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Mechanisms of chromium toxicity, carcinogenicity and allergenicity: Review of the literature from 1985 to 2000

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Laboratory and clinical reports about the pathogenesis of the carcinogenicity and allergenicity of chromium compounds published between 1985 and 2000 have been reviewed as a basis for consideration of the pathogenetic mechanisms involved. There is good evidence from the clinic and the laboratory that Cr[VI] is the ion responsible for most of the toxic actions, although much of the underlying molecular damage may be due to its intracellular reduction to the even more highly reactive and short-lived chemical species Cr[III] and Cr[V]. Exposure to Cr[VI] can result in various point mutations in DNA and to chromosomal damage, as well as to oxidative changes in proteins

and to adduct formation. The relative importance of these effects of chromium ions and of the free oxidising radicals they may generate in the body in causing tumours and allergic sensitisation remain to be demonstrated. Biochemical studies of the DNA-damaging effects and of the pathogenesis of the allergic reactions to chromium ions have not kept up with advances in understanding of the molecular basis of the effects of other carcinogens and allergens. *Human & Experimental Toxicology* (2001) 20, 439–451.

Keywords: chromium; toxicity; allergenicity; carcinogenicity; genotoxicity; biochemical mechanisms

Introduction

Chromium, mainly in the form of various alloys and as soluble salts of Cr[VI] ions, has been in wide industrial use for more than a century. Experience of excessive exposure in the work place has shown that it can act as an acute irritant, as a carcinogen and as an allergen to man,^{37,70} as most commonly seen in association with chromate production, metal plating, alloy manufacture and metal welding and forming processes. The Cr[VI] ion has been accepted as the principal cause of these toxic responses, and Cr[III] compounds, which are also in wide use in certain industrial processes, have been regarded as irritants, but not as carcinogens or allergens in their own right. There have been many studies of the occurrence and prevention of dermatitis, asthma and cancer in these and allied industries. Many experimental and biochemical studies of the mechanisms of these effects have been reported, both to explore their pathogenesis and to try to understand the basic biological processes involved.

The world literature published from 1985 to mid-2000 has been reviewed for information about the mechanisms of the allergenic, carcinogenic and irri-

tant effects of chromium. Papers (totaling 2023) in all languages were retrieved from a search of the Medline, Toxline, Embase and Biological Abstracts databases. They were reviewed for novelty, extent of the information contained, for hypotheses about pathogenesis and experimental or other support provided for the suggestions advanced. The papers cited were considered to provide useful information and ideas without being unnecessarily repetitive.

Our review is focused on theories about the mechanisms of the three major toxic properties of chromium ions, irritancy, carcinogenicity and the related genetic toxicity, and allergenicity, concentrating on biochemical and other mechanisms likely to be involved according to the laboratory and clinical literature. It has not dealt with clinical features of the diseases caused or with hygiene and other practices to prevent or treat them.

Physicochemical properties of chromium and its principal ions

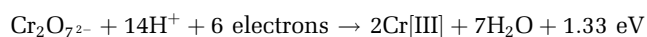
Chromium (atomic number 24, relative atomic mass 51.996) occurs in each of the oxidation states from –2 to +6, but only the 0 (elemental metal form), +2, +3 and +6 states are common. Divalent chromium (+2)

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is unstable in most compounds as it is easily oxidized to the trivalent form by air. Accordingly, only the trivalent Cr[III] and hexavalent Cr[VI] forms are important for human health. Valid generalizations of the biological effects of chromium in its elemental form can not be made.

In the context of this review, it is of great importance to realise that the +3 and +6 oxidation states have very different chemical and hence biological properties. The relationship between the hexavalent and trivalent states of chromium is described by the equation:



Thus, the difference in electric potential between Cr[VI] and Cr[III] reflects the strong oxidizing potential of hexavalent chromium and the substantial energy (1.33 eV) required to oxidize the trivalent Cr to the hexavalent form. Thus, oxidation of Cr[III] never occurs in biological systems. In contrast, reduction of Cr[VI] occurs spontaneously in the organism unless present in an insoluble form. For example, in blood, Cr[VI] is rapidly reduced to Cr[III]. Thus, once Cr[VI] has penetrated the membrane of the red blood cell it is reduced and Cr[III] becomes bound to cellular constituents making it unable to leave the erythrocyte.

Absorption, distribution, excretion and metabolism

Chromium [III] compounds

Oral administration of chromic chloride to humans resulted in 99% of the dose being recovered in faeces while about 94% was recovered after duodenal administration. In both cases, about 0.5% was excreted in urine²⁶ indicating poor absorption of Cr[III] following oral ingestion.

