Estimates of the chromium(VI) reducing capacity in human body compartments as a mechanism for attenuating its potential toxicity and carcinogenicity

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Introduction

At variance with trivalent chromium [Cr(III)], hexavalent chromium [Cr(VI)] is consistently and unequivocally genotoxic in in vitro cellular test systems. The large majority of 436 results reported in the literature with 16 Cr(VI) compounds, up to 1990, were positive in short-term tests evaluating genetic and related effects (1,2). Cr(VI) is carcinogenic to humans (3), but epidemiological studies provide evidence that its carcinogenicity is strictly site-specific. In fact, according to the International Agency for Research on Cancer (IARC), there is sufficient evidence for carcinogenicity in the lung and in the sinonasal cavity, while for other sites ‘no consistent pattern of cancer risk has been shown among workers exposed to chromium compounds’ (3). Review of the epidemiologic literature and other evidence concerning Cr(VI) carcinogenicity that has been published since the IARC review does not alter this conclusion. As cited in the IARC monograph (3), the epidemiologic evidence for increased risk of cancers of the lung and sinonasal cavity is limited to conditions of high exposure, ‘as encountered in the chromate production, chrome pigment production and chromium plating industries’ (3), which in the past decades involved mixed exposures to various Cr(VI) compounds and possibly to other metals as well as organic substances.

Experimental studies in rodents were expected to contribute to the identification of carcinogenic Cr(VI) compounds. However, by analysing the data reviewed by IARC (3), not only none of the six studies performed with metallic chromium or of the 21 studies performed with Cr(III) compounds was positive, but even less than half of the 65 studies performed with Cr(VI) compounds (41.5%) was positive. Moreover, the majority of positive results were generated in studies involving certain exposure routes (subcutaneous, intramuscular, intraperitoneal, intrapleural, intrabronchial) which by-pass important detoxification barriers and do not reproduce any human exposure (2). All patterns of experimental carcinogenicity with Cr(VI) compounds point to the existence of threshold mechanisms, as indicated by the high frequency (58.5%) of negative results, attributable to a complete Cr(VI) detoxification in treated animals, or induction of cancer only under particular conditions, e.g., (a) at extremely high doses, saturating detoxification mechanisms, (b) at implant sites only, and never at a distance from the portal of entry into the organism, (c) in certain targets only, selected by the lack of detoxification mechanisms, or (d) when given in a single massive dose, and not in the same cumulative amount fractionated into smaller doses which can be more easily detoxified (2,4).

Therefore, Cr(VI) is potentially carcinogenic, but a series of hurdles tend to reduce its bioavailability and to limit its effects in the organism. Several studies published in our

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laboratories since 1978 (5) have demonstrated the occurrence of detoxification mechanisms of Cr(VI), mainly based on reduction to Cr(III), which is not carcinogenic and does not bear any toxicological relevance (1–3,6,7).

Based on these studies and on new experiments designed ad hoc, we made quantitative estimates of the overall Cr(VI)-reducing or sequestering capacity of some human body compartments by taking into account the specific Cr(VI) reducing activity of body fluids, cells or organs, and their average volume, number or weight in humans. We use the general term sequestration when intact cells were tested and the term reduction when cell homogenates or their subfractions were tested in the presence of an exogenous NADPH-generating system.

Materials and methods

The Cr(VI) reducing capacity was estimated by multiplying the specific reducing activity of a given organ, cell population, or fluid, expressed as μg Cr(VI) reduced per unit of weight, volume or number, by the average content of the same organ, cell population or fluid in the human body. The Cr(VI) reducing activity was inferred from our previous studies in the case of saliva (8), gastric juice (8,9), epithelial lining fluid (ELF) (10), pulmonary alveolar macrophages (PAM) (10), peripheral lung parenchyma (11) and bronchial tree (12).

