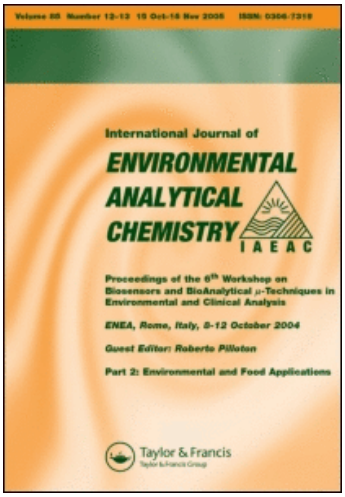

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Blood Levels of Hexavalent Chromium in Rats. "In Vitro" and "In Vivo" Experiments

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For the Cr(VI) selective separation from biological materials we have developed a highly rapid extraction–separation method with liquid anion exchanger as Amberlite LA-1 or LA-2. The analytical determination of Cr(VI) in organic phase was carried out using electrothermal atomic absorption spectroscopy (ETA-AAS).

After i.v. administration of 0.5 and 2.5 mg/kg b.w. of $K_2Cr_2O_7$ in male Wistar rats the biological samples, collected at different times, were immediately analyzed. Cr(VI) was not detected in whole blood one minute after administration of the lower dose. In blood of rats receiving higher dose an incomplete reduction of Cr(VI) was observed.

Such data demonstrate a highly rapid but limited metabolic capacity of hematic compartment to reduce Cr(VI) to trivalent status..

"In vitro" incubation of $K_2Cr_2O_7$ (4 μM) with rat erythrocytes or plasma at 37°C showed a rapid reduction of Cr(VI) in red cells while plasma samples demonstrated a limited reductive power.

These results obtained with a new and specific analytical method, confirmed a trigger role of red cells in Cr(VI) metabolism.

KEY WORDS: Cr(III), Cr(VI), Atomic absorption spectroscopy, blood.

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INTRODUCTION

Trivalent chromium is an essential metal in man and in animals. Both acute and chronic adverse effects of chromium are mainly caused by hexavalent compounds, including carcinogenic effects.[1]

The concentration of chromium in blood has been reported to be 20–30 μg/l with an even distribution between red cells and plasma.[2] As far as the occupational exposure is concerned, the increase in blood values is related mainly to the red cells.[3]

It is well known that hexavalent chromium is the less stable oxidation form of such a metal: when Cr(VI) reacts with organic materials the latter ones reduce it quickly to Cr(III).[1]

These chemical–physical properties have caused some technical and analytical difficulties in the speciation of hexavalent chromium from trivalent form. Consequently the valence state of this metal in biological and related materials is not well known.

The determination of trace amounts of chromium by electro termal atomization–atomic absorption spectrophotometry (ETA-AAS) is now the most popular analytical method in toxicological laboratories, owing to its high sensitivity and possibility to analyze small samples without any chemical pretreatment. At present this technique permitted the determination of total chromium but not the speciation of valence states of this metal (Cr(III) or Cr(VI)).

We have developed a specific method with Amberlite LA-2 (or LA-1), a water insoluble liquid anion exchanger, for the speciation of Cr(VI) in biological materials and in environmental samples.[4,5,6,7] The extraction with Amberlite LA-2 and a successive analysis of Cr(VI) with ETA-AAS permitted to perform rapid speciation of Cr(VI) just after collection of biological samples. This technique gives us the possibility to quantify Cr(VI) in high reducing power compartments as for instance the red blood cells.

In the present work we have performed the determination of Cr(VI) in plasma and in whole blood samples after i.v. administration of potassium dichromate at various doses in rats. By performing "in vitro" experiments we have also studied the ability of plasma, erythrocytes and whole blood to reduce dichromate ions.

MATERIAL AND METHODS

Male Wistar rats weighing 200–220 g (Morini, S. Polo d'Enza, Italy)

were used throughout. The animals were maintained on a standard laboratory diet and water ad libitum.

"In vivo" experiments Groups of treated rats were injected intravenously with potassium dichromate dissolved in saline at doses of 0.5 and 2.5 mg/Kg b.w. of Chromium. Groups of control rats were administered with saline solution only. Femoral vein blood samples (0.5–1.0 ml) were collected in heparinized tubes at 1 min and 5 min after Chromium injection for determination of Cr(VI) in whole blood and in plasma.

The whole blood samples were hemolized immediately after collection.

"In vitro" experiments The incubation of plasma, erythrocytes and whole blood with 0.1 μ/ml of Cr(VI) were performed in a bath kept at $37 \pm 1C°$.

Analysis 100 μl of whole blood or plasma was pipetted into stoppered polyethylene tube, added of 2 ml of bi-distilled water and mixed in a mechanical shaker for 5 sec. After we added 1 ml of LA-2/MIBK, remixed for 1 min and then centrifuged at 2,500 rev/min for 10 min.

A microaliquot (25 μl) of the upper layer (organic phase) was pipetted into a graphite furnace (HGA-500) in atomic absorption spectrophotometry Perkin Elmer mod. 5000, according to the technique previously reported.[4]

In our experimental conditions the sensitivity was 20 ppb Cr(VI). Accuracy and precision of the analysis at various concentration levels were quite satisfactory, the recovery ranging 96.4–97.2% in plasma and 94.7–96.1% in whole blood and the coefficient of variation ranging 4.7–5.2% in plasma and 3.7–5.2% in whole blood.