After human exposure to Cr[III] by inhalation, urinary concentrations of chromium were found to be increased indicating respiratory absorption.^{3,28} However, the extent of the pulmonary uptake of Cr[III] is influenced by the nature of the compound.³⁹ After one volunteer had immersed his hand in tanning liquor for 1 h, monitoring of blood and urine failed to detect dermal absorption of chromic sulphate.³

Studies in experimental animals are compatible with the poor absorption of Cr[III] salts following exposure by oral, dermal and inhalation routes.^{46,67,70}

Chromium [VI] compounds

Following oral administration of sodium chromate in tracer doses to humans, faecal excretion of chromium

indicated that about 10% of the administered dose had been absorbed from the gastrointestinal tract. After duodenal administration approximately half of the administered radioactivity appeared to have been absorbed on the basis of faecal excretion while 10% appeared in the urine during the first 24 h. Reduction of Cr[VI] to the trivalent form was demonstrated.²⁶ Correlation between respiratory exposure to Cr[VI] and urinary excretion of chromium has been demonstrated in welders and in workers in the chrome plating industry.^{4,43} The respiratory uptake rate is unknown, but depends on the solubility of the chromium compound (reviewed by Aitio *et al*⁴). Cr[VI] is reduced in the lower respiratory tract by the epithelial lining fluid and by pulmonary alveolar macrophages. At equivalent numbers of cells, the reducing efficiency of alveolar macrophages by biochemical mechanisms was significantly greater in tobacco smokers than in nonsmokers.⁴⁹

In contrast to Cr[III], which is bound to plasma proteins, such as transferrin, Cr[VI] entering the blood stream is taken up selectively by erythrocytes, reduced, and bound to haemoglobin.^{1,32,40} Reduction of Cr[VI] during transport in the blood is consistent with the finding that only Cr[III] is present in the urine.^{46,48}

Aitio *et al*⁴ reviewed the results of biological monitoring of exposure to chromium (predominately soluble Cr[VI]) and identified three half-lives for excretion of 7 h, 15–30 days and 3 to 5 years. The best estimates for the sizes of these different compartments are 40%, 50% and 10%, respectively.

Retention of chromium on the skin was found following topical application of sodium chromate.⁷

General population exposure

Chromium is ubiquitous in nature; it can be detected in all matter in concentrations ranging from less than 0.1 $\mu\text{g}/\text{m}^3$ in air to 4 g/kg in soils. Naturally occurring chromium is usually present as Cr[III]. Hexavalent chromium in the environment is almost totally derived from human activities.⁷⁰

General effects on humans

Studies in man and experimental animals have established the essential role of "trace amounts" of Cr[III] (50–200 $\mu\text{g}/\text{day}$) for the maintenance of normal glucose metabolism. Such intake by the oral route does not represent a toxicity problem.⁷⁰

In adult human subjects, the lethal oral dose is considered to be 50–70 mg soluble chromates per

kilogram body weight. The clinical features of acute poisoning are vomiting, diarrhoea, haemorrhage and blood loss into the gastrointestinal tract, causing cardiovascular shock.^{56,70} If the patient survives for more than about 8 days; the major effects resulting from oral ingestion of toxic doses of chromium are liver and kidney necrosis.⁷⁰ Although parenteral administration of chromium to experimental animals can lead to teratogenic effects, birth defects have not been associated with human exposure to chromium.¹⁵

Specific toxic effects on humans

Dermal exposure

Chronic ulcers of the skin and acute irritative dermatitis have been consistently reported in workers exposed to chromium containing materials.⁷⁰ Chromates and Cr[VI] released from alloys and chromium-plated objects have been associated with the induction of allergic contact dermatitis (see below). It is generally assumed that Cr[VI] is necessary for the sensitisation, while both chromium [VI] and Cr[III] may cause dermatitis in sensitised individuals.³⁵ Intracellular reduction of Cr[VI] to Cr[III] seems to be a prerequisite for this effect.⁵¹

The strong acid and powerful oxidising properties of soluble chromium ions are regarded as the primary causes of its irritant action on epithelia.

Respiratory exposure

Inhalation of Cr[VI] compounds causes marked irritation of the respiratory tract. Thus, ulceration and perforation of the nasal septum have occurred frequently in workers employed in the chromate producing and hexavalent chromium-using industries.⁷⁰ Cases of sinonasal cancer were reported by the International Agency for Research on Cancer³⁷ possibly indicating a pattern of excess risk for these rare tumours.

Rhinitis, bronchospasm and pneumonia have been reported in workers exposed to Cr[VI] together with impairment of respiratory dynamics during respiration although the precise role of chromium is uncertain as such workers are often exposed to other chemicals.⁷⁰ Nevertheless, bronchial asthma has been reported to be due to exposure to chromates.³⁵

Epidemiological studies of workers in the chromate production industry have consistently shown excess risks for lung cancer. Studies in experimental animals have confirmed Cr[VI] to be carcinogenic by inhalation but not by ingestion or skin contact. There is inadequate evidence in experimental animals and

man for the carcinogenicity of Cr[III] compounds or metallic chromium.³⁷

General mechanisms of chromium toxicity

As a general rule Cr[VI] is much more toxic than Cr[III].