New experiments were carried out for assessing the sequestering activity of human intestinal bacteria or of whole blood, and the reducing ability of red blood cell (RBC) lysates or of liver homogenates. We refer to the previously mentioned papers for technical details. Briefly, 25% liver homogenates were prepared in 0.25 M sucrose–50 mM Tris-HCl buffer, pH 7.4. Heparinized venous blood was used for evaluating the Cr(VI) sequestering capacity of whole blood. For preparing RBC lysates, blood was centrifuged at 2000 g for 15 min, washed three times with phosphate buffered saline (PBS), pH 7.4, taking into account the original volume of blood with distilled water, frozen at −80°C and thawed three times. RBC lysates were centrifuged at 30 000 g for 15 min in order to obtain the corresponding soluble fractions. Intestinal bacteria were obtained from human feces by growing overnight broth cultures (10 ml) in Oxoid nutrient broth. The bacteria were washed by centrifuging three times in the broth cultures at 10 000 g for 15 min and resuspended in PBS, pH 7.4 (1 ml). The number of viable bacteria was assessed by seeding serial dilutions of the concentrated bacteria (1:10^3, 1:10^4, 1:10^5 and 1:10^6) in triplicate Oxoid agar plates.

Varying amounts of sodium dichromate, dissolved in PBS, pH 7.4, at concentrations corresponding to 0.625, 1.25, 2.5, 3.75, 5.0, 7.5 or 10 µg Cr(VI) per vial, were mixed either with liver homogenates (6.25, 12.5, 25 or 50 µl per vial), RBC lysate soluble fractions (6.25, 12.5, 25, or 50 µl per vial), whole blood (100 µl per vial), concentrated intestinal bacteria (100 µl per vial), or equivalent volumes of their diluting solvents. An NADPH-generating system (S9 mix) (13) was added to liver homogenates and RBC lysates. After 60 min of gentle mixing at 37°C, the mixtures were evaluated for the amount of residual Cr(VI), both by using the colorimetric method with s-diphenylcarbazide (10) and/or by assessing mutagenicity in strains TA100 and TA102 of Salmonella typhimurium (13). Due to the turbidity of mixtures, only the mutagenicity test system, used as a ‘biological spectrophotometer’, could be applied in the case of liver homogenates and RBC lysates. The amounts of residual Cr(VI), and consequently the amounts of reduced or sequestered Cr(VI), were calculated from the regression lines relating the initial amounts of Cr(VI) to the loss either of colorimetric reactivity or mutagenic activity.

Results

Table 1 reports the volume, weight or number of selected fluids, organs or cell populations in the human body, along with their ability to reduce or sequester Cr(VI). Accordingly, the overall Cr(VI) reducing or sequestering capacity of fluids, organs or cells was calculated either on a daily basis or in terms of saturating amounts of Cr(VI). The results of these estimates are further summarized in Figure 1.

Saliva

Samples of saliva from five subjects (three males and two females), incubated with Cr(VI) for 5 min at 37°C, reduced 1.4 ± 0.2 μg Cr(VI)/ml (mean ± SD) (8). Since the daily secretion of saliva in humans is in the 500–1500 ml range (14), this mechanism will reduce daily 0.7–2.1 mg Cr(VI).

Gastric juice

The Cr(VI)-reducing activity of gastric juice samples from four fasting individuals was found in an earlier study to be 9.2 ± 0.4 μg/ml fluid (8). Later on, a detailed study regarding the reducing ability in the gastric environment was performed in 17 individuals, both males and females, in whom samples of gastric juice were aspirated at hourly intervals by means of a nasogastric tube (9). The baseline of Cr(VI)-reducing activity of duodenal contents during interdigestive periods (mainly during the night) was 8.3 ± 4.7 μg/ml, i.e. very similar to the one detected in the previous study. After each one of the three daily meals there were periods of 3–4 h during which peaks of 50–60 μg/ml were recorded, averaging 31.4 ± 6.7 μg Cr(VI) reduced per ml gastric juice during these periods. It is known that in a fasting individual the gastric secretion accounts for 1000–1500 ml (15), and in the 4-h period after each meal an average amount of ~800 ml is additionally secreted by the stomach (16). Therefore, the overall Cr(VI) reduction by gastric juice may be estimated to be at least 84–88 mg per day (8.3 μg/ml×1000–1500 ml + 31.4 μg/ml×800 ml×3 meals).

Cr(VI) reduction by gastric juice is due both to thermolabile components of this fluid and to food components (9). Since gastric juice samples were centrifuged before being challenged with Cr(VI) in order to remove gross food residues, the above calculations underestimate the actual Cr(VI) reducing capacity of gastric contents. It is noteworthy that the Cr(VI) reducing activity was evaluated after 60 min of contact at 37°C with gastric juice samples, but time-course experiments showed that the reaction was complete within 10–20 min (9), and at least half of it was accomplished within 1 min only (A.Camoirano and S.De Flora, unpublished data).

