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Effects of Potassium Dichromate on Nucleic Acid and Protein Syntheses and on Precursor Uptake in BHK Fibroblasts[1]

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ABSTRACT

Treatments for 1 to 4 hr with 10^{-4} M potassium dichromate, a soluble hexavalent chromium salt with a strong oxidizing power, markedly reduce DNA and RNA accumulation rates in hamster fibroblasts grown *in vitro* (BHK line), as shown by quantitative spectrophotometric determinations. Such inhibitory action is not immediately evident on the basis of the incorporation rates of labeled nucleosides into DNA and RNA, as dichromate affects also the relative concentrations of labeled precursors in the intracellular pool. Dichromate first stimulates and then inhibits nucleoside (mostly thymidine) uptake, whereas amino acid uptake is immediately inhibited. Actual rates of macromolecular syntheses have been calculated by taking into account the induced changes of soluble precursor concentrations; such normalized rates point out that dichromate induces a sudden blockage of DNA replication, whereas RNA and protein syntheses are secondarily inhibited. The observed cytotoxic effects of dichromate are tentatively referred to the oxidation of cell components by hexavalent chromium and thereby to the interaction of reduced trivalent chromium with specific biological ligands on cell membrane and on DNA.

INTRODUCTION

Several chromium compounds, which have been assessed as carcinogenic agents on the basis of epidemiological and experimental evidence (10, 15, 19), also exert a cytogenetic action on different biological systems (21). Hexavalent chromium compounds induce point mutations in bacteria (22, 29, 31) and yeasts (3), infidelity of DNA replication *in vitro* (27), and cell transformation in hamster fibroblasts cultured *in vitro* (9). Even some trivalent chromium compounds, although devoid of mutagenic activity in bacteria (22, 29, 31), display carcinogenic (15) and cytotoxic (16) actions, induce chromosome aberrations in *Vicia faba* cells (11), and interact with nucleic acids in cultured hamster cells (30) or in purified form *in vitro* (5, 6, 14), modifying their physicochemical and biological properties.

Among hexavalent chromium compounds, potassium dichromate is a strong oxidizing agent showing a marked tendency, once reduced to trivalent chromium by several cell metabolites, to form coordination complexes with different biological ligands, including nucleic acids (14, 20). To understand the mechanisms of chromium cytotoxic action, we have undertaken the study of dichromate effects on nucleic acid and protein metabolism in hamster fibroblasts grown *in vitro*, on the basis of preliminary evidence suggesting that dichromate interferes with DNA duplication and nucleoside uptake (17, 18).

MATERIALS AND METHODS

Cells. Cultures of the established BHK21 hamster fibroblast line, clone 12, originally obtained from Dr. R. Zoletto (Istituto Zooprofilattico delle Tre Venezie, Padua, Italy) are routinely grown in our laboratory at 37° as monolayers, in MEM.[2] The cultures are periodically tested for the presence of *Mycoplasma* (with negative results) by Dr. L. Conventi (Institute of Microbiology, University of Padua, Padua, Italy), with standard selective culture media (1).

Cell Treatment and Labeling. Potassium dichromate ($K_2Cr_2O_7$; Mallinckrodt, Inc., St. Louis, Mo.) is solubilized in sterile twice-distilled water at concentrations of 10^{-1} to 10^{-3} M immediately before use and, afterwards, it is diluted 100 times in prewarmed MEM or BSS to final concentrations of 10^{-3} to 10^{-5} M. Experimental treatments are carried out on exponential cultures at 37° in a climatized room; prewarmed solutions are used to avoid any thermic shock.

After different lengths of treatment, the cultures are rinsed twice with BSS, and the medium containing $K_2Cr_2O_7$ is replaced with normal growth medium. At different intervals after exposure to $K_2Cr_2O_7$, the cultures are incubated for 1 hr with tritiated nucleic acid and protein precursors (Radiochemical Centre, Amersham, England); [^{3}H]dThd (2 Ci/mmole), [^{3}H]Urd (2 to 5 Ci/mmole), and L-[4,5-^{3}H]leucine (0.5 to 1 Ci/mmole) are used at concentrations of 1 μCi/ml.