Cr[VI] enters cells more readily than Cr[III] compounds and is ultimately reduced to Cr[III]. Cr[VI], tested as sodium dichromate, was transported through mammalian cell membranes by the carboxylate, sulphate and phosphate carrier systems; the kinetics of uptake also involved the rate of reduction to Cr[III].^{23,64}

The intracellular reduction of Cr[VI] implies the generation of short-lived species of pentavalent and tetravalent chromium with affinities for cellular constituents that may differ from that of Cr[III].¹⁹ The pentavalent form is stabilised by glutathione.⁴⁰ Thus, the reduction of Cr[VI] is considered to serve as a detoxification process when it occurs at a distance from the target site for toxic or genotoxic effect while reduction of Cr[VI] may serve to activate Cr if it takes place in or near the cell nucleus of target organs.²⁴ The initial binding to cellular macromolecules may therefore involve the pentavalent form.⁵⁴ Once absorbed and retained in biological tissue chromium compounds occur as Cr[III].

Glutathione and cysteine seem to be the most important cofactors for the intracellular reduction of Cr[VI], but ascorbic acid, microsomes in the presence of NAD/NADH, microsomal cytochrome P450, mitochondria and proteins such as haemoglobin and glutathione reductase may also be active in the reduction process.³⁷

Mechanisms of chromium genotoxicity

Cr[VI] compounds of various solubilities in water were consistently active in numerous studies covering a wide range of tests for genetic and related effects. In particular, potassium, sodium and ammonium dichromates and chromates, chromium trioxide, calcium and strontium chromates induced DNA damage, gene mutation, sister chromatid exchange, chromosomal aberrations, cell transformation and dominant lethal mutations in a number of targets, including animal cells *in vivo* and animal and human cells *in vitro* (WHO, 1990).⁷⁰

With purified DNA and isolated nuclei Cr[III] compounds were generally more reactive than Cr[VI] compounds. However, with cellular test systems 12 Cr[III] compounds of various solubilities gave posi-

tive results in only a minority of studies, often under particular treatment conditions or at very high concentrations, which were generally orders of magnitude higher than those needed to obtain the same effects with Cr[VI] compounds. Therefore, some of the positive results could be ascribed to contamination with traces of Cr[VI] compounds. In particular, DNA damage was not observed in the cells of animals administered chromic chloride and micronuclei were not found in the cells of animals given chromic nitrate. The Cr[III] compounds tested generally did not produce DNA damage, gene mutation, sister chromatid exchange or cell transformation in cultured animal and human cells although chromosomal aberrations were often observed with high concentrations of Cr[III] compounds.

Negative results were obtained for Cr[III] compounds in the large majority of tests for DNA damage and gene mutation in bacteria (WHO, 1990).⁷⁰

The activities of Cr[III] and Cr[VI] in acellular (i.e., purified nucleic acids) or subcellular (i.e., cell nuclei) systems have been investigated in several studies. Depurination of calf thymus DNA did not occur with Cr[III]. Assessments of viscosity, ultraviolet absorption spectra and thermal denaturation of purified DNA and RNA showed that, at variance with Cr[VI], which (as an oxidising agent) breaks the polynucleotide chain, Cr[III] is responsible for physicochemical alterations of nucleic acids by interacting with the phosphate groups and nitrogen bases^{65,66} Cr[VI] produced DNA strand breaks, DNA–DNA and DNA–protein cross-links and modified nucleotides, such as 8-hydroxyguanine, indicative of oxygen radical formation.^{5,20,68} However, these reactions do not occur in cell-free systems in the absence of reducing agents and current consensus is that the highly reactive intermediates such as Cr[V] and Cr[IV] formed during cellular Cr[VI] reduction are primarily responsible for the observed genotoxicity.⁴¹ Cellular reducing agents that may be of importance for Cr[VI] reduction includes ascorbate and sulphhydryl compounds such as cysteine and glutathione.^{12,42,60–62} Although hydroxyl, cysteinyl and thionyl radicals may be formed during the reduction of Cr[VI] it is not known if these intermediates are of relevance in chromium induced carcinogenesis.^{18,58,60,68} The oxygen free radicals generated by chromium-mediated reactions activate a transcription factor known as NF- κ B,¹⁴ which is a critical activator of genes involved in inflammation, immunity and apoptosis. Thus, chromium carcinogenicity is proving to be complex matter. Accordingly, although it is now accepted that inhalation exposure to Cr[VI] can cause cancer, the mechanism(s) involved, the involvement of other valence states and the influence of solubility are still very much in dispute.

Chromium and allergic reactions

Principles of allergy and its immunological basis

To understand the way in which chromium compounds may excite allergic responses, it is first necessary to understand modern views about the origins of allergic reactions, their mechanisms and the cells and mediators involved.