Intestinal bacteria

The bacteria isolated from the feces of three male individuals sequestered 3.8 ± 1.7 μg Cr(VI)/10^9 bacteria. The bacterial population in the intestine is huge, i.e. as related to 1 g of intestinal content, 0–10^6 bacteria in the duodenum, 10^9–10^10 in the ileum, and 10^10–10^12 in the cecum (17). The amount of bacteria in feces mostly reflects their concentration in the colon. The average fecal mass is 124 ± 40 g/day, and its dry mass is 21 ± 5 g/day, 14–30% of which is represented by bacteria (14). Accordingly, 2.9–6.3 g of bacteria are eliminated daily with feces. Taking into account that 10^12 bacteria weigh ~1 g and are capable of sequestering 3.8 mg Cr(VI), it can be calculated that 11–24 mg Cr(VI) can be eliminated daily with fecal bacteria.

Liver

Unfractionated homogenates of liver, obtained at surgery from three males, reduced 2.2 ± 0.9 mg Cr(VI)/g wet tissue, after contact for 60 min at 37°C in the presence of S9 mix, as evaluated by assessing the decrease of sodium dichromate mutagenicity in strains TA100 and TA102 of S.typhimurium. Since the average weight of the human liver is 1500 g, not taking into account 400–800 g of blood circulating in this organ (18), we estimated an overall reducing capacity of 3300 mg Cr(VI).

Blood

We evaluated Cr(VI) sequestration by whole blood as well as its reduction by the soluble fraction of red blood cell (RBC)
Chromium(VI) reduction in the human body

Table I. Estimates of the overall chromium(VI) reducing capacity of organs, cell populations and fluids in the human body (see text for details)

<table>
<thead>
<tr>
<th>Organ, cell population, or body fluid</th>
<th>Weight of organs, number/weight/volume of cells, or volume of body fluids</th>
<th>Chromium(VI) reduction or sequestration (mean ± SD)</th>
<th>Overall chromium(VI) reducing or sequestering capacity per individual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saliva</td>
<td>500–1500 ml/day (4)</td>
<td>1.4 ± 0.2 µg/ml (8)</td>
<td>0.7–2.1 mg/day</td>
</tr>
<tr>
<td>Gastric juice</td>
<td>1000–1500 ml/day (fasting) (15) + 800 ml/meal (16) 3400–3900 ml/day (3 meals)</td>
<td>8.3 ± 4.3 µg/ml (9)</td>
<td>8.3–12.5 mg/day during interdigestive periods +25.1 mg/meal &gt;84–88 mg/day (3 meals)</td>
</tr>
<tr>
<td>Intestinal bacteria</td>
<td>2.9–6.3 g eliminated daily with feces (14)</td>
<td>3.8 ± 1.7 µg/10^9 bacteria a</td>
<td>11–24 mg eliminated daily with feces</td>
</tr>
<tr>
<td>Liver</td>
<td>1500 g (18)</td>
<td>2.2 ± 0.9 mg/g liver homogenate a</td>
<td>3300 mg</td>
</tr>
<tr>
<td>Whole blood</td>
<td>4490 ml (males) (14) 3600 ml (females) (14)</td>
<td>52.1 ± 5.9 µg/ml (234 mg (males) 187 mg (females))</td>
<td></td>
</tr>
<tr>
<td>Red blood cells (RBC)</td>
<td>2030 ml (males) (14) 1470 ml (females) (14)</td>
<td>63.4 ± 8.1 µg/ml (128 mg (males) 93 mg (females))</td>
<td></td>
</tr>
<tr>
<td>Epithelial lining fluid (ELF)</td>
<td>37.5–75 ml (20)</td>
<td>23.7 ± 15.9 µg/ml (10)</td>
<td>0.9–1.8 mg</td>
</tr>
<tr>
<td>Pulmonary alveolar macrophages (PAM)</td>
<td>23 × 10^6 PAM (21)</td>
<td>4.4 ± 3.9 µg/10^6 (136 mg)</td>
<td></td>
</tr>
<tr>
<td>Peripheral lung parenchyma</td>
<td>1300 g (18)</td>
<td>0.2 ± 0.07 mg/g lung S12 fraction (11)</td>
<td>260 mg</td>
</tr>
</tbody>
</table>

This study.