Extraction Procedures and Analytical Methods. From labeled cultures different fractions are sequentially extracted as follows. Nucleotides of the intracellular pool are extracted with 5% PCA at 4° for 30 min, and soluble amino acids are extracted with ethanol:acetic acid (3:1) at room temperature for 30 min (12). Nucleic acids are then extracted by differential hydrolysis with PCA, following a modification (4) of the procedure adapted originally by Feinendegen *et al.* (8) to monolayer cell cultures; *i.e.*, RNA is hydrolyzed with 10% PCA at 30° for 1 hr, and DNA is hydrolyzed with 10% PCA at 70° for 2 hr; proteins are finally extracted with 0.3 N KOH at room temperature for 30 min (13). Soluble nucleotides and RNA are measured by UV absorption at 260 nm, DNA is measured by UV absorption at 268 nm, and proteins are measured by UV absorption at 280 nm, with a Hitachi Perkin-Elmer 124 spectrophotometer. The contamination of the protein fraction with nucleic

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[2] The abbreviations used are: MEM, Eagle's minimal essential medium supplemented with 10% calf serum; BSS, Hanks' balanced salt solution; [^{3}H]dTHd, [6-^{3}H]thymidine; [^{3}H]Urd, [5-^{3}H]uridine; PCA, perchloric acid.

acids is determined on Warburg's tables by taking absorption measurements at 260 and 280 nm (13). Radioactivity counting of liquid samples (0.5 ml) of the different fractions is carried out by a Packard Tri-Carb 2425 scintillation counter, with 10 ml Bray's solution.

The selectivity of the nucleic acid and protein extraction procedure has been checked by measuring the radioactivity distribution in the different fractions, after incorporation of [^{3}H]Urd, [^{3}H]dThd, or L-[4,5-^{3}H]leucine. The above procedure allows both quantitative determination and radioactivity counting of different fractions (soluble nucleotides and amino acids, RNA, DNA, and proteins) obtained from the same cell culture. This method is reliable with conditions in the present experiments since no alteration of nucleic acid sensitivity to the hydrolysis with PCA is induced by treatment with $K_2Cr_2O_7$ but is observed only after exposure of BHK cultures to high concentrations of Cr^{3+} (chromium chloride) (16).

Data Processing. The radioactivity counts in the different fractions of a culture are normalized by dividing them by the DNA amount of the same culture, giving values that are referred to as normalized (radio) activities. In the treated cultures, normalized activities are expressed as percentages of control values. Preliminary observations have shown that $K_2Cr_2O_7$ affects the uptake of labeled precursors into the intracellular pool, changing their relative concentrations and inducing, therefore, changes of nucleic acid and protein (radio) activities that do not directly depend on the actual rates of macromolecular syntheses (17, 18). Since the intracellular pool becomes saturated with labeled nucleotides and amino acids in a much shorter time than the present incubation time (2), the original percentage values of nucleic acid and protein normalized activities have been divided by the corresponding percentage of normalized activities of intracellular precursors. Such values therefore are assumed to express the actual rates of precursor incorporation into macromolecular compounds and represent the net levels of RNA, DNA, and protein syntheses after treatment with dichromate.

Chromium Determinations. Wet decomposition of the biological samples is obtained by mineralization at 180° for 2 to 3 hr with nitric acid:sulfuric acid:PCA (3:1:1) (7). Oxidized, hexavalent chromium (Cr^{6+}) is then determined at 540 nm by the colored reaction complex with 1,5-diphenylcarbazide (Riedel-DeHaën AG, Hannover, Germany) in H_2SO_4-acidified solutions (25). Total chromium is determined by the same reaction after oxidation in acid medium with potassium permanganate (25); reduced, trivalent chromium (Cr^{3+}) is calculated by the difference between total and hexavalent chromium contents.

The colorimetric method is sensitive to 0.05 μg chromium per final solution, with 1-cm spectrophotometric cells. Berr's law is followed up to a concentration of 2 μg chromium per ml final solution (or 2 ppm), as shown by the standard calibration curves.

RESULTS

Treatment for 1 to 2 hr with 10^{-4} M $K_2Cr_2O_7$ induces a marked inhibition of DNA and RNA accumulation in BHK cell cultures, whereas, with still longer exposures (4 hr), a complete block of nucleic acid synthesis results, and even cell death is noticed from the eighth hr (Chart 1). In this experiment, 10^7 cells (containing about 10^{-5} μg DNA and 3 × 10^{-5} μg RNA/cell) are treated; no changes of cellular nucleic acid levels are observed after exposure to $K_2Cr_2O_7$, so that the increase in DNA and RNA content per culture follows the growth curve of the treated cells.

