Environmental Health Perspectives
Vol. 92, pp. 87–89, 1991

Panel Discussion: Mechanisms and Health Effects of Chromium*

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The Panel was asked questions that had been prepared prior to the conference as well as queries from the audience, as follows.

In response to a question from R. Bartlett as to how Cr^{VI} can get past the digestive tract without being reduced, M. Costa (New York University Medical Center) stated that it is, in fact, likely reduced. He emphasized that the pharmacokinetics of chromium have not been sufficiently studied.

R. Wedeen (Veterans Administration Medical Center, East Orange, New Jersey) was then asked whether exposure to chromium causes increased renal excretion of chromium and of other compounds, a finding which seemed to be alluded to in Mutti's work (*1*), and if *a*) this is similar to low molecular weight proteinuria and *b*) whether the chromium excretion can be correlated with the air level of chromium. Wedeen replied that he was quoting in his talk from the work of Mutti's group (*1*) that showed both low molecular weight proteinuria and retinol-binding protein in the urine which is not reabsorbed and metabolized. The important point is that when the urinary chromium levels were greater than 15 μg/g urinary creatinine, low molecular weight proteinuria was observed. Whether the air exposure was related with the chromium excretion he did not know. He stated that the problem with the studies of this group (*2*) was that they did not really determine clearance because they did not take into account the protein binding of chromium in the serum.

Another question put to Wedeen was whether data from long-term high-level exposure (4 years) of rats and dogs to chromium in drinking water (which did not result in kidney damage) could be extrapolated to humans. Wedeen replied that the answer is no, especially if you only look at histological damage to the kidney, which is a crude approach. He stated that such data only support his point that no one has looked properly for kidney damage, in animals or humans. He added that the effects in humans must be very subtle, in that not everyone exposed to chromium gets the same disease, and these effects are yet to be discerned. His suspicion remains high from the information that is available, and experiments in animals that have failed to find kidney damage do not convince him to stop looking.

The question was raised as to whether there have been investigations of the effects of simultaneous exposure to lead and chromium on DNA synthesis, as this double exposure is possible for children in a specific Jersey City school. Such studies have apparently not been carried out, but Costa voiced the opinion that this might be an important question as all metals tend to accumulate in the kidney, and lead causes renal cancer in rats and causes intranuclear inclusion bodies. Lead has not been shown to cause cancer in humans, however.

A question was addressed to the entire group as to what compounds potentiate or act synergistically to increase any toxic or carcinogenic effects of chromium. M. Sugiyama (Kurume University School of Medicine) reiterated that vitamin B_2 pretreatment of cells in culture causes dramatic increases of Cr^{V}, which increases the genotoxicity, but the cytotoxicity is not increased; thus the cytotoxicity and genotoxicity are separate and perhaps not closely related. Sugiyama then speculated that the cytotoxicity is the result of membrane damage by chromium. K. Wetterhahn (Dartmouth University) pointed out that she has also observed that glutathione (GSH) is actually protective in terms of cytotoxicity but enhances the genotoxicity of chromium. E. Snow (New York University Medical Center) responded to a question about whether her *E. coli* system had active SOS repair (an error-prone bacterial repair of DNA damage) by stating that the cells in which she carried out mutagenicity studies were wild-type cells which have an SOS repair system that was not turned on in advance and that the involvement of SOS repair is being investigated. She was studying Cr^{III}, and it is not clear how this relates to the SOS system. She emphasized that more work needs to be done with repair mechanisms.

*Moderators: Charlotte Witmer and Iris Udasin. Panelists: Max Costa, Elizabeth Snow, Carroll Snyder, Masayasu Sugiyama, Richard Wedeen, and Karen Wetterhahn. Participants from audience: Roy Albert, Richmond Bartlett, Saul Shupack, and Paul Lioy.

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A question was raised as to whether it is really correct that only the Cr^{VI} form crosses cell membranes. Wetterhahn replied that the tetrahedral geometry of Cr^{VI} allows its transport across membranes by anion carriers. Cr^{III} does get into cells slowly, certainly liver and kidney cells, and eventually it reacts with the DNA, forming different adducts than those formed when Cr^{VI} is reduced *in situ*, Costa pointed out that the essential form of chromium is Cr^{III}, which can chelate fairly stably to a variety of ligands that can aid its transport. C. Snyder (New York University Medical Center) reiterated that the chromium need not get into the cells to cause damage, it can just alter some of the surface proteins, as his evidence shows. For example, if a kidney cell is turned into nonself because the (cell surface) protein is altered next to a major histocompatability protein, and the kidney cell is recognized as nonself by helper T-cells, the T-cells will kill the kidney cell.