The RBC lysate soluble fractions from the same subjects were assayed in the mutagenicity test system. In the presence of S9 mix, 1 ml portions of the RBC lysates reduced 63.4 ± 8.1 µg Cr(VI) within 60 min at 37°C. According to this experimental approach and to the known volumes of RBC in males and females (see Table I), we calculated an overall reduction of 128 and 93 mg Cr(VI), respectively. This is an underestimate of the overall reducing capacity of RBC, since the RBC stromal fraction was removed by centrifugation. These results are comparable to those obtained in a previous study (19), in which an appreciable decrease of Cr(VI) (as chromic acid) mutagenicity by RBC lysates was even observed in the absence of an exogenous NADPH-generating system (i.e. S-9 mix).

Epithelial lining fluid (ELF)
The ELF includes the surfactant and those bronchial and bronchiolar secretions which can be recovered by bronchoalveolar lavage. Amounts of 23.7 ± 15.9 µg Cr(VI) (mean ± SD in 15 individuals, both males and females) were reduced by each ml of ELF after contact for 4 h (10). Since the total volume of ELF has been reported to be between 37.5 and 75 ml (20), its overall reducing capacity can be estimated to be 0.9–1.8 mg Cr(VI) per individual.

Pulmonary alveolar macrophages (PAM)
The total number of these sweeping cells in the normal human lung at any given time is estimated to be 23 billion, with ~50–100 PAM per alveolus (21). We evaluated Cr(VI) reduction by PAM S9 fractions after 30 min at 37°C. Expressed as µg Cr(VI) reduced per 10^6 cells, the results were as follows: 2.4 ± 1.1 (mean ± SD in four non-smokers), 3.6 ± 0.7 (eight exsmokers), 5.8 ± 1.2 (6 current smokers) and 6.3 ± 1.1 (five subjects smoking during the last 2 h before bronchoalveolar lavage). The specific reducing activity of PAM in smokers was significantly higher (P < 0.01) than that in non-smokers and exsmokers. The average Cr(VI) reduction capacity was 4.4 ± 3.9 µg/10^6 PAM in 23 subjects of both sexes (10). In

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**Fig. 1.** Estimates of Cr(VI) sequestration or reduction by organs, cell populations and fluids in the human body. See text for details and explanations.

lytes from three subjects, two males and one female. After contact of sodium dichromate [100 µg Cr(VI)] with 1 ml heparinized venous blood for 60 min at 37°C, an average of 47.9 µg was recovered from plasma, thus indicating that 52.1 ± 5.9 µg had been sequestered by blood cells or reduced by plasma itself. Similar data were obtained by measuring residual Cr(VI) either by colorimetric analysis or in the mutagenicity test system with strains TA100 and TA102 of S.typhimurium. Taking into account the average volumes of blood in males and females (see Table I), we calculated an overall sequestration of 234 and 187 mg Cr(VI), respectively.
the same study we established that the reducing activity of unfractionated PAM homogenates was 1.33 times higher than that of the corresponding S9 fractions (10). Therefore, we introduced this correction factor for estimating the overall Cr(VI) reducing capacity by PAM (136 mg per individual).

Peripheral lung parenchyma

Cr(VI) reduction by S12 fractions of peripheral lung parenchyma was assessed in tissue samples taken at surgery from 71 male subjects. After incubation with sodium dichromate for 60 min at 37°C in the presence of S9 mix, the observed decrease in mutagenicity in strain TA100 of S.typhimurium corresponded to reduction of 0.24 ± 0.07 mg Cr(VI) per g wet tissue (11). Interestingly, the reducing activity was significantly higher (\( P < 0.05 \)) for tissues obtained from 45 smokers (0.25 ± 0.07 mg/g), as compared to 26 non-smokers (0.22 ± 0.06 mg/g), and the 19 individuals who smoked during the last 24 h preceding surgical operation exhibited even greater reduction capacity (0.27 ± 0.06 mg/g) (11). Again, a 1.33 correction factor extrapolating from S12 fractions to unfractionated homogenates was introduced for estimating the overall Cr(VI) reducing capacity of the lungs (260 mg per individual).