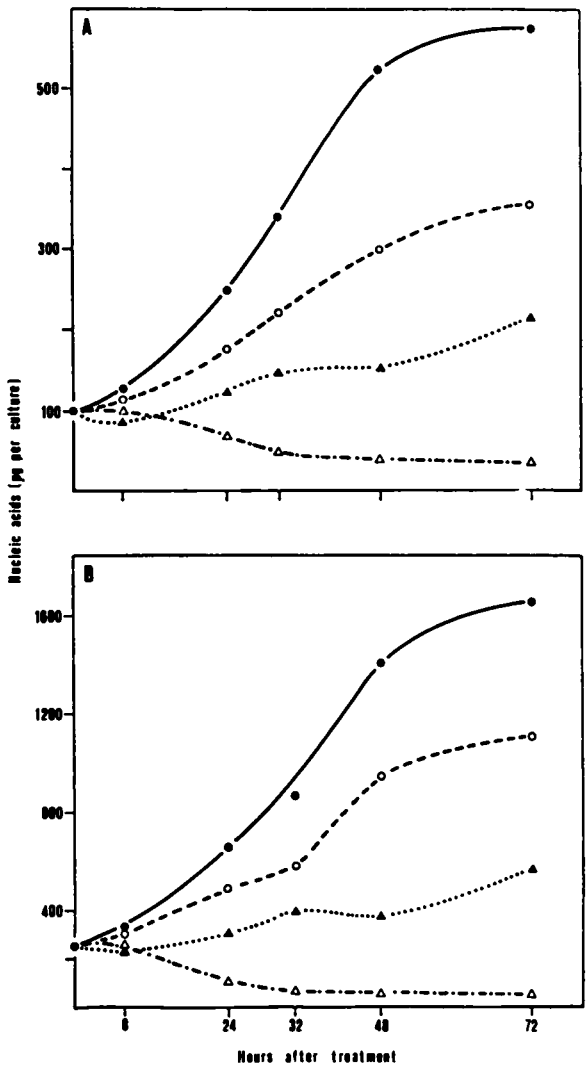

Chart 1. $K_2Cr_2O_7$ effects on nucleic acid accumulation in BHK cultures. The content of DNA (*A*) and RNA (*B*) was determined spectrophotometrically in untreated BHK cultures (●) and in cultures exposed to 10^{-4} M $K_2Cr_2O_7$ for 1 (○), 2 (▲), and 4 (△) hr in BSS.

The assumption of an inhibitory action of $K_2Cr_2O_7$ on nucleic acid synthesis, as suggested by DNA and RNA content determinations, is not consistent with the rates of macromolecular syntheses, as measured by the incorporation of labeled precursors (Chart 2). As a matter of fact, DNA, RNA, and protein normalized (radio) activities are strongly reduced just after $K_2Cr_2O_7$ treatment, but they rise during the hr following this reduction, reaching values comparable to and even much higher than those of controls. Such patterns are particularly evident for DNA (Chart 2*A*) and may be related to a stimulation rather than to an inhibition of nucleic acid synthesis by dichromate.

On the other hand the uptake of labeled precursors into

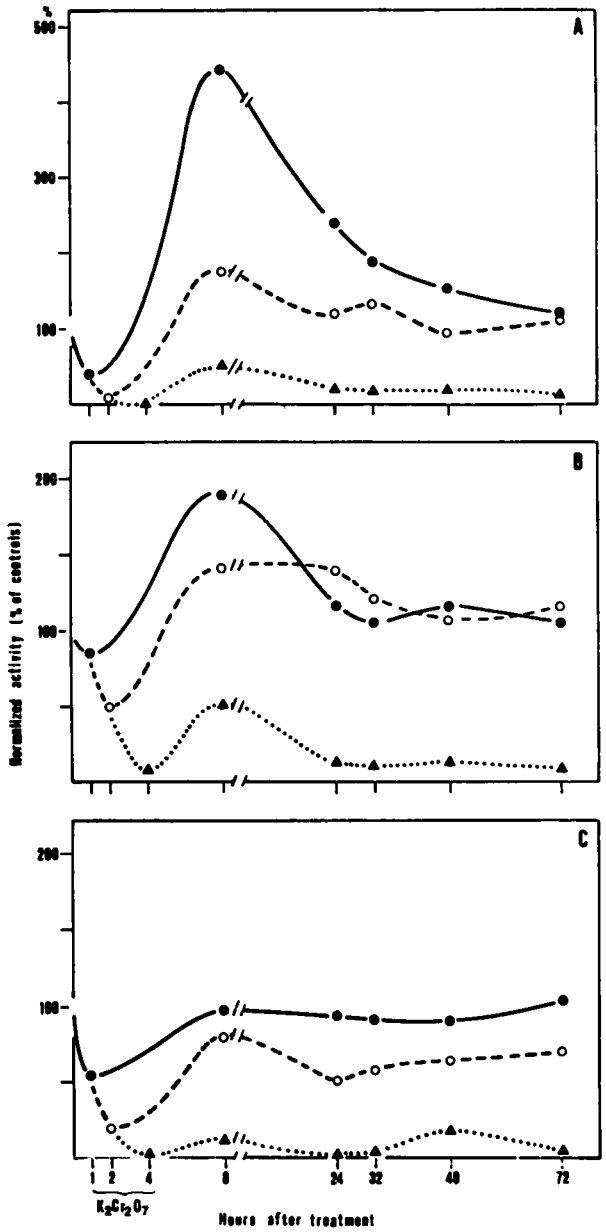

Chart 2. $K_2Cr_2O_7$ effects on the incorporation of labeled precursors into nucleic acids and proteins in BHK cultures. Normalized (radio) activities of DNA (*A*), RNA (*B*), and proteins (*C*) in BHK cultures labeled, respectively, with [^{3}H]dThd, [^{3}H]Urd, and L-[4,5-^{3}H]leucine were determined at different times after treatment with 10^{-4} M $K_2Cr_2O_7$ for 1 (●), 2 (○), and 4 (▲) hr in BSS and are expressed as percentages of control values.

