Inhibition of DNA synthesis in BHK fibroblasts treated in vitro with potassium dichromate

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Summary. Treatment of hamster fibroblasts with potassium dichromate in vitro stimulates tritiated thymidine uptake into the intracellular nucleotide pool. This effect is due to the oxidizing action of hexavalent chromium on the plasma membrane. Dichromate induces also an inhibition of DNA replication, which is due to the interaction of reduced trivalent chromium with specific biological ligands on the DNA molecule.

Environmental contaminants containing metal compounds have been shown to be carcinogenic[1] and mutagenic[2,3] in different biological systems. As for chromium compounds, they significantly increase lung tumor incidence in exposed workers[4,5], are carcinogenic in experimental animals[5,6], induce chromosome aberrations in *Vicia faba* cells[7], point mutations in bacteria[3,8,9] and yeasts[10] and cell transformation in hamster fibroblasts in vitro[11], and are able to interact with purified nucleic acids modifying their physico-chemical and biological properties[12]. These effects have been tentatively attributed to the hexavalent or to the trivalent chromium oxidation state[3,5,12-14].

In order to study chromium effects on nucleic acid and protein synthesis in a hamster fibroblast cell line, we chose potassium dichromate, an hexavalent chromium compound, which is a strong oxidizing agent and has also a great tendency, when reduced to the trivalent state by several cell metabolites, to form coordination complexes which may involve a variety of biological ligands, among which are nucleic acids[13].

Material and methods. Exponential cultures of a hamster fibroblast line (BHK), grown as monolayers in Eagle's basal medium supplemented with 10% calf serum[15], were treated for from 1 to 4 h with potassium dichromate ($K_2Cr_2O_7$, Mallinckroot 6770) in complete