Allergy is defined as an excessive and harmful immune response to an antigen, which may involve either or both specific or cross reactive antibody against the antigen and a cell-mediated reaction. The former is often termed a “humoral” response, because it involves antibodies circulating in the blood stream and present in mucosal secretions; it tends to occur rapidly after exposure, because of the role of preexisting antibody. The latter requires specific sensitised lymphocytes, hence its designation as a “cell-mediated response”; it is also called “Delayed-type hypersensitivity” because of the time required for recruitment and activation of the reactor cells after exposure to the necessary antigen. For reviews see current monographs on immunology, such as Roitt⁵³ and Janeway *et al.*³⁸

Hypersensitivity, although often used as a synonym for *allergy*, should be restricted to the altered state of potential immunological hyperreactivity. The latter represents the potential to react adversely when appropriately exposed, and so is quite distinct from the illness manifested when a hypersensitive individual is exposed to the particular antigen to which there is the abnormal, excessive response.

Development of hypersensitivity

Like any other immune response it requires prior exposure to an appropriate antigen, normally a protein but other sufficiently large molecules can act in the same way, to which an immune response is generated by the body, involving a sequence of “antigen-presenting cells” (dendritic cells), which bind and present the specific immunological determinants (“epitopes”) in a particular way to T lymphocytes at the same time as releasing certain cytokines, especially IL-2. The result is proliferation of the T cells to form unique clones of memory cells and effector cells. The latter may develop into cytotoxic lymphocytes and they may stimulate the formation of clones of B lymphocytes, which secrete antibodies of equal specificity, initially IgM and subsequently of IgG, IgE and other classes. The cells of the immune system are largely stimulated to proliferate and mature by particular patterns of cytokines released by cells activated earlier in the sequence. The lengthy duration of immunological memory depends on the presence of circulating memory T cells, which are stimulated into prolifer-

ation and activity on re-exposure to the appropriate antigen, possibly after many years.

T_H-1 and T_H-2 cells

In the past few years investigations in the mouse, which have subsequently been broadly confirmed in other species, including man, has shown that the initial activated naïve T lymphocytes can develop along two or perhaps three different lines, which control the nature and intensity of the subsequent immune response. Detailed knowledge of the generation, activities and control of the most important T_H-1 and T_H-2 cells is reviewed by Aebischer and Stadler;² the following is a brief and limited summary of the more important features of the system. Their role in allergy is discussed by Maggi.⁴⁵

The class of T_H-1 cells secrete especially IFN γ , TNF α/β , IL-3 and GM-CSF. These cytokines stimulate the proliferation and maturation of active, cytotoxic T cells and so are largely responsible for the development of "cell-mediated" or "delayed-type" hypersensitivity responses. Antibody formation will also occur, mainly of IgG types.

T_H-2 cells secrete particularly IL-4, -5 and -6, as well as IL-3 and GM-CSF, and result in the particular development of B lymphocytes and subsequently plasma cells secreting mainly IgG, IgE and other antibodies. The resultant immune response is dominated by the IgE antibody, which is the basis of immediate-type allergic reactions. IgE antibody is largely bound to cells in tissues, especially mast cells, basophils and eosinophils. Combination of the bound antibody with its specific antigen results in ligation of the bound receptor molecules, activation of the mast cells and the release of their various spasmogenic and inflammatory mediators, thus producing bronchoconstriction, as in asthma, itchy wheals and dermatitis in the skin, etc. A detailed account of mediators involved in asthmatic reactions is given by Barnes *et al.*,⁹ which is broadly applicable to immediate skin reactions, too.

The control of the direction and intensity of the immune response is far more complex than this simple scheme suggests, as there are many ways in which genetic factors influence it, as well as the general state of the individual, the nature of the antigen, the dose and site of exposure to it and whether the immune system is being activated in a particular direction by factors ("adjuvants") that may enhance the response and direct it along a predominantly T_H-1 or T_H-2. Certain patterns of exposure may even lead to tolerance, possibly due to generation of so-called T-suppressor or T_H-3 cells. Those aspects remain the subject of controversy and active research.

Antigenicity and allergenicity

The induction of the initial ("primary") immune response and of the enhanced secondary response reliant on activation of memory cells on a second or subsequent exposure is dependent on several factors unique to each antigen, the genotype and phenotype of the organism, the intensity and magnitude of presentation of the antigen, the route by which the body is exposed to it, and the general physiological state of the organism at that time.

Genetic factors, notably the atopic state in humans, are powerful determinants of whether someone will develop an allergic reaction on exposure to an antigen. The induction of allergy, both primary sensitisation and secondary elicitation are dose related, although a reaction may follow a much lower dose than would be required of a conventional toxicant because of the considerable biological amplification possible by recruitment of immune cells and the synthesis and release of multiple mediators.

Molecular factors

It has been known for many decades that low molecular weight substances are not themselves antigenic, immunogenic, or allergenic. To be recognised by the immune system they must become bound to larger molecules in the body, commonly proteins, although lipids and carbohydrates may sometimes be involved, in order to become antigenic and possibly immunogenic and even allergenic. There is much ignorance still about the detailed factors determining the capacity of a carrier molecule (sometimes called a "Schlepper") to bind a low molecular weight hapten and to lead to an immune response specific to that hapten against the background of the carrier.

