetic gentian violet, and scored for survivors. All colonies visible to the naked eye were counted as survivors.

Chromosome preparations.—Suspensions of CHO cells were prepared by trypsinization of log-phase cultures and diluted with growth medium in 100mm Petri plates. Each plate received about 8 × 10⁵ cells in 10 ml of medium, which was changed 24 h after seeding. At this time 3 × 10⁻⁵ M bromodeoxyuridine (BUdR, Sigma, St Louis, Mo., U.S.A.) was added and the cells were allowed to incorporate the analogue for two division cycles (30 h). Cr compounds (see below) or mitomycin C (Sigma) were added at the same time as BUdR. The medium was not changed until the cells were collected 30 h later. During the last 4 h of treatment, 0-4 μg/ml of colchicine (Merck, Darmstadt, Germany) were added. At the end of treatment, metaphase cells were dislodged by gently pipetting the overlaying medium and collected by centrifuging the suspension at 900 rev/min for 5 min. The cell pellet was suspended in 5 ml hypotonic buffer (1% sodium citrate) at 37°C for 10 min and fixed in ethanol/acetic acid (3/1). Fixed cells were heated at 89°C for 10 min in 1M NaH2PO4 (pH 8) and stained with Giemsa (Korenberg & Freedlender, 1974). All cultures were treated with BUdR so that sister chromatid exchanges (SCE) and chromosome aberrations were scored on the same cell preparations: 1st and 2nd division metaphases could be distinguished because bifilarly BUdR-substituted chromatids stain paler with Giemsa.

Chromium compounds and cell treatments.—Hexavalent chromium (Cr⁶⁺) was tested as K₂Cr₂O₇ (potassium dichromate), K₂CrO₄ (potassium chromate) and Na₂CrO₄.4H₂O (sodium chromate) (Mallinekrod, St Louis, Mo., U.S.A.), as Na₂CrO₃.2H₂O (sodium dichromate) (Riedel De Haén, Hannover, Germany), as CrO₃ (chromic acid) (Merck, Darmstadt, Germany), and as CaCrO₄ (calcium chromate) (BDH, Poole, England). Trivalent chromium (Cr³⁺) was tested as CrCl₃.6H₂O (chromium chloride) and Cr(NO₃)₃.9H₂O (chromium nitrate) (Merck), as CrK(SO₄)₂.12H₂O (chromium potassium sulphate) and Cr(COOCH₃)₃ (chromium acetate) (BDH), and as Cr(NO₃)₃.9H₂O (chromium nitrate) (Riedel De Haén). All chromium compounds were analytical-grade reagents and were soluble in water at concentrations up to 10⁻³M, except CaCrO₄ and Cr(COOCH₃)₃, which were soluble up to 10⁻⁶M and 8 × 10⁻⁸M respectively. Concentrated solutions were made in twice-distilled water, sterilized by filtration through 0-22μm porous Millipore filters, and kept frozen at −30°C. At the time of treatment Cr solutions were diluted in prewarmed culture medium to the final concentrations used for the experimental treatments (see Results section).

Chromium determinations.—Oxidized Cr⁶⁺ was determined spectrophotometrically at 540 nm by the coloured reaction complex with 1,5-diphenylcarbazide (DFCA, Riedel De Haén) in H₂SO₄-acidified solutions (Taras (De Haén) in H₂SO₄-acidified solutions (Taras...
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et al., 1971). Total Cr was determined by the same reaction after oxidation to Cr$^{6+}$ with potassium permanganate in acid medium (Taras et al., 1971). Reduced Cr$^{3+}$ was then calculated by the difference between total Cr and Cr$^{6+}$ content. The colorimetric method is sensitive to 0.01 μg Cr$^{6+}$/ml final solution, with 5-cm spectrophotometric cells. Beer's law is followed up to a concentration of 2 μg Cr$^{6+}$ per ml final solution (or 2 parts in $10^6$), as shown by the standard calibration curves.