the intracellular pool and hence their relative concentration inside the cells are affected by treatment with $K_2Cr_2O_7$ (Chart 3, *A* to *C*). Nucleoside, and most of all thymidine, uptake is clearly stimulated by 10^{-4} M $K_2Cr_2O_7$; the effect is stable and is even enhanced several hr after the end of the treatment (Chart 3, *A* and *B*). On the contrary leucine uptake is inhibited, and the inhibition is limited to the period of dichromate exposure (Chart 3*C*). Therefore, changes of nucleic acid and protein radioactivities are induced that are not solely related to the rates of macromolecular syntheses. Macromolecular radioactivities have been normalized by taking into account the concentrations of labeled precursors in the intracellular pool (*cf.* "Materials and Methods"). Thus, it becomes evident that the radioactivities of nucleic acids rise considerably over those of control values, about 8 hr after treatment (Chart 3, *D* and *E*), only as a consequence of the increased amounts of labeled nucleotides inside the cells. As a matter of fact, $K_2Cr_2O_7$ induces a sudden inhibition of DNA duplication, which afterwards recovers more or less completely (Chart 3*G*). RNA synthesis is less inhibited (Chart 3*H*), whereas protein sythesis is inhibited even less and is secondarily affected (Chart 3*I*).

The differential action of $K_2Cr_2O_7$ on precursor uptake and macromolecular syntheses becomes more evident when treatment with different concentrations of dichromate is prolonged up to 6 to 9 hr. Thymidine uptake is stimulated, even after long exposures, when 10^{-5} M $K_2Cr_2O_7$ is used, whereas such a stimulation progressively declines with 10^{-4} M and is swiftly followed by an inhibition period with 10^{-3} M dichromate (Chart 4*A*). Actual DNA synthesis is exponentially inhibited according to the concentration of dichromate and the length of treatment (Chart 4*B*).

Uridine uptake is increased according to the $K_2Cr_2O_7$ concentration used, and it is stimulated independently on the length of exposure (Chart 5*A*). When cultures are treated with 10^{-3} to 10^{-4} M $K_2Cr_2O_7$ in BSS, a reduced stimulation and a subsequent inhibition can be observed for uridine uptake as well (2). On the contrary leucine uptake is immediately inhibited by 10^{-3} to 10^{-4} M dichromate, even when treatment is carried out in MEM (Chart 5*B*).

Inhibition of RNA (Chart 5*C*) and protein (Chart 5*D*) syntheses is proportionate to the concentration of $K_2Cr_2O_7$ and to the length of treatment. Extrapolation of macromolecular synthesis-inhibition kinetics points out that the pri-

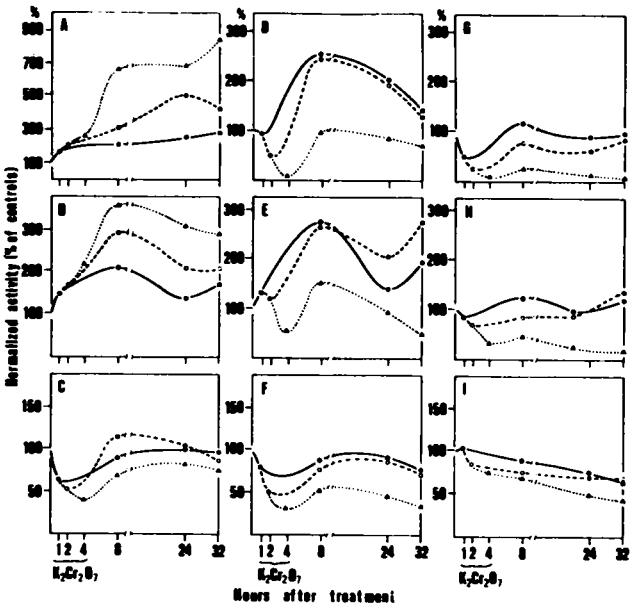

Chart 3. $K_2Cr_2O_7$ effects on precursor uptake and macromolecular syntheses in BHK cultures. Normalized activities of [^{3}H]dThd (*A*), [^{3}H]Urd (*B*), L-[4,5-^{3}H]leucine (*C*) in the intracellular pool and of DNA (*D*), RNA (*E*), and proteins (*F*) were determined in BHK cultures treated with 10^{-4} M $K_2Cr_2O_7$ for 1 (●), 2 (○), and 4 (▲) hr in MEM. The actual rates of DNA (*G*), RNA (*H*), and protein (*I*) syntheses were calculated as specified in "Materials and Methods."