We know most about properties that make proteins themselves antigenic and even allergenic, as helpfully reviewed recently by Huby *et al.*³⁶

The specific features of a protein that make it immunogenic and even more those that make it into an allergen are not well understood. The known factors, which are difficult to quantify, are the following.

Size Larger molecules are more likely to result in an immune response than small ones. Simple inorganic ions will not be immunogenic or allergenic unless they become bound to or otherwise alter ("denature") a sufficiently large protein, i.e., one of molecular weight >about 10000 kDa.

Insolubility or particulate nature Retention at the site of exposure appears important, perhaps because the dendritic (antigen-presenting cells) require some time to ingest and process antigen into the form that excites an immune response.

Denaturation of a protein is a good way of making it an effective antigen and allergen. This process may

expose new antigenic determinants (“epitopes”) against which the response occurs, either conformational changes in the quaternary or tertiary structure of the protein, or due to direct chemical modification, e.g., by oxidation of –SH or other groups, haptenisation by binding of a new chemical, such as a heavy metal, etc. It seems likely that many of these chemical and physical factors act by changing the hydrophobicity and charge of epitopes so that they become recognised by the immune system as “foreign.” A related feature of immunogenicity is the degree of “foreignness” of the protein, i.e., the extent to which its structure (primary or higher order) differs from native proteins in that organism.

Ability of the antigen, when presented as peptides by the APCs, to interact with the “major histocompatibility complex” (MHC) proteins of the individual subject, especially the MHC Class II determinants, which have a vital role in controlling the interplay between antigen–epitope and antigen presenting cell–T lymphocyte interactions.

Route of exposure Exposure via the skin (including the buccal mucosa) and respiratory tract are good ways to excite immune and allergic responses, whereas oral ingestion or parenteral injection are poorly immunogenic. In fact, oral ingestion often leads to a state of tolerance by means that are still disputed, but which may involve the generation of a population of active suppressor T cells.

Thus, the allergenicity of a substance depends on a complex web of chemical and physical features of the material itself, on how they affect the ways in which it is or is not recognised immunologically by the exposed subject and on many factors specific to the individual.

Amongst the critical factors in determining allergenicity are the role of the APCs in generating specific peptides that carry the unique identity, the epitope, of the allergenic substance, and which are able to be recognised by and stimulate T cells by combining with the corresponding unique T-cell receptor.⁸ The specific T-cell epitopes are peptides of 10–15 amino acids in length.⁸ They not only carry the unique identity but they may somehow polarise the T-cell response towards a predominantly T_H-1 or T_H-2 response (e.g.,^{50,59}). Many factors influence the ultimate polarisation towards a T_H-1 or T_H-2 response; the more important include the dose of the epitope, its binding affinity to MHC Class II molecules and its persistence on APCs.⁵²

Allergenicity and low molecular weight haptens

Based on our general understanding of molecular features that predispose to allergenicity, it seems likely that haptens will stimulate an immune and

perhaps an allergic response if certain features occur together.

i) Sufficient density of the haptenic determinant on the carrier molecule.

ii) Almost certainly adequate persistence of the hapten on the peptides processed and presented by APCs to T cells.

iii) The physicochemical nature of the carrier protein and the haptenic peptide in polarising the T-cell response towards a T_H-2 response.

iv) If the carrier protein occurs naturally in the subject, the hapten must modify it sufficiently to overcome the normally dominant tolerance of “self” proteins, by causing sufficient change in the structure (probably tertiary structure) of the endogenous molecule, e.g., by denaturing it, by forcing presentation of a protein normally concealed from the immune system and to which there is not normally tolerance, or by binding to the carrier and so producing a novel epitope to which the body will react.

The overall general conclusions that can reasonably be drawn about allergy and allergenicity, as contrasted with the more common immunogenicity (the capacity to excite an immune response but not one that caused adverse effects), are of limited strength as our fundamental knowledge remains limited.

Allergenicity depends on specific but incompletely defined properties of proteins, either in their native form or modified by the effects of low molecular weight haptens that have acted on them or are bound to them

It is also dependent on genotypic factors in the affected individual, which influence the basic capacity of that person to become sensitised and may subsequently influence his capacity to mount an allergic response and its vigour

The dose and route of presentation of the antigen (or hapten) affect the likelihood of allergy developing, and they have a considerable effect on the nature and severity of the allergic response to a subsequent challenge

We are aware of general principles and of many isolated facts in this area, but the rules describing the response to be expected when a given subject undergoes a sensitising exposure or the nature and strength of the allergic response on subsequent challenge remain to be clarified.

Allergic reactions and chromium occurrence and exposures of humans

Allergic reactions to various chromium compounds have been described (or at least claimed) in industries, in health care, and in the general population.