RESULTS

"In vivo" experiments

The high reducing power of red cells versus dichromate ions constitutes a methodological problem that may alter the "natural" concentration of chromium in samples. In order to minimize this

difficulty we have hemolyzed the whole blood immediately after collection. This step highly limits the reducing power of erythrocytes.

For the determination of Cr(VI) in plasma the problem is the time required (5 min) for the separation of this blood component. In fact, even if the plasmatic matrix has not a markedly reducing power on Cr(VI), the presense of red cells during the centrifugation step alter the "natural" concentration of Cr(VI).

In our experiments Cr(VI) was not detected in whole blood 1 min after administration of the lower dose of $K_2Cr_2O_7$. In blood of rats receiving the higher dose an incomplete reduction was observed (6.2% of Cr(VI) in whole blood after 1 min of administration). In plasma of rats receiving the higher dose 1.9% of Cr(VI) administered was found during the first min.

TABLE I

Cr(VI) in whole blood and in plasma in control rats and I.V. treated with two doses of Cr(VI) as $K_2Cr_2O_7$.

Treatment	Time after treatment (min)	Cr(VI) content			
		Whole blood		Plasma	
		μg	% dose[a]	μg	% dose[b]
Control	5	N.D.	—	not determined	—
0.5 mg/Kg b.w.	1	N.D.	—	not determined	—
2.5 mg/Kg b.w.	1	30.9 ± 4.7	6.18	8.97 ± 3.1	1.79
	5	12.5 ± 3.1	2.25	2.25 ± 0.40	0.45

N.D.--not detectable < 0.02 μg.

[a]Assuming a blood volume of 15 ml.

[b]Mean HT: 65%.

All values are the mean ± S.D. of 7 experiments.

"In vitro" experiments

"In vitro" incubation of $K_2Cr_2O_7$ (4 μM) with rat erythrocytes at 37°C shows a rapid reduction of Cr(VI): about 80% of Cr(VI) is reduced in 60 sec.

The incubation of $K_2Cr_2O_7$ at the same concentration with the whole blood confirmed the high reducing power of red cells: about 70% of Cr(VI) is reduced in 60 sec.

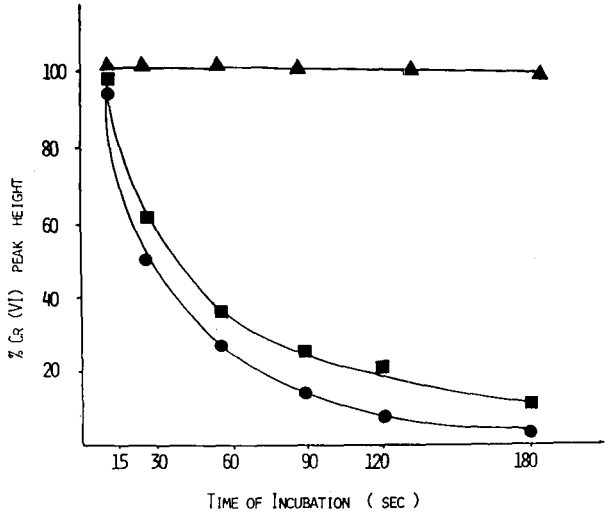

FIGURE 1 Reduction in time of Cr(VI) incubated at 37±0.1°C with plasma, red cells and whole blood.

All results are the mean of 5 experiments.

■ whole blood.
● red cells.
▲ plasma.

Whereas plasma samples demonstrate a limited reducing power: about 20% of Cr(VI) after 20 min of incubation.

TABLE II

"In Vitro" incubation at 37±1°C of plasma with Cr(VI).

Time of incubation (min)	% of Cr(VI) reduced
1	2.1±0.4
2	2.4±0.3
5	7.1±0.6
10	10.5±1.2
15	14.6±1.2
20	19.8±1.4

All values are the mean±S.D. of 5 experiments.

DISCUSSION

Our results indicate that the only problem involved in the speciation of Cr(VI) in the blood compartment is extra-analytical. In spite of the difficulty involved in the determination of the "natural" concentration of Cr(VI) we have been able to obtain valuable information concerning the steps of biotransformation of this metal.

This study demonstrated that the use of both Amberlite LA-2 and ETA-AAS for the determination of Cr(VI) in biological materials represents a highly specific method.

The limits of reliance of this method do not depend so much on the analytical characteristics of this procedure but on the metabolic properties of Cr(VI).

The results obtained with high doses of dichromate confirm a trigger role for red cells in Cr(VI) metabolism. The diffusion of the hexavalent form of chromium across the red blood cells membrane is confirmed to be very rapid: after 1 min from the i.v. administration the reduction of Cr(VI) is about 94% of the dose.

On the contrary the tests of "in vitro" incubation of plasma with Cr(VI) showed a weak capacity of this blood compartment to reduce chromium.

Studies on the hepatic and biliary involvement in Cr(VI) reduction–elimination process are in progress.

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