RESULTS

1. Chromium content and oxidation state of the tested compounds

Data on the cytotoxic and clastogenic effects of the different tested compounds have been referred to the actual Cr contents determined by DFCA in the treatment solutions. With regard to the oxidation state of Cr, it was confirmed that all Cr$^{6+}$ compounds contain only oxidized Cr. On the other hand Cr(NO$_3$)$_3$ produced by Riedel was shown to contain about 2 parts Cr$^{6+}$ per 1000 parts Cr$^{3+}$. This was determined in different solutions prepared from separate stocks never used before, so that accidental contamination in the laboratory can be ruled out. Cr(NO$_3$)$_3$ produced by Merck, and all other Cr$^{3+}$ compounds did not react directly with DFCA, but only after oxidation with sodium permanganate: as the colorimetric method allows one to determine 0.01 μg/ml Cr$^{6+}$ in solutions containing up to 1000 μg/ml Cr$^{3+}$ (see Methods section), contamination with more than 1 part Cr$^{6+}$ in $10^5$ parts Cr$^{3+}$ is excluded.

2. Effects on cell growth

Fig. 1 shows the cytotoxic action on the growth of BHK cell cultures of chronic exposures to Cr$^{6+}$ and Cr$^{3+}$ compounds. The cultures were treated starting from the 24th h after seeding and nucleic acid content was determined at the 5th day,

![Graph showing effects of Cr compounds on BHK cell growth.](https://example.com/graph.png)
when controls became confluent. It can be observed that the different Cr⁶⁺ compounds have quite comparable cytotoxic effects: the 50% inhibiting dose (ID₅₀) is about 0.5 µg/ml Cr⁶⁺. Cr³⁺ compounds are cytotoxic only at much higher concentrations, their ID₅₀ being 50–150 µg/ml Cr³⁺, but CrCl₃ and Merck Cr(NO₃)₃ have little or no cytotoxic action even at 300 µg/ml Cr³⁺, which is the maximum solubility in complete growth medium. Riedel Cr(NO₃)₃ which is contaminated by Cr⁶⁺, is more cytotoxic than Merck nitrate, the ID₅₀ being about 180 µg/ml Cr⁶⁺. On the other hand, chromium sulphate and acetate, though not containing Cr⁶⁺, are weakly cytotoxic.

Data on growth inhibition have also been obtained after acute treatments with Cr⁶⁺ and Cr³⁺ compounds (Fig. 2): BHK cell cultures were exposed only for 4 h, starting from the 24th h after seeding, and were reincubated with normal growth medium up to the 5th day, when controls became confluent. The toxicity curves the different Cr⁶⁺ compounds are again very similar, the ID₅₀ being 6–10 µg/ml Cr⁶⁺. On the other hand, in such treatment conditions all Cr³⁺ compounds are almost inactive up to their maximum soluble concentrations.

3. Effects on cell survival

The effects of chronic exposure to Cr and Cr³⁺ compounds on the survival CHO cell cultures are shown in Fig. Single-cell suspensions were seeded at low cell densities (150 cells/dish) in the presence of Cr, so that treatment was initiated before the cells started to divide. Therefore the inactivation of a single cell produces the loss of a "surviving" macroscopic colony. It can be noted that Cr⁶⁺ compounds inactivate cell survival with quite overlapping kinetics, whereas Cr³⁺ compounds have only limited and more variable effects. The 50% lethal dose (LD₅₀) for Cr⁶⁺ is ~0.15 µg/ml, whereas it is abo
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Fig. 3.—Effects of Cr compounds on CHO cell survival. Single-cell suspensions were seeded in the presence of Cr and were treated up to the 8th day, when survival was measured as macroscopic colonies. Symbols for compounds as in Fig. 1.

60 μg/ml Cr³⁺ for Riedel Cr(NO₃)₃ and 150 μg/ml for chromium acetate, and it is not definable for the other Cr³⁺ compounds, owing to their very low toxicity even at the maximum soluble concentrations.

Cr⁶⁺ survival curves show multi-hit inactivation kinetics, that is, an exponential portion preceded by a rather marked shoulder (Fig. 4). If the straight portion of the curve is extrapolated back to the ordinate axis, the intersection occurs close to 10 (not shown).