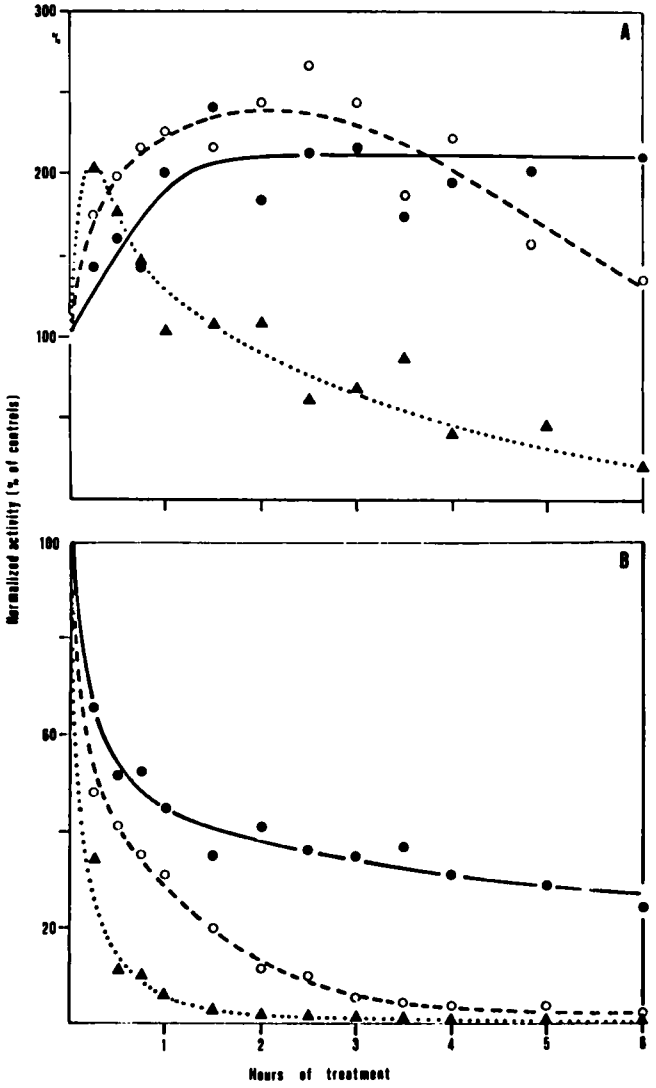

Chart 4. $K_2Cr_2O_7$ effects on thymidine uptake and DNA synthesis in BHK cultures. Normalized activities of [^{3}H]dThd in the intracellular pool (*A*) and normalized rates of DNA synthesis (*B*) were determined in BHK cultures treated for different lengths of time with 10^{-5} M (●), 10^{-4} M (○), and 10^{-3} M (▲) $K_2Cr_2O_7$ in MEM.