Bronchial tree

Peripheral lung parenchyma and bronchial tree S12 fractions from 18 of the above mentioned subjects were compared for their ability to decrease the mutagenicity of sodium dichromate. There was a statistically significant correlation between the two preparations in decreasing Cr(VI) mutagenicity, peripheral lung parenchyma being slightly yet significantly more efficient in accomplishing this process (12). These findings refer to whole bronchial tissue preparations, since it was not possible to dissect sufficient amounts of bronchial epithelium for assessing the endpoints under study. The lack of information on the weight of bronchial tree does not allow us to estimate its overall reducing capacity per individual.

Discussion

The results of the present study provide for the first time estimates aimed at quantitating the ability of organs, cell populations and fluids in the human body to sequester or reduce Cr(VI). It is clear that the reported data do not have absolute precision or universal applicability for several reasons. For example, all estimates were inferred from \textit{ex vivo} experiments which, depending on the specific test design, could overstate or underestimate the reduction or sequestration process \textit{in vivo}. Inter-individual differences in these parameters should also be taken into account to infer the reduction capacity of various compartments in a specific person. Moreover, the overall reducing or sequestering capacity was estimated by hypothetically assuming a uniform distribution of Cr(VI) in the body compartment under evaluation.

Some data, e.g. Cr(VI) reduction in saliva and gastric juice, or its sequestration by bacteria, could be estimated on a daily basis. All remaining data refer to the overall capacity of fluids, organs or cells. Nevertheless, the reported estimates do not reflect rigid saturation levels, unless following massive acute exposure, since there is a continuous production of body fluids and turnover of certain cell populations. For instance, 1–5 million PAM in the human lungs are removed every hour via the mucociliary escalator to be either expectorated or swallowed (22). Taking into account that the mean (± SD) lifespan of human RBC is 107±12 days (14), every day a population corresponding to 1/107th of the total RBC volume, i.e., 19 ml in males and 14 ml in females, will be renewed. Moreover, even in organs characterized by a poor turnover of cells, such as the liver, there is a continuous synthesis of glutathione (GSH) and other reducing molecules which, besides exerting protective effects locally, are exported to other organs. As extensively discussed in previous articles (1,2,4,6,8,23–25), Cr(VI) reduction in biological fluids (e.g. saliva, gastric juice and ELF) and long-lived non-target cells (e.g. RBC and PAM) is expected to greatly attenuate Cr(VI) toxicity, to imprint a threshold character to the carcinogenesis process and to restrict the targets of its potential activity. In fact, Cr(III), the stable reduced form of chromium, is not mutagenic in bacteria (1–3) and does not bear toxicological relevance in the extracellular environment, due to its poor ability to cross cell membranes (4). Provided Cr(VI) may overwhelm a series of hurdles and penetrate into cells, it will be reduced to Cr(V), Cr(IV) and Cr(III) by a complex network of mechanisms, both enzymatic and nonenzymatic, which may also lead to the generation of reactive oxygen species (4,26). Depending on proximity to DNA, these processes may result in DNA damage by these reactive species, or in trapping by cell nucleophiles and consequently in detoxification (4). It is meaningful, in this respect, that tissues which are typical targets for Cr(VI) carcinogenicity in rodents assays, such as the rat skeletal muscle after local implantation (3), have a negligible activity in decreasing Cr(VI) mutagenicity (19). Thus, tissue-specific susceptibility to Cr(VI) tumorigenicity correlates with intrinsically poor capacity for intracellular Cr(VI) reduction.

The investigated mechanisms and quantitative estimates on Cr(VI) reduction may predict and explain the fate of this metal in the human body. The massive reducing and sequestering capacity of the blood explains why Cr(VI) exerts its toxicological consequences only at the portal of entry into the organism, while it is not a systemic toxicant or carcinogen. In fact, Cr(VI) carcinogenicity in humans is restricted to the sinonasal cavity and the lung, whereas excess risks reported for cancer at other sites were inconsistent and not convincingly associated with Cr(VI) exposure (3). In only one out of the 65 carcinogenicity studies in rodents reported by IARC (1990), Cr(VI) was found to be carcinogenic at a distance from the administration site, specifically in rats receiving intramuscular injections of lead chromate, which developed renal carcinomas (27). However, this effect should be ascribed to lead, which typically produces kidney tumors in rodents (28), rather than to chromate.