4. Giant-cell induction and effects on RNA extractability

Microscopic examination of BHK and CHO cell cultures exposed to Cr⁶⁺ revealed that giant cells are induced. After treatment with Cr⁶⁺ concentrations which drastically reduce cell growth and survival, almost pure populations of giant cells can be obtained, both DNA and RNA per cell being greatly increased (Table I). Such an effect can be shown only after treatment with Riedel Cr nitrate and Cr acetate, which have rather marked cytotoxic effects when used at very high concentrations. By contrast, in cultures treated with the other Cr³⁺ compounds, which have much reduced cytotoxicity, the DNA/cell is increased, but the RNA content is much lower than controls. The RNA/DNA ratio is always dramatically reduced after treatment with all Cr³⁺ compounds, whereas it remains almost unchanged after treatment with Cr⁶⁺ (Table I).

The abnormal distribution of optical densities and radioactivities in the different fractions after incubation with [³H]-TdR and [³H]-UR (Table II) indicates that, after treatment with high concentrations of Cr³⁺, RNA is only partially hydrolysed with 10% PCA at 30°C, and is completely extracted at 70°C, thus contaminating the DNA fraction by the usual extraction procedure (see Methods section). RNA can be almost completely and differentially

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extracted at 45°C, but at higher temperatures (53°C) DNA begins to be hydrolysed (Table II). Even soluble-pool nucleotides are not quantitatively extracted with 5% PCA from cultures treated with Cr³⁺.

We have observed that the extractability of RNA is reduced by Cr³⁺ when the cells are treated at the time of seeding or 24 h later, when they are still in monolayer, but is only little affected when crowded, multilayered cell cultures are treated (not shown).

5. Mitotic delay and clastogenic effects

Table III shows the percentages of 1st-division metaphases in CHO cells treated for 30 h with Cr⁶⁺ and Cr³⁺ compounds. All Cr⁶⁺ compounds induce a mitotic delay proportional to the chromium dose: with the concentration of 0.5–1.0 μg/ml Cr⁶⁺ a very marked delay of the cell cycle during the 1st division is observed. Compared to the delays induced by Cr⁶⁺, the effects of Cr³⁺ are much less marked, even if the cells are exposed to very high Cr concentrations (50–150 μg/ml).

The mean number of chromosome aberrations is significantly increased after treatment with all Cr⁶⁺ compounds, in

![Graph](image)

**Fig. 4.—Effects of Cr⁶⁺ compounds on CHO cell survival. Treatment conditions as in Fig. 3. Symbols for Cr⁶⁺ compounds as in Fig. 1.**

### Table I.—Giant cells induced by Cr⁶⁺ and Cr³⁺ compounds in CHO cell cultures

| Treatment*   | [Cr] μg/ml | Cell growth (%) | RNA† | DNA† | RNA + DNA | RNA/DNA |
|--------------|------------|-----------------|------|------|-----------|---------|
| K₂Cr₂O₇      | 0.7        | 100             | 45-7 | 9.2  | 54-9      | 5-0     |
| Na₂Cr₂O₇     | 0.9        | 4               | 135-3| 20-8 | 156-1     | 6-5     |
| K₂CrO₄       | 0.8        | 3               | 127-2| 18-5 | 145-7     | 6-9     |
| Na₂CrO₄      | 0.6        | 5               | 113-2| 19-5 | 131-7     | 6-1     |
| CrO₃         | 0.7        | 6               | 154-3| 29-9 | 184-2     | 5-2     |
| CaCrO₄       | 0.8        | 2               | 140-4| 18-6 | 160-0     | 7-1     |
| CrCl₃        | 262-0      | 1               | 130-0| 26-8 | 156-8     | 4-8     |
| Cr(KSO₄)₂     | 209-0      | 61              | 16-5 | 17-5 | 34-0      | 0-9     |
| Cr(NO₃)₂     | 312-0      | 62              | 18-9 | 15-8 | 32-3      | 1-4     |
| Cr(NO₃)₃     | 312-0      | 2               | 114-9| 54-7 | 169-6     | 2-1     |

* CHO cells were seeded and maintained for 5 days in the presence of Cr compounds. Thereafter nucleic acids were extracted with PCA as detailed in Methods, and the number of cells was determined in parallel cultures.