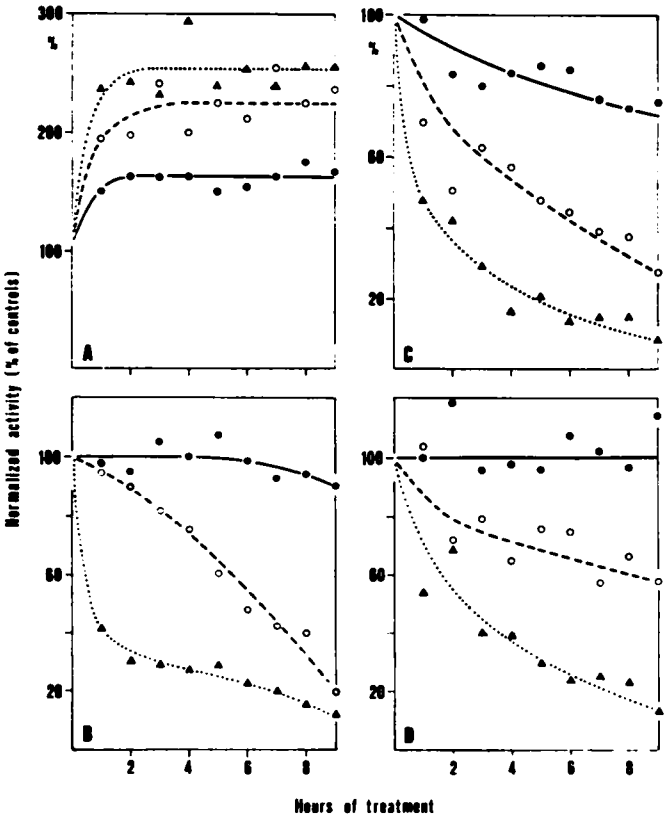

Chart 5. $K_2Cr_2O_7$ effects on uridine and leucine uptake and RNA and protein synthesis in BHK cultures. Normalized activities of [^{3}H]Urd (*A*) and L-[4,5-^{3}H]leucine (*B*) in the intracellular pool and normalized rates of RNA (*C*) and protein (*D*) synthesis were determined in BHK cultures treated for different lengths of time with 10^{-5} M (●), 10^{-4} M (○), and 10^{-3} M (▲) $K_2Cr_2O_7$ in MEM.

Table 1

Differential inhibition of DNA, RNA, and protein syntheses by $K_2Cr_2O_7$ in BHK cell cultures

$K_2Cr_2O_7$ concentration (M)	Time of treatment (hr)	% of macromolecular synthesis inhibition [a]		
		DNA	RNA	Proteins
10^{-3}	1	93	52	45
	3	98	70	60
	6	100	88	75
10^{-4}	1	59	30	0
	3	94	38	21
	6	97	56	25
10^{-5}	1	55	2	0
	3	64	21	4
	6	75	16	0

[a] Percentage of DNA, RNA, and protein synthesis inhibition was calculated by extrapolation of macromolecular synthesis-inhibition kinetics (Chart 4, *B* to *D*).

mary effect of dichromate lies in the blockage of DNA replication, whereas RNA and protein syntheses are secondarily inhibited (Table 1). A differential activity of Cr^{6+} on macromolecular syntheses is always noticed when BHK cells are treated with 10^{-3} to 10^{-5} M $K_2Cr_2O_7$ in MEM or BSS (2).

The concentration of nucleotides in the intracellular pool, as measured by UV absorption, is not appreciably modified even after 6 hr of exposure to dichromate in MEM, but it is significantly reduced when treatment is made in BSS (Table 2). On the other hand, when BHK cells labeled with [^{3}H]dThd or [^{3}H]Urd are exposed to dichromate several hr after the end of labeling, no breakdown of nucleic acids is observed since macromolecular radioactivities remain stable and since no label is detected in the soluble fraction (Table 3).

The kinetics of Cr^{6+} reduction in the medium used for treatment and of Cr^{3+} accumulation in the treated cells (Chart 6) shows that those processes are not instantaneous, as could be assumed on the basis of the well-known oxidizing power of dichromate (15, 20, 24), but that they occur rather gradually and markedly in a dose-dependent manner. In particular, only reduced Cr^{3+} can be detected by the diphenylcarbazide-colored reaction complex in the treated cells, in which reaction a limit value of about 5 ×

10^{-2} μg Cr^{3+} per μg DNA is reached. The amount of cell-linked chromium increases more rapidly the higher the dichromate concentration used (Chart 6), and even more rapidly when cells are treated in BSS, in which the effects on precursor uptake and macromolecular syntheses are more drastic and immediate (2). Moreover, reduction of Cr^{6+} in MEM is quantitatively relevant only with 10^{-5} M $K_2Cr_2O_7$, whereas with higher concentrations of dichromate almost 80 to 90% of Cr^{6+} is stable (Chart 6).

Table 2

Concentrations of nucleotides in the intracellular pool after treatment with $K_2Cr_2O_7$ in BHK cell cultures

The cultures were treated with $K_2Cr_2O_7$ for different lengths of time; afterwards, soluble nucleotides in the intracellular pool were extracted with PCA, and their concentrations were determined by UV absorption, as specified in "Materials and Methods."

$K_2Cr_2O_7$ concentration (M)	Hr of treatment	Nucleotide concentrations in the intracellular pool (% of controls)	
		MEM	BSS
10^{-3}	2	88	75
	4	82	33
	6	85	30
10^{-4}	2	98	90
	4	102	55
	6	100	43
10^{-5}	2	102	99
	4	104	105
	6	98	97

DISCUSSION

Studies that aim to elucidate the mechanisms of chromium cytotoxic action are based on the observation that many chromium compounds, which are carcinogenic in humans and experimental animals (10, 15, 19), are also able to induce point mutations (3, 22, 27, 29, 31) and cell transformation (9). It is generally accepted that the biologi-