Costa was questioned as to whether the linkage to protein (in DNA-protein crosslinks) was the -SH group and whether the linkage site in DNA is known. He replied that the protein -SH is involved and that the phosphate ester group and/or the N7 of guanine appear to be points of attachment for DNA (based on binding constants) in these chromium-mediated DNA-protein crosslinks. He suggested that studies with GSH might shed some light on binding. Wetterhahn stated that in studies of chromium binding to synthetic DNA polymers she found some degree of specificity for chromium binding to deoxyguanine, although this is not absolute. Adjacent adenines and guanines also lead to high levels of chromium bound to the DNA. There appears to be a specificity for purines generally, but the exact site on the DNA is not known.

In response to a question as to the value of speciation of chromium in biological samples, in view of the rapid reduction of Cr^{VI}, the panel agreed that speciation is valuable but difficult to carry out, depending on which oxidation state one is looking at. However, many findings support the premise that Cr^{III} is the form bound to DNA.

A question whether chromium is active in cell transformation assays and whether any specific transformations are correlated with biochemical and genetic findings was answered by Costa by referring to positive results for lead chromate in Syrian hamster embryo cell transformation assays and some positive results for insoluble salts, such as lead chromate, in 10T1/2 cell assays. DNA lesions, such as strand breaks and crosslinks, are present in cultured cells, and such lesions cause these positive results, but specific lesions cannot be yet connected with specific transformations.

When asked whether the uptake of chromium by mitochondria was studied in intact isolated mitochondria or *in vivo*, Wetterhahn replied that chromium is found in both intact mitochondria and in submitochondrial particles in *in vitro* studies and that Cr^{VI} is reduced in mitochondria to Cr^{V} by the electron transport chain. She has not examined mitochondrial DNA for binding of chromium. She added that researchers have shown that chromium accumulates in mitochondria *in vivo*. She also responded to a question about similarities between the extracellular and intracellular reduction of Cr^{VI} and subsequent binding of Cr^{III} by stating that there are different compounds that reduce chromate and that the binding of Cr^{III} following its production extracellularly is different from the binding following intracellular reduction. She reiterated that much of Cr^{VI} in blood is reduced in erythrocytes, although some may be reduced outside of the erythrocyte and then binds to albumin and other proteins.

In response to a question as to whether Cr^{VI} plays a role as a tumor promotor, Wetterhahn replied that it could have such an action by way of its capacity to cause free radical formation. Since the rat is not a good animal model for chromium-induced carcinogenesis, and as inhalation studies have generally been negative, it is difficult to evaluate the possible tumor promotion activity of chromium. Costa added that there are many problems with models for carcinogenesis of chromium, that inhalation studies with chromium in rats are difficult as rats are nose-only breathers. Snyder commented on pellet implant studies conducted at New York University about 20 years ago, which showed that chromium can act as a lung carcinogen in rats; subsequent aerosol studies carried out by the same group showed that about 4% of the treated rats got lung tumors, a high percentage for rats. Whether this was a promotion mechanism is not known.

Sugiyama was asked whether many effects of Cr^{VI} could be attributed to its nonspecific oxidizing action, and thus whether all antioxidants might be expected to protect against the toxicity. His response was that he could not generalize from his studies that all antioxidants would be protective. Extracellular interactions might be important for antioxidants, and he had only studied increased intracellular vitamin E. Wetterhahn agreed that the oxidizing activity of Cr^{VI} is not the sole mechanisms of its toxicity; it is not that nonspecific. The activity of chromium is under kinetic, not thermodynamic, control, so there are compounds that chromium would be expected to oxidize, and yet at physiological pH that doe not happen within a reasonable time scale. She also reminded us that we must distinguish genotoxicity from cytotoxicity citing the samples of glutathione and vitamin B_2 being activating in genotoxicity studies whereas ascorbate and vitamin E are not, and yet glutathione is also considered an antioxidant. She emphasized that one cannot make generalizations about the oxidizing action of chromium and its toxicity.

Wedeen was asked to identify the anatomic end point of the chromium effect on chronic functional deficiency in the kidney. His answer was that decreased renal filtration is the definition of renal disease but that it is difficult to distinguish this effect from that of aging. Renal failure may be multifactorial.