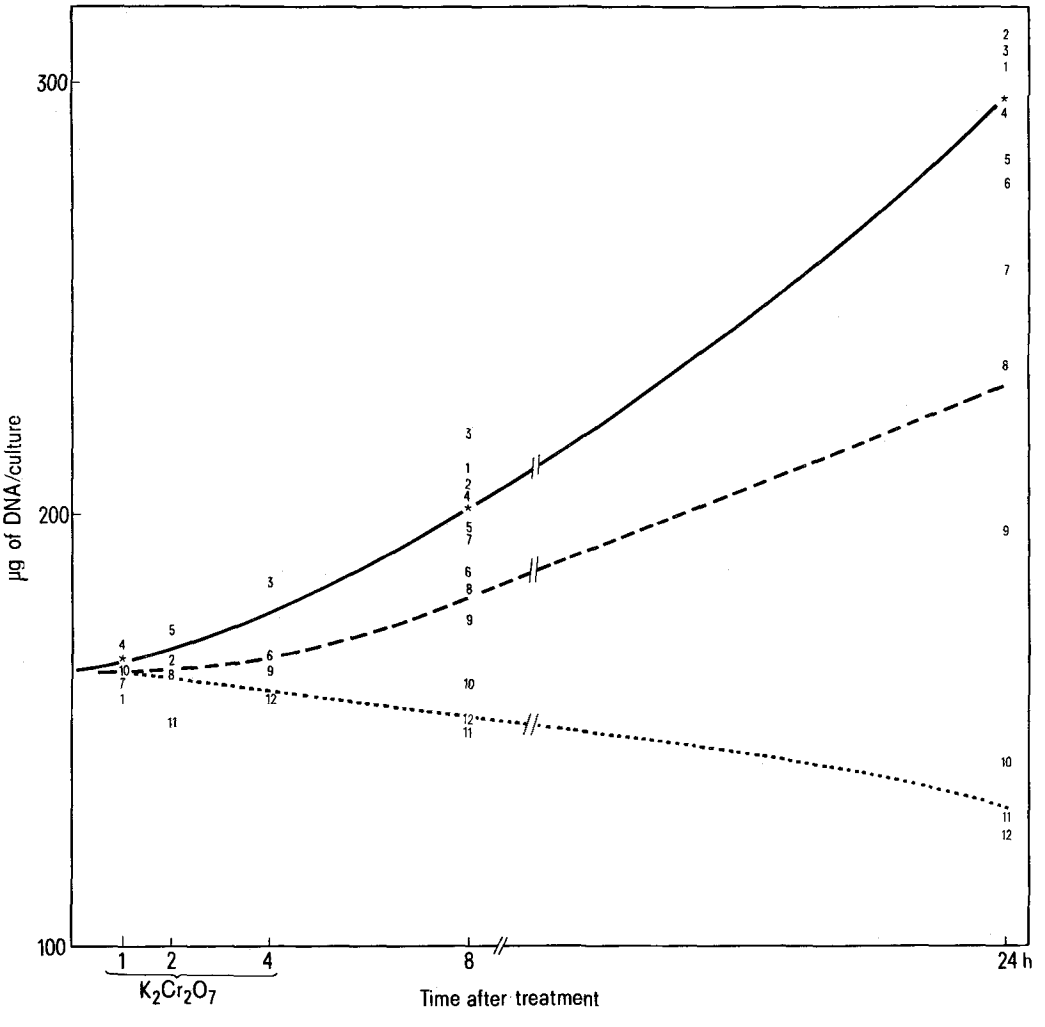

Fig. 1. DNA content in untreated BHK cell cultures (*), and in cultures exposed for 1–4 h to different concentrations of $K_2Cr_2O_7$: 10^{-6} *M*, 1 h (*1*), 2 h (*2*), 4 h (*3*); 10^{-5} *M*, 1 h (*4*), 2 h (*5*), 4 h (*6*); 10^{-4} *M*, 1 h (*7*), 2 h (*8*), 4 h (*9*); 10^{-3} *M*, 1 h (*10*), 2 h (*11*), 4 h (*12*).

growth medium. At the end of the treatment, the $K_2Cr_2O_7$-containing medium was replaced with normal growth medium and the cultures were incubated for one hour with ^{3}H-dT (thymidine-6-H3; Amersham; 2C/m*M*; 1 μC/ml) at different intervals after $K_2Cr_2O_7$ exposure. The intracellular nucleotide pool and the DNA were extracted with perchloric acid[15]. Radioactivity was determined by a Packard Tri-Carb 2425 scintillation counter and DNA was measured by UV absorption at 268 nm using a Hitachi Perkin-Elmer 124 spectrophotometer. The ratios between radioactivity counts and DNA amounts are referred to as specific activities. In the treated cultures, specific activities are expressed as percentages of control values.

Results and discussion. By determining the DNA content in BHK cell cultures treated with $K_2Cr_2O_7$ for from 1 to 4 h, it can be noticed that 10^{-6} *M* and 10^{-5} *M* concentrations have little or no effect on DNA synthesis (Figure 1). On the other hand, a significant DNA synthesis inhibition, proportionate to the duration of treatment, is observed when cultures are exposed to 10^{-4} *M* $K_2Cr_2O_7$. With still higher doses (10^{-3} *M*), a complete