† μg·10⁻⁶/cell.

‡ Riedel De Haen.

§ Merck.
proportion to concentration (Tab. III); the increase is about 10-fold after exposure to 1.0 µg/ml Cr⁶⁺, as sodium dichromate. On the other hand the frequency of chromosome aberrations is doubled after treatment with up to 150 µg/ml Cr⁵⁺, and it is trebled by exposure to 50 µg/ml Cr⁵⁺ only in the case of Riedel Cr nitrate. A detailed analysis of chromosome aberrations shows that single chromatid breaks, gaps, and interchanges prevail, the frequencies of which increase in proportion to the concentration of Cr. Dicentric chromosomes, isochromatid breaks, chromosome and chromatid rings are also induced, but their frequency does not increase linearly with the Cr concentration.

Table IV shows that the frequencies of SCE in 2nd-division metaphases are significantly increased after treatment with all Cr⁶⁺ compounds and with Riedel Cr nitrate, but not with the other Cr⁵⁺ compounds. However the increase of SCE frequency after treatment with Cr⁶⁺ is much lower than that induced by mitomycin C, which was used as a positive control for the response of our cell system to the induction of chromosome damage.

**DISCUSSION**

The increased incidence of lung tumours in workers exposed to Cr is generally attributed to the carcinogenic effect of its hexavalent oxidation form (Browning, 1969; Furst & Haro, 1969; IARC, 1973). Several Cr⁶⁺ compounds, such as Ca, Zn, Sr and Pb chromates, chromic trioxide and mixtures of K dichromate and Na chromate, are capable of inducing tumours in experimental animals (Hueper, 1961; Roe & Carter, 1969; IARC, 1973; Maltoni, 1977). However, even some Cr⁵⁺ compounds, such as Cr oxide as pure salt (Dvizhkov & Federova, 1967) or as residue of roasted chromite ore (Hueper, 1958; Payne, 1960), chromic acetate (Hueper, 1961) and chromic sulphates such as neo chromium and chrome alum (Maltoni, 1977) have been shown to be carcinogenic, although with a lower frequency and with a longer incubation period than Cr⁶⁺ compounds.

For Cr⁶⁺ compounds, a very good correlation has been found between their carcinogenic action and the cyogenetic effects induced in different biological systems: infidelity of DNA replication in vitro (Sirover & Loeb, 1976), interactions with purified nucleic acids (Tamino, 1977), point mutations in bacteria (Venitt & Levy, 1974; Nishioka, 1975; Petrilli & De Flora, 1977) and yeasts (Bonatti et al., 1976), chromosome aberrations (Tsuda & Kato, 1977; Majone & Levis, 1979; Majone & Rensi, 1979) and stimulation of DNA repair synthesis (Raffetto et al., 1977) in mammalian cell cultures, and in vitro cell
### Table III. - Mitotic delays and chromosome aberrations induced by Cr\(^{6+}\) and Cr\(^{3+}\) compounds in CHO cell cultures

