PROBLEMS WITH METHODS FOR MEASUREMENT OF HEXAVALENT CHROMIUM APPLIED TO BIOLOGICAL SAMPLES.

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Hexavalent chromium (Cr(VI)) compounds are conridored to be cytotoxic and gonotoxic agontr. Their biological activity are generally attributed to tho oxidizing power of Cr(VI) and to tho ability of trivalent chromium (Cr(III)) to form substitution inert complexes with different cell components. Several studios about tho valence-reduction of Cr(VI) have been porformed in biological systems (1,2,3). However, tho available methods for Cr(VI)-measurement are not well adapted for such purporos. A main problem is that most of them involvo stops with low pH (less than 5). Such a low pH is unsatirfactory because tho reactivity of Cr(VI) is greatly increased in tho strong reducing cellulrr onvironmont. Tho measurement of Cr(VI) by atomic absorption spoctrophotomotry after a prior extraction with liquid anion-exchanger, may also bo unreliable duo to bad selectivity. We have developed a simple and rapid procoduro for removing reducing compounds from cell-homogenate undor high pH conditions. This method mry eliminate tho problomr connected with low pH during tho rnrlysis of Cr(VI).

Tho dramatic effect of pH on tho roduction rate of Cr(VI) is evident from Fig. 1. At pH higher than 10 chromate is stable for several hours even in tho presence of strong reducing agents.

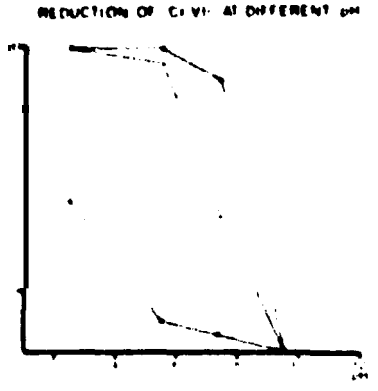

FIGURE 1.

● ——— ● 5 µM Fe^{2+}-ADP (% of complete reaction)
▲ ——— ▲ 1 mM ascorbate
■ ——— ■ 5 mM glutathion

Na_2CrO_4 (100 µM) was preincubated at room temperature in 1 ml 100 mM buffers. The reduction of Cr(VI) was followed at maximum OD (355 - 370 nm) after addition of 10 µl of reducing agents.

The measurement of Cr(VI) in biological materials was performed with the standard diphenylcarbazide (DPC) method (4). Although a very rapid formation of the coloured complex between chromium and DPC, significant quantities of Cr(VI) are reduced by sidereactions at the moment of DPC-acid addition when pH is lowered to about 2. This is evident from Fig.2 where the reduction of Cr(VI) was followed in a microsomal suspension. When a major part of reducing compounds were removed by charcoal-Al_2O_3 (see Fig.3), the same DPC-method gave higher Cr(VI)-values (less reduced) in a parallel incubation. Similar results were seen in experiments with hepatocytes.

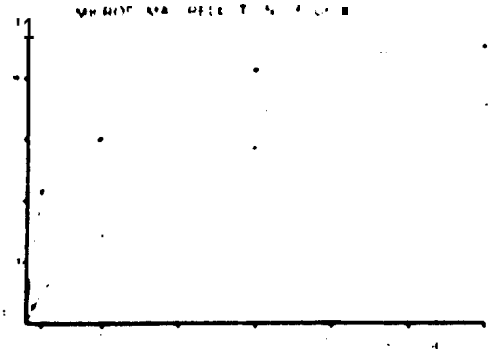

FIGURE 2.

Microsomes 10.S mg protein/ml) were preincubatrd in 150 mM KCl, 20 mM K-HEPES pH 7.20 and 1 mM NADPH under O_2-free conditions et 37 °C. Na_2CrO_4 (20 µM) was then added and Cr(VI) measured in aliquots withdrawn at different incubation times.

◆——◆ One ml aliquot was aired with 100 µl 1 % OPC-2 N H_2SO_4 (1:1) and OD_{540} was measured.

▲——▲ Two ml samle war mixed with 100 µl 2 M Na_2CO_3 and 400 mg charcoal-Al_2O_3. After filtration through cotton, 1 ml filtrate was mixed with 100 µl 1 % DPC - 4 N H_2SO_4 (1:1).