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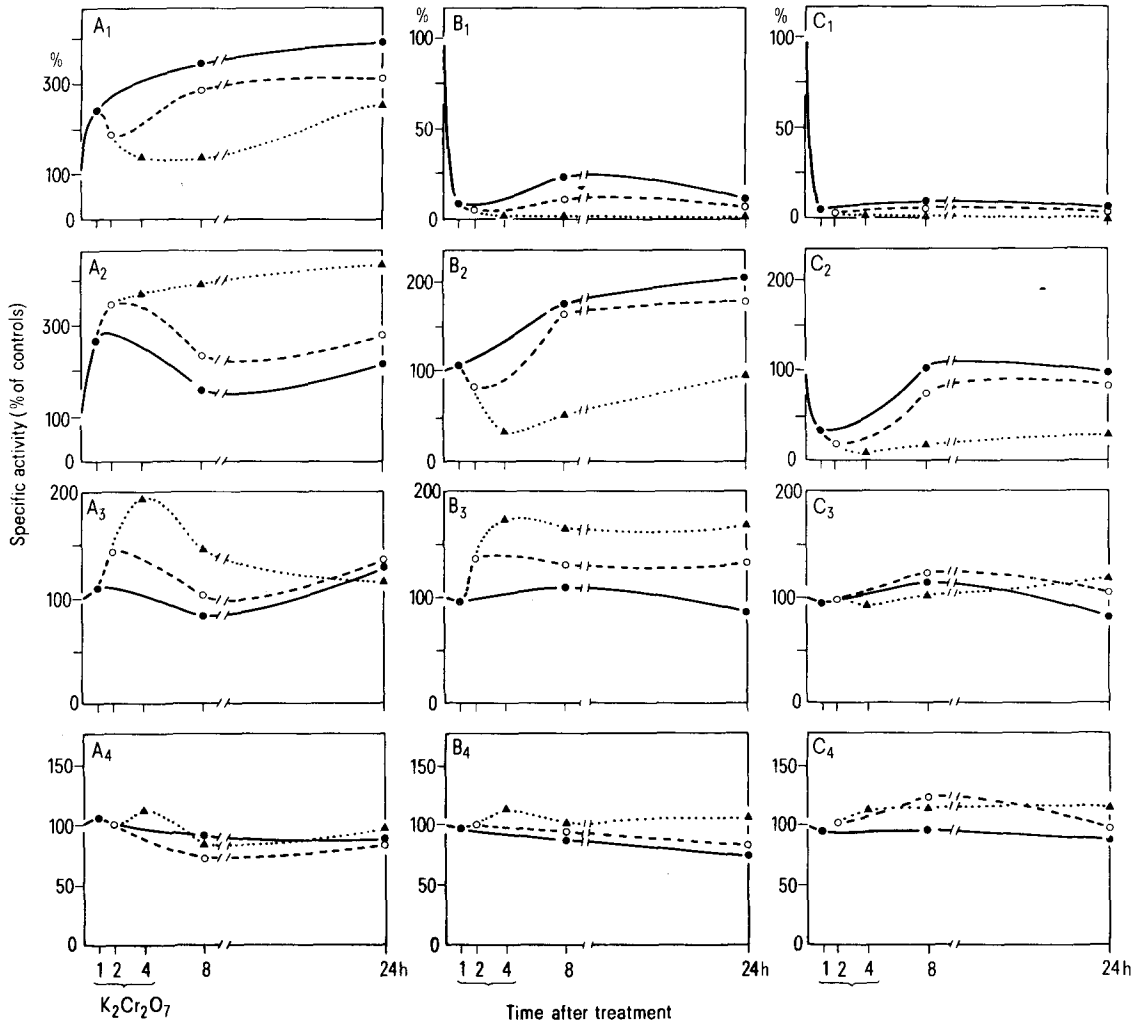

Fig. 2. $K_2Cr_2O_7$ effect on ^{3}H-dT uptake into the intracellular pool and on DNA synthesis in BHK cell cultures. The cultures were treated with 10^{-3} *M* (1), 10^{-4} *M* (2), 10^{-5} *M* (3) and 10^{-6} *M* (4) $K_2Cr_2O_7$ for 1 h (filled circles), 2 h (empty circles) and 4 h (filled triangles). After labelling with ^{3}H-dT at different times after $K_2Cr_2O_7$ exposure, the specific activities of ^{3}H-dT in the nucleotide pool (A) and of DNA (B) were determined. DNA specific activities were normalized as specified in the text in order to obtain the actual rates of DNA synthesis (C).

block of DNA synthesis, as well as a delayed cell death, is revealed by the progressive decrease of DNA content.

These effects of $K_2Cr_2O_7$ on DNA synthesis in BHK cultures are confirmed by the values for ^{3}H-dT incorporation into DNA only after 10^{-3} *M* and 10^{-6} *M* treatments, while the intermediate doses, which have been shown either to inhibit DNA synthesis (10^{-4} *M*) or to leave it unaffected (10^{-5} *M*), lead to a considerable increase of DNA specific activities (Figure 2B). The discrepancy between the two sets of data can be overcome by taking into account the fact that $K_2Cr_2O_7$ also interferes with ^{3}H-dT uptake into the intracellular pool, that is stimulating it when used at concentrations higher than 10^{-6} *M* (Figure 2A). DNA specific activity changes are therefore induced (Figure 2B) which do not directly depend on the actual DNA synthesis rates.