| Treatment         | [Cr] \(\mu g/ml\) | Metaphases counted* | 1st division (%) | Chromosome aberrations | Chromatid aberrations | Isochromatid breaks† | Total |
|-------------------|-------------------|---------------------|------------------|------------------------|------------------------|--------------------|-------|
|                   |                   |                     |                  | Dicentrics | Rings | Gaps | Breaks | Rings | Inter-changes |                     |                 |                   |
| K\(_2\)Cr\(_2\)O\(_7\) | 0-1               | 99                  | 15-1             | 2          | 1     | 6    | 4      | -     | 1           | 3                 | 17               |
|                   | 0-3               | 122                 | 30-3             | 7          | -     | 8    | 7      | 2     | 5           | 7                 | 36               |
|                   | 1-0               | 93                  | 78-5             | 8          | -     | 9    | 6      | -     | 4           | 5                 | 32               |
|                   | 0-1               | 43                  | 93-0             | 2          | -     | 23   | 21     | -     | 5           | 5                 | 56               |
| Na\(_2\)Cr\(_2\)O\(_7\) | 0-3               | 100                 | 25-0             | 9          | 7     | 16   | 18     | -     | 7           | 1                 | 58               |
|                   | 1-0               | 80                  | 77-5             | 11         | 5     | 26   | 30     | 2     | 5           | 1                 | 80               |
| K\(_2\)CrO\(_4\)   | 0-25              | 51                  | 100-0            | 6          | 8     | 78   | 65     | -     | 10          | 2                 | 169              |
| Na\(_2\)CrO\(_4\)  | 0-25              | 100                 | 95-0             | 3          | 3     | 6    | 19     | -     | 1           | 9                 | 41               |
| CrO\(_3\)         | 0-1               | 80                  | 82-5             | 8          | 1     | 15   | 25     | 4     | 2           | 5                 | 60               |
| CrO\(_3\)         | 0-25              | 100                 | 94-3             | 3          | 3     | 27   | 19     | -     | 1           | 9                 | 62               |
| CaCrO\(_4\)       | 0-5               | 88                  | 100-0            | 23         | 1     | 24   | 33     | 7     | 11          | 3                 | 102              |
| CrCl\(_3\)        | 5-0               | 93                  | 29-0             | 11         | -     | 5    | 3      | -     | 1           | 1                 | 21               |
| CrCl\(_3\)        | 50-0              | 90                  | 32-0             | 3          | -     | 15   | 3      | 2     | 7           | 1                 | 31               |
| Cr(NO\(_3\))\(_3\) | 50-0              | 99                  | 34-3             | 3          | 3     | 20   | 18     | 2     | 2           | 3                 | 51               |
| Cr(NO\(_3\))\(_3\) | 150-0             | 100                 | 35-8             | 3          | 1     | 5    | 3      | 1     | 1           | 1                 | 15               |
| KCr(SO\(_4\))\(_2\) | 150-0             | 94                  | 51-1             | 1          | 3     | 9    | 4      | 1     | 2           | 1                 | 21               |
| Cr(COOCH\(_3\))\(_3\) | 5-0               | 88                  | 45-0             | 7          | 4     | 14   | 9      | 2     | 1           | 1                 | 1                |
| Cr(COOCH\(_3\))\(_3\) | 20-0              | 82                  | 48-7             | 5          | 2     | 16   | 11     | 3     | 1           | 1                 | 39               |

* Total at 1st and 2nd division. † Including replicated single-chromatid breaks. ‡ Riedel De Haen. § Merck.
transformation (Fradkin et al., 1975; Tsuda & Kato, 1977). Such a correlation is on the contrary still obscure when the cytogenetic effects of Cr\(^{3+}\) compounds are examined. As a matter of fact they have always given negative results when tested for the induction of point mutations in bacteria (Venitt & Levy, 1974; Nishioka, 1975; Petrilli & De Flora, 1977), and for the stimulation of DNA repair synthesis in mammalian cells (Raffetto et al., 1977). Furthermore, addition of a strong oxidizing agent to several inactive Cr\(^{3+}\) compounds resulted in a dose-effect mutagenic response with the Salmonella test system, due to oxidation to the active hexavalent state (Petrilli & De Flora, 1978b), whereas incubation of mutagenic Cr\(^{6+}\) compounds with reducing agents or metabolic systems, such as liver microsomal fractions and erythrocyte lysates, caused complete loss of mutagenicity, which was ascribed to reduction of the metal to the inactive trivalent form through a simple oxidoreductive reaction (Petrilli & De Flora, 1978a). Also, the clastogenic action of Cr\(^{6+}\) on cultured hamster cells is suppressed by the addition of a reducing agent (Tsuda & Kato, 1977). However, Cr\(^{3+}\) compounds have been shown to interact with purified nucleic acids (Eisinger et al., 1962; Huff et al., 1964; Danchin, 1975; Tamino, 1977) to produce chromosome aberrations in plant (Gläss, 1956) and animal cell cultures (Raffetto et al., 1977; Majone & Rensi, 1979) and to induce cell transformation in vitro (Raffetto et al., 1977). Furthermore, the alteration of DNA replication fidelity seen in the presence of CrCl\(_2\) (Sirover & Loeb, 1976) could be attributed to Cr\(^{3+}\), because divalent Cr compounds are extremely unstable unless carefully protected from oxidation to Cr\(^{3+}\), which takes place very easily in air, water and biological systems (Mertz, 1969).