Occurrence

The diverse exposures involved have been:

INDUSTRIAL	electroplating — chromates manufacture of refractory tiles, etc for high-temperature processes — chromates metallurgy for alloys used in tools and in stainless steels — Cr mist inhibitors in cooling towers ore refining paints, pigments and rust-resistant coatings — various chromates and oxides tanning — dichromates fluxes and alloys in welding — chromates and Cr catalysts — Cr metal and various oxides and chromates wood preservatives — Cr and chromates recording tapes (Cr[IV] as CrO ₂) component and impurity in cements (dye mordant)
HEALTH CARE	prosthetic implants — Cr in alloys mineral supplements for human and animal consumption (glass cleansing in laboratories histological stains)
PUBLIC	“chromium”-plated jewelry clothing components via water in soil and loess

Exposures

The principal forms of chromium and chromium compounds to which people may be exposed are:

- chromium metal, almost always in alloys.
- Cr[III] as chromic dioxide (Cr₂O₃), chromic chloride (CrCl₃) or more often as stable trivalent complexes with organic and inorganic ligands.
- Cr[VI] as chromic oxide (CrO₃) and various chromates and dichromates (CrO₄²⁻), including a number of alums.

Although the net solubilisation of Cr compounds from implanted metal alloys is low, it does occur.^{22,27}

There is good evidence from humans and from animal studies that inorganic trivalent Cr[III] compounds are very poorly absorbed from the skin, lung and gastrointestinal tract.^{22,27,33}

Hexavalent inorganic chromium compounds are better absorbed; the uptake depends on the chemical nature and aqueous stability of the compound, and

on the rapidity with which it reacts with local proteins and other substances.²⁷

As hexavalent Cr compounds are powerful oxidising agents, in biological matrices, such as the fluid phase in the gastrointestinal tract and lung, and on the surface of the skin, hexavalent Cr compounds are rapidly reduced to the trivalent state by reaction with available reducing groups. They also readily enter cells, where they are reduced in the same way and by a number of mitochondrial and other electron transfer processes. The resulting Cr³⁺ compounds become complexed with glutathione and probably other -SH donors, phosphate ions, and with a variety of proteins, lipids and perhaps carbohydrates.^{21,22,27,33} The complexation of chromium compounds on body surfaces and within cells has not been well characterised.

The chemical reactions occurring when chromium oxides, silicates and other compounds in welding fumes are inhaled do not appear to have been reported.

Thus, when humans and animals are exposed by any route to chromium compounds, or to chromium metal, there are many chemical reactions that may affect the nature of the chromium compounds, which are very rapidly formed *in situ* and after uptake into the body and into cells.

The principal factors affecting the nature of the exposures that occur are the initial valency state of the chromium, as hexa- are reduced to trivalent forms, and there is ready oxidation of many biological molecules associated with speedy complexation of the trivalent forms with a wide variety of naturally occurring substances that contain reducing groups, including -SH, amino acids, lipids and carbohydrates. Complexation with inorganic phosphate also occurs, the resulting chromium phosphate probably being relatively unreactive and stable.

This aspect of the biological chemistry and reactivity of chromium compounds is very complex and is poorly understood. It is important in the present context because our ignorance means that we do not know what are the particular antigens or epitopes important in the various allergic reactions to chromium.

Allergy to chromium

Extensive human experience over many years has shown that hexavalent chromium compounds, especially the water soluble forms, most readily produce sensitisation and allergic reactions, probably because of their reactivity.⁵⁵ Formation of the ultimate hapten is considered to involve Cr[III], probably because of its ready complexation with large biological molecules, at least according to experimental studies. The equally well established immunological reac-

tions to metallic chromium almost certainly involve the initial production of soluble trivalent chromium ions.

There are reports of contact dermatitis to the tetravalent form in CrO₂.^{22,27}

Skin allergy

Hexavalent chromium in dichromate, or as chromium trioxide, sensitised every person in a human maximisation test,²⁷ and almost everyone in typical patch tests. This means that it is an extremely potent sensitising agent. Contact dermatitis has been a serious problem in industries where solutions of Cr[VI] salts are handled, as well as in workers exposed to stainless steel welding fumes.

It is important that a high proportion of the general population shows a positive reaction on skin patch testing to dichromate — from 10% to 12% has been claimed.^{22,27} The population incidence may be falling in Scandinavia, possibly due to reduced exposure to chromium-plated objects.²²

In practice both the initial sensitisation phase and the subsequent elicitation of a contact dermatitis reaction on challenge can be caused by very small quantities of chromium compounds, including the amounts released from chrome-tanned leather and from solid alloy implants.

The evidence that trivalent chromium compounds are the basis of the ultimate skin allergen comes from knowledge of the brief existence of hexavalent ions in biological environments, the cross reactivity of hexavalent Cr-sensitised people to trivalent Cr salts and the typical laboratory responses of lymphocyte transformation and migration inhibition on exposure of cells from Cr[VI]-sensitised subjects to trivalent Cr compounds (*loc. cit.*). Oral ingestion of Cr[VI] salts by sensitised subjects has led to a positive skin response, and it is known that only Cr[III] will be absorbed and circulate.^{21,22,27}

There is a limited literature on gum reactions around dental implants containing chromium alloys, which have been attributed to contact allergy, and a very few claims have been made of generalised eczematous reactions to chromium released from orthopaedic implants.^{22,27}

The skin reactions are typical of a delayed hypersensitivity in their clinical features, time course and histological findings on biopsy.^{22,27}

There do not appear to have been specific reports of the minimum period of exposure required to cause sensitisation, or of the minimum concentration of Cr[VI] ions involved.