Since the intracellular pool becomes saturated with ^{3}H-dT in a much shorter time than our incubation time[16], the DNA specific activities have been normalized by dividing their original values by the corresponding ^{3}H-dT specific activities. Such normalized values (Figure 2C) therefore express the actual rates of ^{3}H-dT incorporation into DNA and represent the net levels of the DNA synthesis after $K_2Cr_2O_7$ treatment. By this procedure, it becomes evident that the DNA synthesis inhibition induced by protassium dichromate is almost complete and irreversible when cells are exposed to 10^{-3} *M* concentrations, while on the other hand it is more or less pronounced and reversible, according to the duration of treatment, when 10^{-4} *M* concentrations are used. This effect on DNA duplication can be attributed to the action of reduced trivalent chromium, as this represents the only chromium oxidation state present inside the cell even after treatment with hexavalent chromium compounds[13,17]. Moreover, DNA synthesis inhibition is the primary chromium effect on cell macromolecular syntheses, since higher $K_2Cr_2O_7$ doses or longer exposures are required to reduce RNA and protein syntheses to comparable levels[15].

The present results point out that potassium dichromate also independently affects ^{3}H-dT incorporation into the intracellular pool, increasing its uptake across the plasma membrane and thereby producing higher ^{3}H-dT specific activities. This effect is not observed after treatment with trivalent chromium compounds[17] and is most probably related to the oxidizing action of hexavalent chromium on the plasma membrane, which leads to coordination complexes directly involving cell ligands. As in the present experimental conditions potassium dichromate does not inhibit endogenous nucleotide synthesis, unlabelled thymidine concentration in the intracellular pool thus remaining unchanged[15], the observed increase in ^{3}H-dT specific activity seems to be due to an actual stimulation of the mechanisms responsible for nucleoside transport across the plasma membrane[18].

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Effect of photoperiod on early changes in the neonatal rat pineal gland

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Summary. It is concluded that photoperiod has little direct effect on the mitotic activity or morphological development of the pineal parenchymal cell of the rat in the early postnatal period.

If rats are blinded, development of gonads is impeded in intact animals but not in pinealectomized animals[2]. This indicates that pineal function is influenced by the presence or absence of light. At birth, the rat pineal is not complete in its development, since mitotic activity of parenchymal cells is still occurring[3], parenchymal cells have not reached their full size[4] and do not have their full complement of enzymes[5–7]. The following experiment was performed to determine whether or not conditions of lighting could influence some of the anatomical aspects of pineal gland maturation in the immediate postnatal period in the rat.

Materials and methods. Newborn albino rats and their mothers were exposed to one of 3 lighting conditions: normal (12 h of light; 12 h of dark); continuous light (24 h of light) or dark (1 h of light; 23 h of dark). All rooms were air conditioned (25–26 °C) and food and water were provided ad libitum. Tritiated thymidine was injected intraperitoneally at a dosage of 0.5 μC/g body weight on day 1, 7, 14 or 21. Rats were killed 6 h, 7 or 14 days after injection. At euthanasia the pineal gland was fixed in Bouin-Hollande fixative for 24 h and then processed for paraffin sectioning at 5 microns. The sections were dipped in Kodak NTB2 liquid emulsion for autoradiography, exposed for 6–8 weeks, developed and then stained in hematoxylin.

Over 6000 cells from sections through the middle of each pineal gland were studied to determine the percentage of parenchymal cells labeled with tritium and the percentage of parenchymal, neuroglial, endothelial and ependymal cells present. By counting the number of parenchymal cells in 10 ocular grid fields and using the percentage of parenchymal cells present, a rough estimate of the average parenchymal cell size was determined. Results were tested for significance using unpaired Student's *t*-test and correlation coefficients.

Results. Rat pups kept in the dark showed the best weight gain and by day 14 were significantly ($p < 0.01$) heavier than pups from the other groups. Pups kept in

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