Very marked differences of cytotoxic (Levis et al., 1978a, b; Luciani et al., 1979) and clastogenic (Majone & Rensi, 1979) activity between Cr\(^{6+}\) (as potassium di-
chromate) and Cr\(^{3+}\) (as CrCl\(_3\)) have been seen in different mammalian cell lines. The present data on the cytotoxic action of 11 water-soluble Cr compounds show that all Cr\(^{6+}\) compounds inhibit growth of BHK cells and reduce survival of CHO cells to levels comparable to those obtained only after exposure to Cr\(^{3+}\) concentrations 100–1000-fold higher.

It must be noted that survival and growth-inhibition curves obtained with the different Cr\(^{6+}\) compounds are almost overlapping when cell inactivation is referred to the actual concentrations of Cr\(^{6+}\) determined by DFCA in the treatment solutions, indicating that the cytotoxic effect is most probably due to the presence of the oxidized metal. On the other hand, marked differences of cytotoxic activity are noticeable among Cr\(^{3+}\) compounds. In particular Riedel Cr nitrate is comparatively more active than the same salt manufactured by Merck, though the presence of a 2 × 10\(^{-3}\) contamination with Cr\(^{6+}\) in the former compound accounted for its cytotoxic effect. Cr sulphate and acetate, though not containing detectable amounts of Cr\(^{6+}\), are more toxic than CrCl\(_3\) and Merek Cr nitrate. Even such small cytotoxic action is therefore related to properties of these compounds other than the presence of Cr, whatever its oxidation state.

CHO survival curves to the different Cr\(^{6+}\) compounds are very similar to those obtained with BHK cells treated with K\(_2\)Cr\(_2\)O\(_7\) (Levis et al., 1978a); namely, they are classical multi-hit curves with an initial, rather pronounced shoulder followed by an exponential portion.

Giant cells were seen after treatment of mammalian cell cultures with Cr\(^{6+}\) (Majone, 1977) and are the consequence of the blockage of the cell cycle without a sudden inhibition of macromolecular syntheses, in particular of RNA and protein syntheses (Levis et al., 1978b). In the present experiments almost pure giant-cell populations are obtained with the rise of DNA and RNA per cell, provided that treatments are made with Cr concentra-

A specific effect of Cr\(^{3+}\) compounds is the resistance of nucleic acids, especially of RNA, to hydrolysis with PCA, which was attributed to the stabilization of nucleic acid tertiary structure by Cr\(^{3+}\) (Levis et al., 1978a) in accordance with the modifications of the physico-chemical properties of purified RNA seen in the presence of CrCl\(_3\) (Huff et al., 1964; Tamino, 1977). Such interpretation seems very unlikely on the basis of the present data, as the reduced extractability of RNA is found even after treatment with Cr\(^{6+}\) concentrations at which cell growth and survival are only partially affected. Also the extractability of soluble pool nucleotides is altered, which is due to dehydration by PCA and not to its hydrolytic action. Moreover, such alterations are noticeable when monolayer cultures are treated, but are much less marked when crowded, multilayer cultures are exposed. Therefore, the alteration of cell membrane by Cr\(^{3+}\), or tanning of extracellular matrix proteins, would more probably be involved, which could affect only the superficial layer of cells and give rise to a reduced permeability to PCA and a reduced extractability of nucleotides and nucleic acids at low temperatures.

The present data on the clastogenic effects of Cr show that both Cr\(^{6+}\) and Cr\(^{3+}\) are able to induce chromosome aberrations, whereas Cr\(^{3+}\) is absolutely incapable of inducing SCE, only Cr\(^{6+}\) being active. As we have already noted (Majone & Levis, 1979), in spite of the sensitivity of CHO cells to the induction of SCE by mitomycin C, the frequency of SCE hardly doubles after exposure to Cr\(^{6+}\) compounds.

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