Respiratory system

Asthma is a well-reported finding in some subjects exposed to welding fumes from stainless steel.^{22,27}

Hexavalent chromium compounds in the fumes are considered the cause of the reaction.

Challenge studies to confirm the specific nature of the allergen have been described.²¹ There was an effect level on challenge with inhaled Cr[VI] as CrO₃ as low as 2 µg/m³.

The minimum risk level of Cr[VI] in inhaled air is said to be 0.02 µg/m³.⁶

Autoimmunity

A few contentious reports have suggested that exposure to chromium compounds may result in the formation of anti-DNA antibodies, although the relationship between them and clinical disease is unclear.^{11,29}

Many other disorders have been linked to exposure to chromium compounds, including hepatic and renal damage and cancers, but there is no reason to associate them with specifically immunological mechanisms.

General immunotoxicity

A brief report notes that a group of 15 workers exposed to lead chromate dust by inhalation at work, and who had raised blood and urine chromium levels compared to controls, had reduced levels of circulating CD4+ helper, activated B and natural killer cells. Correlation analysis was used to claim that the effects were due to chromium exposure and not to the concomitant increased body burden of lead, but the analysis is primitive and the conclusion may best be regarded as tenuous.¹³

Experimental immunotoxicity

Cr[VI] as sodium dichromate solution (25–250 µg/m³) inhaled daily for up to 90 days by rats resulted in increased weight of the spleen (specific cause not determined) and increased antibody-forming cells on immunisation with sheep red cells. This is a classic and well-founded test of a T-cell-dependent, humoral antibody response of the immune system to a novel challenge.³⁰

The finding might represent an action on initial antigen uptake, on the extent of the immune response at any of several of levels by an “adjuvant-like” action, or a diminution of the activity of T-suppressor cell mechanisms.

Subsequent experiments¹⁷ showed that brief inhalation of soluble or insoluble chromate (Cr[VI]) 360 µg Cr/m³ by the rat led to reduction in the production of cytokines by alveolar macrophages, which is a possible indicator of an immunosuppressive action.

Specific investigations of the allergenic effects of chromium compounds

Disappointingly little work has been reported compared to the wealth of investigations into the carcinogenic activity of chromium compounds.

In contact sensitivity reactions, keratinocytes are the cells first exposed to the allergen. They may respond by gaining the characteristics of antigen presenting cells and by producing cytokines that amplify and modulate the subsequent immune response.

Experiments *in vitro* compared the effects of potassium dichromate (Cr[VI]) 2 µg/ml with other known allergenic metals Ni and Co in a tissue culture medium on fresh human keratinocytes.³⁴

The dichromate treatment up to 48 h led to increased production of TNF α and Heat Shock Protein 72. The latter might just reflect toxicity, but the former response might represent activation of the keratinocytes in a way that would enhance immunological reactivity, perhaps along the T_H-1 allergenic pathway.

Granchi *et al*³¹ examined peripheral blood mononuclear cells (PBMC) from 15 patients with aseptic loosening of hip prostheses made from Co–Cr hip prostheses (type not stated).

Resting PBMC released more TNF α than cells from normal subjects. On stimulation with an extract of “chromium powder” in culture medium (metal ion nature and concentration not stated) IL-6 release from patient PBMC was increased more than controls, and so were GM-CSF and TNF α . Only the “chromium” led to a particular increase in IL-6 production as compared to exposure to “cobalt” extract. There was also a nonsignificant increase over controls in PBMC with the T_H-1 phenotype on FACS analysis.

The proportions of various lymphocyte subsets were not significantly different in the patients compared to the controls.

The suggestion is made that the long continued exposure of the patients to chromium ions released from the implants had led to a prolonged hypersensitivity response. The evidence is weak!

There is a prevalent view, mainly in Scandinavia, that “cement dermatitis” is a sensitisation reaction to Cr[VI] compounds in cement, and that reducing the content of Cr[VI] to less than 10 ppm would prevent this distressing and disabling condition.²² Despite a number of encouraging reports of a considerable reduction in the occurrence of this condition on compulsory addition of a small quantity of ferrous sulphate to cement to reduce Cr[VI] to Cr[III],²² later work has not supported the beneficial effect and so has not confirmed the hypothesis.¹⁶ This may be due more to confounding by the multiplex nature of the

causes of an industrial dermatitis than to an error in the basic hypothesis.

Other information

Potassium dichromate 0.5% gave a clear positive result in the murine local lymph node assay used to predict the sensitising potential of chemicals.¹⁰ The effect was dose related.