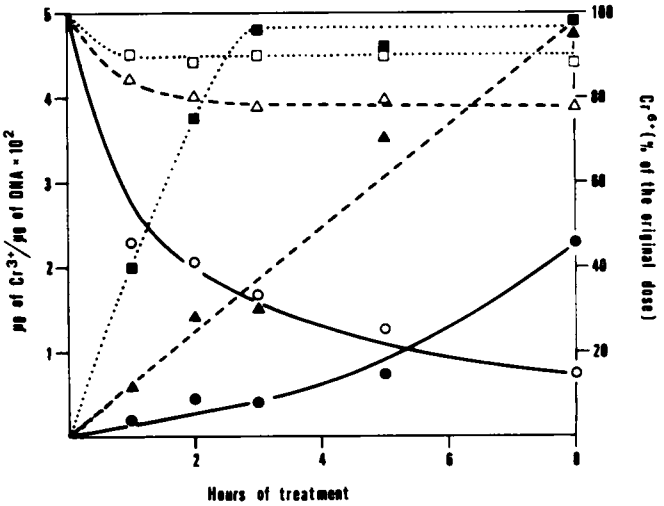

Chart 6. Cr^{6+} reduction in $K_2Cr_2O_7$ solutions and Cr^{3+} accumulation in BHK cultures. Cr^{6+} levels in the growth medium (*open symbols*) and Cr^{3+} levels in the cells (*closed symbols*) were determined spectrophotometrically by the diphenylcarbazide complex reaction in BHK cultures treated for different lengths of time with 10^{-5} M (●; ○), 10^{-4} M (▲; △) and 10^{-3} M (■; □) $K_2Cr_2O_7$ in MEM.

Table 3

Stability of RNA and DNA in BHK cell cultures after treatment with $K_2Cr_2O_7$

BHK cell cultures, incubated for 3 hr with 0.2 μCi [^{3}H]Urd or [^{3}H]dThd per ml and then transferred for 16 hr to unlabeled growth medium, were treated with $K_2Cr_2O_7$ for different lengths of time in MEM or BSS. At the end of treatment, soluble nucleotides in the intracellular pool and macromolecular nucleic acids were extracted with PCA, and their radioactivity was determined, as specified in "Materials and Methods."

$K_2Cr_2O_7$ concentration (M)	Solution	Hr of treatment	Nucleotide pool (cpm)		Nucleic acid normalized activity	
			[^{3}H]Urd	[^{3}H]dThd	RNA	DNA
None		2	112	34	1.431	16.707
		4	117	30	1.339	13.437
		8	135	36	1.208	13.055
10^{-5}	BSS	2		37		14.665
		4		37		14.123
		8		29		13.316
10^{-4}	BSS	2	84	31	1.308	13.316
		4	59	33	1.157	12.285
		8	49	31	1.265	12.450
	MEM	2		37		15.251
		4		31		14.704
		8		47		14.845
10^{-3}	BSS	2	81		1.326	
		4	47		1.211	
		8	51		1.241	
	MEM	2		35		14.913
		4		34		12.613
		8		33		13.000

cal activity of Cr^{6+} compounds is related to their strong oxidizing tendency (15, 20, 24); this has been suggested to result in the formation of aldehydes and epoxyaldehydes that may be the actual mutagenic and carcinogenic agents (26). However, some Cr^{3+} compounds also, although devoid of oxidizing power, are carcinogenic in animals (15), interact with nucleic acids purified *in vitro* (5, 6, 14), interfere with macromolecular syntheses (16), induce chromosome aberrations (11), and modify some physicochemical properties of nucleic acids in cultured mammalian cells (30). On the other hand Cr^{3+} represents the only stable form of chromium that can be detected inside the cells, even after exposure to Cr^{6+} compounds, by the standard colorimetric reaction (Chart 6) as well as by the atomic absorption spectrophotometry (7, 20). The lack of mutagenic activity reported for Cr^{3+} in bacteria (22, 29, 31) may be attributed to its low ability to pass through the plasma membrane (24) and its even lower ability to pass through the cell membranes of bacteria.

The present results point out that potassium dichromate, a Cr^{6+} salt with a strong oxidizing power, reduces the accumulation of nucleic acids in cultures of BHK hamster fibroblasts, as determined spectrophotometrically on the basis of the growth curves (Ref. 18; Chart 1). When $K_2Cr_2O_7$-treated cells are incubated with labeled precursors, alterations of the radioactivity levels are noticed for nucleotide and amino acid pools as well as for nucleic acid and protein macromolecular fractions (Charts 2 and 3); *i.e.*, the radioactivities of amino acid and protein fractions are reduced, especially just at the end of treatments, whereas those of nucleotides are markedly increased. As for nucleic acid activities, they are lower than those of controls throughout the observation time after a 4 hr exposure to $K_2Cr_2O_7$, whereas, for shorter exposures they are reduced only at the end of treatments reaching, afterwards, levels much higher than those of controls.