In the present study, ~200 mg Cr(VI) were estimated to be sequestered by whole human blood. About half of this figure was found to be reduced in the presence of the soluble fraction of RBC lysates. This difference is likely due to the fact that preparations of RBC lysates involve elimination of their stromal fraction by centrifugation and loss of biochemical activities, which are partially restored by adding an exogenous NADPH-generating system. In addition, blood plasma has been reported to reduce up to 2 \( \mu g \) Cr(VI) per ml blood (29,30). Since the mean plasma volume is 2640 ml in adult males and 2130 ml in adult females (14), the overall Cr(VI) reducing capacity of blood plasma can be estimated to be 4.9 and 4.2 mg, respectively. A recent investigation (31) examined the rate and capacity of Cr(VI) reduction in human blood \textit{in vitro} and reported findings that agree well with data generated in the present study. The major detoxification occurs
Chromium(VI) reduction in the human body

in RBCs, where since many years it is known that Cr(VI) is selectively accumulated (32) and reduced to Cr(III) (especially by GSH) and bound to low-molecular weight components and chiefly to hemoglobin (33–38). These mechanisms also help to explain why all chromium detectable in the urine of exposed individuals is Cr(III) (3,39).

Formidable barriers hamper the potential toxicity of Cr(VI) when introduced by the oral route or swallowed either following reflux from the respiratory tract or following impact onto the proximal aerodigestive tract of inhaled Cr(VI) associated with large-size particles. After reduction in the oral cavity by saliva, which would be sufficient to detoxify Cr(VI) contained in several liters of drinking water at the standards recommended in several countries (0.05–0.1 mg/l), an efficient reduction occurs in the stomach, accounting for at least 84–88 mg per day. A further hurdle is represented by intestinal bacteria which, as estimated just from the aliquot of bacteria eliminated with feces, will sequester daily 11–24 mg Cr(VI). Intestinal bacteria are known to contain high amounts of GSH (40), which efficiently reduces Cr(VI) (4). A Cr(VI) reducing role has been also ascribed to enterobacterial enzymes, such as nitroreductases (4). Since bacterial GSH is released extracellularly (40), an additional Cr(VI) reducing activity is also likely to occur in the extracellular environment of the intestinal lumen. All these mechanisms explain the poor absorption of Cr(VI) in the intestine (41,42). In case some amount of Cr(VI) may escape detoxification, it will penetrate into the blood of the portal vein and, in case even this barrier may be overwhelmed, it will reach the liver, which is capable of reducing grams of Cr(VI) daily. As a consequence, Cr(VI) is poorly toxic by the oral route. In fact, only few lethal effects were observed in episodes of accidental ingestion of high doses of Cr(VI) by humans (6). For instance, following acute ingestion in the range between 8 and 20 g of Cr(VI) compounds, eight subjects survived and three subjects died (6). The LD₅₀ in rodents is >50 mg Cr(VI)/kg body weight (43).

The defence mechanisms are even more effective after repeated ingestion of smaller Cr(VI) doses, which can be readily detoxified and, therefore, do not result in cumulative phenomena, including mutagenicity and carcinogenicity. For instance, a family accidentally exposed to Cr(VI) at 1 mg/l in well water for three years was reported to have no adverse effects (44). Dogs fed 11.2 mg/l Cr(VI) for 4 years (45), and rats fed 25 mg/l for 1 year (46) or 134 mg/l for 6 months (47) exhibited no adverse effects from these chronic exposures. Similarly, a recently published investigation showed a lack of genotoxic effects after oral administration of potassium dichromate at up to 20 mg of Cr(VI) per liter of drinking water, or administration of a similar amount by gavage, in either the mouse bone marrow micronucleus assay or the rat in vivo—in vitro rat hepatocyte DNA repair assay (48). In addition, a three-generation study of drinking water exposure to potassium chromate in rats failed to demonstrate treatment-related increases in carcinogenicity following lifetime exposures (47). This observation is in agreement with the fact that no consistent pattern of excess risk for cancer of the digestive tract emerged from a large number of epidemiologic studies of Cr(VI) workers (3), despite the fact that massive exposures by inhalation presumably involved swallowing of Cr(VI). Furthermore, no exposure-related increase in stomach cancers or overall cancer mortality was reported in a recent evaluation of a residential population in China exposed for up to 6 years to Cr(VI) in drinking water derived from an aquifer contaminated by effluent from a chromate alloy facility (49).