Many studies have been published into the chemical effects of various chromium compounds on DNA in cells in attempts to understand the mechanism of its carcinogenic action.

A few are mentioned here only because they bear on possible chemical reactions between chromium compounds and cellular macromolecules, and so may be related to the nature of the hapten and the ultimate epitope underlying chromium-induced immunological sensitisation.

i) Liu *et al*⁴⁴ used electron paramagnetic resonance spectroscopy to show that Cr[VI] applied to the skin of the living rat was rapidly reduced to Cr[V]. Others have shown that form is able to generate OH \cdot radicals by Fenton-like reactions and to be capable of causing DNA damage.⁵⁷

ii) The speedy reduction of intracellular Cr[VI]/Cr[V] to Cr[III] in V79 cells was proven by single cell micro X-ray absorption spectroscopy,²⁵ a finding reported independently by Myers,⁴⁷ who also showed the probable sequential one electron reduction of Cr[VI] to Cr[III] by human tissues.

Taken together, and generalising, these two papers show the anticipated reduction of higher valency states of chromium to Cr[III] *in vivo*.

iii) Analogous results are available showing that Cr[VI] leads to the generation of free radicals in cells *in vitro* and in the rat *in vivo* — both directly,⁶⁹ and indirectly by demonstrating the protective effect of desferrioxamine on DNA single strand breaks and on lipid peroxidation in rat hepatocytes.⁶³

iv) Various experiments have described the formation of DNA–protein cross-links *in vitro* and *in vivo* on exposure to Cr[III] and Cr[VI] compounds (e.g.,^{21,71,72}).

These may be important as the source of novel epitopes that could account for the appearance of anti-DNA antibodies after exposure to chromium compounds, and which might be the source of the epitope(s) underlying sensitisation.

Conclusions about chromium and allergenicity

There is long experience in man of allergic sensitisation causing dermatitis on cutaneous exposure to

Cr[VI]>Cr[III] or Cr[IV] compounds, and more limited but clear evidence of asthmatic responses sometimes after respiratory exposure to the same compounds.

Following sensitisation, the allergic responses behave like other typical allergic reactions in terms of dose–response relationship, duration, occasional cross-reactivity with other metals, e.g., Ni, and the nature of the response, which is indicative of delayed hypersensitivity. There is only very limited evidence of IgE antibody formation, which would be expected to underlie the asthmatic reactions, but it has been described.

General experience rather than specific enquiry suggests that the individual genotype, notably a family history of atopy, will increase the likelihood of an allergic reaction to Cr compounds developing after appropriate exposure, but there do not appear to have been specific studies of genotypic markers. As Cr[III] and Cr[VI] are powerful allergens in man, the genotype may not have much practical influence.

The epitope(s) involved in the allergic reactions have not been examined. As Cr compounds may oxidise many thiol-containing compounds, amino acids, lipids and carbohydrates, both when isolated and forming part of macromolecules, there are many candidates for the sensitising epitomes, including covalently bound Cr compounds, altered protein structure, and cross-linked protein–DNA complexes.

Simplistic animal studies have shown that inhalation exposure to Cr[VI] may cause a systemic increase in antibody formation to injected sheep red cells.

Whether industrial or other exposures to chromium compounds may generally influence the immune responses of the body is not known.

It would be fair to point out that knowledge of the allergies caused by chromium compounds has not kept up with advances in our understanding of the pathogenesis and mechanisms of allergies in general.

Questions that seem worth consideration and perhaps investigation might include —

- Why are there so few clinical reports of exacerbation of chromium allergies at one or more distant sites after exposure of sensitised subjects by another route, e.g., it appears rare for

there to be an asthma attack after oral or cutaneous exposure despite evidence of absorption of chromium ions into the blood stream? Is this just clinical underreporting or is specifically local exposure required? If the latter, in what way does local exposure trigger a response, when systemic exposure does not? Might there be different antigens/epitopes at different sites?

- Why is Cr[VI] such a powerful sensitising agent on the skin and yet seemingly a relatively weak one by the respiratory route — judged by the paucity of reports of asthma?

Again, is this clinical underreporting or a genuine difference in potency?

- How do chromium ions act as allergens?
- Is the effective epitope Cr ion covalently bound to a protein or other macromolecule, or is it a protein (or other macromolecule) whose tertiary structure is modified by the action of Cr ions on thiol bonds and other reducing groups? Is the epitope always the same in different individuals and at different sites in the body?
- Does the proven action of Cr[VI] on cytokine release by keratinocytes make it a powerful adjuvant as well as an allergenic epitope? Is that the reason why it is such a potent allergen (on the skin)?
- What is the relative importance of cell-mediated and humoral (IgE) immune mechanisms in the effect of a challenge on sensitized subjects?

Answering some of these points might aid the definitive diagnosis and permit more effective treatment of chromium allergies, as well as providing much information about basic mechanisms of immune and allergic responses.

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