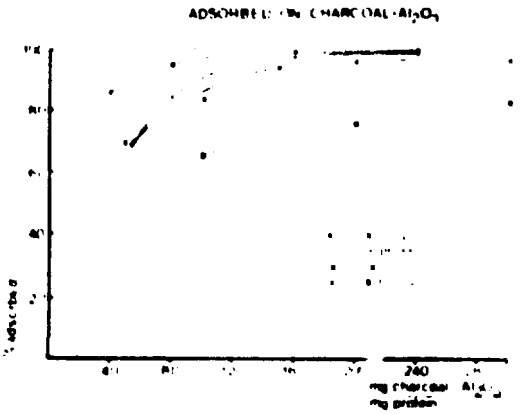

FIGURE 3.

Freshly prepared liver homogenate (2 a) protein) was incubated In 3 ml 100 mM KCl and 20 mM K-HEPES pH 7.20. Chromium (20 µM) was added either as Cr-51 labelled $CrCl_3$ or as Na_2CrO_4 end incubated under O_2-free conditions in 3 hours at 37 °C. After this incubation all chromium is present as Cr(III)-complexes. tho samples were then mixed with 100 µl 2 M Na_2CO_3 and different quantities of charcoal - basic aluminium oxide (1:1). A11 particles were removed by fil- tration through cotton. Protein (Lowry), OD_{260} end Cr-51 were determined before and after tho treatment.

tho ability of charcoal-Al_2O_3 to adsorb proteins and other OD_{260} absorbing materials (nucleotides and several metabolites) from liver-homogenate is shown in Fig.3. It is also interesting to notethat Cr(III) is differently adsorbed, dependent whether the source is $CrCl_3$ or Na_2CrO_4 reduced in the homogenate. When Na_2CrO_4 was addod to the homogenate at pH about 10.5 (no reduction occured), the recovery of Cr(VI) was 98-100 % after the charcoal-Al_2O_3 treatment.

A further selective extraction of Cr(VI) from the water-phase with liquid anion-exchanger was not successful as significant quantities of Cr(III) are present as anionic complexes, co-extracted with Cr(VI) (Tab.1). The quantity of these complexes was highest when Cr(VI) previously was reduced in the homogenate. A one-step ion-pair extraction of chromate was therefore not adequate for measurement of Cr(VI) by AAS. Generally. selectivity tests with $CrCl_3$ in this connection may be misleading.

RECOVERY OF CHROMIUM IN ORGANIC PHASE AFTER ION-PAIR EXTRACTION

Ion-exchanger	Cr(VI)	Cr(VI) → Cr(III)	Cr(III)
LA2/MIBK (1:1)	96 ± 3	7 6 ± 0 7	2 4 ± 0 2
10% MTOA i cyclohexan	97 ± 2	8 0 ± 1 0	2 1 ± 0 2

TABLE 1.

The figures are mean with O.d. from 4 experiments.
The samples were prepared from 2 rl medium (150 mM KCl and 20 mM K-HEPES pH 7.20) with 60 mM Na_2CO_3 and 20 µM chromium added either as Cr-51 labelled Na_2CrO_4 • 2 mg homogenate (Cr(VI)), or Na_2CrO_4 previously reduced in 2 mg liver homogenate (Cr(VI) → Cr(III)), or 2 mg liver homogenate preincubated with Cr-51 labelled $CrCl_3$ (Cr(III)). A11 samples were mixed with 400 mg charcoal-Al_2O_3 and filtered through cotton. The filtrates were diluted 10^3x wrth dest. water maturated with the organic solvent, and pH adjusted to about 5 with HCl. The extraction was performed with 1 ml ion-exchanger and Cr-51 counted in tho organic layer. LA 2 / methylisobutylketone (MIBK) was washed 3 a in 6N HCl before use. MTOA - methyltrioctylammonium chloride.

REFERENCES.

1. J.E. Gruber and K.W. Jennette: Metabolism of the carsinogon chromate by rat liver microsomes. Biochem. Biophys. Res. Commun. 1978 , 82, 700-706.
2. O. Ryberg and J. Alexander: Inhibitory actron of hexavalent chromium (Cr(VI)) on the mitochondrial respiration and a possible coupling to the reduction of Cr(VI). Biochom. Phrrmrcol. 1984, 33, 2461-2466.
3. P. Richelmi and C. Baldi: Blood levels of hexavalent chromium in rats. 'In vitro' and "in vivo' experiments. Intern. J. Environ. Anal. Chem. 1984, 17, 181-186.
4. E.B. Sandell: Colorimetric determination of traces of metals. Interscience Publishers Inc. Now York 1959, 392-397.

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