A series of recent studies indicate that the relatively high reducing capacities related to oral ingestion are effective in (a) dramatically attenuating chromium absorption into the blood; (b) preventing any measurable entry of chromium in the hexavalent form into the blood, (c) preventing any increase in a putative marker for blood uptake of Cr(VI) (DNA–protein cross-links in peripheral lymphocytes) and (d) controlling the absorption, distribution and excretion of Cr(III) complexes that are formed following ingestion of Cr(VI) at plausible concentrations in drinking water (39,50–53). Each of these studies monitored total chromium concentrations in RBCs, plasma and urine over time to assess bioavailability and potential Cr(VI) uptake into the blood, using RBC chromium levels as a primary biomarker for such uptake. Human volunteers in these studies ingested water containing Cr(VI) or Cr(III) at concentrations up to 10 mg/l for as long as 17 consecutive days. In sum, these studies suggest that ingestion of Cr(VI) in drinking water at plausible chronic exposure concentrations (i.e. at or below 0.5 to 2 mg/l, when yellow discoloration becomes apparent) results in minimal uptake of chromium into the blood. Further, the RBC and plasma chromium concentration patterns following exposures to Cr(VI) in drinking water at up to 10 mg/l suggest that >99.7% of the ingested dose is reduced in the gastrointestinal tract prior to absorption into the blood. Thus, the pharmacokinetics of Cr(VI) at plausible chronic doses in drinking water mimics that of organic complex forms of Cr(III) (e.g. vitamin forms such as the tripicolinate or the glucose tolerance factor), likely due to formation of such complexes upon contact with organic reducing agents present in the gastric contents, tissues and blood.

In addition to well-known non-specific defense mechanisms of the respiratory tract (22), reduction in the ELF and in PAM supports the view that Cr(VI) encounters efficient detoxification barriers following inhalation. This is consistent with the premise that even for the primary target organ of Cr(VI) in humans there is likely a threshold for adverse effects including cancer, that is related to the reduction capacities of intrinsic defense mechanisms (54). The ELF layer has an average thickness in the 0.54–1.07 μm range over the 70 m² of alveolar surface of each individual (20). This fluid is particularly rich in antioxidants, such as GSH, vitamin C (ascorbic acid), vitamin E (tocopherol), superoxide dismutase, catalase, albumin, ceruloplasmin, transferrin, lactoferrin and other proteins (55). In rats the Cr(VI) reducing activity of ELF has been mainly ascribed to ascorbic acid (56), but in humans GSH levels are particularly high in ELF (57). Even more important is phagocytosis and reduction of Cr(VI) by PAM, that we estimated to reduce 136 mg/day/individual. The reducing activity of rat PAM was of the same order of magnitude as that of human PAM from non-smokers, i.e., around 2 μg Cr(VI)/10⁶ cells (58). Peripheral lung parenchyma showed an appreciable Cr(VI) reducing capacity (260 mg/individual), although it appears to be 12.7 times lower than that of the liver, which is also known to be more efficient than the lung in repairing DNA damage (59). It is also noteworthy that Cr(VI) is capable of inducing its own pulmonary metabolism (60). On the whole, there is evidence that the human respiratory tract has considerable defence mechanisms towards Cr(VI), although they are not as outstanding as those of the digestive tract. These processes unequivocally support the existence of
threshold mechanisms, which could be overwhelmed only under conditions of massive exposure by inhalation, as it occurred in certain work environments prior to the implementation of suitable industrial hygiene measures.

In conclusion, the herein reported quantitative estimates of Cr(VI) reduction in organs, cell populations, and fluids in the human body provide a rationale for predicting and interpreting the health effects of Cr(VI), including acute toxicity and long-term effects, as related to the portal of entry into the organism. At the same time, these data may be useful to regulatory agencies when setting Cr(VI) standards in the workplace or in the environment.

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References

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Protection Agency. Downloaded from National Library of Medicine online service, 25 January, Washington DC.