For estimation of the actual rates of nucleotide incorporation into nucleic acids in the $K_2Cr_2O_7$-treated cells, macromolecular radioactivities must be normalized by taking into account the altered radioactivities of nucleotide pools. Our results were normalized on the basis of data showing that, also in the treated cells, the intracellular pool becomes saturated with labeled nucleotides within 10 to 15 min, which interval is much shorter than the present incubation times, and that, after their entrance into the cells, all nucleosides are made available for macromolecular syntheses (2). By this procedure the inhibitory action of dichromate on nucleic acid syntheses, as estimated from normalized macromolecular radioactivities, turns out to be consistent with the inhibition detected on the basis of the growth curves.

The action of dichromate on nucleoside uptake is characterized by a stimulation phase, which is observed during the first hr of treatment in complete growth medium (Charts 4 and 5). When BHK cells are exposed for longer times to high dichromate concentrations (10^{-3} to 10^{-4} M), an inhibition phase of nucleoside uptake follows, and this is particularly evident when treatment is made in BSS (2). An inhibition of endogenous nucleotide synthesis, leading to reduced intracellular nucleotide concentrations prior to the incubation with labeled nucleosides, could induce the observed stimulation of labeled nucleoside uptake by means of simple diffusion mechanisms. Spectrophotometrical determinations of nucleotide pool concentrations (Table 2) reflect almost exclusively ribonucleoside-triphosphate levels, particularly the ATP level (12), so that a specific reduction of thymidine and uridine derivatives would be undetectable by this procedure and would require a further qualitative analysis of pool composition. In any case a reduced concentration of nucleotides in the soluble fraction is observed, even by UV absorption, when BHK cells are treated with 10^{-3} to 10^{-4} M $K_2Cr_2O_7$ in BSS (Table 2).

The kinetics of nucleoside uptake could be explained, assuming that the onset of the inhibition phase depends on the progressive damage of membrane structures by chromium, which eventually blocks even passive nucleoside entry. The accumulation of trivalent chromium in the treated cells is gradual and dose dependent (Chart 6) and more rapid when treatment is carried out in BSS, in which the onset of nucleoside uptake inhibition is likewise anticipated (2); in any case a limit value of about 5×10^{-2} μg Cr^{3+} per μg DNA is always reached. The different time at which the shift from the stimulation to the inhibition of nucleoside uptake takes place may therefore reflect the moment when linked chromium reaches the critical dose-determining cell membrane denaturation. On the other hand dichromate does not induce nucleic acid breakdown (Table 3), so that the observed inhibition of labeled nucleoside uptake is not to be explained by an increase of intracellular nucleotide concentration.

Alternatively, the action of $K_2Cr_2O_7$ on the uptake of nucleosides could lie in an interaction of Cr^{6+} with specific receptors involved in the transport of precursors across the plasma membrane, *e.g.*, the nucleoside permeases (12, 23). The stimulation-inhibition patterns observed for nucleoside uptake in the cells treated with $K_2Cr_2O_7$ resemble those obtained by directly treating *in vitro* purified enzymes such as phosphoglucomutase with metal ions, in particular with Cr^{3+} (28). Such patterns could not be explained, assuming that Cr^{6+} acts progressively activating the transport mechanisms, which are subsequently inactivated (*e.g.*, denaturated) when linked Cr^{3+} has accumulated over a critical level.

An interaction of chromium with the nucleoside permeases is suggested by the specific patterns observed for the uptakes of the different DNA and RNA nucleosides into BHK cells treated with dichromate and by the kinetics of thymidine and deoxycytidine uptake; chromium acts on both the saturable, facilitated portion (on the permeases) and the linear portion (the simple diffusion) of the uptake (2). At the concentrations of nucleosides used with conditions in the present experiments (0.5 μM, corresponding to 1 μCi [^{3}H]dThd and [^{3}H]Urd per ml with a specific activity of 2 Ci/mmole), most of the nucleoside uptake is due to facilitated diffusion, and only a little occurs by simple diffusion (2, 12, 23), so that the observed modifications of the uptake can be mainly referred to the alteration of permease function. In any case, the stimulation of nucleoside uptake represents a specific effect of Cr^{6+}; it has never been observed after treatment with Cr^{3+} (chromium chloride), which is devoid of oxidizing power and whose active concentrations always exert an inhibitory action (16).

As for the action of $K_2Cr_2O_7$ on macromolecular syntheses, it is characterized by a sharp and sudden blockage of DNA replication, whereas RNA and protein syntheses seem to be secondarily inhibited (Charts 4 and 5; Table 1). Such a pattern could be due to a differential action of dichromate on the enzymes involved in nucleic acid and protein syntheses. However, on the basis of BHK survival curves to $K_2Cr_2O_7$ (16) and of physicochemical studies of DNA treated *in vitro* with Cr^{3+} (5, 6) and Cr^{6+} (30), a stabilization of the DNA molecule can be suggested as the final effect of dichromate, which could account also for the differential inhibition of macromolecular syntheses (16).

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