R. Albert asked whether the mucosol lining of the respiratory tract reacts quickly enough to reduce Cr^{VI}

to serve as a protective barrier against chromium for this tract by preventing its absorption. Snyder replied that the mucous lining does not go all the way down in the respiratory tract and that this lining probably does not serve as a good barrier for small chromate particles, although the size of the inhaled chromate particles is not really known. He added that he thought that there were some human alveolar tumors from chromium. Albert cited the mucosal protective effect on formaldehyde. Costa commented that the competition for reduction and absorption is a matter of concentration and solubility of the specific chromate; particles may produce local effects. Wetterhahn replied that the uptake into cells is very rapid, and thus, although there may be some reduction extracellularly, reduction by the mucosa will not be sufficiently rapid as a competing reaction to prevent the entry of Cr^{VI} into cells. Snow also explained that some binding of Cr^{III} to protein may be very rapid but not necessarily very tight so that this relatively loosely bound Cr^{III} could still be available for further toxic reactions. It was the consensus that the extracellular reduction in the mucosal lining is not so rapid as to impair the uptake into cells.

The question was raised whether very large doses of Cr^{VI} are required to bypass the extracellular binding of Cr^{VI} and whether these are really related to doses to which the public may be exposed. The levels of chromium to which humans are exposed are not known, but Snyder explained that the doses required for his immunological responses were not high, and humans would perhaps be exposed to nearly such levels (1 mg/L in the drinking water). How much is reduced in the stomach in humans is not known, so the intestinal exposure to the trivalent versus the hexavalent state is not known. Another point was made by Costa's group that in his work of exposure to Cr^{VI} in drinking water, some chromium got into the red blood cells, indicating the presence of Cr^{VI} in the blood stream as Cr^{VI} and thus not all was reduced in the gastrointestinal tract. Bartlett queried whether some of the Cr^{III} could get reoxidized in the digestive tract, but Costa did not think this was possible.

Snyder was also asked about the immunological effects of long-term exposure to chromium. This is not yet known, but it can be speculated that there may be resultant autoimmune diseases. It was then suggested that Jersey City may have an increased incidence of lupus, but Wedeen replied that this is not true. He also commented on an immune response to low doses of mercury which he has presented, which is a T-cell polyclonal release in the rat, which is a little different from the mechanism Snyder proposed. Snyder pointed out that T-cells are accessory cells in the antibody response, and therefore they may sometimes respond to altered cell protein, antigen, not by killing the cell but by increasing the antibody response. He added that this is rampant speculation, but things are starting to be tied together as to why there is kidney damage not only from chromium but from other metals as well, and why we are getting enhanced immunological response.

It was also noted by Sugiyama that no lipid peroxidation was found by addition of 1 mM chromate to cells in culture, although addition of Cr^{VI} to liver homogenates causes lipid peroxidation. This indicates that in cell culture situations the Cr^{VI} is immediately reduced to Cr^{V} and Cr^{III} and so does not cause the same effects as *in vitro*. Even though free radicals may be produced, there is no lipid peroxidation, so that the association of free radical formation and lipid peroxidation requires additional research.

It was asked whether the use of moist soil for experiments would be an improvement over previous exposures, and the answer was that such experiments are not now planned, as the reversal of the drying effects takes place in the animal. Snyder suggested that he may try Cr^{III} with the same immunological experiments he is now carrying out with Cr^{VI}. The similarity of Cr^{III} geometry (with water or DNA as ligands) to cisplatinum was briefly discussed, but there is no relevant similarity of these compounds and their effects, especially in regard to the renal toxicity of cisplatinum, as compared with the Cr^{III} in the nucleus. Wedeen also emphasized that the Cr^{III} form of chromium may not be much less toxic than the Cr^{VI}, particularly in the kidney, as tubular cells have the unique capacity to allow many compounds to cross membranes that would not be allowed by other cells. Thus Cr^{III} may be able to cross renal cells despite the fact that only Cr^{VI} is transported across other membranes.

When asked about the half-life of reduced chromium, Costa explained that Cr^{III} stays in the red cell for the life of the cell and at cell lysis the chromium is released and excreted as Cr^{III}. Weeden commented that his observations indicate that the $t_{1/2}$ for chromium in humans appears to be about 30 days; at least the blood levels and excretion levels drop to about 50% in 30 days.

The study of the species of chromium found *in vivo* is very difficult, it was emphasized, due to changes during the extraction processes. Costa emphasized that with Cr^{VI} and with other metals, distribution studies are very difficult because of ligand exchange reactions. It was also commented that the results of distribution studies for Cr^{III} are better than those of other metals because of the relative inertness of Cr^{III}. It is still difficult, however, and the speciation of chromium at various locations in the body is not definitely known.

The panel members agreed that the research to define the ultimate toxin/carcinogen of chromium was of the utmost importance and that this is related to the speciation and pharmacokinetics of chromium in mammalian tissues. It also appears that renal effects of chromium have not been well studied and in humans this is also a high priority.

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